Guidance on the Biocidal Products Regulation

Volume III: Human health
Parts B+C: Assessment and evaluation
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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.

[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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1 Abbreviations

Standard term / Abbreviation	Explanation
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
APF	Assigned Protection Factors
ARfD	Acute Reference Dose
a.s.	Active substance
ATP	Adenosine-tri-phosphate
AUC	Area under the curve
BMD	Benchmark dose
BPC	Biocidal Products Committee (ECHA body)
BPD	Biocidal Products Directive. Directive 98/8/EC of the European Parliament and of the Council on the placing on the market of biocidal products
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
b.r.	Biocidal Residue
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.
CAR	Competent Authority Report, also known as the assessment report
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 the consumer exposure model
Cmax	Peak plasma concentration
CNS	Central nervous system
DBP	Disinfection By-Product
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

Standard term / Abbreviation	Explanation
DNEL	Derived No Effect Level
DRA	Dietary Risk Assessment
DRAWG	Dietary Risk Assessment Working Group
EATS	Estrogen, Androgen, Thyroid, Steroidogenesis
EC_{50}	Median effective concentration
EFSA	European Food Safety Agency
EN	European norm
EPA (USA)	Environmental Protection Agency of the United States of America
FAO	Food and Agriculture Organization
FCM	Food contact material
FDA	U.S. Food and Drug Administration
GLP	Good laboratory practice
GSD	Geometric standard deviation
HEEG	Human Exposure Expert Group (under BPD)[4]
HI	Hazard index
HQ	Hazard quotient
IC_{50}	Median immobilisation concentration or median inhibitory concentration 1 (explained by a footnote if necessary)
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
Ka	Acid dissociation coefficient
Km	Michaelis constant, describes the substart 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
LoD	Limit of detection
LoQ	Limit of quantification
M	Molarity
MMAD	Mass median aerodynamic diameter
mmHg	Millimeter(s) of mercury, a unit of pressure equal to 0.001316 atmosphere
mN/m	Millinewton(s) per metre, a unit of torque
mol	Mole(s)

[4] Note: Under BPR replaced by the AdHoc Working Group on Human Exposure

Standard term / Abbreviation	Explanation
MOS	Margin of Safety
MRL	Maximum residue level
MS	Mass spectrometry
MSCA	Member State Competent Authority
MTD	Maximum tolerated dose
NAEL	No Adverse Effect Level
nm	Nanometre(s)
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
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)
PBPK	Physiologically based Pharmacokinetic
PEC	Predicted environmental concentration
pKa	Negative decadic logarithm of the acid dissociation constant (describes how acidic (or not) a given hydrogen atom in a molecule is)
PNEC	Predicted no effect concentration
PPE	Personal Protective Equipment
PPP	Plant Protection Product
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
RDT	Repeated dose toxicity
RMM	Risk Management Measures
RPE	Respiratory Protective Equipment
SDS	Safety data sheet
SD	Standard deviation
SME	Small and medium-sized enterprise
SoC	Substance of concern
SOPs	Standard Operating Procedures developed by the Residential Exposure Assessment Work Group for Residential Exposure Assessments (for the U.S. EPA Office of Pesticide Programs)
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

Standard term / Abbreviation	Explanation
TK	Toxicokinetics
TG	Technical guideline
TGD	Technical Guidance Document
TM	Biocides Technical Meeting, an established subsidiary body responsible for the implementation of the Biocidal Products Directive, together with the European Commission.
TNsG	Technical Notes for Guidance
TTC	Threshold of toxicological concern
TWA	Time weighted average exposure by inhalation.
UDS	Unscheduled DNA synthesis
Vmax	Maximum velocity, reflects how fast the enzyme can catalyze the reaction
VMP	Veterinary Medicinal Product
w/w	Weight per weight ratio
w/v	Weight per volume ratio
WHO	World Health Organisation
WoE	Weight of evidence

1 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.

Standard term / Abbreviation	Explanation
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 those involved in manufacturing, handling and/or packaging of actives 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.
LoD, LoQ - limits of detection and quantitation	are levels below which the biocide cannot be accurately detected (LoD) or measured (LoQ).
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, use of disinfectants in a restaurant by regular employees). A clear definition of the use and user is required to distinguish between professionals and non-professionals. It is assumed that non-professionals will 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.

Standard term / Abbreviation	Explanation
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 dose descriptor (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 rote of application, but are also derived for a specific time-frame. In order to convert systemic/internal reference values into route-specific external ones, the former have to be corrected by the corresponding rate of (dermal, inhalative or oral) absorption, or an estimate thereof.
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.

Standard term / Abbreviation	Explanation
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).
static monitoring	is sampling of background atmospheric concentrations or deposition.
surrogates or tracers	- e.g. strontium salts, dyes, fluorescent agents - are used in surveys and studies to enable analysts to trace the exposure pattern.
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.

1 Table of references

2 Only the "short references" are given within the guidance text.

SHORT REFERENCE	FULL NAME AND/OR LINK
ECHA Guidance on the Application of the CLP criteria	Available at https://echa.europa.eu/guidance-documents/guidance-on-clp
ECHA Guidance Vol I Parts A+B+C	Guidance on the Biocidal Products Regulation, Volume I: Identity of the active substance/physico-chemical properties/analytical methodology – Information Requirements, Evaluation and Assessment. Parts A+B+C Available at https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation
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

ECHA Guidance Vol V Disinfection By-Products	Guidance on the Biocidal Products Regulation, Volume V, Guidance on Disinfection By-Products
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
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
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
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
REACH Guidance R.5	Guidance on information requirements and chemical safety assessment Chapter R.5: Adaptation of information requirements, available at https://echa.europa.eu/guidance-documents/guidance-on-reach
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
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
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
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
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
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
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

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
OECD GD 203	OECD Guidance Document No. 203 on an Integrated Approach on Testing and Assessment (IATA) for skin corrosion/irritation
OECD GD 263	OECD Guidance Document No. 263 on an Integrated Approach on Testing and Assessment (IATA) for serious eye damage and eye irritation
OECD 497	OECD Guideline No. 497: Defined Approaches on Skin Sensitisation
WHO/IPCS, 2012	Guidance for Immunotoxicity risk assessment for chemicals by WHO/IPCS (WHO/IPCS, 2012)
OECD TG	OECD Test Guidelines for testing of chemicals; available at https://www.oecd.org/env/ehs/testing/seriesontestingandassessmentadoptedguidanceandreviewdocuments.htm
OECD IATA for phototoxicity	OECD draft guidance document on an integrated approach on testing and assessment (IATA) for phototoxicity (2024) https://www.oecd.org/chemicalsafety/testing/draft-guidance-document-integrated-approach-on-testing-and-assessment-for-phototoxicity.pdf
OECD overview on genetic toxicology TGs (2017)	Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015, ENV/JM/MONO(2016)33 Series on Testing & Assessment No. 238 https://www.oecd.org/publications/overview-on-genetic-toxicology-tgs-9789264274761-en.htm
HERA guidance	HERA guidance document Methodology, February 2005
EMA-CVMP guidance	EMA-CVMP guidance "Guideline on risk characterisation and assessment of maximum residue limits (MRL) for biocides" (EMA/CVMP/SWP/90250/2010)
JECFA ARfD guidance	Joint FAO/WHO Expert Committee on Food Additives (JECFA); Guidance document for the establishment of Acute Reference Dose (ARfD) for veterinary drug residues in food https://cdn.who.int/media/docs/default-source/food-safety/jecfa/guidance-document-arfd-2017.pdf

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2

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) is given in BPR Annex VI.

The evaluating CA 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 or not the (representative) biocidal product complies with the criteria for authorisation set down in Article 19(1)(b) or whether the active substance may be approved.

This guidance explains how to perform the risk assessment to evaluate the effects on human health.

Assessment

The risk assessment process, in relation to human health entails a sequence of actions which is 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 and indirectly via the environment) may be exposed.

(3) **Risk characterisation:** estimation of the acceptability, incidence and severity of the adverse effects likely to occur in a human population due to actual or predicted exposure to a 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.

Possible results of the risk assessment for biocidal active substances:

- Recommendation to approve an active substance for use in biocidal products, where necessary subject to certain requirements.
- Recommendation not to approve an active substance for use in biocidal products.

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 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 based on representative monitoring data and/or on model calculations. 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. Preference should be given to the best and most realistic information available.

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 toxic 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 (the outcome of the hazard characterisation).

The exposure levels are derived based on available monitoring data and/or model calculations. The NOAEL/LOAEL values are determined on the basis of results from animal testing or available human data.

For some substances, it is not possible to derive an AEL value. As an example, for genotoxic substances it is considered prudent to assume that a threshold exposure level cannot be identified (see chapter 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 the 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 it has an impact on TK and metabolism.

The decision whether a substance presents a risk to human health is taken on the basis of whether exposure level exceeds AEL/AEC. 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 heavily 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 the *Guidance on the BPR: Volume III Human Health, Part A Information Requirements.* 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, if available or obtainable. At each stage, integrated requirements for further testing must be developed, using professional 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 procedure:

- **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 both animal data and human data are available, as a general rule, well reported relevant human data for any endpoint is given preference in the risk assessment. 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 not usually 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, the *Guidance on the BPR: Volume III Human Health, Part A Information Requirements* needs to be considered together with this Guidance as well as with the *Guidance on the Application of CLP*. 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, as part of step 3 and using the *Guidance on the BPR: Volume III Human Health, Part A Information Requirements*, 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[5], the QSAR Toolbox[6] and US EPA CompTox Chemicals Dashboard[7] 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. Literature databases should also be considered.

1.2 Evaluation of data

In all stages of effects assessment, it is very important to evaluate the adequacy and completeness of the data. This is particularly important for existing substances where there may be a number of test results 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 guidance provided in this section, the *Guidance on information requirements and chemical safety assessment, Chapter R.4 (Evaluation of available information)* provides further guidance for assessing the relevance, reliability, and adequacy of the information.

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

[5] http://www.echemportal.org
[6] http://www.qsartoolbox.org
[7] https://comptox.epa.gov/dashboard/

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, please see the Guidance on information requirements[8].

1.2.2 Adequacy of data

The adequacy of data can be considered to 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 attached to the most reliable and relevant.

1.2.3 Reliability of data

For biocidal active substances, tests conducted according to the EU Test Methods Regulation (Regulation (EC) No 440/2008) and in compliance with the principles of GLP will be available, and consequently many of the issues addressed in this section will not be relevant.

For some existing biocidal 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[9] or U.S. EPA Test Guidelines[10]) and appropriately reported should be considered most reliable for risk assessment. The scoring system developed by Klimisch *et al*. (1997)[11] 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

[8] 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.
[9] http://www.oecd.org/env/ehs/
[10] https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances
[11] Klimisch, H. et al. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regulatory toxicology and pharmacology: RTP 25 1 (1997): 1-5

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.

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 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 are 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 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 are not very 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 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) 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, have to be 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).

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 must not be conducted specifically for the purpose of BPR. It is emphasised that testing with human volunteers is strongly discouraged but when good quality data are already available, they may be used in well justified cases.

***In vitro* data**

It can be expected that some of the available data have been derived from studies conducted *in vitro*. The usefulness of these studies will be determined by their adequacy in the 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 Guidance Document on Good *In Vitro* Method Practices (GIVIMP)[12] . 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 the 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).

[12] 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

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.

The dose-response relationships in the animal studies (or the severity of the effect, when only a single dose was tested) are also assessed as a part of the risk assessment process. These assessments are taken into account at the risk characterisation stage when a judgement is made of the likelihood of occurrence of an adverse effect in humans at a particular 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 Guidance Document on Good *In Vitro* Method Practices (GIVIMP)).

For some studies, *in 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.

(Quantitative) Structure-Activity Relationships ((Q)SARs)

When experimental data do not exist for a given endpoint, or when data are limited, the use of Structure-Activity Relationships (SARs) may be considered. It should be noted that SAR techniques and methods, particularly for QSARs models are not well developed for application in risk assessment especially in relation to long-term mammalian toxicity. The SARs which are used for the risk assessment purpose are usually more of qualitative

nature and are not addressing quantitative aspects.

QSAR models are usually developed to give binary results; the substance is predicted to have or not have a particular property. If the substance is predicted to have that property, the result of a QSAR prediction is considered as positive. Similarly, if the substance is predicted not to have a particular property, the result of the QSAR prediction is considered negative.

If the applicability domain is appropriate, QSAR models could be used as part of a WoE approach, when considered alongside other data. QSAR can also be used as supporting evidence when assessing the toxicological properties by read-across. Positive and negative QSAR modelling results can be of value in a read-across assessment. For more information on QSARs and grouping of substances, see ECHA (2022) Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.6: Guidance on QSARs and grouping of substances.

SARs may be of value in indicating a potential hazard, toxicokinetic properties or the need for further testing.

1.2.4 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. As this would rarely be the case, further considerations are given in chapters 1.2.4.1 to 1.2.4.3. These chapters 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 chapter 1.2.4 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.

1.2.4.1 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.

1.2.4.2 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.

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 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.

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 nonapproval of the active substance as specified and reducing the concentration of the impurity to a level at which it would not impact the toxicity.

1.2.4.3 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 Chapter 1.2.4.2.

1.2.5 Considerations on specific effects

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

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[13]

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.

1.3 Toxicokinetics

Toxicokinetic data of a substance are needed for the interpretation of toxicological findings and hence in the risk assessment process. 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 assessment factors. 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 Pharmacokinetic (PBPK) modelling.

Section 1.8 on TK within the ECHA Guidance Vol III Part A should be considered together with this section for the assessment of TK. Further information is also available in REACH Guidance R.7c

1.3.1 Definitions

Toxicokinetics (TK) is used to describe the time-dependent fate of a substance within the

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

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:

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).

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 recirculation. 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 recirculation 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 in 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 1 provides an overview of different types of data that can be considered for the estimation of oral/GI absorption.

Table 1: 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.

Data source	What it tells us
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 2 provides an overview of the type of data that can be considered for the estimation of respiratory absorption.

Table 2: 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 can not 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 highly lipophilic compounds in the viable epidermis are the most resistant to penetration.

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. When test data is not

available, default values suggested in (EFSA, 2017[14]) can be used as a first step.

For the purpose of estimating dermal absorption for biocidal active substance and products, the principles described in the EFSA Guidance Document for dermal absorption (EFSA, 2017[14]) should be followed, complemented with the OECD Guidance on Dermal Absorption (OECD, 2004; OECD, 2022[15]).

Table 3 provides an overview of the type of data to be considered for dermal absorption estimation.

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 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, 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.

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

Table 3: 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

[14] EFSA Journal 2017;15(6):4873; doi: 10.2903/j.efsa.2017.4873
[15] https://one.oecd.org/document/ENV/JM/MONO(2011)36/REV1/en/pdf

Data source	What it tells us
	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.

Data source	What it tells us
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 concentrations, 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 3 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, 2017[14]) 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.

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. 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. If information of such interactions is available, it 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*.

In following the EFSA guidance on dermal absorption (2017)[14], 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[16], 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 (2017)[14].

Where dermal absorption to animals such as livestock 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.

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 is 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.

PBPK 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.

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

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

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-testes 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 4 provides an overview of data that can be considered for estimation of distribution.

Table 4: 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 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 detoxicification; 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 5 provides an overview of the data that can be used for estimation of excretion.

Table 5: 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 counter-balances 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 6 provides an overview of data that can be considered for the estimation of

accumulation.

Table 6: 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.
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. At least some of the substance becoming systemically bioavailable is referred

to as systemic exposure.

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).

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. It is advised to consider systematically the possible substrates for non-linear kinetics, especially for repeated dose testing.

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.

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 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 PBPK 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, QSAR 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. 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 may 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 process and to identify sensitive populations. For a discussion on the applicability of physiologically-based kinetic modelling for the development of assessment factors 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.

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 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.[17]

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[18]) 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.

[17] 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.

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

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.

1.3.12 Using toxicokinetic information

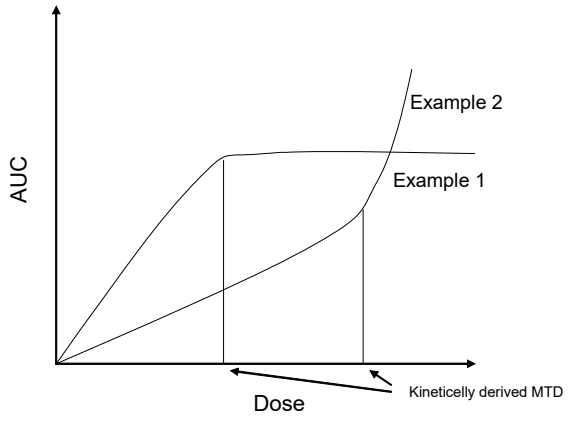
1.3.12.1 Dose setting for repeated dose studies

TK data, especially information on absorption, metabolism, and elimination, are useful in designing repeated dose toxicity (RDT) 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 RDT 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 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 assessment factors (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 recirculation, 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:

Yquantity of interest
aspecies-independent scaling coefficient (fitting data points to form a curve)
BMbody mass
ballometric 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 predicts the total amount of a substance administered by one route that would produce the same systemic toxic response as that obtained for a given amount administered by another route.

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;
- 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 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

The section on Acute Toxicity, Section 1.7. of the ECHA Guidance Vol III Part A should be considered together with the elements described in this section for the assessment of 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 the 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 LC_{50}, 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;
- to 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 a 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, it should be considered for low volatility substances, which are defined as 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 the 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

Guidance on the application of read across on grouping approaches is provided in:

- the REACH Guidance R.6.
- the Read Across Assessment Framework.

(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 QSAR 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.

Examples of available QSAR systems for acute toxicity are available in the REACH Guidance R.7a.

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

1.4.2.1.2 Testing 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 the 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);
- unreferenced data reported in secondary sources (e.g. toxicology handbooks);
- 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 Commission Regulation (EC) No 440/2008; tests to study effects on particular organs/systems such as the cardiovascular system).

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 the 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 local 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 or NOAEL. 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 (see Section 1.2.3), the relevance and the adequacy of the data 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, gender, 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 h are often achieved using Haber's Law ($C \times t = k$). 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 *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:

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

The C × t approach uses two animals per C × 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 useful 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). Studies on humans should not be performed for the purposes of BPR.

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 particular 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 compromises 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 thought to be of low toxicity on the basis of single exposure studies in animals will not cause adverse effects in humans.

1.4.4 Concluding on suitability for Classification and Labelling

The Guidance for the implementation of the CLP Regulation shall be followed with regard to the use of the data for classification and labelling. If the data available is not sufficient, additional testing will 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 posed by a substance 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 then it is unlikely that lower dose levels will have been investigated and in this case identification of the 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 the most 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. In this situation, it may not be appropriate to determine a threshold level from this 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 particular 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 *Risk Characterisation for local effects*.

The elements described in this section should be considered together with other guidance presented in Table 7.

Table 7: 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
	ECHA Guidance on the Application of the CLP criteria	3.2. Skin corrosion/irritation
	OECD Guidance Document No. 203 on an Integrated Approach on Testing and Assessment (IATA) for skin corrosion/irritation	
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
	ECHA Guidance on the Application of the CLP Criteria	3.3. Serious eye damage/eye irritation
	OECD Guidance Document No. 263 on an Integrated Approach on Testing and Assessment (IATA) for serious eye damage and eye irritation	

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 CLP Regulation.

The following definitions are taken from the Guidance on the Application of the CLP Criteria:

- **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, pruritis and/or pain and impair function causing symptoms such as cough, pain, choking, and breathing difficulties. The evaluation is based primarily on human data.

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 Guidance on the Application of the CLP Criteria:

- 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).

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, whatever type of study provides the information.

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, small numbers of subjects.

Data from animal studies in accordance with internationally accepted test methods will usually give very good information on the skin or eye irritancy of a substance in the test species. It is generally assumed that when tested in animals according to internationally accepted test methods, substances which are irritant to animals will be skin and/or eye irritant in humans, and those which are not irritant to animals will not be irritant in humans. Data of 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. However, inconsistent results from more than one similar studies increases the uncertainty in using data from animal studies.

The data obtained from *in vitro* studies may include many dose levels and replicates: when such a study has a well-defined mechanistic basis and indicates that a substance is expected to be irritating, this may be sufficient for hazard identification.

1.5.5 Concluding on suitability for Classification and Labelling

In order to conclude on classification and labelling, all the available information needs to be taken into account. The Guidance on the Application of the CLP Criteria should be followed.

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 *Risk Characterisation for local effects*).

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 *Risk Characterisation for local effects* and the guidance listed in table 8.

Table 8: 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
ECHA Guidance on the Application of the CLP Criteria	3.4. Respiratory or skin sensitisation
OECD Guideline No. 497: Defined Approaches on Skin Sensitisation	

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. In this Section the endpoints discussed 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 Guidance on the Application of the CLP Criteria:

- **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 skin and respiratory sensitisation is induction of specialised immunological memory in an individual by exposure to an allergen. 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

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 Guidance on the Application of the CLP Criteria:

- 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 Weight of Evidence and the potency assessment of skin and respiratory sensitisation.

1.6.3 Remaining uncertainty on sensitisation

The following situations may increase the uncertainty on 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 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.

- 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 needs 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).

1.7 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 (See also Section 4.1.4 Refinement of risk characterisation).

1.7.1 Definition of repeated dose toxicity

Repeated dose toxicity comprises the adverse general toxicological effects (i.e. excluding reproductive, genotoxic or carcinogenic effects) occurring as a result of repeated daily exposure to a substance for a part of the expected lifespan (sub-acute or sub-chronic exposure) or for the major part of the lifespan for chronic exposure.

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 system
- pathological alterations in organs and tissues as examined macroscopically and microscopically.

Repeated dose toxicity studies may also examine parameters, which have the potential to identify specific manifestations of toxicity such as neurotoxicity, immunotoxicity, endocrine mediated effects, reproductive toxicity and carcinogenicity.

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 (OECD, 2009).

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 effects as a consequence of systemic toxicity or secondary to systemic distribution of the substance or its active 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 lead to the identification of NOAEL. 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 potentially may also 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, potential cumulative effects 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 risk 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

The potential toxicity of a substance for which no or limited data are available on a specific endpoint can, in some cases, be evaluated by read-across from structurally or mechanistically related substances for which experimental data exists. The read-across approach is based on the principle that structurally and/or mechanistically related substances may have similar toxicological properties. Note that there are no formal criteria to identify structural alerts for repeated dose toxicity or for read-across to closely related substances.

Based on structural similarities between different substances, the repeated dose toxicity potential of one substance or a group of substances can be extended (read-across) to a substance for which there are no or limited data.

A mode of action identified for a substance and/or group of substances and causally related to adverse effects in a target organ can be extended (read-across) to a substance for which a similar mechanism or mode of action has been identified, but where no or limited data on repeated dose toxicity are available. In such cases, the substance under evaluation may reasonably be expected to exhibit the same pattern of toxicity in the target organs and tissues.

(c) (Q)SAR systems

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

Overall, (Q)SAR approaches are currently not well validated for repeated dose toxicity and consequently 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 today 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 QSAR modelling is that dose-response information, including NOAEL, is not provided. Similarly, a validated QSAR 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, QSAR models could be used as part of a WoE approach, when considered alongside other data, provided the applicability domain is appropriate. Also, QSARs can be used as supporting evidence when assessing the toxicological properties by read-across within a substance grouping approach, providing the applicability domain is appropriate. Positive and negative QSAR 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

(a) *In vitro* data

Available *in vitro* data alone is currently not useful for regulatory decisions such as risk assessment and C&L. However, such data may be helpful in the assessment of repeated dose toxicity, 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.

Generic guidance is given in the REACH Guidance R.4 and R.5 for judging the applicability and validity of the outcome of various study methods, assessing the quality of the conduct of a study, reproducibility of data and aspects such as vehicle, number of replicates, exposure/incubation time, GLP-compliance or comparable quality description.

(b) Animal data

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

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

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.

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.

Chronic toxicity studies:

The 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.

The 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, the 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, which 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.

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.

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.

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.

Other studies providing information on repeated dose toxicity:

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 repeated dose toxicity studies can provide information on potential reproductive toxicity and on carcinogenicity (e.g., pre-neoplastic lesions).

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 (draft 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.

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.

The following general guidance is provided for the evaluation of repeated dose toxicity

data and the development of the WoE, considering also all other information (including non test methods):

- 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 in determining the most relevant NOAEL.

If sufficient evidence is available to identify the critical effects with regard to dose-response relationships and relevance for humans, and the target organs and/or tissues, greater weight should be given to specific studies investigating this effect in the identification of the NOAEL. The critical effect can be local or systemic.

Data from repeated dose toxicity studies not performed according to conventional guidelines and/or GLP may still provide relevant information but requires careful and critical evaluation.

Data from non-guideline studies can be considered similar to data generated by corresponding test methods if all the following conditions are met:

- adequate for the purpose of classification and labelling and/or risk assessment;
- adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding OECD/EU test methods;
- Exposure duration comparable to or longer than the corresponding OECD/EU test method if exposure duration is a relevant parameter; and
- adequate and reliable documentation of the study.

If the above conditions are not met, non-guideline studies may contribute to the overall weight of the evidence but cannot be used to conclude a substance as being adequately tested for repeated dose toxicity.

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.

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 compared to those for the 28-day and 90-day dermal and inhalation protocols.

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.

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.

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.

Table 9: List of *in vivo* repeated dose toxicity test guideline studies

Test Subacute	Route	Name
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

Test Subacute	Route	Name
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	Chronic toxicity studies

Table 10: 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	Detailed clinical observations Functional observations (sensory reactivity to stimuli of different types, grip strength, motor activity, more specialized tests on indication) Ophthalmological examination Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, platelet count, blood clotting time/potential)

Test	Design	Endpoints (general toxicity)
	of administration	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) Delayed neurotoxicity of organophosphorus substances: 28-day repeated dose study	Exposure for 28 days At least 3 dose levels plus control At least 12 birds per group Species: domestic laying hen	Detailed clinical observations Body weight and food/water consumption 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	As described in OECD TG 443	As described in OECD TG 443

Test	Design	Endpoints (general toxicity)
reproductive toxicity study		
OECD TG 414 (EU B.31) Prenatal developmental toxicity study	Exposure at least from implantation to one or two days before expected birth At least 3 dose levels plus control At least 20 pregnant females per group	Clinical observations Body weight and food/water consumption Macroscopical examination 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/developmental 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

Test	Design	Endpoints (general toxicity)
		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 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. However, human volunteer studies should not be performed for the purposes of the BPR.

The following types of human data may be available however:

- 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.
- Controlled studies in human volunteers, 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 Specific system/organ toxicity

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 is the induction of adverse effects in the central or peripheral nervous system, or in sense organs. It is useful for the purpose of hazard and risk assessment to differentiate sense organ specific effects from other effects which lie within the nervous system. A substance is considered "neurotoxic" if it induces a reproducible lesion in the nervous system or a reproducible pattern of neural dysfunction.

1.7.3.2.2 Introduction

A hierarchical approach is recommented in the investigation of the potential neurotoxicity of substances. The starting point for the testing strategy should be exposure considerations, *in vitro* data and SAR and should proceed via data already available from base set tests to more specific testing. Any indications of specific or non-specific neurotoxicity in the acute and repeated dose toxicity tests are important. If there are SAR alerts or available information on the substance or similar substances, and repeated dose toxicity testing is needed, it would be prudent to investigate neurotoxicity within the repeated dose test.

The OECD/EU oral 28-day and 90-day tests examine a number of simple nervous system endpoints, e.g. clinical observations of motor and autonomous nervous system activity and histopathology of nerve tissue, which should be regarded as the starting point for evaluation of potential neurotoxicity. The standard 28-day and 90-day tests measure only some aspects of nervous system structure and function, while e.g. learning and memory and sensory function is not tested, or only superficially tested. SAR considerations may prompt the introduction of additional parameters to be included in standard toxicity tests or the immediate request of studies such as delayed neurotoxicity.

1.7.3.2.3 Structure-activity considerations

Structural alerts are only used as a positive indication of neurotoxic potential. Substance classes with an alert for neurotoxicity may include organic solvents (for chronic toxic encephalopathy); organophosphorus compounds (for delayed neurotoxicity), carbamates (for cholinergic effects), pyrethroids (sodium channel inhibitors) and neonicotinoids (disruptors of neural transmission).

1.7.3.2.4 Assessment of available information or results from initial testing

Signs of neurotoxicity in standard acute or repeated dose toxicity tests may be secondary to other systemic toxicity or to discomfort from physical effects such as a distended or blocked GI tract. Nervous system effects alone seen at dose levels near or above those causing lethality should not be considered as evidence of neurotoxicity. 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), and a distinction should be made between specific and non-specific signs of neurotoxicity.

Neurotoxicity may be indicated by the following signs:

- morphological (structural) changes in the central or peripheral nervous system or in special sense organs;
- neurophysiological changes (e.g. electroencephalographic changes);
- behavioural (functional) changes;
- neurochemical changes (e.g. neurotransmitter levels).

The type, severity, number and reversibility of the effect should be considered. Generally, a pattern of related effects is more persuasive evidence of neurotoxicity than one effect or a few unrelated effects.

It is important to ascertain whether the nervous system is the primary target organ, and whether the neurotoxic effects are reversible. The potential for such effects in exposed humans (i.e. the exposure pattern and estimated level of exposure are "acute") should be considered in the risk characterisation. Also reversible effects may be of high concern depending on the severity and nature of effect. Effects observed in experimental animals that appear harmless might be of high concern in humans depending on the setting in which they occur, e.g. sleepiness in relation to operation of machinery. The overt effect being transient does not exclude that a permanent lesion has occurred. The nervous system possesses reserve capacity, which may compensate for damage, but the resulting reduction in the reserve capacity is an adverse effect. Compensation may be suspected if a neurotoxic effect slowly resolves during the lifespan. This could be the case for developmental neurotoxicants. Irreversible neurotoxic effects are of high concern and usually involve structural changes. However, at least in humans, lasting functional effects (e.g. depression, involuntary motor tremor) are suspected to occur as a result of neurotoxicant exposure without morphological abnormalities.

1.7.3.2.5 Cholinesterase inhibition

The inhibition of brain acetylcholinesterase activity and clinical signs are considered to be the primary endpoints of concern in toxicological studies on compounds that inhibit acetylcholinesterases.

Inhibition of erythrocyte acetylcholinesterase is also considered to be an adverse effect, insofar as it is used as a surrogate for brain and peripheral nerve acetylcholinesterase inhibition, when data on the brain enzyme are not available. The use of erythrocyte acetylcholinesterase inhibition as a surrogate for peripheral effects is justified for acute exposures resulting in greater acetylcholinesterase inhibition in erythrocytes than in the brain. However, reliance on inhibition of erythrocytic enzyme in repeated dose studies might result in an overestimate of inhibition on peripheral tissues, because of the lower rate of re-synthesis of the enzyme in erythrocytes than in the nervous system.

Plasma acetylcholinesterase inhibition is not considered relevant.

For brain and erythrocyte acetylcholinesterase inhibition, statistically significant inhibition by 20% or more represents a clear toxicological effect and dismissing such findings should be justified. Statistically significant inhibition of less than 20% or inhibition above 20% without statistical significance should trigger a more detailed analysis of the data. The toxicological significance of these findings should be determined on a case-by-case basis, considering also dose response.

Certain substances and effects are best investigated in particular species. Pyridine derivatives are neurotoxic to humans and primates but not to rats. Organophosphorus compounds are a group with known delayed neurotoxic properties, which need to be assessed in a specific test for delayed neurotoxicity, to be performed preferentially in the adult laying hen according to the EU B.37 / OECD TG 418 (Delayed neurotoxicity of organophosphorus substances following acute exposure) and the EU B.38 / OECD TG 419 (Delayed neurotoxicity of organophosphorus substances: 28-day repeated dose study). Such studies are specifically required for biocidal substances of similar or related structures to those capable of inducing delayed neurotoxicity. If anticholinesterase activity is detected, a test for response to reactivating agent may be required.

1.7.3.2.6 Further neurotoxicity testing

If the data acquired from the standard systemic toxicity tests are inadequate or provide

indications of neurotoxicity which are not adequate for risk characterisation, the nature of further investigation will need to be considered. Additional guidance is provided in the ECHA Guidance Vol III Part A.

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, 2012).

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 recognized 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 Guidance for Immunotoxicity risk assessment for chemicals by WHO/IPCS (WHO/IPCS, 2012) 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.

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 of the approach to assessment of potential immunotoxicity is that many immunotoxic substances can be identified via the standard tests for systemic toxicity, particularly if the relevant additional measures of the 28-day and 90-day test guidelines are used. 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.

The need for further testing to examine immunotoxicity in more depth, repeated dose test will be considered on a case-by-case basis. Substances with SAR indications of potential immunotoxicity, but no indications from the repeated-dose test results, may also need to be considered for further testing for immunotoxicity. The timing of any further testing to investigate immunotoxicity will be influenced by the level of concern in relation to both the observed/expected effects and the potential for human exposure. The severity of the effect, its implications for human health and which human populations are exposed (e.g. workers and/or consumers) will be influencing factors.

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, immunoglobulin concentrations if determined).

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 BPR Guidance Vol III Part A.

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, in the workplace, overload related effects can be avoided 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 Section 2.3).

1.7.4.1 Threshold of the critical effect

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.

The NOAEL is typically used as the starting point in deriving the threshold level (AEL, ADI). In case a NOAEL has not been achieved, a LOAEL may be used. BMD may also be used as the starting point.

The selection of NOAEL/LOAEL is usually based on the dose levels used in the most relevant toxicity study, without considering the shape of the dose response curve. Therefore, the NOAEL/LOAEL may not reflect the true threshold for the adverse effect. On the other hand, the BMD is a statistical approach for the determination of the threshold and relies on the dose response curve. The use of such approaches to estimate the threshold should be considered on a case-by-case basis. For further guidance see Section 2.

1.7.4.2 Other considerations

When testing is not technically possible, approaches such as QSAR, 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 dose descriptor. 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 dose descriptor (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 and/or a dose- and time-response relationship, biological relevance, reversibility, and the normal biological variation of an effect that may be shown by representative historical control values.

1.7.6 Concluding on suitability for Classification and Labelling

In concluding on classification and labelling, the ECHA Guidance on the Application of the CLP criteria should be used.

1.7.7 Concluding on suitability for risk assessment

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

The dose descriptor 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).

1.8 Mutagenicity

For the assessment of mutagenicity, sections 1.5 and 1.6 of the ECHA Guidance Vol. III Part A should be considered together with the elements described in this section.

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.

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.

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 the introductory section 1.2.3. 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 (Reichstein, 2023[19]; Brendt, 2021[20]).

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

[19] Reichstein, IS et al. “Replacing animal-derived components in in vitro test guidelines OECD 455 and 487.” The Science of the total environment vol. 868 (2023): 161454.
[20] Brendt, J et al. “Is a liver comparable to a liver? A comparison of different rat-derived S9-fractions with a biotechnological animal-free alternative in the Ames fluctuation assay.” The Science of the total environment vol. 759 (2021): 143522.

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, and 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:

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

Detailed guidance on the assessment of the non testing data for mutagenicity is provided in Section R.7.7.4.1 of REACH Guidance R.7a (2025[21]).

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 11: *In vitro* test methods

Test method	Genotoxic endpoint measured	EU/OECD

[21] Full reference to be included. The Guidance is currently under PEG consultation, draft available at https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach.

	Principle of test method and special considerations	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 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 (DRPs) 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 12: 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 mutations and chromosomal rearrangements (the latter specifically in the LacZ plasmid mouse model)	OECD TG 488

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

gene mutation assays	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.	EU B.58
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

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2 **Table 13: 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	OECD TG 483 EU B.23

Test method	Genotoxic endpoints measured Principle of the test method	EU/OECD guideline
	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.	
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
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 the OECD overview document of the genetic toxicology test guidelines (OECD,

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

2017[24]).

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.
  - 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?
  - Was the test system sensitive to the nature of the genotoxic changes that might have been expected? For example, some *in vitro* test systems will be sensitive to point mutations and small deletions but not to mutagenic events that create large deletions.
  - 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

[24] OECD, Overview on Genetic Toxicology TGs : https://www.oecd.org/publications/overview-on-genetic-toxicology-tgs-9789264274761-en.htm

(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?

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. For each test type and each genotoxic endpoint, there should be a separate WoE analysis. 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 in order 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 the 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 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.

Negative *in vitro* genotoxicity testing

In general, substances that are negative in the full set of *in vitro* tests are considered to be 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 of industrial chemicals. 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).

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.

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 the OECD overview document of the genetic toxicology test guidelines (OECD overview on genetic toxicology TGs, 2017).

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 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.

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, 2017[25]). 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 alone 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

[25] EFSA, European Food Safety Authority (2017) Clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017 15(12):5113 [25 pp.] Available online: https://doi.org/10.2903/j.efsa.2017.5113

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 involving studies to focus on tissues at sites of contact, such as the glandular stomach and the duodenum (oral administration) or the lung (inhalation), must be considered. 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.

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, and thereby save on 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, 2011[26]). 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 overview on genetic toxicology TGs, 2017). 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 overview document of the genetic toxicology test guidelines (OECD overview on genetic toxicology TGs, 2017) 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 the overview document of the genetic toxicology test guidelines (OECD overview on genetic toxicology TGs, 2017), 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).

[26] 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 2011 9(9):2379 [69 pp.] Available online: www.efsa.europa.eu/efsajournal

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 recommendations on how to demonstrate bone marrow exposure can be found in EFSA, 2017[27] and OECD overview on genetic toxicology TGs, 2017.

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.

[27] EFSA, European Food Safety Authority (2017) Clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017 15(12):5113 [25 pp.] Available online: https://doi.org/10.2903/j.efsa.2017.5113

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.

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.

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

The available data should be considered using the CLP criteria and Section 3.5 "Germ Cell Mutagenicity" of the ECHA Guidance on the Application of the CLP criteria.

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. EFSA has provide guidance on the risk assessment of aneugenic substance (EFSA, 2021[28]).

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)

[28] EFSA, European Food Safety Authority (2021) Scientific Opinion on the guidance on aneugenicity assessment. EFSA Journal 2021;19(8):6770, 27 pp. https://doi.org/10.2903/j.efsa.2021.6770

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 (anti-oxidants 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, 2015[29]).

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.

- the OECD overview document of the genetic toxicology test guidelines (OECD overview on genetic toxicology TGs, 2017) 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).

[29] Guérard M, Baum M, Bitsch A, Eisenbrand G, Elhajouji A, Epe B, Habermeyer M, Kaina B, Martus HJ, Pfuhler S, Schmitz C, Sutter A, Thomas AD, Ziemann C and Froetschl R (2015) Assessment of mechanisms driving non-linear dose-response relationships in genotoxicity testing. Mutat Res Rev Mutat Res 763:181-201.

- 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 dose descriptors 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 (ECHA, 2018[30]).

1.9 Carcinogenicity

The section on Carcinogenicity of the ECHA Guidance Vol III Part A should be considered together with the elements described in this section.

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 ECHA Guidance on the Application of the CLP criteria). 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 extrapolation from structurally similar substances (read-across). The determination of the carcinogenic potential of a chemical is based on a WoE approach. 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

[30] 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

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 genetic alterations indirectly following interaction with other cellular processes (e.g. secondary to the induction of oxidative stress).

Non-genotoxic modes of action include:

- epigenetic changes, i.e. effects that do not involve alterations in DNA 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,

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 directly genotoxic or not. 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.

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.

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 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.

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 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 principle 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. The degree of reliability for each study on the carcinogenic potential of a substance should be evaluated. 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, the ECHA Guidance on the Application of the CLP criteria should be used.

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 dose descriptor 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.

If the available information would lead to classification for reproductive toxicity, substances are allocated to one of two (sub)categories:

- Category 1: Known or presumed human reproductive toxicant
  - Category 1A: the classification of a substance in is largely based on evidence from humans.
  - Category 1B: the classification is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development.
- Category 2: Suspected human reproductive toxicant.
  - Some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1

Within each category, adverse effects on sexual function and fertility, and on development, are considered separately. In addition, effects on or via lactation are allocated to a separate single hazard category. Effects on or via lactation can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

If the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments.

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)[31], 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

[31] 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.

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).

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 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 1Chemical grouping or read-across and (Q)SAR models and

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. QSAR 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 QSAR prediction. Another limitation of QSAR modelling is that dose-response information (e.g. NOAEL) required for risk assessment is not provided. A negative result from current QSAR 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-fetal crosstalk and biotransformation which may differ depending on the organ and the estimation of the point of departure 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 weight of evidence 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[32].

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[33] or OECD test guidelines 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)

Several other assays, or combinations thereof, have been proposed to predict (specific aspects of) developmental (neuro)toxicity[34], 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, as well as internationally agreed testing methods by OECD.

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 a classification decision and risk assessment. *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

[32] See CLP Annex I, 3.7.2.5.4.

[33] 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

[34] 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)

from the assessment of endocrine disruption. For endocrine activity, there is the additional requirement for the biologically plausible link between endocrine activity and adversity. While negative results from *in vitro* tests, QSAR predictions and/or *in chemico* assays do not provide enough confidence for regulatory decision making to demonstrate absence of a reproductive hazard[35], they may provide valuable support for read across justification and contribute to a weight of evidence 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);[36]
- 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[37]), 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 for anti-oestronicity);

[35] 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.

[36] 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).

[37] OECD TG 408 has 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.

- Hershberger bioassay in rats: a short-term screening assay for (anti)androgenic properties (EU B.55, OECD TG 441 and GD 115);
- Studies on juvenile/peripubertal animals.

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

- mechanistic studies;
- toxicokinetic studies (EU B.36, OECD TG 417);
- studies in fish (e.g. Fish Sexual Development Test (OECD TG 234));
- studies in amphibians (e.g. Amphibian Metamorphoses Assay (OECD TG 231) or Larval Amphibian Growth and Development Assay (EU C.53, OECD TG 241));
- studies in other non-mammalian species.

1.10.2.3 In vivo reproductive toxicity tests:

1.10.2.3.1 Prenatal development toxicity study (OECD TG 414) (two species)

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.

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 Guidance on the Biocidal Products Regulation, Volume III Part A.

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 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 OECD TG 443 with additional investigation for cognitive functions (see also Guidance on the Biocidal Products Regulation, Volume 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.

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 OECD TG 443. Testing for cognitive functions needs to be added if DNT is investigated via OECD TG

443. 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 extended one generation study in accordance with OECD TG 443 (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.

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) should be addressed and concluded under developmental toxicity. This is because in OECD TG 443, 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[38].

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.

Positive treatment-related effects in a developmental neurotoxicity study are relevant to developmental toxicity hazard classification and the human health risk assessment, providing a NOAEL/LOAEL, 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 human or it can be clearly demonstrated that the effects are solely secondary non-specific effects (secondary non-specific consequence of maternal toxicity, see section 1.10.2.3). Note that if the developmental neurotoxic effects are mediated via endocrine activity, they are relevant also for the assessment of endocrine disruption in

[38] RAC Guidance Note: Addressing developmental neurotoxicity and neurotoxicity under the current CLP hazard classes

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 than 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 CLP Regulation does not refer to higher sensitivity of offspring as compared to parental generation in the classification criteria for developmental toxicity or for effects on or via lactation.

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 critical appraisal of the adequacy of controls, the quality and relevance of the effects and an assessment of the exposure. 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 1,000 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. In addition, the aim of classification is to identify the intrinsic toxic properties of the substance on reproduction and do not take into consideration specific exposure scenarios. There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding inclusion within the criteria of a specific dose as a limit dose. However, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. In practise, specification of the actual 'limit dose' will depend upon the test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by the oral route, an upper dose of 1 000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level.

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 classification and labelling

In order to conclude on a proper classification and labelling, all the available information

needs to be taken into account. For the legal reference, see Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures and for the more detailed guidance, see Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures.

1.10.5 Concluding on suitability for risk assessment

In order to be suitable for risk assessment, appropriate threshold levels (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

The section on Endocrine disruption within the ECHA Guidance Volume III Part A, should be considered together with the elements described in this section for the assessment of endocrine disruption.

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[39], together with a specific guidance on how to assess whether active substance would meet the ED criteria[40]. 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[41] 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;*
>
> *(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:

- '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;

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[39] 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

[40] ECHA/EFSA (2018) Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

[41] The same definition is used for Plant Protection Products, as established in Regulations (EU) 2018/605 and (EU) 2023/707.

- '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[42] as a separate hazard class with sub-categorisation[43]. The assessment under BPR requires a conclusion regarding the ED properties of the active substance, without sub-categorization.

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[44] 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, 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 by estrogen (E), androgen (A), thyroid (T) and steroidogenic (S), (EATS). 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 is a broader scientific agreement on the interpretation of the effects observed on the investigated parameters.

[42] 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.
[43] 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.
[44] 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.

For non-EATS modalities there are some considerations available, but there are no endorsed assays for testing. Nevertheless, non-EATS modalities are valid reasons to consider that a substance meets ED criteria.

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 guidance is available for the assessment of endocrine disruption in the context of the BPR and the PPPR (ECHA/EFSA, 2018), and additional guidance is available for the implementation under CLP[45]. Both guidance documents build in turn on the OECD GD 150 (2018) *Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption*. This OECD GD 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:

1. Level 1: Existing data and existing or new non-test information;
2. Level 2: *in vitro* assays providing data about selected endocrine mechanism(s)/pathways(s);
3. Level 3: *in vivo* assays providing data about selected endocrine mechanism(s)/pathway(s);
4. Level 4: *in vivo* assays providing data on adverse effects on endocrine relevant endpoints;
5. 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.

Relevant data for the assessment of endocrine disrupting properties for human health is made on the basis of an assessment of the total weight of evidence 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.

[45] Draft CLP guidance covering ED properties is available: https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-clp

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 weight of evidence 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 (ECHA/EFSA, 2018). 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 (OECD, 2018) 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 thus (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 and a disturbance of estrous cyclicity or increases in weight or changes in histopathology of the follicular cells of the thyroid gland
- 'Sensitive to, but not diagnostic of, EATS parameters' measured *in vivo* 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))

Note: the bullet points above are from the CLP draft guidance that is yet to be finalised. The draft is available at https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-clp.

The text will be adapted to be the same as the final CLP Guidance.

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 (ECHA/EFSA, 2018).

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 (ECHA/EFSA, 2018):

- ***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 intact animal model (e.g. Hershberger assay OECD TG 441 (OECD, 2009d) or fish short-term reproduction assays OECD TG 229 (OECD, 2012a)) 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.

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 2017.

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 see ECHA/EFSA ED Guidance, section 4 (ECHA/EFSA, 2018).

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 Guidance (2018). Currently, 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 KERs and should always be assessed. Additional guidance on performing the mode of action analysis is provided in the ECHA/EFSA ED guidance and the CLP guidance.

In the weight of evidence considerations in the MoA framework (adopted also by the AOP 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[46]. It should be noted that the presence of an AOP in the AOPwiki does not necessarily indicate its relevance or reliability. 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.

[46] aopwiki.org

Special consideration 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.

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 fetal 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 (Mughal, 2018; Päkkilä, 2015; Demeneix, 2019; Korevaar, 2018), cognitive and neurobehavioral defects (Gilbert et al, 2012), and hearing loss (Crofton, 2004; Mughal, 2018; Hendrichs, 2010). In adults, THs are responsible e.g., for maintenance of cellular metabolism and cardiovascular functions (Yamakawa, 2021; Mullur, 2014).

Additional guidance on the assessment of the thyroid modality 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 disruptors 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 preformed 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 disputing 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 weight of evidence 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.

As the BPR requires a conclusion on the ED properties of an active substance, 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.

1.11.5 Concluding on Classification and Labelling

In 2023, a separate hazard class for endocrine disruption has been introduced under CLP, with two categories. The allocation to Category 1 or 2 depends on the strength of the available evidence, i.e. on how convincing the evidence for criteria (a) and (b) is, and whether a clear endocrine (pattern of) changes are identified:

- *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 safe level considering a weight of evidence 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 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 ECHA Guidance Vol III Part A.

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 14), there are currently no validated test methods to evaluate photosensitive or photogenotoxic potential of chemicals (OECD IATA for phototoxicity). 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 14: 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, 2012[47]).	**1st Tier** • 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 2nd Tier.
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 2nd tier according to SCCS 2021[48]: *As a second tier, the biological effects can be further evaluated on a reconstructed human skin model with some barrier properties.*	**2nd Tier** • Conclude on photoirritation potential based on the result. • If positive proceed to 3rd Tier.
Pharmacokinetic 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.	**3rd Tier** 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

[47] ICH Guidance S10 on Photosafety Evaluation of Pharmaceuticals, 25 August 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

[48] **SCCS 2021:** 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

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.

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 IATA 2024.

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. 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 (see Section 1) and, where possible, dose descriptors (NOAEL, LOAEL, NOAEC, LOAEC, BMD) are established.

- A systemic effect is normally observed distant from the site of first contact when the substance 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 critical NOAELs for each of the relevant exposure time frames can be used to establish AEL values. Indications of route-specific sensitivity and dose-response relationship shall be 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 (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, reference values are derived for the most critical effect(s).

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 identification of it. In case of uncertainty, assuming a non-threshold mode of action is the prudent choice.

For risks related to exposure to carcinogens or mutagens at work, Directive 2022/431[49] requires that occupational exposures are avoided/minimised as far as technically feasible. The approach to controlling workplace exposure should comply with this minimisation requirement.

2.2.1 Hazard Information underlying the derivation of AEL(C)s

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 should be available to support route-to-route extrapolation in the risk characterisation where needed or to address species specific mechanisms if relevant.

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.

2.2.1.2 Acute toxicity

The exposure to biocidal products takes place mostly via the dermal route and by inhalation. Inhalation exposure is especially relevant where volatile active substances are applied indoors. The oral route has to be considered as well, and this can be significant for the general public following accidental/intentional ingestion, in particular by young children.

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

[49] 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

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.

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 RC is performed for sensitisation, as the proposed quantitative methodologies for dermal sensitisation are not considered sufficiently protective and need 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 chapters 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).

2.2.1.7 Carcinogenicity

If a threshold mode of action is identified, a dose descriptor 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.

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 assessment factor 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.

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 assessment factor.

Fertility and developmental effects are relevant in considering repeated exposure, but effects on fertility have been reported already following short-term exposure, and 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 and reference values. 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 threshold for endocrine disruption as such 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 must be considered as potentially significant 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, dose descriptors 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 14.

Table 14: Time frames relevant for setting and applying an AELs[50]

AEL	Relevant toxicity studies	Relevant time frame of human exposure
Acute AEL	Single dose studies designed to determine dose descriptors* or repeated dose studies with acute effects, e.g. • acute neurotoxicity • 28-day/90-day studies • developmental toxicity	≤ 24 h
Medium-term AEL	Repeated-dose studies, e.g. • 28-day/90-day studies • 90-d neurotoxicity • 12-month dog, depending on nature of effects • 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, depending on nature of effects	> 6 months (min. 3 months)

* Data from LD_{50} studies can be considered supportive if appropriate acute effects were investigated

In selecting the dose descriptor 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 14 provides guidance on deciding the relevant studies, but in addition:

- For acute effects, the dose descriptor can also come from a repeated dose study when the critical effect 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

[50] The time frames are based on Doe et al., 2006.

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 dose descriptor is lower than the one based on a chronic toxicity study. The one-year dog study is more relevant for the derivation of the medium-term AEL.

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 intervals, 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 often are 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 and dose descriptors, whereby a set of relevant dose descriptors is established to cover the different exposure time frames and routes.

2.3.2.1 Identification of Dose Descriptors for systemic effects

Dose descriptor 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/or mechanisms of action. The observed threshold dose or effect level in a toxicity test is influenced by the sensitivity of the test system 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, 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.

Selecting the most relevant dose descriptors

Considering the relevance of the study setup 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 critical dose descriptor for AEL 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 dose descriptors, 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.

As a general rule, if several relevant NOAELs (or other dose descriptors) are available, the one that would result in the lowest AEL for a given time frame should be chosen. The lowest dose descriptor may not always result in the lowest AEL value as this also depends on the assessment factors used in deriving the reference value.

Using NOAEL as the dose descriptor 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 chapter 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, but this information may not be used in deriving safe levels.

Benchmark dose (BMD)

As an alternative to using NOAEL/LOAEL as dose descriptor, the BMD methodology can be used. 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 often taken as the point of departure 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 point of departure 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 More et al., 2022[51].

2.3.2.2 Identification of Dose Descriptor for local effects

Irritant, corrosive and sensitising effects are normally driven more by concentration than a (systemic) dose, and the dose descriptor for these properties is normally set as a

[51] https://doi.org/10.2903/j.efsa.2022.7584

concentration where possible. For guidance on this, see Section 4.4.2.

2.3.3 Modification of Dose Descriptor (Determination of absorption rates and bioavailability)

In some situations, it may be necessary to convert a dose descriptor into a correct starting point. 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 conditions;
4. Differences in respiratory volumes between experimental animals and humans, considering the activity level of both animals and humans.

Modification of a dose descriptor 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 dose descriptor 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 most critical and relevant dose descriptors. 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 and a 10-fold factor for intraspecies variation; both factors can be further divided to toxicokinetic and toxicodynamics factors.

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, while human volunteer studies should not be performed for the purposes of the BPR. Human monitoring data can be requested for products already authorised for use under 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. Depending on the information, using human data can lead to higher or lower overall assessment factors, compared to using animal data only. In any case, 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.

2. The human (sub-)population exposed

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 in 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 pharmacokinetic (PBPK) modelling

When considered of sufficient reliability, data from PBPK modelling can be used to refine the assessment factors. PBPK models provide a documentable, scientifically defensible means of bridging the gap between animal bioassays and human risk estimates.

2.3.5 Assessment factors for local effects

For local effect 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 3.2 should normally not be changed. The kinetic factor 3.2 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. 3.2) 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 assessment factor 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. 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 assessment factors 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%[52]. Rounding should happen as late as possible in the assessment process.

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 the cumulative effect 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 assessment factor of 3 for duration extrapolation to chronic scenarios has been applied, and an additional assessment factor 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.

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, information should be considered that was not available when setting the SCL.

For oral effects, setting a reference value would normally not be relevant (see Section 4.4.2.1.2) but case-by-case consideration is needed.

[52] 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.

In some cases, only a NOAEC could be set, without deriving an AEC. This should be the case e.g. for dermal effects.

However, as indicated above, for irritation/corrosion and sensitisation the derivation of dose descriptor is difficult and in most cases a qualitative risk assessment will be performed (see Section 4.4.2).

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.

A dermal AEC should normally not be derived, as it is preferable not to set a defined limit for acceptable exposure due to local dermal effects. 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.

2.3.8 Derivation of External Reference Values for Exposure via Food

An external reference value for exposure via food is needed, if residues in food or feed are expected to arise from the use pattern of a biocidal product.

- **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[53]). 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[54]). 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[55]),
- the principles for ADI and ARfD setting in plant protection products,
- the current guidance in selecting the critical dose descriptors and appropriate assessment factors.

JMPR has given detailed consideration to the use of particular toxicological end-points

[53] WHO 1987, International Programme on Chemical Safety, Principles for the safety assessment of food additives and contaminants in food. Environmental Health Criteria 70

[54] 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

[55] 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

that are most relevant to establishing ARfDs (reviewed by Solecki et al., 2005[56]), with a focus on interpreting effects that have been problematic when deciding whether an effect is relevant to an 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). Much of this guidance is relevant to the setting of ARfD in biocides.

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.

As these values may not be used in the assessment of an active substance in a particular PT, a standard phrase could be included where relevant: "The value was not used in the current assessment as no consumer exposure via food is expected in the PT/uses assessed".

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, as recommended by Art. 95 of REACH Regulation and ECHA Management Board Decision 18/2013. 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.

The study in the most sensitive and relevant species resulting in the most appropriate dose descriptor should be selected from the complete toxicology dataset, considering also the relevance for human exposure in terms of duration and pattern of exposure.

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

[56] Solecki R et al. Guidance on setting of acute reference dose (ARfD) for pesticides. Food and Chemical Toxicology 43 (2005) 1569–1593

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. 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 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 dose descriptor 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.

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).

2.4.1 Non-threshold carcinogens

As required by the Directive 2004/37/EC (amended in Directive (EU) 2022/431) on the protection of workers from the risks related to exposure to carcinogens or mutagens at work ("Carcinogens and Mutagens Directive"), workplace exposure to carcinogenic substances (Cat 1A or 1B) must be avoided or minimised as far as technically feasible. As a general rule, a risk for the general public from secondary exposure to a non-threshold carcinogenic biocidal substance is also unacceptable.

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. 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 process 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.

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 *Biocides Human Health Exposure Methodology*[57]

Note that there are several references in this section to Biocides Human Health Exposure Estimation Methodology (see above) for detailed information on the methodology and the reader is advised to read this section in conjunction with the document on methodology.

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 process therefore requires:

- Information of 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

[57] Available at: http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/human-exposure

formulations and the corresponding exposure via different routes of each exposed population.

3.2.2 Patterns of use / exposure scenario

For the purpose of 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).

Industrial users are in essence a subcategory of the professional users: they are professional users involved in manufacturing, handling and/or packaging of actives or products in industry as well as those using biocidal products in their own processes at industrial settings, for example, manufacturers of timber cladding using wood preservatives or food companies using disinfectants. Professional users are using end-products outside industry.

In some cases, 'trained professionals' may be considered separately from 'professionals'; see Section 3.2.2.1.

The terms 'industrial users' and 'professional users' are used to indicate the area where a task is performed: within or outside industrial settings.

3.2.2.1 Industrial and professional users

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.

Some workers will have limited knowledge and skills to handle hazardous biocidal products – particularly if the use of biocidal products is not routinely required in their workplace. This could take place due to e.g. incidental use of slimicides, insecticides, irregular disinfection and use of products containing preservatives. The exposure of these users might be similar to non-professional users.

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.

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. The non-professional pattern of use is expected to have a lower frequency and/or duration of use.

The consumer exposure assessment should normally address the intended uses of the product. However, since consumers may not accurately follow instructions for use, the assessment should include reasonably foreseeable use patterns in line with the intended use but not strictly following the instructions. For example, consumers may experience relatively high exposures when they use biocidal products in poorly ventilated indoor areas.

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 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 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 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 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[58] (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 other than that which 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. As absorption data for the different routes of exposure are often not available, the calculation of systemic body burdens is subject to a high degree of uncertainty and requires expert judgement.

The second approach is to use route-specific external exposure data and compare that to limit values for each relevant route of uptake. These external values can be calculated from the systemic limit value (e.g. AEL) using relevant absorption data for each route of uptake.

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[58] 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.

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 "Default Human Factor Values for Human Health Exposure Assessment" within the Biocides Human Health Exposure Estimation Methodology should also be consulted.

The most appropriate way of assessing total systemic exposure is by biomonitoring, however, the measured levels of a substance or its metabolites are dependent on numerous factors which can result in inaccuracy/uncertainty of the method. Hence, biomonitoring and interpretation of its results is only reliable if detailed pharmacokinetic information on the substance/compound is available. For exposure assessment a biocidal product is usually considered, containing the active substance.

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.

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 a risk, no further refinement of the exposure estimate is required. If 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.

If the Tier 1 assessment shows unacceptable risk, a refined exposure estimate is required.

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 Biocides Human Health Exposure Estimation Methodology document.

If Tier 2 shows unacceptable risk, a third iteration will be required.

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.

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 on the basis of the robustness/representativeness of the measured and modelled data for the situation and conditions under consideration.

3.2.5.1 Product specific exposure data

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. 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.

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 3.1);
- Method of application or task: where and how the product will be used (see Section 3.2);
- Expected exposure controls (see Section 3.3.1);
- Refinement of exposure assessment if risk is not acceptable (see Section 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 no risk is identified, no further refinement is needed. If 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,
- 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.

In the following overview table (Table 15), the most relevant data requirements for primary (direct) exposure assessment are listed:

Table 15: Overview of requirements for primary (direct) exposure assessment

Data Requirements	Priority	Comment
Product		
- physical properties	Essential	liquid / solid / in-situ generation / particle size, aerosol, volatility
- package details	Essential	volume, material, closure, bulk delivery
- formulation details	Essential	active substance and co-formulants
- site inventory	Desirable	amount, delivery frequency

Data Requirements	Priority	Comment
- 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
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

Data Requirements	Priority	Comment
- duration of task	Essential	event duration
- quantity used	Essential	not always relevant
- area / volume treated	Essential	not always relevant
- timing	Desirable	Season/ weather conditions
Post-application phase		
- task	Essential	description
- 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	
Data may be better expressed as ranges and likely values, rather than as single values.		

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2 ### 3.3.1 Duration and frequency of use

3 The frequency and duration of a task are major determinants of exposure. The frequency
4 of a task is critical in deciding whether the exposure is chronic or acute. It should be
5 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 has to 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

When undertaking an exposure assessment the assessor should seek to ensure that exposure to a biocide is prevented or controlled. 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 is included in appendix 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 when the products are supplied with appropriate gloves. 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:

- Guidance Document on Characterising and communicating uncertainty in exposure assessment
- Principles of Characterising and Applying 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.

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 Residential environment

Assessing exposure for the residential environment covers residents or bystanders who are present during or following the use of a biocidal product. The post application phase is particularly important for non-professional exposure assessment 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 Biocides Human Health Exposure Methodology. 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 the environment may occur by consumption of food and drinking water, inhalation of air and ingestion of soil. It 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 to the overall calculation of indirect exposure from the environment, in three specific 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 still has to be considered on a case-by-case basis.

1. Estimating Dietary Risk from Transfer of Biocidal Active Substances into Foods Non-professional Uses (see Section 5.2) 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).

2. Estimating Transfer of Biocidal Active Substances into Foods – Professional Uses is relevant for:

- PT 3 (Veterinary hygiene products);
- PT 4 (Food and Feed area disinfectants);

- PT 8 (Wood preservatives);
- PT 12 (Slimicides);
- PT 14 (Rodenticides);
- PT 18 (Insecticides, acaricides & products to control arthropods);
- PT 19 (Repellents & attractants).

3. Estimating Livestock Exposure to Biocidal Active Substances 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 taken into account. 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.

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.

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 16: Recommended pragmatic acceptance criteria for human exposure studies

Essential Requirements	Desirable Requirements	Rejection criteria
Aims of survey or study strategy[59]	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
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

[59] GLP compliance of studies into exposure to biocidal products is at the moment no generic demand in the EU, as it is in the USA and Canada. Some Member States require GLP-compliant studies for pesticides.

Essential Requirements	Desirable Requirements	Rejection criteria
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

Notes on Table 16

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 the following table (Table 17). 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 17: 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

Data item	Desirable amount of detail to be recorded
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 controls	Presence of local ventilation for inhalation risks, ideally with some comment on 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

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3.7 Selection of indicative exposure values

The following general rules should be used in selecting indicative exposure values from exposure data (see also Appendix 3-2).

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 are typically less than a factor of 2. 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.

It is important to note that the rules defined above only address the sampling uncertainty associated with each data set. The use of any generic data model is also subject to scenario and extrapolation uncertainty reflecting the degree of analogy between the assessment scenario and the circumstances represented by the data model. The strength of this analogy requires expert evaluation and might justify the use of a higher percentile.

Appendix 3-1: Refinement of exposure estimates

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.

Figure 2: Hierarchy of controls

A figure will be included with the content depicted below.

(Most effective)

Elimination – physically remove the hazard

Substitution – replace the hazard

Engineering controls – isolate prople from the hazard

Administrative controls – change the way people work

PPE – protect the worker with PPE

(Least effective)

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. 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. 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

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 in order 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 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.

3.8 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.

3.9 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 by the use of 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 of time, 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. Important information on gloves relates to those materials that are effective and over what duration they are effective. 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 374*" does not give sufficiently concrete information to ensure that the risk can be adequately controlled.

Appendix 3-2: Confidence Intervals for Percentiles of Exposure Distributions

The correct selection and use of exposure percentiles in risk assessment is essential to avoid excessive conservatism whilst also providing reassurance that highly exposed

workers are incorporated into the assessment. As uncertainty increases with small datasets, generally a higher percentile such as 90th, 95th or maximum exposure value will be used in place of a more moderate one such as a 75th percentile. Alternatively, a confidence interval may be calculated for a percentile to indicate the level of precision in the value and this supplementary information is considered in the assessment.

Assuming that a sample of *n* exposure measurements has a lognormal distribution with a geometric mean of exp (μ) and a geometric standard deviation of exp (σ) then an estimate of the *p*th percentile is given by:

$$\exp \{ \mu + z_p \sigma\}$$

Where z_p is the *p*th percentile from a standardized normal distribution N(0,1). For example, $z_{75} = 0.6745$, $z_{90} = 1.2816$.

An approximate standard error of log(*p*) can be calculated as:

$$\sqrt{\sigma^2 n^{-1} + z_\alpha{}^2 \sigma^2 (2n)^{-1}}$$

1-α% confidence intervals for exposure percentiles can then be calculated using the following formula:

$$\exp\left(\mu + z_p \sigma \pm z_{\frac{\alpha}{2}} \sqrt{\sigma^2 n^{-1} + z_p{}^2 \sigma^2 (2n)^{-1}} \right)$$

Example

A sample of size 10 with geometric mean 20 and GSD 5 has a 75th percentile of exp{log(20) + 0.6745 $\times$ log(5)} = 5.88.

The standard error of the log 75th percentile is $(\log(5)^2/10 + 0.6745^2 \times \log(5)^2 / 20)^{0.5}$ = 0.245.

A 90% confidence interval for the 75th percentile is then given by exp(log(5.88) $\pm$ 1.6449 $\times$ 0.245).

Often, rather than assuming a lognormal distribution, an empirical estimate of a percentile will be taken directly from the ranked exposure data. In these cases an approximate 90% confidence interval for the percentile is given by:

$$\text{Lower endpoint: } p / \exp\left(1.6449 \sqrt{\sigma^2 n^{-1} + z_p{}^2 \sigma^2 (2n)^{-1}} \right)$$

$$\text{Upper endpoint: } \quad p \times \exp\left(1.6449 \sqrt{\sigma^2 n^{-1} + z_p{}^2 \sigma^2 (2n)^{-1}} \right)$$

Tables 18 and 19 give the multiplicative values required to obtain a 90% confidence interval for a 75th and 95th percentile of a variety of geometric standard deviations and sample sizes. For example, for an empirical 75th percentile of 100 mg/min from a dataset

of 50 measurements with a GSD of 6 a 90% confidence interval for the percentile is 63 mg/min (100 /v1.59) to 159 mg/min (100v×v1.59). Confidence intervals become wider (less certain) with greater exposure variability and narrower with increasing sample size.

Table 18: Scaling factors to obtain a 90% confidence interval for a 75th percentile with a variety of sample sizes and GSDs

		Geometric standard deviation								
		2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sample size	**5**	1.75	2.45	3.10	3.71	4.31	4.88	5.45	5.99	6.53
	10	1.49	1.88	2.22	2.53	2.81	3.07	3.31	3.55	3.77
	20	1.33	1.56	1.76	1.93	2.08	2.21	2.33	2.49	2.56
	50	1.20	1.33	1.43	1.51	1.59	1.65	1.71	1.76	1.81
	100	1.13	1.22	1.29	1.34	1.39	1.43	1.46	1.49	1.52

Table 19: Scaling factors to obtain a 90% confidence interval for a 95th percentile with a variety of sample sizes and GSDs

		Geometric standard deviation								
		2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sample size	**5**	2.19	3.45	4.78	6.15	7.55	8.99	10.45	11.93	13.44
	10	1.74	2.40	3.02	3.61	4.18	4.72	5.25	5.77	6.28
	20	1.48	1.86	2.19	2.38	2.75	3.00	3.23	3.45	3.67
	50	1.28	1.48	1.64	1.78	1.90	2.00	2.10	2.19	2.27
	100									

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:

$$0.25 \text{ mg/kg/d} \times 10 = 2.5 \text{ mg/kg/d}$$

If the operator weighs 60 kg then active substance contamination would need to exceed:

$$2.5 \text{ mg/kg/d} \times 60 \text{ kg} = 150 \text{ mg/d}$$

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:

$$150 / 0.005 = 30{,}000 \text{ mg} = 30 \text{ g}$$

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:

$$30 \text{ g} / 30 \text{ min} = 1 \text{ g/min}$$

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 20.

Table 20: 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)	1,000
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 Biocides Human Health Exposure Estimation Methodology document.

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 are 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 preferable.

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 end result (via sensitivity analysis). If data quality is adequate, a probabilistic analysis is preferred, at least to underpin a deterministic presentation of the results.

Appendix 3-5 Principles for design of rinsing experimental studies

Introduction

The objective of this Appendix is to outline the principles that should be taken into consideration when performing PT 3 or PT 4 rinsing studies (trials). Rinsing studies should demonstrate the efficiency of rinsing, i.e. the removal of the active substance and its degradation products according to the residue definition. The rinsing factor is a parameter that can be used at Tier 2 for dietary risk assessment.

This Appendix was written following the considerations stated in the draft guidance on Estimating transfer of biocidal active substances into foods – Professional uses, especially in point 5.9.

Only rinsing with water is detailed in this Appendix as it is the common practice. However, if solvent is intended to be used for rinsing of the intended biocidal product, it must be demonstrated that no solvent residues are expected on the treated surface. For instance, in the case of a highly volatile solvent, the applicant could provide a solid justification based on the volatile nature of the solvent to substantiate the absence of residues on the treated surface.

Test procedure

Tested surface materials

The nature of the test surface material should be representative for the intended uses of the biocidal product. For example, if the product is used for the disinfection of equipment/materials in professional kitchens or in food processing plants, non-porous surface materials (e.g. inox and/or HDPE) would the appropriate materials for the demonstration of the effectiveness of rinsing. Furthermore, if the product is used for surface disinfection in livestock buildings, the choice of test surface materials may be different. In that case a porous material (e.g. concrete and/or wood) seems more appropriate. With regard to uses, the choice of the test surface materials should be justified and considered as a worst case.

In all cases, it is preferable to use standardized test surface materials which means that characteristics should be defined for each type of material, to ensure harmonized assessment. As a minimum, material composition should be required (e.g for concrete: bare concrete breeze block, 5 cm thick, with an average cement load of 500 g).

Test item

In general, the test item must be identical to the claimed biocidal product in the registration dossier (in terms of composition and formulation).

In some cases, an aqueous solution containing only active substance may be considered worst case but this depends on the composition of the biocidal product. The absence of impact of the formulation tested on the adhesion of the active substance and consequently the effectiveness of the rinse should be justified based on the product's composition.

The chosen dilution must reflect the product's intended worst-case application conditions, i.e. resulting in highest surface residues. The test item should be stored under appropriate conditions for the study duration and applied soon after preparation or

mixing.

Application of biocidal product: surface treatment

The biocidal product/test item should be applied according to the intended use. The maximum concentration claimed on the biocidal product label should be used for treating surfaces resulting in maximal surface residue levels. The frequency, duration and contact time of the application of biocidal treatment should be justified and in accordance with the intended uses.

Verification of the doses applied (recovery check)

After biocidal treatment, the recovery of the maximum biocide residue at the surface should be determined. This should correspond to the level that is expected after biocidal treatment. The approach used to achieve this should be detailed.

According to ARTFood guidance, biocide residues are wiped off a defined surface area with dry pads or pads soaked with an appropriate solvent. The biocide residues from the pads are extracted and biocide residues are measured in the extract.

Particular attention should be given to substances exhibiting surface-active characteristics, such as quaternary ammonium compounds. In such cases, rinsing with an appropriate solvent could be more suitable or solvent-extracted in an ultrasonic bath, if possible. In this step, all the amount of active substance applied in the treatment should be recovered taking into account the uncertainties of the method used. The choice of the solvent should be justified. Different solvents may be compared in order to choose the optimal one.

Rinse procedure

For experimental setup, a horizontal position for the surface material when treating with biocidal product and rinsing is worst case and representative of the conditions of use for most applications. In some cases, rinsing with an appropriate solvent could be more suitable than with water for the experiment. Please note that for rinsing (water or solvent), it is necessary to specify the duration of the rinsing step and the volume of water/solvent used.

Treated surface should typically be rinsed with water and rinsing water should be analysed for biocide residues.

The rinsing method should be in line with the intended use of the biocidal application.

Biocide residues remaining on treated and rinsed surfaces can be analysed via wiping tests or using a solvent.

If a solvent is used, after rinsing with water, the treated surface should be then rinsed with solvent or solvent-extracted in an ultrasonic bath and the rinsing solvent should be then analysed in order to double check the efficiency of the rinsing.

The choice between performance of wiping tests and/or using a solvent must be justified.

Analytical methods

Validated analytical methods should be used to quantify residues of the active substance in solvent and in water. The biocide residue is the residue according to the residue definition. It can be the active substance or it can be a combination of active substance and degradation and/or reaction products.

The analytical method should be validated according to the ECHA Guidance Vol I Parts A+B+C.

However, even if the guidance SANTE/2020/12830 was not endorsed by the WG APCP, this guidance is more appropriate for the validation of this kind of analytical method. In such cases, the use of an appropriate solvent could be more suitable.

The limit of quantification (LOQ) must be given. If MRLs are set for the test substance, the LOQ must not exceed them.

Controls

In order to be sure that the surfaces are not contaminated with biocide residues at T0 (before biocidal treatment), the determination of biocide residues at T0 is essential. According to the ARTFood guidance, a wiping test should be used to determine the background level for the experiment.

A negative control (applying a solution without biocide product) must be performed for each tested surface material.

Number of samples

At least five replicates and one negative control must be conducted per protocol modality or stage (e.g. for each surface material tested, for successive rinses).

Number of rinsing

The frequency and duration of rinsing must be justified and in accordance with the intended uses.

Considerations for data reporting

Condition of storage

Sampling, analysis dates and storage conditions of samples must be specified. Deviations from the study protocol and their impact on the results should also be mentioned. To facilitate sample identification, a unique code or number should be assigned to each sample.

Storage stability of the biocide residues under the storage conditions should be demonstrated. The samples should be analysed as soon as possible and no longer than 30 days after sampling, otherwise storage stability studies should be provided (information on storage stability studies can be found in OECD 506[60]).

Materials

[60] OECD 506 : Stability of Pesticide Residues in Stored Commodities

All information appropriate and relevant to provide a complete and thorough description and identification of the test substances used in rinsing testing must be reported.

Results

For each sample analyzed (on treated surfaces and rinsing water), results should be presented in units of mass (µg, mg...) per unit of surface or volume. To facilitate interpretation of the results, a recovery ratio in % should be calculated in relation to the initial active substance concentration applied.

The use of a correction factor (to account for recovery of the biocide residue) is not recommended. However, if the rinsing data are needed to be supported with a factor of correction, it should be justified and its calculation should be detailed. Results without any correction should also be provided.

GLP

Concerning experimental phase, if studies cannot be performed under GLP, an alternative strategies can be pursued: application of EN ISO/IEC 17025.

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.

This guidance focuses on the assessment of risks associated with the active substance, but the principles can be adapted to assessing the risk related to residues or substances of concern, as relevant.

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 in particular in relation to cumulative and synergistic effects (see section 4.3.2).

In the interest of harmonising the assessments under different regulatory frameworks, especially in borderline cases, the conclusions under other regulatory frameworks should be taken into consideration to support the assessment.

4.1.1 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/EC), 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;
- Personal protective equipment (respiration, skin, eyes) where exposure cannot be prevented by other means.

Apart from substance or process specific risk management measures, good industrial 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 cloths) 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 (REACH Guidance R.13).

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).

4.1.2 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.

The effect of formulation will also depend on the active substance and 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. 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.

4.1.3 Local or systemic risk characterisation

Whether local or systemic effects are more critical depends on several factors including 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 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. This means that local or systemic effects may be more critical for different products containing the same active substance, and/or different intended uses or PTs.

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.

4.1.4 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.

An uncertainty analysis can provide more accurate estimates for hazard or exposure side, or information on the uncertainties; see REACH Guidance R.19.

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.

If the second tier still shows risk, risk management measures may be required.

For non-professionals, risk reduction by personal protection measures 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) and has residual risks controlled through control measures. 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 will 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

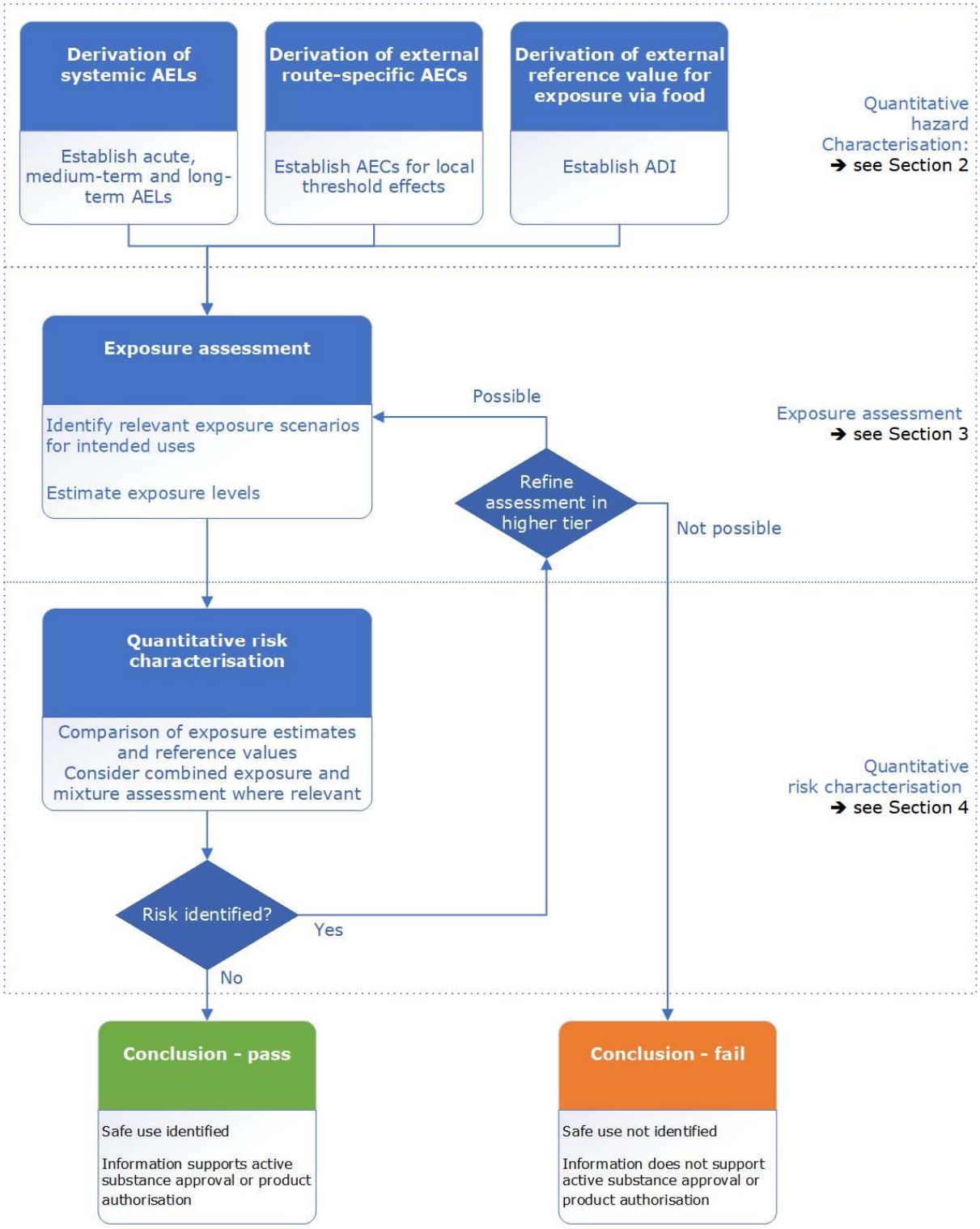
4.2.1 Introduction

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.

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.

Figure 3: Concluding on safe uses in quantitative hazard and risk characterisation



1

2 In quantitative risk characterisation, exposure estimates are compared to the
3 corresponding AEL for each use and relevant time-frame. A tiered approach has to be
4 followed according to the same principles as described in Chapter 3.2.4. Where the
5 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.

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 Chapter 4.5 for further guidance on the refinement possibilities.

4.2.2 Exposure via food

For exposure via food, please refer to chapter 5 of this Guidance. Further guidance is also available on the ECHA Website[61].

If exposure via food is possible, derivation of ADI and ARfD is necessary (see Section 2.3.8).

4.2.3 Corrosive substances

For corrosive concentrations, 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 substances, 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 systemic effects, or 2) it is possible to justify that exposure is negligible due to a thorough description of technical 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.

The systemic risk characterisation should cover all routes of exposure and is performed in addition to the qualitative assessment.

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61 https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/assessment-of-residue-transfer-to-food

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.

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 21.

The TTC concept has been incorporated in the risk assessment processes 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 Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment[62] 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.

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.

[62] https://doi.org/10.2903/j.efsa.2019.5708

Table 21: 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[63])
Cramer Class II	540	9	
Cramer Class I	1800	30	

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

[63] Munro IC *et al*, 1996. Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food and Chemical Toxicology, 34, 829–867. DOI: 10.1016/s0278-6915(96)00049-x

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:

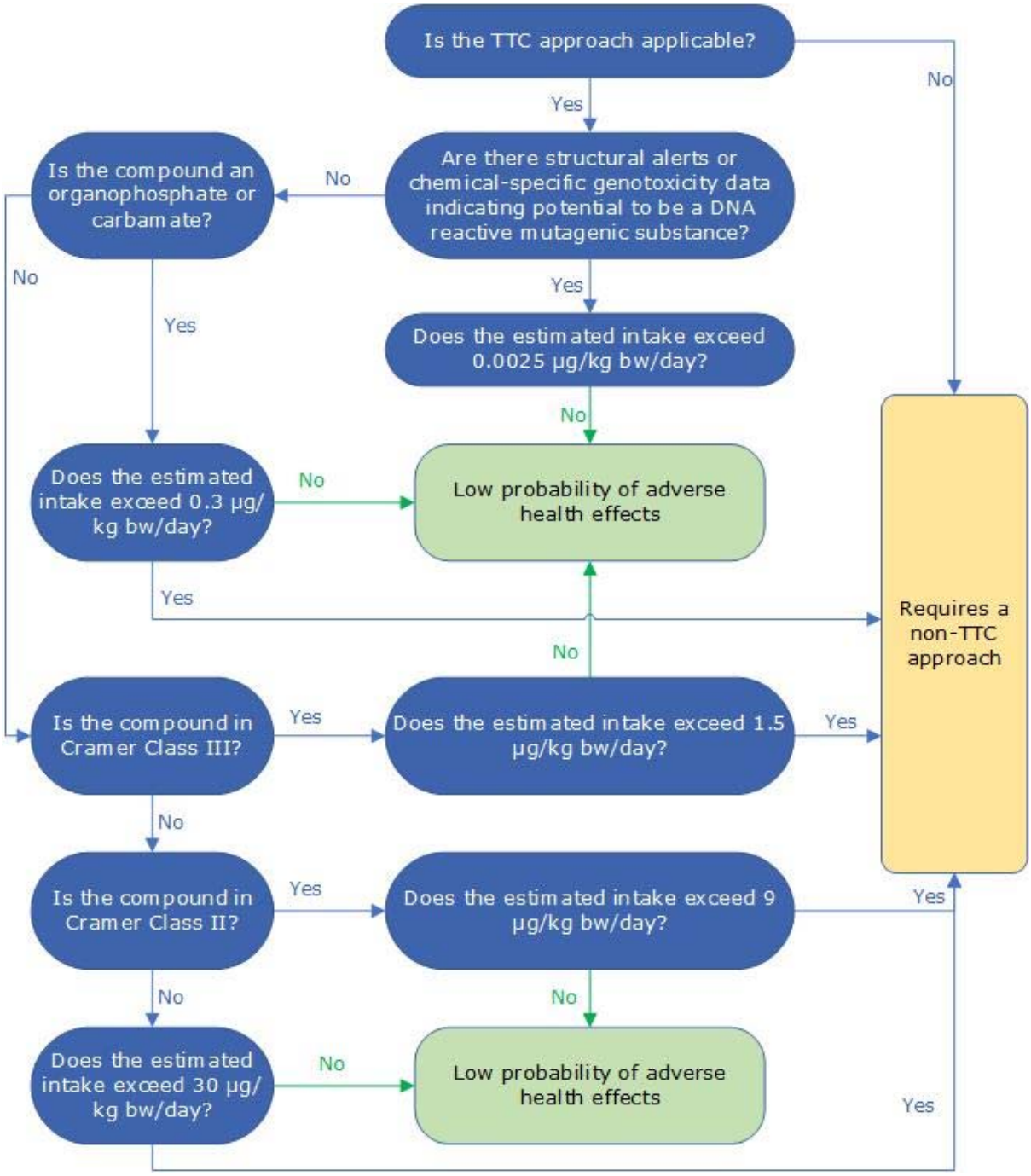
- High potency carcinogens: aflatoxin-like, azoxy- or N-nitroso substances and benzidines;
- Steroids;
- Substances with a potential for bioaccumulation according to the analysis of EFSA 2012, including substances like polyhalogenated-dibenzodioxins, -dibenzofurans and -biphenyls.

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

Within the process of 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 have the same mode of action and/or 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 situations 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.

  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 e.g. 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 aggregare exposure via several or all these sources and routes, where the biocidal use being assessed is only one contributing factor.

  Aggregate exposure would normally be assessed only in specific situations 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**: combined exposure to multiple 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, the substances can be very different while for cumulative exposure the mode of action and/or the target organ are the same.

  Combined exposure would normally need to be assessed for a biocidal product that contains more than one active substance and/or substances of concern.

'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 Guidance on the Application of CLP Criteria.

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 in the beginning of this Guidance, as well as Section 4.3.1 Terminology.

4.3.3 Introduction to combined exposure

The first step 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:

- Concentration (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. 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 can not be ensured with certainty, but the conservative approach of concentration (dose) addition can be used as a first tier.
- 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.

For **interaction**, one must assume one of the two possibilities:

- Synergism: the combined effect of two chemicals is greater than dose addition or independent action.
- 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 4.3.5. In addition, PBPK 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.

For biocidal products, the hazard assessment relies generally 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.

The same assessment principles can be used in other situations where combined exposure 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) 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.

Before the tiered assessment, two preliminary steps are performed to a) verify acceptability of each substance in the mixture(s) one by one, and 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. 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 is provided as an option for completeness while most often information would not be sufficient to follow it.

Noting that Tier 1 is most conservative and higher tiers become more realistic, one may also proceed to higher tiers to verify if the requirement of PPE and RMMs identified in lower Tiers is necessary. 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 other reference values such as OEL or DNEL, the information used in setting these values need to be considered. OELs and DNELs are often route specific, most commonly derived for inhalation.

Figure 5 presents an overview of the methodology, and an example of applying the methodology is provided in Section 4.3.10.

For the classification of mixtures under the CLP Regulation, see also the *Guidance on the Application of CLP Criteria*.

A fully quantitative risk assessment is required for SoCs in Band C and D[64], corresponding to classifications as Carc (1A, 1B, 2), Repr (1A, 1B, 2), Lact (H362), Muta (1A, 1B, 2), STOT RE (1, 2). 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

[64] 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 4.11.6Concluding on suitability for risk assessment (under 4.11 Endocrine disruption).

existing value such as DNEL, AOEL or OEL. These values should be used only if they are validated by a European authority (e.g. ECHA, EFSA or a Member State Competent Authority for biocides).

- 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 assessment. 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. For such cases, and where necessary based on the information available, the possibility of an assessment for a specific endpoint/mode of action can be considered according to Tier 3 described below. For an acceptable outcome, the assessment should cover at least the hazard properties and modes of action triggering the classification that results in placing the substance in Band C or D. It is expected that only in rare cases, information would be missing for risk assessment, while relevant information would nevertheless be available for performing the Tier 3 assessment.

DNELs from safety data sheets and REACH Registration dossiers are not considered to be validated as they are not generally 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 snap-shot 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. Each recommendation for a DNEL needs to be considered case-by-case.

RAC sets reference DNELs that are used as a basis for characterising risk in Applications for Authorisation. 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 DG EMPL) 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].

[65] https://echa.europa.eu/applying-for-authorisation/evaluating-applications; see the row 'Reference DNELs'.
[66] https://echa.europa.eu/oels-activity-list

4.3.4 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 assessment 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 assessment for the use.

4.3.5 Preliminary step b) Synergistic effects

This step is performed to confirm whether synergistic effects are identified or there is convincing evidence that justifies assuming synergy. The modes of action of the substances should be reviewed, taking into account all available information, including literature, to identify potential mixture effects and synergy.

If the conclusion of the assessment is that synergy is demonstrated, a safety factor for synergy (SYN) is applied on the basis of all available information. The SYN should normally be between 1 (no impact) and 10 (very conservative), depending on the information available.

4.3.6 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, deviating from this only where synergistic effects were identified in preliminary step b).

The HI is calculated as follows:

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.

4.3.7 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:

$$\mathbf{HI_{target\ organ} = (SYN) \times \Sigma\ (internal\ exposure\ /\ AEL)}$$

➔ If each $HI_{target\ organ} \leq 1$ 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 3.

Note: If there is no target organ or mode of action in common, dose addition is not confirmed and the risks are covered by the preliminary step a).

4.3.8 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:

$$\mathbf{HI_{target\ organ} = (SYN) \times \Sigma\ (internal\ exposure\ /\ AEL_{target\ organ})}$$

➔ If each $HI_{target\ organ} \leq 1$ 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.

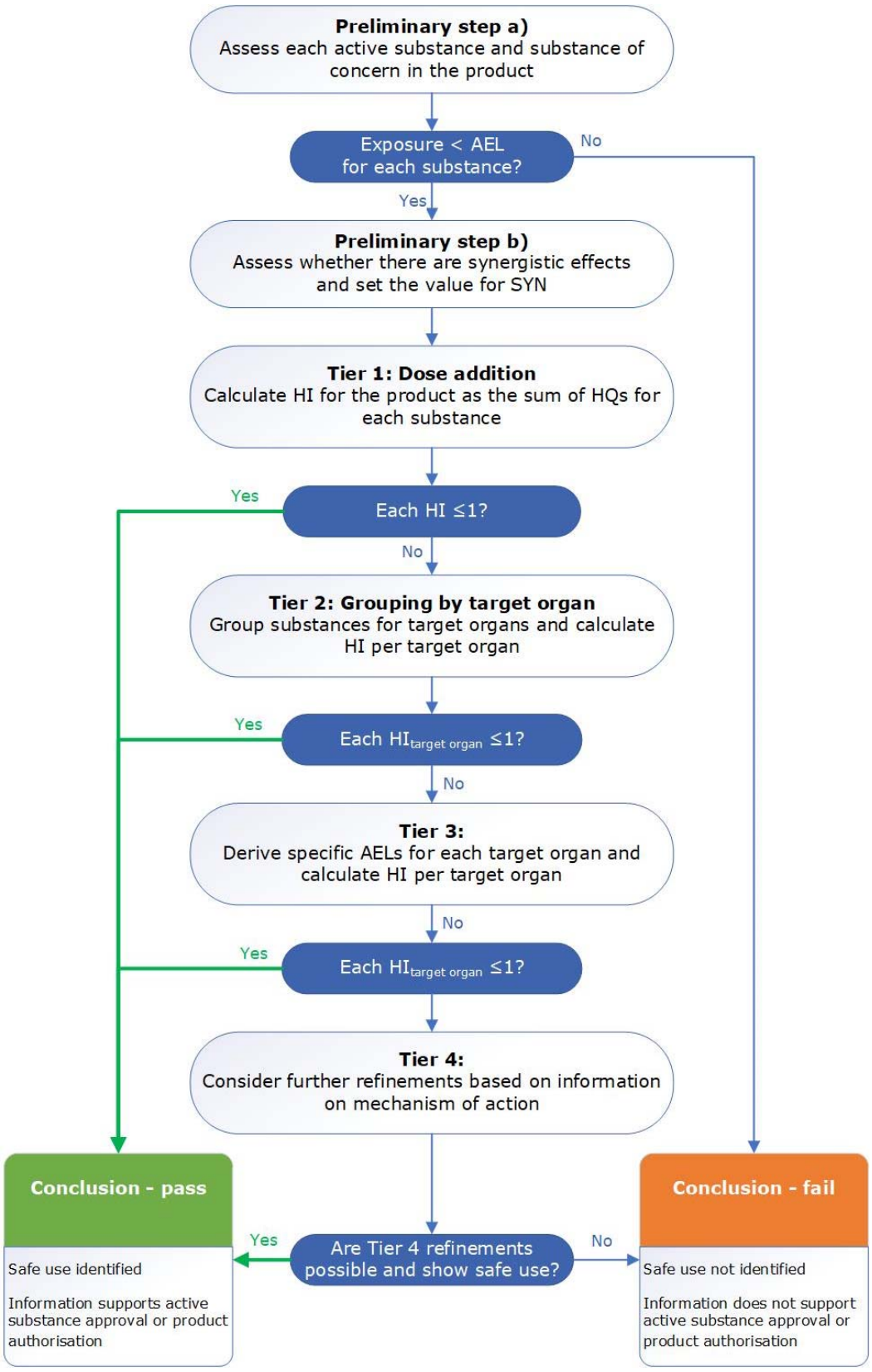
4.3.9 Tier 4: Mechanism of action

If available, information on the mechanism of action can be used to further refine the assessment.

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.

Figure 5: Overview of RC for combined exposure assessment


Preliminary step a)
Assess each active substance and substance of concern in the product
Exposure < AEL for each substance?
No
Yes
Preliminary step b)
Assess whether there are synergistic effects and set the value for SYN
Tier 1: Dose addition
Calculate HI for the product as the sum of HQs for each substance
Yes
Each HI ≤1?
No
Tier 2: Grouping by target organ
Group substances for target organs and calculate HI per target organ
Yes
Each HI target organ ≤1?
No
Tier 3:
Derive specific AELs for each target organ and calculate HI per target organ
No
Yes
Each HI target organ ≤1?
Tier 4:
Consider further refinements based on information on mechanism of action
Conclusion - pass
Safe use identified
Information supports active substance approval or product authorisation
Yes
Are Tier 4 refinements possible and show safe use?
No
Conclusion - fail
Safe use not identified
Information does not support active substance approval or product authorisation

4.3.10 Example of risk characterisation for combined exposure

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 22. 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 22: RC for combined exposure – preliminary step

		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.

Preliminary step b) Synergistic effects

No indications of synergy 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 22 is applicable. The results of the assessment by dose addition are shown in Table 23.

Table 23: 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 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 24 and the HI for each target organ or mode of action is calculated in Table 25.

Only long-term effects and long-term exposure will be considered because acute exposure is safe in Tier 1.

Table 24: Target organs for each substance – Tier 2

Target organ (mode of action)	AS	SoC1	SoC2
Liver	✓	✓	✓
Thyroid	✓	✓	
Kidney	✓		✓
Eye (cataract)	✓		
Fertility		✓	

Table 25: 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 chapter 2.3.4 apply.

Table 26: 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 27: 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		0.05	0.02	0.02		

(mg/kg bw/day)						
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:

- 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.

For a substance where reference values are derived and quantitative assessment is possible but for some endpoints a qualitative assessment is triggered (e.g. local effects), it may not be straightforward to identify the critical effect for the relevant exposure patterns, or to decide which approach would be more protective. 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 should 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 aneugenic substances that are not clastogenic nor causing gene mutations. 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 (BPR Article 5(1), exclusion criteria) 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 (BPR Article 5.1, exclusion criteria) 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.

Non-professional use and risk for the general public from secondary exposure to these substances would normally be unacceptable.

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 assessment factor to cover for the severity of effect might be used (e.g. if the starting point 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, please see section 2.4.1. The DMEL methodology 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) is then compared to the exposure estimate to conclude whether the risk is as low as reasonably practicable.

4.4.2 Local effects (irritation/corrosion, sensitisation) – 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 section x.x *Hierarchy of controls and the STOP principle*), 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 weight of evidence 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 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 th 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, only classification is considered. 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 between 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 (including NOAEC) with the concentration of that substance in a biocidal product or a dilution.

4.4.2.1.2 Decision logic for performing (semi-)quantitative or qualitative local RC

Table 1 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.

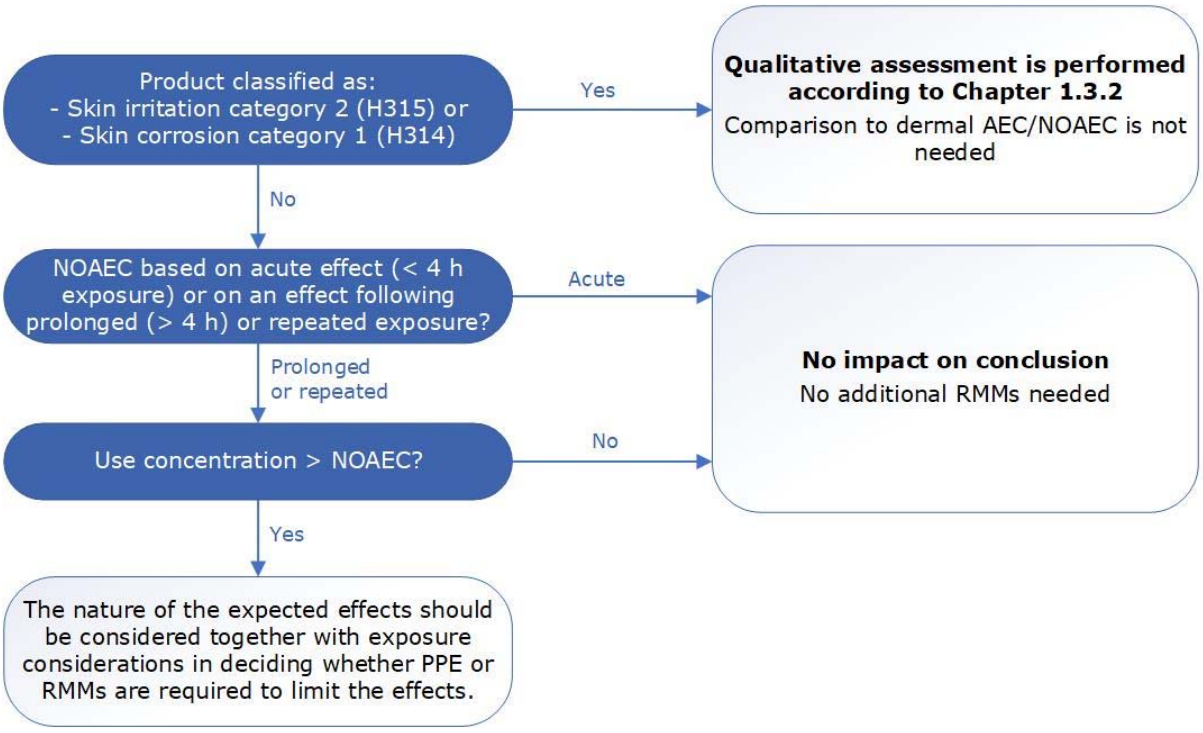
Table 28: Decision logic for local RC

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) • 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. 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, 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*)* • H371 - *May cause damage to organs* (gastrointestinal tract) • H372 - *Causes damage to organs* (gastrointestinal	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 frequency of exposure. Furthermore, the experimental design may not be corresponding to human oral exposure (e.g. testing by gavage).

		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*	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*	Not applicable
Eye	Irritation	Performed only if classification is triggered as one of the	Not applicable

	Corrosion	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*	

Figure 6. Decision tree for performing local RC for dermal irritation/corrosion effects



Where classification (of the product or the in-use dilution) is a trigger for local RC in the principles described in Table 28 and Figure 6, harmonised classification of co-formulants in that product is not required for triggering the local RC. In the absence of an existing entry in Annex VI to CLP or a RAC opinion on harmonised classification, it is sufficient to have a (CLH) classification proposal (including a proposal for an SCL only) or self-classification or classification derived from the data available for active substances and co-formulants, together determining the classification 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[67].

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, such as repeated exposure under occlusive dressing.

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, quantitative RC may be possible. 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 not 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 Guidance on information requirements and chemical safety assessment Chapter 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.

➔ Exception to this rule: If a reliable quantitative assessment shows no risk, it may be possible to make a case-by-case decision whether the qualitative assessment

[67] https://echa.europa.eu/regulations/clp/classification

can be disregarded. Such a situation would be more likely 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.

In some situations, the available information might not allow for the performance of a reliable (semi-) quantitative assessment. The qualitative assessment then has to be relied on. It is therefore imperative to consider also the reliability of the (semi-) quantitative assessment.

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.

Assessment factors 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 assessment factors 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 local 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 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 result in the concentration to increase. 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 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 *Guidance on information requirements and chemical safety assessment Chapter 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

[This brief chapter is still being drafted]

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 assessment factors in *Guidance on information requirements and chemical safety assessment Chapter 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).

Assessment factors 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 assessment factors) 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 assessment factors. 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 (see also Section 4.5).

In line with the *Guidance on information requirements and chemical safety assessment Chapter R.19 (uncertainty analysis)*, 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 assessment factors 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 the *Guidance on information requirements and chemical safety assessment, Part E: Risk Characterisation* should be considered.

The steps in the assessment are described in sections 1.3.1 and 1.3.2.

The examples in Appendix 4-5 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 Appendix 4-5
- 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 worstcase 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 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 5, and PPE in Tables 3 and 4.

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 2: *Examples of qualitative arguments that can be used to support acceptability or non-acceptability*

Support for acceptable risk	**Support for non-acceptable risk**
reversible effect	irreversible and/or severe effect[68] (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): - low amount used per event - low vapour pressure - low aerosol formation (liquid or solid) - high viscosity of product (less aerosol formation and potential for splashes) - high ventilation expected, e.g. due to outdoor use or a use for which high ventilation is standard - no 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 (approximate information): - high amount used per event - high vapour pressure - high aerosol formation (liquid or solid) - low viscosity of product - low ventilation expected (e.g. non-professional indoor use) - direct contact with skin, eye, GT expected - high exposure level compared to adverse effect concentration (LOAEC) or NOAEC, if available

[68] 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 degree of operational RMMs already in use or recommended and compliance expected - High level of containment - Easy maintenance - Minimization of manual phases - Local exhaust ventilation	operational RMMs cannot be applied or compliance not expected
high degree of organisational RMMs already in use or recommended and compliance expected - Permit to work procedures - Trained workers - Intensive supervision of workers regarding correct use of RMM	necessary organisational RMM not applicable
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 4.1.2) 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, it is not appropriate to apply the same control strategy to irritants as to strong sensitisers, and life-threatening consequences require the most stringent measures.

Tables 3 and 4 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 5 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 2 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.
- Strong skin sensitisers, moderate respiratory sensitisers and corrosives (including corrosives to the respiratory tract and severe eye irritants) with significant potency can cause serious, irreversible effects at relatively low concentrations.
- Moderate skin sensitisers can cause significant, possibly irreversible effects at less low concentrations.
- The least stringent measures concern moderate irritants and products/in use-dilutions which cause skin dryness. These are moderate, reversible effects at relatively high concentrations.

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 4.1.1).

Table 3. *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 2.

Effects	**Acceptable frequency and duration of exposure**[69]	**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.

[69] Duration of potential exposure can be shorter than task/use/process

Effects	Acceptable frequency and duration of exposure[69]	Acceptable degree of exposure
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

Table 4. *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 2. In concluding on acceptability, one should consider also what is an acceptable degree of exposure for non-professionals (Table 3).

Effects	Acceptable frequency and duration of exposure[70]	Acceptable degree of exposure[71]	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			Appropriate respirator mandatory unless complete containment is verified for all phases of the operation

[70] 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.
[71] The degree of exposure can be reduced by PPE, or by RMMs such as requiring LEV.

guidance			
Skin corr. 1A (H314)			Face shield Chemical goggles 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)		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 Chemical goggles 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)			Face shield Chemical goggles
Corrosive to the respiratory tract, EUH 071		High level of containment, practically no exposure; no splashes, no (liquid or solid) aerosol formation Example: brief contact with technical RMM and	Substance/task appropriate respirator

		PPE	
Skin sens. 1B, H317, or Skin Sens 1 (H317) and potency evaluated as "moderate" according to CLP guidance		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)			Substance/task appropriate protection (select from box above)
Skin irrit 2, H315 EUH066 - Repeated exposure may cause skin dryness or cracking	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) Substance/task appropriate gloves Protective coverall
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)

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 5 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 29: 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
	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

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4.4.2.3.3 Examples on risk characterisation for local effects including sensitisation

Example 1: Qualitative risk assessment for local effects

Primary exposure: use of product

Hazard		Exposure							Risk	
C&L	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Potential degree of exposure	Relevant RMM & PPE	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 10L water (=1%)	skin Eye (splashes, hand to eye transfer)	2 / year; Few minutes or less per day	n.r.	labelling as eye irritant child proof closure instructions for use packaging reducing risk for eye exposure by splashes washing of hands after use	Acceptable since: +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 (↑↓)

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	
C&L	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Potential degree of exposure	Relevant RMM & PPE	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	n.r.	labelling as eye irritant child proof closure instructions for use packaging reducing risk for eye exposure by splashes washing of hands after use	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 (↑↓)
As above			professionals	As above		not daily, but ≥ 1 / week Few minutes or less per day	n.r	labelling as eye irritant child proof closure instructions for use minimizing exposure packaging reducing risk for eye exposure by splashes washing of hands	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 (↑↓)

Hazard		Exposure					Risk	
					after use			

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2 **B) Primary exposure: use of application solutions**

Hazard		Exposure							Risk	
C&L	additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	frequency and duration of potential exposure	Potential degree of exposure	Relevant RMM & PPE	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/m^3 (~5ml/m^3) 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	~ 100 ml/m^2 masonry surface ~ 97 µl/m^3 air	labelling as eye irritant child proof closure instructions for use washing of hands after use washing of face/eye after accidental exposure	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	As above	Like for general public + instructions for use minimizing exposure for professionals	Acceptable: +Reversible effect +low intensity: outdoor use, low intensity compared to additional hazard	

Hazard	Exposure					Risk	
						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

Primary exposure: use of product

Hazard		Exposure							Risk
C&L	Additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	Frequency and duration of potential exposure	Potential degree of exposure	Relevant RMM & PPE	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	6 g / person	labelling for eye damage, child proof closure instructions for use packaging reducing risk for eye exposure by splashes washing of hands after use	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	
C&L	additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	frequency and duration of potential exposure	Potential degree of exposure	Relevant RMM & PPE	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 RT	few minutes per day or less	n.r.	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	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 (↓)

Abbreviations: IBC-intermediate bulk container; CIP – cleaning in place

B) Primary exposure: use of application solutions

Hazard		Exposure							Risk	
C&L	additional relevant hazard information	PT	Who is exposed?	Tasks, uses, processes	Potential exposure route	frequency and duration of potential exposure	Potential degree of exposure	Relevant RMM & PPE	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 RT	Very low frequency More than few minutes but equal to or less than few hours per day	n.r.	Technical and organisational RMM adequate for the low hazard category are achievable use of appropriate gloves, eye protection, filter mask	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 (↓)

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5 Dietary risk assessment

5.1 Estimating Transfer of Biocidal Active Substances into Foods – Professional Uses

5.1.1 INTRODUCTION

Regulation (EU) No 528/2012 (BPR – Biocidal Product Regulation) requires that a risk assessment is performed for biocidal products. Whenever food contamination results from the use of a biocidal product, a dietary risk assessment (DRA) should be performed.

The principles outlined in the CA-March17-Doc.7.6.c-final (interim approach) should be taken into consideration in order to assess whether biocide residues should be further explored. If it is concluded that the assessment is required, this draft guidance document provides the methodology for the estimation of the possible biocide residue transfer into food as a result of professional use of biocidal products. To this end, the amount of biocide residue in food is estimated and compared to a pre-defined trigger value. If the trigger value is exceeded, further evaluation will be required to decide whether the biocide dossier has to be handed over to the Maximum Residue Limit (MRL) setting authority. This document describes methods for estimating biocide residues in food for various professional use scenarios. Due to the complex nature of the use scenarios, model calculations are in certain cases not possible. Instead, biocide residue measurements would be needed. Dietary risk characterisation is not addressed in this guidance document.

For further information to be provided by the applicant and information on risk assessment from other regulatory areas, see Appendix V.

Biocidal products are divided into 22 product types (PTs) (Annex V of BPR), some of which are applied by professional users in areas or on objects where food is produced, stored and/or processed (prepared). In this way, biocidal active substances (a.s.) and/or their degradation products can be transferred into food.

Based on representative uses submitted in the course of EU-wide biocidal active substance evaluations and product authorisations, a number of scenarios have been identified by way of which food can be exposed to biocidal active substances used by professionals:

- Disinfectants and preserved cleaners in the food and drink industry (PT 4, PT 1)
- Aseptic packaging (PT 4)
- Food contact materials treated with or incorporating biocides (e.g. PT 4, PT 12)
- In-can preservatives (PT 6)
- Pest control in the food and drink industry (PT 14, PT 18)
- Storage protection in the food and drink industry (PT 18)
- Treated wood (PT 8)
- Drinking water disinfectants (PT 5)

Other professional use scenarios are unlikely to lead to dietary exposure, but this has to be considered on a case-by-case basis.

For each of these scenarios listed above, possible methods for estimation of biocide residue transfer into food will be discussed in this document. For the listed scenarios in particular, the possibility of biocide residue transfer into food must be considered and addressed either by an assessment or a waiver in the form of a Justification for Non-Submission of Data detailing the reasons for the waiver.

Biocidal products may contain formulants that are substances of concern. Substances of concern may be equally or more hazardous to human health than the active substance itself. An assessment for substances of concern in a biocidal product (formulation) must therefore be performed according to CA-Nov14-Doc.5.11[72].

Particular attention should also be paid to the formation of disinfection by-products (DBPs). A separate guidance on how to evaluate DBPs and their formation has been developed (Guidance on Biocidal Products Regulation (BPR guidance): Volume V Disinfection By-Products). Currently the BPR guidance focuses on PTs 2, 11 and 12 and does not specifically address assessment of DPBs formed in food. However, the BPR guidance presents a strategy for risk assessment of DBPs which should be followed for the DRA of biocidal products used in areas or on objects where food is produced, stored and/or processed (prepared), if relevant.

Under Article 5(1) of the BPR, active substances that are classified as, or meet the criteria to be classified as, carcinogenic category 1A or 1B, mutagenic category 1A or 1B, reprotoxic category 1A or 1B (in accordance with the Regulation (EC) No 1272/2008 for Classification, Labelling and Packaging of substances and mixtures (CLP)), and/or meet the criteria for being persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) according to Annex III to Regulation (EC) No 1907/2006 and/or have endocrine-disrupting properties should not normally be approved. Such active substances should not be allowed for use in biocidal products unless this would have a negative impact on society compared to the risk to humans and the environment of not using the biocidal product; or the risk is negligible; or the active substance is considered essential (Article 5(2) of the BPR). This section of the guidance does not apply to active substances with such classifications for health hazard.

OVERVIEW OF BIOCIDE RESIDUE ASSESSMENT

In order to determine biocide residues in food, the assessment follows the approach which is outlined in figure 7:

Step 1

In the first step, it is assessed whether the use of the biocidal product may lead to transfer of biocide residues to food (see chapter 3). When no transfer of biocide residues to food is expected, a biocide residue assessment is not considered necessary.

Step 2

When transfer of biocide residues to food is expected, the nature of the residue needs to be identified (see chapter 4).

Step 3

[72] Available at https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b

It must be verified whether derived toxicological reference values for the active substance cover the degradation products identified in Step 2. For this purpose, the intrinsic properties and in particular the available information on degradation, toxicokinetics and the overall toxicity profile of the active substance need to be taken into consideration. This information can usually be retrieved from the applicant's dossier.

If the toxicological information shows that an active substance and/or its toxicologically relevant degradation product(s) do not become systemically available and that primary irritation/corrosion at the site of first contact is the only relevant effect observed, a local risk assessment rather than a systemic DRA is required. A biocide residue with irritating properties that is transferred into food can exert its irritating effect on the consumer who eats this food. Therefore, local effects should be addressed in the biocide residue assessment. For general guidance on the assessment of such local effects, see Section 4.4.2.

Based on the results of the nature-of-residue study and the toxicological data on the active substance and its degradation products, a decision is made as to which compounds of the residue are included in the biocide residue definition for DRA (see Chapter 4)

Step 4

Once the biocide residue relevant for DRA is defined, transfer of biocide residues into food is estimated based on this residue definition. An overview on assessment approaches that are appropriate for various biocidal uses is given in Chapter 5. Refinement of biocide residue transfer estimates based on additional information is possible.

Step 5

Estimated biocide residue levels in food are compared to the trigger value. When transfer of biocide residues to food is the result of professional use, the food is placed on the market and may be subject to Maximum Residue Level (MRL) setting and enforcement. According to the EU Commission's interim approach on MRL setting[73], an MRL assessment is necessary "*if there are indications that (i) measurable residue levels can be found in food as a result of the use of the biocidal product for which authorisation is requested and (ii) the applicant fails to demonstrate that these residue levels do not pose a risk to health*." The term "*measurable residue levels*" is not further specified in the interim approach. In this guidance, it is interpreted as a value of 0.01 mg biocide residue/kg food (from here on referred to as "trigger value") [74]. If the Limit of Quantification (LOQ) of the analytical method for food/feed is higher than 0.01 mg/kg, the trigger value corresponds to the LOQ.

When it can be shown that biocide residues in food do not exceed the trigger value, no MRL evaluation is required, unless the biocide residue is particularly toxic. However, a dietary risk assessment will still be required as part of product authorisation. When biocide residues in food exceed the trigger value, the EU Commission's interim approach on MRL setting applies (CA-March17-Doc.7.6.c-final).

Step 6

In the case that MRLs, MLs (Maximum Levels) or specific migration limits (SMLs) are available for the active substance from uses other than biocides (plant protection

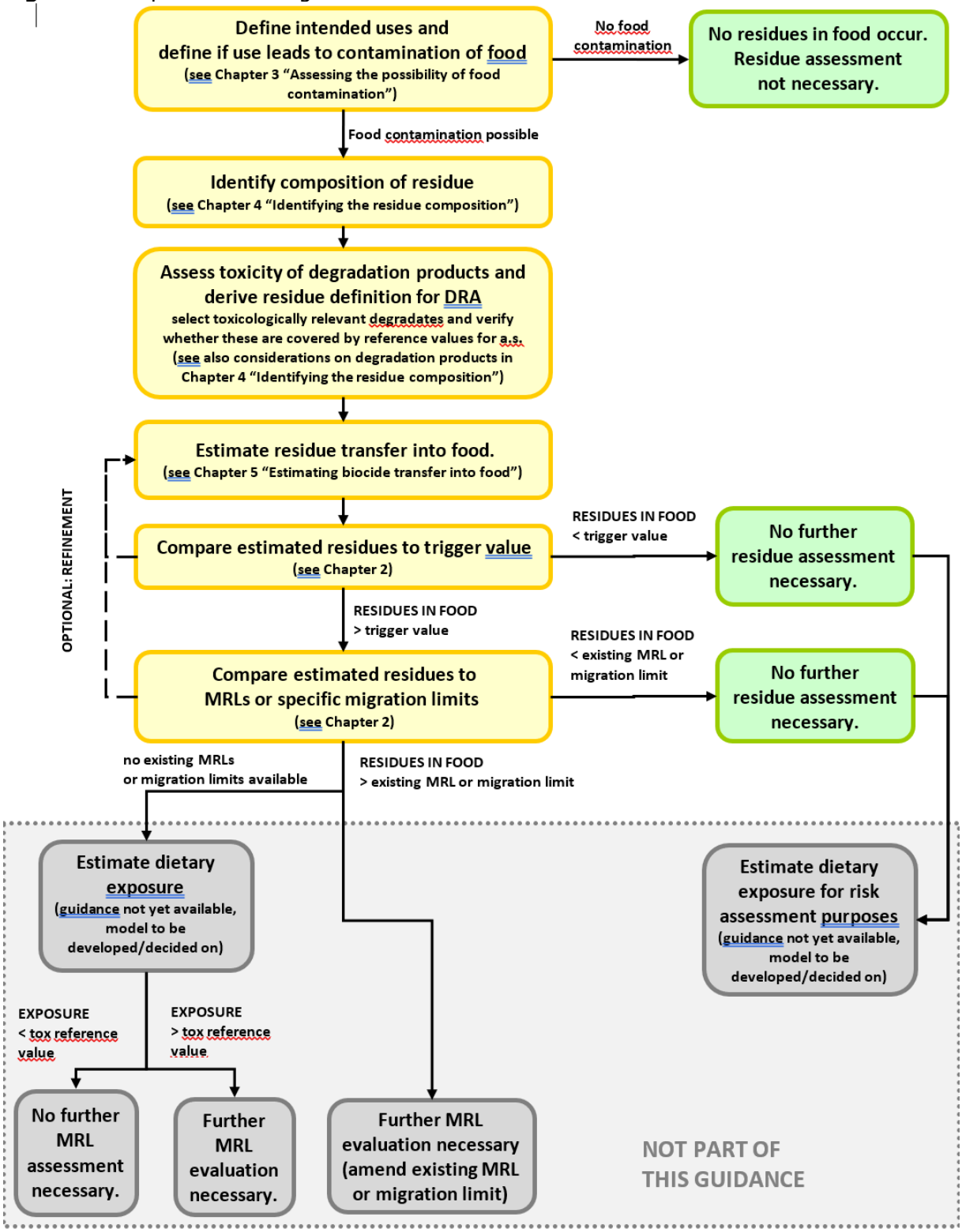
[73] see footnote 4

[74] A value of 0.01 mg a.s./kg food is also used in the regulatory framework for plant protection products, where residue levels at or below this value are considered safe and no MRL evaluation is needed.

products, veterinary medicines, contaminants or food contact materials), estimated biocide residues are compared to this value. If the existing limit covers the expected residues from the biocidal use, no MRL evaluation is required. Nevertheless, a dietary risk assessment will still be required as part of the biocidal product authorisation. If existing limits are exceeded, it must be evaluated whether the existing MRLs, MLs or SMLs could be amended accordingly (not part of this guidance).

If no existing MRLs, MLs or SMLs are available further evaluation will be required, which is not part of this guidance. The EU Commission's interim approach on MRL setting should be followed.

Figure 7. Steps in Assessing Residue Transfer into Foods



5.1.2 ASSESSING THE POSSIBILITY OF FOOD CONTAMINATION

In the first step of DRA, it is assessed whether the use of the biocidal product may lead to a contamination of food. Some biocidal products are designed to preclude food contamination. The product may carry on its label instructions to the user to avoid food contact (e.g. "Keep away from foodstuff, eating utensils or food contact surfaces.") and/or may be formulated in a way that food contamination is unlikely (e.g. a rodenticide

in a bait box; a gel spot application that prevents splashes). If the Applicant concludes that food contamination can be excluded due to label instructions and/or special product formulations, the Applicant must submit a Justification for Non-Submission of Data listing the arguments that led to this conclusion. On the basis of the Justification, the Competent Authority evaluates whether the argumentation is valid. If this is the case, biocide residues in food do not have to be further evaluated.

Label restrictions can generally be accepted as risk management measures, unless the restrictions appear impractical or not plausible. Misuse of any type (e.g. accidental or deliberate) should not be considered in the assessment.

With regard to professional users, it is realistic to assume that label restrictions will be observed. The group of professional users includes pest control operators and trained service personnel, who have received appropriate training and have hands-on experience in the use of biocidal products in food areas.

5.1.3 IDENTIFYING THE BIOCIDE RESIDUE COMPOSITION and DEFINING THE BIOCIDE RESIDUE RELEVANT FOR DRA

Before biocide residues in food can be estimated, it must be determined which toxicologically relevant compounds should be included in the biocide residue definition for DRA. This may include the active substance, one or more of its degradation products or a combination of both.

To identify the composition of the biocide residue, nature-of-residue studies with radiolabelled compounds designed to simulate realistic use conditions of the biocidal product must be performed. It is recommended to follow OECD test guideline 507, "Nature of the Pesticide Residues in Processed Commodities-High Temperature Hydrolysis" taking into consideration the adjustments described in this chapter. Applicants may propose other methods for assessment as long as they are substantiated, well documented and in line with the general principles of the guidance.

A nature-of-residue study must always be performed, unless one of the following waiving arguments applies.

Waiving of the nature-of-residue study is possible if physical-chemical properties indicate that the active substance is stable (solubility, log P_{ow}, volatility, biodegradability, light sensibility, pH, pKa) .

If it can be reasonably justified that the active substance will always be at ambient conditions during and after application, the hydrolysis studies that are part of the core data set for biocidal active substances can be used to identify the biocide residue composition and to define the biocide residue relevant for DRA. If degradation is observed in these studies and if it can be reasonably justified that no new degradation products are likely to be formed at higher temperatures (e.g. on the basis of thermal stability data), studies at higher temperatures (according to OECD TG 507) are not necessary. The biocide residue relevant for DRA is then defined on the basis of the hydrolysis studies of the core dossier.

If the formation of significant amounts of additional relevant degradation products at higher temperatures cannot be ruled out, a nature-of-residue study based on OECD TG 507 (or similar) should be performed. The study conditions of OECD TG 507 should be adapted to the use conditions of biocidal products. To this end, the following must be kept in mind; degradation of the active substance can occur during (i) the application of the biocidal product, (ii) between application and biocide transfer to food (e.g. when biocide treated equipment is rinsed) and (iii) after biocide transfer to food (e.g. during food processing and/or preparation). To cover degradation that occurs after biocide residue transfer into food, the study design must consider the common food processing conditions. OECD TG 507 defines three different hydrolysis conditions to simulate most processing practices (see Table 4-1 lines 1-4). In addition, for biocides, any other relevant degradation conditions that occur during or after application of the biocidal product must be covered (e.g. high temperatures, pH, pressure, enzyme activity). For example, biocides contained in machine dishwashing detergents are exposed to elevated temperatures (70°C) and changes in pH (7 and 11) throughout a machine wash cycle of approximately 215 minutes. These conditions are different from those seen during food processing and must therefore be built into the design of the study. On the other hand, single experiments can be waived if a condition does not apply to the use of the biocide under evaluation.

Table 4-1: Required conditions for nature-of-residue studies for biocides

Temperature (°C)	pH	Time (min)	Process represented
90	4	20	Pasteurisation
100	5	60	Baking, Brewing, Boiling

120	6	20	Sterilisation
Any other relevant conditions occurring during or after application of the biocidal product.			

The presence of the food commodity is not required for the nature-of-residue studies.

Where appropriate, these studies should be conducted with exaggerated amounts of radiolabelled active substance. The values of the measured amounts of active substance and degradation products are then adjusted to the actual use conditions of the biocidal product.

Regarding the characterisation and identification of degradation products, the principles reported in OECD TG 507 apply. For example, degradation products that make up less than 10 % of the total residue do not need to be identified and require no additional toxicological information unless there is reason to believe that they are of toxicological concern, for example due to their chemical structure.

Based on the results of the nature-of-residue study and the toxicological data, a decision is made as to which degradation products are included in the biocide residue definition for DRA. Degradation products that have been found in sufficient quantities as metabolites in the toxicology studies submitted as part of the core data set are already considered in setting the ADI/ARfD. For other degradation products it should be assessed whether the reference values of the active substance (parent) cover their toxicity profile. Read-across, QSAR, TTC or other predictive models can be used to conclude on the adequacy of the parent ADI or ARfD with respect to the degradation products. The OECD (2009) guidance document on the Definition of Residues may be useful in deciding how to proceed. Additional documents covering this topic, e.g. EFSA (2012) "Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment" and EFSA (2016) "Guidance on the establishment of the residue definition for dietary risk assessment" are currently under discussion in the EU, but have not been endorsed yet. Also, consideration should be given to already existing residue definitions for the same active substance in other frameworks such as veterinary medicines (VMP) or plant protection products (PPP).

5.1.4 ESTIMATING BIOCIDE RESIDUE TRANSFER INTO FOOD

Once the biocide residue relevant for DRA is defined, transfer of biocide residues into food is estimated based on this residue definition. The following sections describe methods for estimation of biocide residue transfer into food for the different use scenarios. These include calculation models, rinsing/wiping trials, migration studies and biocide residue studies. Although not specifically mentioned in the chapters, conducting biocide residue studies is always an acceptable approach (see chapter 5.8).

Performing biocide residue studies for all foods would not be practical. Instead, biocide residue studies should be conducted for a selection of representative food commodities that cover all foods. In case specific conditions apply, for example if the biocidal product's intended use is limited in a way that only certain foods are exposed, biocide residue studies can be limited to the specified foods.

Biocide residue studies in stored foods are a central element of both MRL evaluations and DRA. The definition of representative foods therefore falls within the scope of ARTFood as well as the MRL-setting authority and a final agreed list of representative foods will be compiled in collaboration of the two groups. ARTFood has prepared a first proposal for representative foods, which is presented in Appendix II. It may serve as a starting point for discussions with the MRL-setting authority.

It should be noted that potential transfer into food can be reduced by the introduction of risk management measures.

5.1.4.1 Disinfectants and Preserved Cleaners in the Food and Drink Industry

PT 4 biocidal products are used for the disinfection of food and feed areas, including equipment, containers, consumption utensils, counter tops and other surfaces and pipework associated with the production, transport, storage or consumption of food, feed or beverages (including drinking water) for humans and animals. Following this disinfection process biocide residues remaining on treated surfaces may possibly be transferred into food that gets in contact with these surfaces.

Substances most commonly used for disinfection are highly reactive substances (for example active chlorine, hydrogen peroxide, peracetic acid, quaternary ammonium compounds) or other substance likewise iodine, amphoterics (mostly for meat processing operations), alcohols, aldehydes and halogen carboxylic acids (mostly in breweries).

Cleaning and disinfection of surfaces and equipment/machinery takes place in the food, drink and milk industries (FDM sector), in slaughterhouses, butcheries (meat, poultry), fish trade, catering kitchens and canteens, milking parlour systems, food shops and the food transport sector (tankers/vessels). An overview is given in table 5.1-1.

In the food industry, the use of hand disinfectants (PT 1) is common to ensure proper hygiene. If disinfected hands get in contact with food, food can become contaminated. An assessment model for hand disinfectants can be found in section 5.1.4.4. It should be noted that many of the active substances used in hand disinfectants evaporate quickly due to their high vapour pressure (e.g. ethanol, propanol) and are therefore not expected to lead to significant biocide residues in food. No biocide residue calculation needs to be performed in these cases. Other active substances used in hand disinfectants are highly reactive. For these, their degradation products rather than the active substance itself need to be considered in the assessment (as defined in the biocide residue relevant for DRA).

For the purpose of this guidance, professionals are the people working in the relevant

sectors. The level of training is likely to vary. For example, disinfection in catering kitchens and canteens might be performed by the chefs, by specialised cleaning personnel or by unskilled workers depending on whether the machines, floors or kitchen utensils need to be cleaned.

Table 5.1-1Cleaning and disinfection in the FDM sector - examples

Cleaning and disinfection of	CIP/ non-CIP treatment*	Cleaning procedure, Application method
Closed machinery (such as pipe work and tanks/vessels)	CIP	1. remove food from machinery 2. rinse machinery with clean water 3. (optional) detergent wash and rinse 4. rinse installation circuit with disinfection solution 5. drain 6. rinse again with potable water
	Non-CIP	Clean disassembled machinery with ready-to-use sprays or wipes , foams, spraying, fogging, or soaking.
Slaughterhouses and butcheries	Non-CIP (CIP used only in exceptional cases)	Clean disassembled machinery (mainly surfaces) by spraying (low and high pressure), foaming, soaking (dipping) and manual brushing
		Clean flat surfaces (e.g. counter tops) by wiping, soaking or manual brushing
Catering kitchens and canteens (including kitchens in restaurants, hospitals, nursing homes, public institutions), food shops, bakeries, fast food retailers	Non-CIP	Clean flat surfaces (e.g. counter tops) by wiping, soaking or manual brushing
		Clean utensils and dishes in dishwashers
Milking parlour systems (including milking machinery, pipework and milk containers)	CIP	1. empty system 2. circulate water through the equipment 3. add disinfectant to circulating water 4. flush with clean water after disinfection to remove any residues of the biocidal product

* CIP = cleaning in place (i.e. by means of circulation of the disinfectant through the system)

5.1.4.1.1 Food Contamination with Biocides

Biocide residues in food have been reported as shown e.g. by occurrence data (see Appendix III1.1 (Quaternary ammonium compounds) and III1.2 (Chlorate) for more information). Contamination of food with biocide residues generally takes place after application of disinfectant, for example when put on a surface that contains biocide residues either because surface rinse did not take place, surface rinse was ineffective or evaporation of volatile biocide residues was not complete. It is unlikely that

contamination of food takes place during actual application, i.e. when a surface is disinfected while food/feed is still left on a neighbouring surface (e.g. when cleaning coolers or storage rooms).

Disinfection of surfaces not directly in contact with food such as walls, floors, ceilings is generally not considered a scenario of concern. A label restriction stating that food should not come into contact with treated surfaces usually suffices to exclude biocide residue transfer into food. In cases where the disinfectant is applied via nebulising, fumigation, spraying or any other type of application that leads to contamination of neighbouring surfaces, the label restriction must require coverage of all food contact surfaces prior to use as well as thorough cleaning of food contact surfaces after use. Please refer to chapter 5.4 (Pest control in the food and drink industry) for more details.

5.1.4.1.2 Scenarios

Regarding the cleaning and disinfection of machinery in the FDM sector, several scenarios can be considered, depending on the type of food processed in the machinery (liquid or solid) and whether the machines are dismantled before cleaning or not.

Liquid foods in closed machinery (volume based dosing, rinsing, no drying)

Closed machinery for liquid foods like beer and soft drinks includes mixing and storage tanks, pipes/pipelines, filtration equipment, filling machines, kegs and draft installations (taps) which come into contact with beverages. Presumably the machinery is not dismantled before cleaning and disinfection. After treatment, the machinery is rinsed with water and drained. Any remaining rinsing water left in the machinery is mixed with the liquid food. Systems for liquid foods are never dried after rinsing. Dosing in closed machinery is expressed on a volume basis (e.g. 5-10% of the installation volume).

Solid foods in closed machinery (volume based dosing, rinsing, no drying)

Solid foods that are transportable in pipes (e.g. preparation of sausages, preparation of ice cream, aseptic filling machines) will not be as effectively mixed with residual disinfectant as liquid foods. As a worst case, it can be assumed that the food hardly mixes with the rinsing water and the first stream of solid food pushes the remaining rinsing solution out of the machinery. Hence the first food that comes out of the machine contains higher levels of biocide residues than subsequent food. For this reason, cleaning manuals for machinery might specify an amount of food that has to be discarded, before the food has sufficient quality (i.e. no residues > LOQ, or according to internal references).

Solid foods in closed machinery (volume based dosing, rinsing & drying)

For closed machinery that is air dried after treatment and rinsing, any biocide residues present will be on the inner surfaces of the machinery. As a worst case, it can be assumed that the first food that comes out of the machine contains all the biocide residues that are left on the food contact areas in the machinery. For this reason, cleaning manuals for machinery might specify an amount of food that has to be discarded, before the food has sufficient quality (i.e. no residues > LOQ, or according to internal references).

In this context particular attention should be given to substances exhibiting surface-active characteristics, such as quaternary ammonium compounds.

Solid foods in open or closed machinery and on surfaces (area based dosing, rinsing & drying)

Open machinery includes slicing machines, sawing machines in butcheries and conveyor lines. Some of this machinery is dismantled for cleaning and disinfection. After treatment, the biocidal product is rinsed off with water and the equipment parts are air dried before reassembly. Any biocide residues present will be on the surface. As a worst case, the first food that is taken through the machinery will take up the biocide residues that are left on the food contact areas in the machinery. In this context, particular attention should be given to substances exhibiting surface-active characteristics, such as quaternary ammonium compounds.

For open machinery and for some closed machinery, dosing is area based (e.g. 100 ml biocidal product/m^2). For area based applications, dose rates are determined in a laboratory setting on a flat surface. In practice, users will never calculate the actual area of the equipment in question. Manual application to small areas is generally performed until the whole area is visually covered. For larger areas, generally foam or spray applicators are used until run-off. Based on expert information, 6-40 ml biocidal product/m^2 is left on walls after draining.

Solid foods in open machinery and on surfaces (area based dosing, evaporation, no rinsing)

Area based dosing followed by evaporation is generally only used on small open machines, like slicing machines. After treatment, the biocidal product is left to evaporate and is not rinsed off.

5.1.4.1.3 Biocide residue assessment approach

In order to develop a model suitable to estimate biocide residues in food, it is necessary to know internal volumes or internal areas of food producing machinery as well as the amounts of food that come into contact with the disinfectant. This information is highly dependent on type of food, the size and design of the equipment etc., making it difficult to obtain reliable information or to set standardised default values.

Nevertheless, screening models for different scenarios are proposed as first tier approach (see 5.1.5), calculating a worst-case estimate of biocide residues in food. The information for the models has been obtained from various sources, e.g. authorisation procedures, questionnaires and guidelines[75].

For higher tier assessment experimental data will become necessary. Although measurements in food give a good indication of biocide residues that can be expected, several different foods have to be analyzed and agreements have to be made on which foods to analyze and a what stage in the food processing. Therefore, as a second tier, it is sufficient to analyze the biocide residues in the rinsing water and the inner surfaces of food equipment in order to determine whether the trigger value is exceeded. Equivalents of the trigger value of 0.01 mg biocide residue/kg food have been derived for rinsing water and surface areas (see appendix I.1).

5.1.4.1.4 Tier 1 – Screening models for estimating biocide residues in food

Calculation models are proposed below for estimating transfer of biocide residues into foods, covering the conditions for closed/open containers (section 5.1.4.1), CIP systems

[75] Chapter 6 in ECHA Guidance Vol III Parts B+C, Version 4.0, December 2017 (Section 5.3 of the current Guidance).

(pipes and closed containers) (section 5.1.4.2), open food preparation areas (section 5.1.4.3) as well as for hand disinfection (section 5.1.4.4).

For screening assessment calculation models should be chosen to cover all scenarios relevant for the intended use. For example, for a biocidal product to be used in a CIP application, calculation should be performed for a model system consisting of closed containers in combination with pipes (see example in section 5.1.4.2).

5.1.4.1.4.1 Disinfection of closed or open containers

Description of scenario

A container (open or closed) used for the production, transport or storage of foodstuffs is disinfected with a biocidal product (automatically or manually). Biocide residues remaining on the disinfected surface are transferred into food that is filled into that container after the disinfection process.

Assumptions

- Depending on their function the size of such containers is highly variable, e.g. bottles for storage and transport of beverages; or tanks/vessels as part of food producing machinery. In the calculation the type of container should be selected as appropriate (see default values in Appendix I Table I-1 and Appendix I.3). Containers with a large surface-to-volume ratio represent the worst case.

- 100 % of biocide surface residue is transferred to food (liquid or solid) in contact with the surface. The property of the container inner surface material (stainless steel, plastic, glass, etc.) is not considered.

- The composition of the food (level of protein, fat, carbohydrate) is not taken into account. As worst case a food density of 0.06 g/mL is assumed in the calculations (representing the worst-case for liquid, semi-solid/paste-like and solid foods). The density could be adjusted according the selected type of food if feasible (depending on the intended application area of the biocidal product, e.g. the density of milk in the milk industry or the density of ice cream in the gastronomy). For more information on food density see Appendix I.6.

- Containers are considered to be fully filled.

- Within one container an even distribution of biocide residues in the food can only be assumed for liquids. As a worst case for solid (powdered, granular) and semi-solid (paste-like) foods the first layer of solid and semi-solid (paste-like) foods in contact with the container/machinery inner surface is considered in the calculations (default 1 cm layer, see Appendix I).

Estimation of biocide residue transfer into food

The calculations below assume as a first tier that the parent compound is not degraded and thus the concentration of the active substance in the biocidal product is used for the estimation of biocide residue (b.r.) levels in food.

$$R_{food}\ [\text{mg b.r./L}] = C_{b.r.} \times DS_{surface} \times A_{inner\ surface} \times TF \times RF \div V_{container}$$

$$R_{food}\ [\text{mg b.r./kg}] = R_{food}\ [\text{mg b.r./L}] \div \rho_{food}\ [\text{g/mL}]$$

Where:

R_{food} Biocide residues transferred into food [mg b.r./L or mg b.r./kg]

$C_{b.r.}$ Concentration of a.s. in in-use disinfection solution [mg/L] as first tier.

$DS_{surface}$ Amount of disinfection solution remaining on surface [L/cm^2 or L/m^2] (for default values see Appendix I)

$A_{inner\ surface}$ Inner surface area of container [cm^2 or m^2] (for default values see Appendix I)

$V_{container}$ Volume [L] of container (e.g. bottle, vessel, tank) (for default values see Appendix I)

ρ_{food} density of food [g/mL] (default value for liquids + semi-solids + solids, see Appendix I, Table I.1 and Appendix I.6)

TF Mass transfer efficiency factor (fraction of the biocide residue transferred from inner container surface into food) (default: TF = 1)

RF Refinement factor (default RF =1)

Refinement options (Tier II)

- Justified product specific data on the rinsing efficiency (for the appropriate material) may reduce the refinement factor to RF < 1.

- Data on degradation of the active substance. As the nature-of-residue study and the toxicological potency of the degradation products has already been assessed in step 2 and step 3, the active substance concentration in the equation above could directly be replaced by the biocide residue concentration (according to the biocide residue definition for DRA).

- Data on evaporation of biocide residues.

Example 5.1-1: Disinfection of water bottles

Intended use: The biocidal product is used for the disinfection of water bottles. The in-use disinfection solution is a 1.5% dilution of the concentrated biocidal product (2000 mg a.s./L).

Estimation of biocide residue transfer into food

$C_{b.r.}$ (biocidal product specific information)	30 mg a.s./L (= 1.5% × 2000 mg a.s./L) = 30 mg b.r./L as first tier
$DS_{surface}$	2×10^{-6} L/cm^2 (default, see Appendix I, Table I-1)
$V_{container}$	1 L (default, see Appendix I, Table I-1)
$V_{1\ cm\ inner\ layer}$	0.725 L (default see Appendix I, Table I-1)
$A_{inner\ surface}$	725 cm^2 (default, see Appendix I, Table I-1)
$\rho_{food,\ liquid}$	0.789 g/mL (default for liquids, see Appendix I, Table I-1 and Appendix I.6)
$\rho_{food,\ semi\text{-}solid}$	0.496 g/mL (default for semi-solids, see Appendix I, Table I-1 and Appendix I.6)
TF	1
$RF_{Tier\ I}$	1

(1) Calculation (for liquid filling of bottle = even distribution of biocide residues in food))

R_{liquid} [mg b.r./L] = $C_{b.r.} \times DS_{surface} \times A_{inner\ surface} \times TF \times RF_{Tier\ I} \div V_{container}$

= 30 mg b.r./L × 2×10^{-6} L/cm^2 × 725 cm^2 × 1 × 1 ÷ 1 L

= 0.044 mg b.r./L

R_{liquid} [mg b.r./kg] = R_{liquid} [mg b.r./L] ÷ $\rho_{food,\ liquid}$

= 0.044 mg b.r./L ÷ 0.789 kg/L = 0.056 mg b.r./kg

(2) Calculation (for semi-solid filling of bottle = inhomogeneous distribution of biocide residues in food)

$R_{food,\ semi\text{-}solid}$ [mg b.r./L]= $C_{b.r.} \times DS_{surface} \times A_{inner\ surface} \times TF \times RF_{Tier\ I} \div V_{1\ cm\ inner\ layer}$

= 30 mg b.r./L × 2×10^{-6} L/cm^2 × 725 cm^2 × 1 × 1 ÷ 0.725 L

= 0.059 mg b.r./L

$R_{food,\ semi\text{-}solid}$ [mg b.r./kg] = R_{food} [mg b.r./L] ÷ $\rho_{food,\ semi\text{-}solid}$

= 0.059 mg b.r./L ÷ 0.496 kg/L = 0.119 mg b.r./kg

The trigger value of 0.01 mg b.r./kg is exceeded.
See Chapter 2 on how to proceed with the assessment.

Example 5.1-2: Disinfection of ice cream machines

Intended use: The biocidal product is used for the daily disinfection of ice cream machines. The in-use disinfection solution is a 1.5% dilution of the concentrated biocidal product (2000 mg a.s./L).

Estimation of biocide residue transfer into food

$C_{b.r.}$ 30 mg a.s./L (1.5% × 2000 mg a.s./L) = 30 mg b.r./L as first tier (biocidal product specific information)

$DS_{surface}$ 2×10^{-6} L/cm^2 (default, see Appendix I)

V 1.7 L (default, see Appendix I, Table I.3)

$A_{inner\ surface}$ 102 cm^2 (default, see Appendix I, Table I.3)

$\rho_{ice\ cream}$ 0.54 g/mL (default applicable for ice cream, see Appendix I.6, Table I.6-2)

TF 1

$RF_{Tier\ I}$ 1

Calculation

$R_{ice\ cream}$ [mg b.r./L] = $C_{b.r.} \times DS_{surface} \times A_{inner\ surface} \times TF \times RF_{Tier\ I} \div V$

= 30 mg b.r./L × 2 × 10^{-6} L/cm^2 × 102 cm^2 × 1 × 1 ÷ 1.7 L

= 0.0036 mg b.r./L

R_{food} [mg b.r./kg] = R_{liquid} [mg b.r./L] ÷ $\rho_{ice\ cream}$

= 0.0036 mg b.r./L ÷ 0.54 kg/L = 0.0067 mg b.r./kg

The trigger value of 0.01 mg b.r./kg is not exceeded. No quantifiable biocide residues are expected in food from the described application.

Example 5.1-3: Disinfection of wine barrels

Description of scenario
Disinfection of empty wooden wine barrels (prior to filling with wine) by combustion of substance X (precursor substance). Treated barrels will contain gaseous combustion product XO (= active substance) that will rapidly dissolve during filling of barrels with wine. The active substance XO does not degrade any further and thus the biocide residue relevant for DRA is equivalent to active substance XO.

Assumptions
- Application rate: 5 g biocidal product /225 L barrel corresponding to 10 g active substance XO released in a 225 L barrel (biocidal product specific information)
- Fraction of active substance bound to wine (F): 100% (default value = worst case).
- Volume of wine barrel (V_{barrel}): 225 L (default, see Appendix I.3)

Estimation of biocide residue transfer into food
m_{XO}: 10 g amount of substance XO released in barrel (mg b.r.)
F100 %
V_{barrel}225 L
$\rho_{liquid,\ wine}$0.96 g/mL (default applicable to wine, see Appendix I.6, Table I.6-2)

Calculation
R_{wine} [mg b.r./L]= $m_{XO} \times F \div V_{barrel}$
= 10000 mg b.r.x 100% ÷ 225 L
= 44 mg b.r./L
R_{wine} [mg b.r./kg]= R_{wine} [mg b.r./L] ÷ $\rho_{liquid,wine}$
= 44 mg b.r./L ÷ 0.96 kg/L = 45.8 mg b.r./kg

The trigger value of 0.01 mg b.r./kg is exceeded.
See Chapter 2 on how to proceed with the assessment.

5.1.4.1.4.2 CIP systems: disinfection of pipes and closed containers

Description of scenario

A closed food processing machine is automatically disinfected with a biocidal product (Clean-in-place system). Residual biocidal product remaining on the disinfected machine´s inner surfaces is transferred into food that is processed in that machinery after the disinfection process.

Assumptions

- Dimensions of pipes and containers are variable. In the calculation the type of pipe and container should be selected as appropriate (see list of proposed default values in Appendix I, Table I-1 and Appendix I.3).

- 100% of biocide surface residue is transferred into food in contact with the surface.

- The property of the pipe and container inner surface material (stainless steel, plastic joints etc.) is not considered.

- The composition of the food (level of protein, fat, carbohydrate) is not taken into account. As worst case a food density of 0.496 g/mL is assumed in the calculations (representing the worst-case for liquid and semi-solid foods). The

density could be adjusted according the selected type of food if feasible (depending on the intended application area of the biocidal product, e.g. the density of milk in the milk industry).

- Pipes and vessels are considered to be fully filled.

- Within a CIP system an even distribution of biocide residues in the food can only be assumed for liquids. As a worst case for solid (powdered, granular) and semi-solid (paste-like) foods the first layer of solid and semi-solid (paste-like) foods in contact with the container/machinery inner surface is considered in the calculations (default 1 cm layer, see Appendix I).

Estimation of biocide residue transfer into food

The calculations below assume as a first tier that the parent compound is not degraded and thus the concentration of the active substance in the biocidal product is used for the estimation of biocide residue (b.r.) levels in food.

R_{food} [mg b.r./L] = $C_{b.r.} \times DS_{surface} \times A_{inner\ surface} \times TF \times RF \div V_{pipe+cont}$

R_{food} [mg b.r./kg] = R_{food} [mg b.r./L] $\div$ ρ_{food} [g/mL]

Where

R_{food}	Biocide residues (b.r.) transferred into food [mg b.r./L or mg b.r./kg]
$C_{b.r.}$	Concentration of a.s. in in-use disinfectant solution [mg/L] as first tier
$DS_{surface}$	Amount of disinfection solution remaining on surface [L/cm^2 or L/m^2]
$V_{pipe+cont}$	Volume of pipe and container [cm^3] ($V_{pipe} = \pi R_{pipe}^2 \times L_{pipe}$)
L_{pipe}	Pipe length [cm] ($L_{pipe} = V_{Pipe} \div \pi R_{pipe}^2$)
R_{pipe}	Inner radius of pipe [cm]
$A_{inner\ surface}$	inner surface area [cm^2 or m^2] ($A_{inner\ surface} = 2 \pi R_{pipe} \times L_{pipe}$)
ρ_{food}	density of food [g/mL] (default value for liquids + semi-solids, see Appendix I, Table I.1 and Appendix I.6)
TF	mass transfer efficiency factor (fraction of the biocide residue transferred from inner pipe or container surface into food) [default value = 100%]
RF	Refinement factor [$RF_{Tier\ I}$ = 1 for screening assessment without rinsing]

Refinement options (Tier II)

- Justified product specific data on the rinsing efficiency (for the appropriate material) may reduce the refinement factor to RF < 1.

- Data on degradation of the active substance. As the nature-of-residue study and the toxicological potency of the degradation products has already been assessed in step 2 and step 3, the active substance concentration in the equation above could directly be replaced by the biocide residue concentration (according to the biocide residue definition for DRA).

- Data on evaporation of biocide residues.

Example 5.1-4: Disinfection of pipes

Intended use: The biocidal product is used for the daily disinfection of pipe systems in the food industry. The in-use disinfection solution is a 3% dilution of the concentrated biocidal product (10 g a.s./L).

Estimation of biocide residue transfer into food

$C_{b.r.}$ 300 mg a.s./L (3% of 10000 mg/L) = 300 mg b.r./L as first tier (product specific information)
$DS_{surface}$ 2×10^{-6} L/cm² (default, see Appendix I)
R_{pipe} 0.5 cm (default, see Appendix I)
V_{pipe} 1 L ≙ 1000 cm³ (default, see Appendix I)
L_{pipe} $L_{pipe} = V_{Pipe} \div R_{pipe}^2 = 1273$ cm (default, see Appendix I)
$A_{inner\ surface}$ $A_{inner\ surface} = 2\ \pi\ R_{pipe} \times L_{Pipe} = 4000$ cm² (default, see Appendix I)
ρ_{food} 0.496 mg/mL (default for liquids and semi-solids, see Appendix I, Table I-1 and Appendix I.6)
TF 1
$RF_{Tier\ I}$ 1

Calculation

R_{food} [mg b.r./L] = $C_{b.r.} \times DS_{surface} \times A_{inner\ surface} \times TF \times RF \div V_{Pipe}$
= 300 mg b.r./L × 2×10^{-6} L/cm² × 4000 cm² × 1 × 1 ÷ 1 L
= 2.4 mg b.r./L

R_{food} [mg b.r./kg] = R_{food} [mg b.r./L] ÷ ρ_{food}
= 2.4 mg b.r./L ÷ 0.496 kg/L = 4.8 mg b.r./kg

The trigger value of 0.01 mg b.r./kg is exceeded.
See Chapter 2 on how to proceed with the assessment.

Example 5.1-5: Disinfection of a CIP system

Intended use: The biocidal product is used for the daily disinfection of closed food producing machinery (CIP system). The in-use disinfection solution is a 3% dilution of the concentrated biocidal product (10 g a.s./L).

Estimation of biocide residue transfer into food

$C_{b.r.}$ 300 mg a.s./L (3% × 10000 mg/L) = 300 mg b.r./L as first tier (biocidal product specific information)
$DS_{surface}$ 2×10^{-6} L/cm² (default, see Appendix I)
ρ_{food} 0.496 mg/mL (default for liquids + semi-solids, see Appendix I, Table I-1 and Appendix I.6)
TF 1
$RF_{Tier\ I}$ 1

Pipe system

R_{pipe} 5 cm (default, see Appendix I)
L_{pipe} 2000 cm (default, see Appendix I)
V_{pipe} $V_{pipe} = L_{pipe} \times R_{pipe}^2 = 2000$ cm × 25 cm² = 50 000 cm³ = 50 L
$A_{inner\ surface\ pipe}$ $A_{inner\ surface} = 2\ \pi\ R_{pipe} \times L_{pipe} = 2\ \pi \times 5$ cm × 2000 cm = 62 832 cm²

Container

V_{cont} 900 L (default, see Appendix I)
$A_{inner\ surface\ cont}$ 5.94 m² = 59400 cm² (default, see Appendix I)

Calculation

For the calculation, a model machine consisting of pipes and two containers is considered (see Appendix I).

R_{food} [mg b.r./L] = $C_{b.r.} \times DS_{surface} \times (A_{inner\ surface\ pipe} + 2 \times A_{inner\ surface\ cont}) \times TF \times RF \div (V_{pipe} + 2 \times V_{inner\ surface\ cont})$
= 300 mg b.r./L × 2 × 10^{-6} L/cm^2 × (62 832 cm^2 + 2 × 59400 cm^2) × 1 × 1 ÷ (50 L + 2 × 900 L)
= 0.059 mg b.r./L
R_{food} [mg b.r./kg] = R_{food} [mg b.r./L] ÷ ρ_{food}
= 0.059 mg b.r./L ÷ 0.496 kg/L = 0.119 mg b.r./kg

The trigger value of 0.01 mg b.r./kg is exceeded.
See Chapter 2 on how to proceed with the assessment.

5.1.4.1.4.3 Disinfection of open food preparation areas

Description of scenario

Open food preparation areas and conveyor belts in the food industry, restaurants or canteens are cleaned manually or semi-manually followed by disinfection with spray or wiping products.

Assumptions

- 100% of biocide surface residue is transferred to foods in contact with the surface.
- The property of the food preparation surface material (stainless steel, plastic cutting crushers, etc.) is not considered.
- The composition of the food (level of protein, fat, carbohydrate) is not taken into account.
- No additional rinsing step is considered in Tier I.

Estimation of biocide residue transfer into food

The calculations below assume as a first tier that the parent compound is not degraded and thus the concentration of the active substance in the biocidal product is used for the estimation of biocide residue (b.r.) levels in food.

R_{food} [mg b.r./kg] = $C_{b.r.} \times AR \times SWR_{food} \times TF \times RF$

Where

R_{food}	Biocide residue transferred into food [mg b.r./kg]
$C_{b.r.}$	Concentration of a.s. in in-use disinfectant solution [mg/L] as first tier
AR	Application rate [L/cm^2]
SWR_{food}	Surface area-weight-ratio, i.e. food surface area in contact with food preparation area related to food weight (default SWR_{food} = 2000 cm^2/kg, see Appendix I)
TF	mass transfer efficiency factor (fraction of the biocide residue transferred from surface area into food) (default value = 1)
RF	Refinement factor (default RF = 1)

Refinement options (Tier II)

- Justified product specific data on the rinsing efficiency (for the appropriate

material) may reduce the biocide residue factor to RF < 1.

- Justified biocide residue mass transfer efficiency. Experimental data for appropriate materials may reduce the transfer factor to TF < 1).

- Data on degradation of the active substance. In that case the $C_{b.r.}$ reflects the concentration of the biocide residue (according to the residue definition for DRA) in the the disinfection solution [mg/L]. As the nature-of-residue study and the toxicological potency of the degradation products has already been assessed in step 2 and step 3, the active substance concentration in the equation above could directly be replaced by the biocide residue concentration (according to the residue definition for DRA).

- Data on evaporation of biocide residues.

Example 5.1-6: Disinfection of food preparation area

Intended use: The biocidal product is used for the daily disinfection of open food preparation areas in professional kitchens. The in-use disinfection solution is a 3% dilution of the concentrated biocidal product (10 g a.s./L).

Estimation of biocide residue transfer into food

$C_{b.r.}$ 300 mg a.s./L (= 3% × 10 000 mg a.s./L) = 300 mg b.r./L as first tier
AR1 × 10^{-5} L/cm^2 (biocidal product specific value)
SWR_{food}2000 cm^2/kg (default, see Appendix I)
TF1 (default)
$RF_{Tier\ I}$1 (default)

Calculation
$R_{food} = C_{b.r.} \times AR \times SWR_{food} \times TF \times RF$
$= 300 \text{ mg b.r./L} \times 1 \times 10^{-5} \text{ L/cm}^2 \times 2000 \text{ cm}^2\text{/kg} \times 1 \times 1$
$= 6 \text{ mg b.r./kg}$

The trigger value of 0.01 mg b.r./kg is exceeded.
See Chapter 2 on how to proceed with the assessment.

5.1.4.1.4.4 Hand disinfection

Description of scenario

Product Type 1 (PT 1) biocide products (BP) are used for disinfection related to human body hygiene, including antiseptics used in topical application on intact human skin surface to prevent infections. These comprise hygienic hand disinfection and surgical hand disinfection.

In the framework of the DRA and the estimation of food and feed exposure to PT 1, only hygienic hand disinfection is considered relevant to develop in this guidance (no food contamination is expected from surgical uses).

Two types of products can be distinguished: Hand rub products and Hand wash products.

- Hand rub products**:** these products are applied on dry skin of the hands, then hands are rubbed intensively, the disinfectant is left on the skin and let to dry. On the one hand, this may involve BPs exhibiting rapid evaporation (vapour pressure

> 0.1 Pa). Since the disinfectant will evaporate before handling food, the transfer from treated hands to food is considered negligible. On the other hand, for leave-on products not likely to evaporate, the calculation model for "Hand disinfection for human hygiene" as described below may be applied for estimating residue levels in food. Regarding Disinfection By-products (DBP) potentially formed, no assessment can be conducted until an EU harmonized guidance on DPB linked to PT 1 uses becomes available.

- Hand wash products: These antimicrobial soaps for hygienic hand wash are applied on wet skin and are rinsed off with water, after hands are rubbed intensively. These products contain in general non-volatile compounds. Hand disinfection may be carried out by professionals, e.g. at food processing facilities or in hospitals. Regarding this kind of BP, the transfer from treated hands to food is considered relevant, and a dietary exposure assessment is considered necessary.

Hence, a scenario to estimate residue transfer into food prepared by hands treated with PT 1 in the framework of professional uses is derived hereafter[76].

Assumptions

- The application rate, expressed as g or mL of BP for inside and outside of both hands, is considered to estimate the exposure. The applied amount of soap depends on the product in use and is given by the information provided by the applicant and communicated on the label (e.g. 3 g biocidal product for both hands).

- The default value of portion of hand that can be in contact with food for a professional user is 0.75, which represent the inside and outside of both hands (see Appendix I, Table I-1).

- A retention factor of 1% after rinsing can be used for hand wash products (see Appendix I, Table I-1).

- Transfer factor from hand to food: 50% (see Appendix I, Table I-1 for default values)

- Exposure of professional users (see Appendix I, Table I-1)

- The frequency of hand contact with food is not included in the calculation, as the amount that is transferred to food should be the same after each disinfection.

- The default amount of food that can be touched by disinfected hands is 1 kg. (see Appendix I, Table I-1)

Estimation of biocide residue transfer into food

$$R_{food} = AR \times C_{a.s.\ in\ bp} \times H_{food\ contact} \times TF \times RF \div m_{food}$$

Where:

R_{food} Biocide residues transferred into food [mg b.r./kg]

AR Application rate for both hands [g or mL BP]

[76] This scenario was developed in accordance with the PT 19 as agreed on at ARTFood meeting in November 2019.

$C_{a.s.\ in\ bp}$ Concentration of a.s. in the BP [%]
$H_{food\ contact}$ Portion of hand in contact with food [%]
TF transfer factor (fraction of biocide residue transferred from hand surface to food) [%]
RF retention factor (fraction of biocide residue retained on hands after hand washing) [%]
m_{food} amount of food that is touched by disinfected hands [kg]

Refinement options (Tier II)

Study data to support refined transfer factor

Example 5.1-7: Hand disinfection

Intended use: An antimicrobial soap is used by a professional user for washing and disinfecting hands. The user is working in the food and drink industry and may touch food items with his hands.

Estimation of biocide residue transfer into food
AR3 g (biocidal product specific information)
$C_{a.s.\ in\ bp}$0.9% (biocidal product specific information)
$H_{food\ contact}$0.75 (default value, see Appendix I, Table I-1)
TF50% (default value, see Appendix I, Table I-1)
RF1% (default value, see Appendix I, Table I-1)
m_{food}1 kg (default value, see Appendix I, Table I-1)

Calculation
$R_{food} = AR \times C_{a.s.\ in\ bp} \times H_{food\ contact} \times TF \times RF \div m_{food}$
$= 3\ g \times 1000 \times 0.9\% \times 0.75 \times 50\% \times 1\% / 1kg = 0.101$ mg b.r./kg food

The trigger value of 0.01 mg b.r./kg is exceeded.
See Chapter 2 on how to proceed with the assessment.

5.1.4.1.5 Tier 2 – Rinsing and wiping tests

In food production, hygiene codes apply, which are specific for industry, restaurants, butcheries, the transport sector etc.[77] In most countries such codes often specify that machinery be rinsed after cleaning and disinfection[78] (exceptions are biocidal products which evaporate quickly such as ethanol or propanol) and further stipulate that the tasks performed (e.g. cleaning, disinfection, rinsing, checking of rinsing performance) have to be registered by the user and checked by the inspection service.

An effective rinsing process will remove most of the active substance and its degradation products, so that no biocide residues are to be expected in food. If the efficiency of the rinsing process for a biocidal product is supported by data submitted with the application, experimental data on biocide residues in food do not have to be submitted. In proving the efficiency of the rinsing process, it must be taken into account that some active substances and/or their degradation products have low water solubility or high surface

[77] Examples include Regulations (EC) No 852/2004, (EC) No 853/2004, (EC) No 882/2004 and (EC) No 183/2005
[78] G. Wildbrett. Reinigung und Desinfektion in der Lebensmittelindustrie. Behr's Verlag. Kapitel 14.1, S. 343

activity and will therefore not be efficiently removed through rinsing (e.g. quaternary ammonium compounds).

Rinsing in large closed systems is never expressed on volume basis but on a circulation time basis. Circulation time is the time required for a liquid to go through the whole system. It depends on the components of the system (pipelines are filled completely with rinsing water, but storage/mixing tanks are not filled completely) and the pumping capacity of the system. Required rinsing is expressed as x times the circulation time. X is determined by biocide residue measurements in the discharged rinsing water. Because cleaning of closed systems is generally performed automatically with only one programme for all objects, x is set for the most difficult component to clean. This means that the other components in the system are rinsed with exaggerating amounts of rinsing water.

In CIP systems, the pH, conductivity and turbidity of a range of fluids are commonly checked in-line. pH sensors can be used to control additions of acid or alkali while conductivity sensors serve to monitor levels of dissolved salts (e.g. caustic substances used in bottle-washing treatments). The quality of process water can be monitored with turbidity sensors to optimise re-use of cleaning water.

5.1.4.1.5.1 Data requirements for biocidal products that are rinsed off

For biocidal products that are rinsed off specific experiments are required showing that rinsing with water is effective and that the biocide residue (according to the biocide residue definition for DRA) does not stick to the surface considering the most common materials used in food producing equipment/machinery/surfaces[79]. For the biocide residue removal experiment, biocidal treatment and rinsing are conducted as per label instructions. Effectiveness of rinsing is assessed by measuring the biocide residues in the last rinsing water (if possible) and on the treated surface of two representative pieces of equipment (milking machine and ice-cream machinery).

To determine biocide residues on treated surfaces, a wiping test is performed just before biocidal treatment, immediately after biocidal treatment and after the appropriate rinsing process. The wiping tests before and after biocidal treatment serve as controls. The wiping test before biocidal treatment provides the background level for the experiment. If the background level is already higher than the maximum allowable concentration (see "criteria for acceptance of rinsing tests"), the wiping test is not appropriate. The wiping test just after biocidal treatment gives an indication of the maximum biocide residue level after application. It should correspond with the level that is expected after biocidal treatment. If this is not the case, then wiping might not be efficient and/or the analytical method performance might be insufficient. In the biocide residue removal experiment, biocide residues are wiped off a defined surface area with dry pads or pads soaked with an appropriate solvent (Kells and Solomon, 1995). The biocide residues from the pads are extracted and biocide residues are measured in the extract. Possible solvents are water at high temperature (e.g. 60-100°C), acid (e.g. 0.01 M HCl in water), alkali (e.g. 0.01 M NaOH) and/or any common appropriate extraction solvent (e.g. methanol, ethyl acetate, acetonitrile, acetone, hexane) depending on the compound to extract. The biocide residue in the extract is then calculated back to mg b.r./m^2 of the original

[79] Stainless steel is the most common material, although copper is also used in breweries and distilleries, rubber for conveyor belts (transporting fruits/vegetables) and possibly also plastic for tubing (e.g. polyethylene). Other possible materials are galvanised iron, zinc, lead, tin, glass, wood (cutting boards/chopping blocks), concrete/cement (floors) and painted surfaces (FAO, 1985).

surface.

The wiping test will give an indication on whether the biocide sticks to the surface material and if rinsing effectively removes potential biocide surface residues. The wiping test will give no information about biocide residues that may be trapped in hard-to-reach places within the machinery, but do not adhere to the inner surface. However the trapped biocide residues will most likely be pushed out of the machinery with the first food that is processed following the cleaning procedure and will commonly be discarded. As geometry of machineries is highly variable (no EU standards available) the supplier of the machinery should provide information on effective disinfection and cleaning procedures for the individual equipment and the necessity to discard first food. As common practice in the food industry critical control points need to be defined and monitored (HACCP concept[80]). Special attention should be given to machinery that contains membranes (e.g. used in processing of milk, wine, beer and juice), as the biocide residue situation and subsequent rinsing behaviour may differ from that of plain surfaces within the equipment.

Requirements for the biocide residue removal experiments include information on surface activity of the active substance and its degradation products (as included in the biocide residue definition for DRA) and the biocidal product, a validated analytical method and specifications on the rinsing process (given on the biocidal product label). The analytical method should be able to determine the biocide residue (according to the biocide residue definition for DRA) in water and the wipe extract. The quantification limit of monitoring methods should be given as well as minimum frequency of checking rinsing efficiency and how to perform this check.

In the biocide residue removal experiment for machinery, actual machinery rather than models should be used. Wiping tests must be performed at the food inlet and outlet of the equipment. If possible, the biocide residues in the last rinsing water must be measured, which is generally only possible for closed machinery. Rinsing efficiency is not only influenced by the physico-chemical properties of the biocide residue, but to a large extent by physical factors such as the amount of valves and tubing, the different materials the machine consists of and the general complexity of the machine. A model setup would therefore not be able to adequately reflect rinsing conditions. The trials must be performed with two representative pieces of equipment, ice-cream machinery and milking machinery. Trials on two types of equipment ensure that both manual cleaning (ice-cream machinery) and automated cleaning (milking machinery) scenarios are covered. Automated cleaning processes are generally set to limit water usage, which can result in reduced rinsing efficiency and higher biocide residues. In addition, milking equipment represents the most standardised equipment available. Small equipment such as ice-cream machinery represents the worst case, since the magnitude of biocide residue in food is determined by the ratio of food volume and equipment surface area and because in small equipment, the pipe work is often not emptied properly following disinfection.

For biocidal products restricted to certain machinery (e.g. brewery installations), it is left up to the Applicant to submit rinsing efficiency data on either representative equipment or the equipment intended to be treated. In the latter case the choice of equipment must be accompanied by a justification.

For biocidal products restricted to use on counter tops where food is prepared for commercial use (bakeries, butcheries, fast food retailers, etc.), it is sufficient to submit biocide residue removal experiments for a flat surface on which the food is normally

[80] HACCP (Hazard Analysis and Critical Control Points) refers to a preventive approach commonly applied in the food industry to identify and subsequently reduce food safety hazards in the food production process.

prepared.

For dishwashing detergents used in professional kitchens (e.g. canteens, restaurants), it is sufficient to submit biocide residue removal experiment data for dishes normally used to prepare and serve foods.

The biocidal product label must either note minimum rinsing water amounts (as % of installation volumes or as L/m^2) or provide instructions for monitoring rinsing efficiency (for examples of test or indicator strips see Appendix III.2).

5.1.4.1.5.2 Data requirements for biocidal products that are left to evaporate

For biocidal products that are left to evaporate specific experiments are required showing that biocide residues have evaporated completely at the ambient use temperature from the surface in the time period given on the label. Even for active substances with a high vapour pressure, biocide residues can occur in closed parts of complex machinery.

For the biocide residue removal experiment, biocidal treatment and evaporation are conducted as per label instructions. Effectiveness of evaporation is assessed by measuring the biocide residues on the treated surface using a wiping test as indicated in section 5.1.4.1.

Requirements for the biocide residue removal experiments include information on surface activity of the active substance and its degradation products (as included in the biocide residue definition for DRA) and the biocidal product, a validated analytical method to determine the biocide residue (according to the biocide residue definition for DRA) in the solvent or wipe extract and information from the label which gives a dose rate in g or mL biocidal product/m^2 and a specification of the evaporation period. The representative piece of equipment for these experiments is the slicing machine.

For biocidal products restricted to certain machinery, it is left up to the Applicant to submit biocide residue removal data on either the representative equipment or the equipment intended to be treated. In the latter case the choice of equipment must be accompanied by a justification.

For biocidal products restricted to use on counter tops where food is prepared for commercial use (bakeries, butcheries, fast food retailers, etc.) it is sufficient to submit biocide residue removal data for a flat surface on which the food is normally prepared.

The biocidal product label should provide instructions for monitoring evaporation efficiency.

5.1.4.1.5.3 Criteria for interpretation of rinsing and wiping tests

The following are the criteria for interpretation of rinsing tests for volume and area based biocidal treatment. Their derivation is explained in Appendix I.

Trigger concentration in rinsing water: 0.01 mg b.r./L

AND

Trigger amount of biocide residues on inner surface of machinery and on a flat surface after rinsing: 0.06 mg b.r./m^2.

The trigger values must be adapted to proportionally lower levels for substances with

ADIs and/or ARfDs < 0.01 mg/kg bw.

If the trigger values are not exceeded, no further DRA is necessary and no MRLs need to be set.
If one or both of the trigger values are exceeded, the rinsing process can be improved. If this fails to bring biocide residues at or below the level of both trigger values, further evaluation will be required. This evaluation starts with estimating biocide residue levels in foods. These are then compared with existing MRLs, MLs or SMLs (if available) and used to perform a preliminary estimate of consumer exposure via food (model to be developed, not part of this guidance). If quantifiable biocide residues are expected, the EU Commission's interim approach on MRL setting applies (CA-March17-Doc.7.6.c-final).

Example 5.1-8: Disinfection of a closed food processing installation (CIP scenario)

Intended use: Brewery tanks are treated once daily with an a.s. concentration of 3% of the installation volume.

Exposure Estimation
The goal is to show that:
Rinsing with water effectively removes most biocide residues from the inner surface of machinery

This is done by:
Measuring biocide residues in rinsing water **AND**
Measuring biocide residues on the inner surface of machinery

Rinsing is considered effective if:
The biocide residue concentration in rinsing water is ≤ 0.01 mg b.r./L **AND**
biocide residues on the inner surface of machinery after rinsing are < 0.06 mg b.r./m^2.

Experiment
The experiment is conducted using the two representative food processing installations milking machinery and ice-cream machinery.

The following steps must be performed for both representative food processing installations:
1. The biocidal treatment is performed as per label instructions. This involves:
- Emptying the installations of any remaining food.
- Rinsing the installations with clean water.
- Running the disinfectant solution through the installation at an a.s. concentration of 3% of the installation volume.
- Draining the disinfectant solution from the installation.
- Running potable water through the installation for the circulation time given on the biocidal product label. If no circulation time is given on the label, biocide residues are measured at several time points throughout the rinsing process until the acceptable biocide residue concentration is reached.

2. biocide residues are quantified. This involves:
- biocide residues are measured in accordance with the biocide residue definition for DRA in the last rinsing water that is drained from the system using a suitable validated analytical method. These biocide residues are expressed as mg b.r./L
- After the last rinsing water is drained from the installation, pads soaked in an appropriate solvent are used to wipe a defined area at the food inlet and outlet of the installation. The biocide residues are then extracted from the pads and biocide residues are measured in accordance with the biocide

	residue definition for DRA using an appropriate validated analytical method. biocide residues in the extract are calculated back to mg b.r./m^2 of the original surface. → If the biocide residue concentration in rinsing water is ≤ 0.01 mg b.r./L and the biocide surface residues after rinsing are < 0.06 mg b.r./m^2, the rinsing procedure is considered effective for the biocidal product. No further MRL assessment is necessary, provided the ADI and ARfD is above 0.01 mg/kg bw. → If these values cannot be met, attempts could be made to improve the rinsing process.

5.1.4.2 Aseptic packaging

Disinfection techniques are used along the entire production line in the food industry (including e.g. PT 4 or PT 6 uses). Besides ensuring a safe processing environment on surfaces and equipment, they are also used in aseptic packaging operations to sterilise containers and their lids before they are filled with food. Sterilisation techniques allow extension of the shelf-life of food, keeping it safe without refrigeration. Aseptic packaging has increasingly replaced traditional heat-based techniques (e.g. canning). A disadvantage of heat-based techniques is that they are limited to robust packaging materials and change product characteristics, often decreasing the quality of the food. With the development of aseptic packaging, it became possible to increase shelf-life and ensure food safety of delicate products without substantial loss in quality and to use heat-sensitive containers (such as PET bottles) that would not withstand the temperatures of conventional sterilisation. Aseptic packaging improves food quality by retaining the food's colour, texture, taste and nutrition and is routinely used to package juices, milk and other beverages, desserts, toppings, but also non-homogenous foods.

5.1.4.2.1 The Aseptic Packaging Procedure

Aseptic packaging replaces the sterilising effect of heat with disinfectants in wet or dry operations. The most common disinfectants used are peracetic acid and hydrogen peroxide. Container and lid are sterilised using disinfectants before they are filled with ultra-high-temperature treated food. The entire operation takes place in a closed circuit of sterilised machinery that ensures aseptic conditions throughout the entire process.

Package types used in aseptic packaging include cartons (e.g. brick and gable top shapes), bottles, cups and pouches (stand-up and flat types). Aseptic carton packages are typically a mix of paper/cardboard (79%), polyethylene (24%) and aluminium (6%). The outer and inner layers are often polyethylene coatings, but can also be metalized films. The main component paper/cardboard provides stability to the package. Polyethylene provides a barrier to microorganisms and ensures that liquid stays in the package. Aluminium protects against air, light and odours. Pouches and other flexible packages are film laminates composed of different combinations of materials such as polypropylene (PP), polyester, polyolefins, polyethylene terephthalate (PET) and polyethylene (PE). An aluminium layer is often added. PET and HDPE (high-density polyethylene) are used for bottles. Liquid foods (e.g. milk, juices) are generally packaged in carton packages or in bottles. Semi-liquid foods (e.g. pudding, apple sauce) are generally packaged in carton packages, pouches or plastic cups. Chunky foods (e.g. cut fruit) are generally packaged in carton packages or plastic cups. Carton-based packages can also be used to package liquid foods. Depending on the company, aseptic packaging methods may differ somewhat. But the basic steps are the same. Examples are given in the following paragraphs.

Preformed packaging (e.g. bottles) treated with hydrogen peroxide

For dry sterilisation, gaseous hydrogen peroxide is channelled through the bottles. Following the sterilisation process, all hydrogen peroxide is replaced with sterile air to remove any biocide residues. The exterior of the bottle is simultaneously treated. The sterilised bottles are then filled with the foodstuff and sealed with caps that have been sterilised either with gaseous hydrogen peroxide or in a peracetic acid bath.

For wet sterilisation, a mixture of peracetic acid and steam is guided through the packaging. The exterior of the container is simultaneously treated with liquid disinfectant. This is followed by a mandatory water rinse. Then the bottles are filled and sealed with caps that have been sterilised either with gaseous hydrogen peroxide or in a peracetic acid bath.

Unformed packaging treated with hydrogen peroxide

Flat, unformed packaging material is passed through a heated hydrogen peroxide bath. Residual hydrogen peroxide is then removed with a water rinse or a stream of hot air. Alternatively, pressure rollers may be used to force the disinfectant from the packaging. The packaging material is then simultaneously formed and filled with food product.

5.1.4.2.2 Biocide residue removal efficiency

When disinfectant residues remain in the packaging, it is likely to be distributed evenly in liquid foods and less well in solid foods. An effective biocide residue removal procedure is mandatory in all filling operations, and, if effective, will remove most of the biocide residue. As long as the Applicant can prove the efficiency of the biocide residue removal process for the biocidal product, data on biocide residues in food do not have to be submitted. To ensure an effective biocide residue removal process in practice, the product label should indicate biocide residue removal requirements and indicate the minimum frequency of checking biocide residue removal efficiency and how to perform this check.

Representative packaging

Biocide residue transfer to food occurs when the biocide residue-removal method was ineffective, e.g. it did not reach all interior surface areas or insufficient amounts of water or air were used. In containers with corners, indentations etc. there will be a greater chance of biocide residue accumulation because the biocide residue removal procedure may not reach corners and indentations.

Bottles and unformed carton-based packages represent the worst case. For bottles the critical characteristics are their narrow neck which provides only a small opening for rinsing water or air, the indentations at the bottom where biocide residues can settle. For carton-based packages, the critical characteristics include the many folds in the flat material that allows them to later take on a finished shape. The folds provide a number of nooks where biocide residues can settle.

The sterilisation techniques for bottles and carton-based packaging are sufficiently different to justify requiring biocide residue removal experiments on both. For example, bottles are treated in their finished shape while carton-based packaging is generally treated in its flat, unformed shape. Bottles are treated using a steam-disinfectant mixture or gaseous disinfectant, whereas carton-based packaging may be passed through a disinfectant bath. For carton-based packages, the pressure roller technique may be used for biocide residue removal. Biocide residue removal efficiency should therefore be tested on bottles and carton-based packages using all applicable biocide residue removal techniques. For composite cartons, all three biocide residue removal techniques (water rinse, hot air, pressure rollers) should be applied. For plastic bottles, due to their shape, only water rinse and hot air can be used.

Biocide residue removal experiments

Experiments are required showing that the biocide residue removal process is effective in removing the active substance and its degradation products (according to the residue definition for DRA) from the interior surface of the packaging. The experiment should be performed under the same conditions as in an actual aseptic packaging operation, using representative packaging, equipment, procedure and disinfectants that are used under realistic conditions. Specifications on the biocide residue removal process should be indicated on the biocidal product label.

In case the last step of the aseptic packaging procedure is a water rinse, effectiveness of rinsing is assessed by measuring the biocide residues in the last rinsing water and on the treated surface. If another procedure is used for biocide residue removal (e.g. hot air, pressure rollers), biocide residues should be measured on the treated surface only (after treatment and the biocide residue removal process).

To check the effectiveness of the biocide residue removal procedure on the treated surface, the entire inner surface of the packaging is rinsed with water and wiped off with a pad soaked in water. Biocide residues in the rinsing water and the pad are analysed for biocide residues (in accordance with the biocide residue definition for DRA) using a validated analytical method. For biocide residues with unknown water solubility and/or surface activity, an appropriate solvent is used instead of water. Solvents need to be capable of removing the biocide residue in question from the inner surface of the packaging without disintegrating the packaging itself. Biocide residues in the water, solvent or pad extract must be calculated back to mg b.r./m^2. The biocide residue removal experiment for a treated surface is presented in Figure 8.

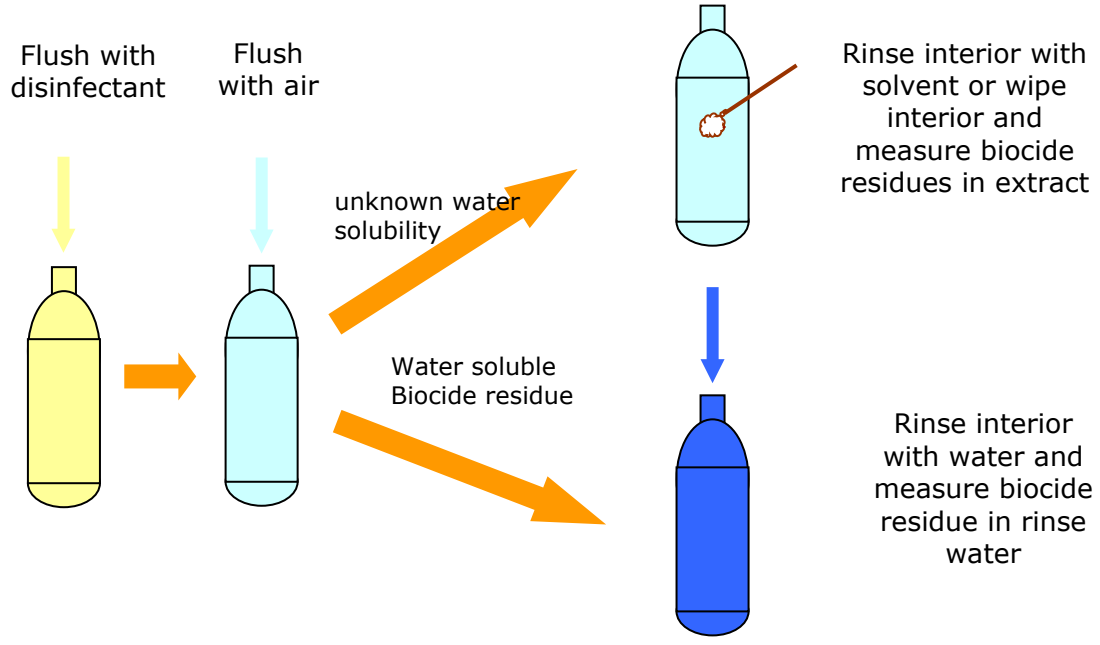


Figure 8 Schematic representation of the experiment testing residue removal efficiency of a

5.1.4.2.3 Criteria for acceptance of biocide residue removal experiments

The following is the criterion for the acceptability of biocide residue removal tests. Its derivation is explained in Appendix I.

Maximum acceptable amount of biocide residues on food contact surface of package after residue removal: 0.17 mg b.r./m^2.

AND

Maximum acceptable concentration in rinsing water: 0.01 mg b.r./L
(only where the last step of the aseptic packaging procedure is a water rinse)

The threshold levels must be adapted to proportionally lower levels for substances with ADIs and/or ARfDs < 0.01 mg/kg bw.

If the trigger values are not exceeded, no further DRA is necessary and no MRLs need to be set, provided the ADI and ARfD is > 0.01 mg/kg bw. If one or both trigger values are exceeded, the rinsing process can be improved. If this fails to bring biocide residues at or below the level of the trigger values, further evaluation will be required. This evaluation starts with estimating biocide residue levels in foods. These are then compared with existing MRLs or migration limits (if available) and used to perform a preliminary estimate of consumer exposure via food (model to be developed, not part of this guidance).

If quantifiable biocide residues are expected, the EU Commission's interim approach on MRL setting applies (CA-March17-Doc.7.6.c-final).

Example 5.2-1: Aseptic packaging

Intended use: Packaging material is treated with a disinfectant prior to being filled with food.

Exposure Estimation
<u>The goal is to show that:</u>
Biocide residue removal procedure effectively removes most biocide residues from the interior surface of the package.

<u>This is done by:</u>
Measuring interior biocide surface residues as water-solubility of biocide surface residues is not known.

<u>Rinsing is considered effective if:</u>
Biocide surface residues after are < 0.17 mg b.r./m^2 **AND**
The biocide residue concentration in rinsing water is ≤ 0.01 mg b.r./L

Experiment
The experiment is conducted using the two representative packages plastic bottle and carton-based composite packaging. For bottles, experiments are conducted using water rinse and using hot air to remove biocide residues. For carton-based composite packaging, experiments are conducted using water rinse, hot air and pressure rollers.

The following steps must be performed for each representative packaging/ biocide residue removal procedure combination:
1. The biocidal treatment is performed as per label instructions. This involves:
   - Flushing packaging with the disinfectant solution as per label instructions.
   - Draining the disinfectant solution.
   - Applying all biocide residue removal procedures for the time specified on the biocidal product label. If no time is given on the label, biocide residues are measured (in accordance with the biocide residue definition for DRA) at several time points throughout the biocide residue removal process until the acceptable biocide residue concentration is reached.
2. Biocide residues are quantified. This involves:

- Biocide residues are measured in accordance with the biocide residue definition for DRA in the last water rinse that is drained from the package using a suitable validated analytical method. These biocide residues are expressed as mg b.r./L
- Cheesecloth pads soaked in an appropriate solvent are used to wipe the entire inner surface area of the package. The biocide residues are then extracted from the pads and are measured in the extract using an appropriate analytical method (in accordance with the biocide residue definition for DRA). Biocide residues in the extract are calculated back to mg b.r./m^2 based on the surface sampled.

→ If the surface residues after rinsing are < 0.17 mg b.r./m^2 for all representative package/residue removal procedure combinations AND the residue concentration in rinsing water is ≤ 0.01 mg b.r./L, the residue removal procedures are considered effective for the biocidal product. No further DRA is necessary and no MRLs need to be set, provided the ADI and ARfD is > 0.01 mg/kg bw.
→ If these values cannot be met, attempts could be made to improve the rinsing process.

5.1.4.2.4 Other uses of disinfectants in food packaging

Next to aseptic packaging, there are additional uses of disinfectants in the food packaging industry. Examples are the conservation and disinfection of wine barrels with sulphur dioxide and the disinfection of bottles used in non-aseptic packaging processes. For these additional uses, it is up to the Applicant to present suitable data and/or information to show that biocide residue levels from the biocidal application are below the trigger value. The argument must consider the specific biocidal application (e.g. burning of sulphur chips in barrels), and may include monitoring data, model calculations (e.g. see example 5.1-3) and/or literature research.

5.1.4.3 Food contact materials treated or equipped with biocides

5.1.4.3.1 Description of uses

For the purpose of surface protection, biocides (e.g. PT 4) may be incorporated into food contact materials (FCM). The biocides are slowly released over time for the purpose of protecting the surface of the material from microbial damage. Examples of biocide-treated FCM are plastic cutting boards, ceramic or plastic kitchenware, refrigerator linings, membrane filters for beverages or processing water, kitchen counter tops and coatings for packaging paper and other materials. Biocidal active substances and their degradation products (included in the biocide residue definition for DRA) can migrate from FCM into foods.

In addition, FCM may contain biocide residues due to the presence of biocide residues in components used in the production of the FCM. Examples include in-can preservatives (PT 6) that may be present in coatings, printing inks, plastics and adhesives, and slimicides (PT 12) present in paper and cardboard, due to use of this type of biocidal products in paper pulp.

For more guidance on the assessment of in-can preservatives, please see the "DRAWG Opinion on identifying worst-case exposure scenarios for PT 6 biocidal products in order to minimise the number of scenarios to be assessed for dietary risk" (see Appendix VIII).

For slimicides (PT 12) applied in the production of paper for food packaging, please refer

to TAB entry TOX 45[81] which states: *it is proposed to estimate the biocidal active substance transfer from food using data if available, and otherwise by a theoretical worst case scenario. This proposal should be seen as an interim approach until a more clear procedure will be defined by the Commission*".

5.1.4.3.2 Biocide residue assessment approach

For the estimation of biocide residues in food derived from FCM, migration studies according to the provisions set out in the FCM framework need to be conducted. Migration studies are used to determine the release rate of the active substance and its degradation products (as included in the biocide residue definition for DRA) into the food. Since the release rate depends on many factors, such as the intended use (e.g. contact time and temperature, type of food), the active substance itself, the material of which the FCM is made, it would be impossible to set a default. Migration studies on the other hand give reliable values for the release rate of the biocide residue from the FCM. The data requirements on migration are listed in chapter 5 of the Note for Guidance for the Preparation of an Application for the Safety Assessment of a Substance to be Used in Plastic Food Contact Materials[82]. For non-plastic materials, adjustments might have to be made. Food simulants for the migration studies should be chosen according to Annex III of Regulation (EU) No 10/2011.

According to the FCM guidance, migration studies should be conducted with a surface to volume ratio of 6.0 dm^2 test sample per kg food or L simulant. This value might not be applicable to all biocide scenarios involving an FCM. "6.0 dm^2/1 kg food" describes the ratio at which food is in contact with its packaging. But FCM also include items such as counter tops and cookware. Different surface to volume ratios might have to be used for these items. For example, for a counter top, a contact ratio of "0.2 m^2/kg food" may be used, which would reflect the area of food preparation used in chapter 5.6.1 of Guidance on BPR, Volume III, Part B and C (non-professional guidance).

In line with the current approach for FCM, small packaging sizes are not considered in biocide residue assessment. If the FCM approach is amended to include small packaging sizes, this should be adopted for biocide residue assessment as well.

5.1.4.4 In-can preservatives

PT 6 biocidal products are used to preserve products other than foodstuffs, feedstuffs, cosmetics, medicinal products or medical devices (BPR, Annex V). Although PT 6 uses do not include the direct preservation of food or feed, contact of food or feed with in-can preservatives may occur via the use of PT 6 biocidal products in the context of professional biocidal uses. Examples include:

- in-can preservation of insecticides (PT 18) and subsequent dietary exposure via biocide residues on food storage/preparation surfaces

- industrial or institutional cleaners or disinfectants (PT 4) and subsequent dietary exposure via biocide residues on food storage/preparation surfaces

- production of food contact materials or components thereof, e.g. paper, coating, polymer dispersions (PT 12)

[81] Technical Agreements for Biocides, Human Health (TOX), Version 2.0, November 2018
[82] Note for Guidance for the Preparation of an Application for the Safety Assessment of a Substance to be Used in Plastic Food Contact Materials. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.21r

- production of feed packaging, e.g. paper, coating, polymer dispersions (PT 12)

For estimating transfer of PT 6 biocide residues into food the biocide residue assessment approach depends on the use of the preserved product. For example if the PT 6 active substance is contained in a component of a food contact material the approach detailed in section 5.3 should be followed. For more guidance on the assessment of in-can preservatives please see the "DRAWG Opinion on identifying worst-case exposure scenarios for PT 6 biocidal products in order to minimize the number of scenarios to be assessed for dietary risk" in Appendix VIII.

For PT 6 biocidal products used in the production of feed packaging, dietary exposure occurs via transfer of biocide residues from feed packaging to feed, subsequent uptake and metabolism by livestock animals and possible transfer of biocide residues to edible animal matrices. For biocide residue assessment of this scenario please refer to Example 2.3 in Section 5.12.4.7.1.3.

Biocidal products are also used for the preservation of lubricants for the operation of conveyor belts in the food industry. Without preservation, lubricants would spoil rapidly, leading to frequent replacement and impacting workers' health and the environment negatively83.

The development of an assessment model for the use of biocidal products in lubricants is neither considered feasible nor necessary. Conveyor belts are used in virtually all sectors of the food industry transporting a wide variety of foods from chocolates to slabs of meat with very different surface and contact areas with the conveyor belt. Different sectors use different models and sizes of conveyor belts, making it impossible to make a general estimate of the amount of lubricant that can end up on their surfaces. Furthermore, depending on the food (small versus large pieces) all or very little of the lubricant on a conveyor belt may come into contact with food. It is therefore not feasible to set default values that can be entered into a model to arrive at a sensible biocide residue estimate in food.

Possible biocide residues in food from this use are expected to be infrequent and low, since lubricants are used on the joints and not on top of the conveyor belt where food is transported. Therefore, only traces of lubricant may occasionally end up on the food contact surface of conveyor belts.

In addition, it is expected that many food industry sectors in the EU use H1 certified lubricants in their operations, which have been assessed safe for occasional food contact under U.S. regulation84. In the U.S., lubricants for use in the food industry are regulated by the FDA (Food and Drug Administration). Lubricants that have been assessed safe for occasional contact with foods are classified as H1 lubricants and only those lubricants are allowed for uses where occasional food contact is possible[85]. There is, however, currently no comparable regulation in the EU[86].

Note: For guidance on how to assess the disinfection of conveyor belts, please see chapter 5.1.4.3 – Disinfection of open food preparation areas.

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[83] http://www.vsi-schmierstoffe.de/regelwerke/biozide-und-fungizide.html (accessed 24.01.2020)

[84] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=178.3570

[85] https://de.wikipedia.org/wiki/H1-Schmierstoff (accessed 24.01.2020)

[86] https://de.oelcheck.com/wiki/Schmierstoffe_in_der_Lebensmittelindustrie (accessed 24.01.2020)

5.1.4.5 Pest control in the food, feed and drink industry

For many professional applications of pest control products in the food, feed and drink industry (e.g. PT 14, PT 15, PT 18), it is not necessary to develop a model to estimate biocide residue transfer to food/feed. Pest control products for professional use carry label restrictions that ensure that no contact with food/feed occurs. For professional users (e.g. service companies specialised in pest control) it is realistic to assume that label restrictions will be observed. Pest control products are generally not applied to food/feed contact surfaces. However, if a product is sprayed or volatilised, droplets/vapours may settle/condense on food/feed contact surfaces. For such products, a label instruction preventing exposure of food/feed contact surfaces must be applied. Exposed food/feed contact surfaces can therefore be covered before biocidal treatment, so that biocide residues settle/condense on the covers instead of the food/feed contact surface. In addition to this general phrase the label must contain specific instructions. For example, the type of material the covers must be made of has to be specified to ensure that they are not permeable to the biocidal product or the biocide residue (as defined in the biocide residue for DRA). The instructions must also contain information on the amount of time the covers must stay in place after the end of treatment to ensure that all droplets/vapours have settled/condensed before covers are removed. After covers are removed, food/feed contact surfaces should be thoroughly cleaned as an additional precautionary measure. As this is an additional safeguard, no data on the efficiency of the cleaning procedure are considered necessary. The label should however list the type of detergent that is efficient in removing biocide residues. If biocide residue transfer is precluded through label restrictions, no MRLs are set and no DRA is required.

Some pest control products, e.g. insecticide fumigation applications, are intended to enter food/feed processing machinery and are likely to leave biocide residues on the food/feed contact surfaces in the machinery. These biocide residues are difficult to remove so that subsequent food/feed production batches may contain high biocide residues. In fact, complete removal of biocide residues could require several hours of running production. In order to evaluate this scenario, biocide residue trials in food/feed under representative conditions are required in collaboration with the MRL authority. A list of representative food/feed on which trials are required has not yet been established (see also Appendix II).

5.1.4.6 Storage protection in the food, feed and drink industry

Airspace treatments such as fogging and fumigation are used in storage protection of food/feed commodities. The distinction between plant protection product (PPP) and biocidal product uses (usually PT 18 biocidal products) depends on the type of food/feed that is stored. Uses in storage areas for food/feed in its unprocessed state or food/feed which has undergone only minimal processing (e.g. drying) fall within the scope of the PPP legislation (Reg. (EC) No. 396/2005). Uses in storage areas for food/feed with higher degrees of processing are considered biocidal uses. In the residue assessment for PPP, the conduction of residue studies in stored goods (post-harvest treatment), is a well-established procedure[87]. To be in line with this, uses that fall under the biocide legislation should be subject to similar requirements since the use is essentially the same and only differs in the type of food/feed. Model calculations are furthermore not a practical approach because conditions in storage facilities are variable (e.g. size of the facility, amount and type of food/feed stored). The default values required for model calculations would therefore be impossible to set. For airspace treatments, residue data

[87] See Guidelines for the generation of residue data required under Directive 91/414/EEC and Regulation (EC) No 396/2005: Appendices B (SANCO 7029/VI/95 rev. 5) and D (SANCO 7525/VI/95 rev 10.3) http://ec.europa.eu/food/plant/protection/pesticides/publications_en.htm

on food/feed will therefore be required in most cases to determine whether the trigger value is exceeded. It should be noted that residue data will be required for a range of representative foods/feeds. For different food matrices, specific validated analytical methods are needed, which are able to measure the biocide residue in accordance with the biocide residue definition for DRA.

5.1.4.7 Treated wood and agricultural scenarios

To ensure its durability, wood is often treated with wood preservatives (PT 8 biocidal products). A variety of wooden materials are used in the production, storage, transport and preparation of foods. Therefore it must be considered that biocide residues of wood preservatives can leach from the treated wood and be carried over into foods. The following scenarios that may be relevant for biocide residue assessment are discussed below:

- wooden kitchen articles (section 5.7.1)
- wooden packaging materials (section 5.7.2)
- wood chips used in the preparation of smoked food (section 5.7.3)
- wooden posts used in training systems for vining plants (section 5.7.4)

5.1.5 Wooden materials in kitchenware products

Wooden materials such as spoons (cutlery), cutting boards or surfaces with direct food contact (countertops) are used in kitchens while processing raw or cooked meals. In commercial settings such as canteens, restaurants or shops, wooden materials are generally not used as a consequence of the rules on food hygiene (Reg. (EC) No 852/2004). However, these articles can be common in household settings, for which no specific EU rules apply. Instead national standards may be available in individual member states[88].

In case wood treated with a wood preservative (PT 8 biocidal product) is designated to be used for kitchenware products, potential transfer of biocide residues into food should be measured.

5.1.6 Wooden packaging materials

Packaging made of wood (as in wooden pallets, wooden crates, wooden boxes, or any other packing material made of wood) can be used to contain the following food categories: fruit and vegetables, fishery, wine and liquors, oils, cheese and milk derivatives, meat and meat products, bread and bakery products, pulses, nuts and dried fruits, tea.

At EU level there are no specific rules for wood as food contact material. However the general principle of the FCM Regulation (EC) No. 1935/2004 applies, i.e. any FCM must be sufficiently inert not to be transferred to food in quantities that would endanger human health or bring unacceptable changes in the composition of food or deterioration in its organoleptic properties.

The treatment appropriate for wooden packaging material used in international

[88] For example, according to FEDEMCO (Federación Española del Envase de Madera y sus Componentes, Spanish association for wooden packaging) these wooden materials have to comply with the same rules as wooden packaging for commercial trade which are stated in section 5.7.2.

commercial trade (outside EU) is addressed by FAO standard ISPM No. 15. This standard requires that all wooden packaging materials (such as pallets or crates) have to be heat treated[89]. The treated wood must be marked with an approved logo to show that requirements are met. This guideline is implemented in Directive 2000/29/EC. For heat treatment, wooden packaging material must be heated to achieve a minimum temperature of 56°C for a minimum duration of 30 continuous minutes throughout the entire profile of the wood (including at its core). As this standard does not require biocidal product use, no biocide residues are expected in food or feed traded internationally in these wooden materials.

Wooden packaging material that is not internationally traded (inside EU) may contain wood preservatives. In case wood treated with a wood preservative (PT 8 biocidal product) is designated to be used as packaging material for food, potential transfer of biocide residues into food should be estimated. Examples for such uses may be the storage and transport of fruit/vegetables in wooden boxes (see Example 5.7-1) or food transported on wooden pallets (see Example 5.7-2)

Example 5.7-1: Storage and transport of fruit/vegetables in wooden boxes

Description of scenario/Intended use: Boxes for storage and transport of fruit are manufactured from wood treated with a wood preservative (maximal application rate 0.122 g a.s./m^2). After placing fruit in the boxes biocide residues are transferred from the impregnated wood onto fruit.
[Note: For active substances with a high vapour pressure (i.e. > 0.1 Pa)[90] the scenario may not apply, as the volatility of the active substance and its degradation products (as defined in the biocide residue for DRA) may prevent biocide residue transfer to food.]

Assumptions
The calculations below assume as a first tier that the parent compound is not degraded and thus the concentration of the active substance in the wood is used for the estimation of biocide residue (b.r.) levels in food.

-. Concentration of active substance in the surface layer of the treated wood as first tier
- Mass transfer efficiency factor TF = 10% (For derivation of this default value see Appendix I. A lower value must be supported by study data.)
- The scenario applies to unwrapped raw foods that are commonly stored and transported in wooden boxes (e.g. fruits and vegetables such as apples, cucumbers etc).
- Contact surface of apple with the wooden box: $A_{contact}$ = 5 cm^2 per apple. (default)
- Average number of apples per kilo: N_{apples} = 6 apples per kilogram (default)
- Penetration depth of biocidal product into wood: $d_{penetration}$ = 1 mm (default)
- Contact duration of apple with treated wood: $t_{contact}$ = 6 months (default)
- The scenario is considered applicable for both acute and chronic exposure assessment.

Estimation of residue transfer into food
$C_{b.r.,\ surface}$0.122 g a.s./m^2 (=0.0122 mg a.s./cm^2) = 0.122 g b.r./m^2 as first tier (biocidal product specific information)
$A_{contact}$5 cm^2 (default, see Appendix I)
$N_{apples\ per\ kg}$6 kg^{-1} (default, see Appendix I)
TF10 % (default, see Appendix I)

[89] Fumigation with methyl bromide, which is another treatment method mentioned in the FAO standard ISPM No. 15, is no longer possible due to phase out under the Montreal Protocol on Substances that Deplete the Ozone Layer (http://ozone.unep.org/en/treaties-and-decisions/montreal-protocol-substances-deplete-ozone-layer).
[90] ConsExpo Paint Products Fact Sheet, https://www.rivm.nl/bibliotheek/rapporten/320104008.pdf

$d_{penetration}$1 mm (default, see Appendix I)
$t_{contact}$ 6 months (default, see Appendix I)

Calculation
If experimental data are provided to refine penetration depth and contact duration, the equation is as follows:
$R_{apple} = C_{b.r., surface} \times TF \times (t_{contact} \div 6 \text{ months}) \times (1\text{mm} \div d_{penetration}) \times A_{contact} \times N_{apples\ per\ kg}$
$= 0.0122$ mg b.r./cm^2 × 0.1 × 5 cm^2/apple × 6 apples/kg
$= 0.0366$ mg b.r./kg

Without refinement, the equation is simplified: $R_{apple} = C_{b.r., surface} \times TF \times A_{contact} \times N_{apples\ per\ kg}$

The trigger value of 0.01 mg b.r./kg is exceeded.
See Chapter 2 on how to proceed with the assessment.

Example 5.7-2: Wooden pallets

Description of scenario/Intended use:
The biocidal product is a wood preservative for industrial use. In the intended use it is specified that treated wood may be used to manufacture pallets intended for transport and storage of food and feedstuff. Thus indirect contact of treated wood with food and/or feedstuff may occur.

Assumptions
- Food (wrapped in packaging material) is placed on wooden pallets treated with wood preservative. Thus food is in indirect contact with the treated wood. (An additional intermediate paper or cardboard layer between packaged food and wood is not considered.)
The calculations below assume as a first tier that the parent compound is not degraded and thus the concentration of the active substance in the biocidal product is used for the estimation of biocide residue (b.r.) levels in food.
- Mass transfer efficiency factor TF = 10% (For derivation of this default value see Appendix I. A lower value must be supported by study data.)
- The scenario applies to wrapped foods that are commonly stored and transported on wooden pallets (e.g. wrapped raw and processed foods of plant and animal origin).
- Contact surface of packaged food with treated wood: 300 cm^2 for 1 kg foodstuff (assuming a food layer of 3.3 cm thickness).
- Penetration depth of biocidal product into wood: $d_{penetration}$ = 1 mm (default)
- Contact duration of food with treated wood: $t_{contact}$ = 6 months (default)
- The scenario is considered applicable for both acute and chronic exposure assessment.

Estimation of biocide residue transfer into food
$c_{b.r., surface}$0.075 g a.s./m^2 (=0.0075 mg a.s./cm^2) (biocidal product specific information) = 0.075 g b.r./m^2 as first tier
$A_{contact}$300 cm^2/kg food (assuming a food layer of 3.3 cm height)
TF2% (supported by experimental data)
$d_{penetration}$1 mm (default, see Appendix I)
$t_{contact}$ 6 months (default, see Appendix I)

The TF of 2% used in this example was experimentally determined as described below (rough outline only, in practice a complete study report will be necessary).
- Planks treated according to intended use and left to dry (detailed description was provided on wooden plank dimensions, application method, application rate, drying

time etc.)
- Experimental setting:
  - Justified choice of foodstuffs (representative food/feed based on food composition, texture, surface area etc)
  - Foodstuffs (in paper or cardboard based packaging) placed in direct contact with the pallet planks, i.e. without an additional intermediate protecting paper or cardboard layer.
  - Test conditions: temperature 15°C, relative air humidity 65%, test period: 6 months (default duration of transportation and temporary storage of food on wooden pallets)
  - Sample analysis
    - Wood samples: 1 mm surface layer was shaved off, untreated (control) and treated planks
    - Food samples: Exposed packaging material was cut out and sampled. Subsequently, 30-50 g of the food product adjacent to the underlying packing was sampled.
    - Samples were taken after 0, 1, 2, 3 and 6 months
    - analytical method for biocide residues (in accordance with the biocide residue definition for DRA) in wood and food matrices (information on method and validation data to be submitted)
- Results: After 6 months biocide residues were detected in all food products analysed in this study (highest concentration 0.0279 mg b.r./kg).
- Conclusion: Measured biocide residues in food support the assumption that 2% of the surface biocide residues are transferred into food.

Calculation

If experimental data are provided to refine penetration depth and contact duration, the equation is as follows:

$R_{food} = c_{b.r.,\ surface} \times TF \times (t_{contact} \div 6\ months) \times (1\ mm \div d_{penetration}) \times A_{contact}$

$= 0.0075\ mg\ b.r./cm^2 \times 0.02 \times 300\ cm^2/kg$

$= 0.045\ mg\ b.r./kg$

Without refinement, the equation is simplified: $R_{apple} = C_{b.r.,\ surface} \times TF \times A_{contact} \times N_{apples\ per\ kg}$

The trigger value of 0.01 mg b.r./kg is exceeded.
See Chapter 2 on how to proceed with the assessment.

5.1.7 Preparation of smoked food

The use of wood as fuel in the preparation of smoked foods can be a source of contamination of such foodstuff with chemicals present in the wood. However, the wood for such uses should not be treated with chemicals, according to FAO/WHO GEMs food contaminants (ALINORM 08/31/41 paragraph 109 and its appendix VI). Thus this is not considered a scenario of concern.

5.1.8 Vining plants / training systems

Certain horticulture varieties (i.e. tomatoes, pepper, beans, cucumber, and grapes) as well as fruit trees and olive trees are grown using training systems (also known as espalier systems, trellising systems) which consist of wires that run horizontally between wooden posts (other materials are also employed). Training plants to grow up the wires supports the plant, keeping the fruit and foliage off the ground. Training systems are also used for decorative purposes, to save space and to hold nets protecting crops against extreme weather conditions (e.g. hailstorms) or against birds. Further information can be

found in Appendix IV. Only treated wood of use class 4 (European standard EN 335) must be used for wooden posts which are in direct contact with soil. Wood of use class 4 is mainly preserved with inorganic compounds which may leach and subsequently be translocated into the diverse parts of the plant via the rooting/vascular system. The lifespan of a wooden post in an espalier system is about 20 years with the maximal leaching rate expected in the first few years of use. In those years, young plants are in the growing stage and no significant harvest of their fruits occurs (an exception are annual plants). However, fruits that are harvested during that time may contain biocide residues of the wood preservative. In addition, persistent substances can accumulate in the soil and be quantified in crops even after several years. Indeed, a biocide residue study submitted in the context of product authorization shows that transfer of biocide residues from treated wood into fruit is a valid scenario that must be assessed. Similarly, cultivation of vegetables in treated wooden boxes or wood framed areas filled with soil, can lead to biocide residues in food.

The Applicant must demonstrate that use of the wood preservative under evaluation does not lead to biocide residues (in accordance with the biocide residue definition for DRA) in raw agricultural commodities that exceed the trigger value[91] (raw agricultural commodity defined in Annex I of EC 396/2005). This may be achieved for example by providing experimental data. Required information may be obtained from rotational crop studies (according to OECD test guidelines 502 and 504). Useful information may also be available in the environmental section of the dossier. When biocide residues in raw agricultural commodities exceed the trigger value, further evaluation will be required. This evaluation starts with estimating biocide residue levels in foods. These are then compared with existing MRLs, MLs or SMLs (if available) and used to perform a preliminary estimate of consumer exposure via food (model to be developed, not part of this guidance). If quantifiable biocide residues are expected, the EU Commission's interim approach on MRL setting applies (CA-March17-Doc.7.6.c-final).

5.1.8.1 Drinking water and water used in food processing

In order to protect public health PT 5 biocidal products are used to disinfect drinking water for both humans and animals (BPR Annex V). Disinfected water may be directly consumed or it may be used in food/feed processing. As the disinfection process involves direct contact of water with the biocidal product a biocide residue assessment has to be performed.

Below the biocide residue assessment of biocidal products applied by professional users is described covering direct consumption of drinking water by humans (section 5.8.1) and water used in food processing (section 5.8.2). The biocide residue evaluation should include both uses of the disinfected water, unless the intended use of a biocidal product is restricted to a specific use.

Biocide residue assessment regarding drinking water for livestock animals is described in Section 5.3, while assessment of drinking water disinfectants applied by non-professional users is addressed in Section 5.1.

More information on the evaluation of *in-situ* generated active substances, which are commonly applied in drinking water disinfection, is given in the BPC-WG Recommendation on *in-situ* generated active substances[92].

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[91] see footnote x

[92] Recommendation of the BPC Working Groups, In situ generated active substances – Risk assessment and implications on data requirements for active substances generated in situ and their precursors,

The disinfection of water with biocidal products (e.g. all oxidative biocidal disinfectants) leads to the inevitable formation of disinfection by-products (DBPs). The nature and amount of DBPs is related to the composition of the water (i.e. the organic matter in the water) and it is not possible to predict beforehand which compounds will be formed and at which concentrations.

This hampers a straightforward quantitative risk assessment based on comparisons with toxicological reference values. Therefore, the formation of DBPs should be addressed qualitatively in the product assessment report and recommendations to minimize the formation of DBPs should be provided, for example via label instructions. A separate guidance document on how to evaluate DBPs and their formation has been developed (ECHA Guidance Volume V Disinfection By-Products). This approach should be followed for the DRA of PT 5 biocidal products. Currently the guidance focusses on PTs 2, 11, and 12 and does not specifically address assessment of DPBs formed in drinking water and water used in food processing. Nevertheless, certain parts of the ECHA Guidance Volume V Disinfection By-Products can also be used for DRA of PT 5 biocidal products.

5.1.8.1.1 Drinking water

Disinfection of drinking water by professional users may occur in local water supply works as well as in other water purification plants/units (e.g. as part of a food processing factory). In addition professional disinfection of drinking water may occur in the context of water storage e.g. for sea-going vessels.

The Drinking Water Directive (Council Directive 98/83/EC on the quality of water intended for human consumption) must be followed for drinking water disinfectants used to disinfect drinking water at all stages before it is drawn from the tap. Drinking water disinfectants that are used at any point after that are within the scope of the BPR.

Biocide residues of disinfectants that are added directly to drinking water are estimated by assuming that they are present in the water in the amount of the application rate given on the label as first tier. As the nature-of-residue study and the toxicological potency of the degradation products has already been assessed in step 2 and step 3, the active substance concentration could directly be replaced by the biocide residue concentration (according to the biocide residue definition for DRA).

5.1.8.1.2 Water used in food processing

In the food sector drinking water is commonly used as food ingredient and as processing aid. Water may be utilized for cooling, heating and cleaning processes, for washing and sorting of fruits and vegetables, for dilution of concentrates, for glazing of foods during deep freezing etc.

When processing food it is important to maintain a proper water quality in order to meet the required hygiene standards. In this context drinking water disinfectants are applied in water purification plants (e.g. public water works, units contained within a food processing plant). As disinfected water gets in direct contact with food or on food contact surfaces during food processing it is possible that disinfectant residues are transferred onto food. For instance, the EFSA CONTAM Panel has attributed the presence of chlorate residues in food to the use of chlorinated water for food processing and to the disinfection of surfaces and food processing equipment coming in contact with food (for background information see Appendix III.1.2 Occurrence data: Chlorate).

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https://echa.europa.eu/documents/10162/13564/situ_as_precursors_wg_recommendation_+2017_en.pdf/0c6aee50-5c29-bccc-3836-bb033a015144

Modelling the scenario of biocide residue transfer from disinfected water to foods during food processing appears quite complex. In addition, it appears difficult to design biocide residue studies analyzing transfer of biocide residues reflecting all potential scenarios. Therefore, the following approach is proposed for biocide residue evaluation (in line with the COM interim approach for establishment of biocide MRLs[93])

1. Identify degradation products, assess their toxicological potency relative to parent and derive a biocide residue definition for DRA as indicated in step 2 and 3

2. Identify representative food commodities in which biocide residues are most likely to occur (and indicate critical sampling points in the process of food production, processing and storage)

3. Provide validated analytical methods to quantify biocide residues (in accordance with the biocide residue definition for DRA) in these food commodities (according to ECHA Guidance Vol I Parts A+B+C analytical methods are required for PT 5 biocidal active substances, but these should be extended to the degradation products included in the biocide residue definition for DRA.)

4. Collect occurrence data on biocide residues in food by applicant/stakeholders

5.1.8.2 Principles for design of experimental studies

The following section outlines some principles that should be taken into consideration when performing experimental studies (trials), such as measurement of biocide residues in rinsing water, on surfaces, or in the food itself

- Relevant biocide residue: Study needs to cover the biocide residue as defined in the biocide residue definition for DRA (this may include the active substance and toxicologically relevant degradation products, by-products and excipients);

- Analytical method: A valid analytical method is needed in order to perform measurements. All compounds that comprise the relevant biocide residue have to be accounted for;

- Time frame: To define a time frame for the trial, the degradation rate/reaction rate as well as the label instructions can be taken into account. When degradation/reaction occurs, a minimum time frame of 2x the half-life might be appropriate. The conditions of degradation/reaction compared to the conditions in the treated area must be considered. If no degradation/reaction occurs, the frequency of application according to label instructions can serve as a guide;

- Number of trials: Measurements should be performed at various time points to adequately capture the degradation of the active substance throughout the treatment period;

- Site selection, site requirements: Trials should be performed under realistic circumstances (e.g. on representative food processing machinery) or under conditions reflecting realistic circumstances. The material treated and the application rate must reflect the intended use of the biocidal product;

- Application of biocidal product: Trials should be performed using the highest proposed rate of application and using the formulation under evaluation. In cases

[93] CA-March17-Doc.7.6.c-final

where multiple applications are intended, this should be reflected in the trial;

- Choice of food/feedstuffs: representative food/feed based on food/feed composition, texture, surface area etc. should be used for the trial.

- Sampling: Sampling should occur under as realistic circumstances as possible (e.g. reflecting critical points within the food production chain). Since biocide residue levels will vary within the treated area or in the treated food/water and also over time, several samples have to be obtained. Conditions and time period of storage should be considered as well. For example, for stored food, samples from the food layer in direct contact with the treated surface and samples from the inner layers of food should be obtained. For water stored in treated tanks, samples should be taken at various time points to account for the maximum period the water is stored within the treated tank.

5.1.9 AGGREGATE RISK ASSESSMENT

Please refer to Section 4.3.2 for more information.

5.1.10 REFERENCES TO CHAPTER 5.1

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Appendix 5-1

Appendix 5-1: (I) Default Values

Table I-1

No.	Description	Default Values	Background Information Remarks	References
5.1. Disinfectants and preserved cleaners in the food and drink industry				
1	**maximum acceptable concentration of biocide residue in rinsing water**	0.01 mg/L	The trigger value must be adapted to proportionally lower levels for substances with ADIs and/or ARfDs < 0.01 mg/kg bw.	This value is derived from the trigger value of 0.01 mg b.r./kg food, which determines whether an MRL assessment is needed. Therefore, if biocide residues remain at or below this level, no further MRL evaluation is needed. The derivation is explained below this table. The biocide residue refers to the biocide residue definition for DRA
2	**maximum acceptable amount of biocide residue on inner surface of machinery after rinsing**	0.06 mg/m^2	The trigger value must be adapted to proportionally lower levels for substances with ADIs and/or ARfDs < 0.01 mg/kg	This value is derived from the trigger value of 0.01 mg b.r./kg food, which determines whether an MRL assessment is needed. Therefore, if biocide residues remain at or below this level, no further MRL evaluation is needed. The derivation is explained below this table. The biocide residue refers to the biocide residue definition for DRA
3	**mass transfer efficiency (TF)**	100%	Worst case assumption	
4	**density of food (ρ_{food})**	Worst case default values for con-version of "mg/L food" into "mg/kg food" Liquid foods ρ_{food} = 0.789 g/cm^3 Semi-solid foods ρ_{food} = 0.496 g/cm^3 (*) Solid foods ρ_{food} = 0.06 g/cm^3	For more details on the derivation of the default values and values for more specific food groups (e.g. ice cream) see Appendix I.6 Density of food. (*) value applies to PT 4 screening scenario CIP systems:	Charrondiere R., Haytowitz D, Stadlmayer (2012) FAO/INFOODS Density Database Version 2.0 (2012). FAO, Rome, Italy E-ISBN 978-92-5-107346-9 (PDF). http://www.fao.org/3/ap815e/ap815e.pdf

		(**)	disinfection of pipes and closed containers (**) value applies to PT 4 screening scenarios disinfection of closed or open containers and disinfection of open food preparation areas as well as PT 1 hand disinfection	
5	**distribution of biocide residues in containers/machinery**	<u>for solid and semi-solid foods:</u> 1 cm layer of food in contact with container/machinery inner surface for liquid foods: even distribution	Within one container an even distribution of biocide residues in the food can only be assumed for liquids. For solid (powdered, granular) and semi-solid (paste-like) foods an even distribution may only be assumed if blending, mixing, or shaking occurs. As a worst case for solid and semi-solid foods the first layer of food (i.e. 1 cm layer) in contact with the container/machinery inner surface is considered in the calculations. Similarly for pipes with a diameter > 2 cm a 1 cm layer of	expert judgement

			food in contact with the pipe inner surface is considered.	
6	**amount of liquid remaining on wall/surface after draining**	2×10^{-6} L/cm^2 (=20 mL/m^2)	Biocidal product (in-use solution) left on treated surfaces (open surfaces, container inner surfaces, pipe inner surfaces) after draining: assumption of 20 μm film thickness on treated surface corresponding to 2×10^{-6} L/cm^2. The same default value is applied in the ARTFood Guidance on Non-professional Uses. Additional information provided by Industry reported values in the range of 6 to 40 mL/m^2 (see Appendix I.2).	BPR Vol III Parts B+C, section 5.6.4.2
7	**volume (V) and inner surface area ($A_{i.s.}$) of bottle**	V = 1 L; Ai.s. = 725 cm^2 V (1 cm inner layer)	1L-bottles are commonly used for beverages. It was agreed in ARTFood not to consider smaller package sizes (although also commonly used) to be in line with the FCM approach that considers 1 kg food	

		= $A_{i.s.}$ × 1 cm = 725 cm^3 = 0.725 L	packaged in a volume of 1 L (= 6 dm^2) for both adults and children. The volume of the 1 cm inner layer of food may be considered for solid an semi-solid foods, in which an even distribution in the food cannot be assumed.	
8	**volume (V) and inner surface area ($A_{i.s.}$) of commercial beverage container**	V = 50 L; $A_{i.s.}$= 0.9 m^2	Size of commercial beverage container commonly used in food service	
9	**volume (V) and inner surface area ($A_{i.s.}$) of small tank**	V = 1000 L; $A_{i.s.}$= 6.0 m^2 V (1 cm inner layer) = $A_{i.s.}$ × 0.01 m = 0.06 m^3 = 60 L	Size of s**mall tank** used in food industry The volume of the 1 cm inner layer of food may be considered for solid an semi-solid foods, in which an even distribution in the food cannot be assumed.	Wildbrett (2006)
10	**volume (V) and inner surface area ($A_{i.s.}$) of Large tank**	V = 140 000 L; Ai.s. = 167.2 m^2 V (1 cm inner layer) = $A_{i.s.}$ × 0.01 m = 1.672 m^3 = 1672	Size of large tank used in food industry The volume of the 1 cm inner layer of food may be	Wildbrett (2006)

		L	considered for solid an semi-solid foods, in which an even distribution in the food cannot be assumed.	
11	**volume (V) and inner surface area ($A_{i.s.}$) of model pipe**	V= 1 L $A_{i.s.}$ = 4000 cm^2	Model pipe (narrow pipe reflecting worst-case): Radius: R_{pipe} = 0.5 cm; Volume: V_{Pipe} = 1L Length: $L_{pipe} = V_{Pipe} \div R_{pipe}^2$ = 1000 $cm^3 \div \pi (0.5\ cm)^2$ = 1273 cm Inner surface area: $A_{\text{inner surface}} = 2\ \pi\ R_{pipe} \times L_{Pipe}$ = 2 π × 0.5 cm × 1273 cm = 4000 cm^2	
12	**volume ($V_{model\ CIP}$) and inner surface area ($A_{i.s.\ CIP}$) of model CIP machinery**	V = 1850 L; $A_{i.s.}$ = 181632 cm^2 = 18.16 m^2	Model CIP machinery based on survey data for gelatin production machinery: Model system consisting of (A) pipes (radius 5 cm, length 2 m) $V_{pipe} = L_{pipe} \times R_{pipe}^2$ = 2000 cm × 25 cm^2 = 50 000 cm3 = 50 L; $A_{i.s.pipe} = 2\ \pi\ R_{pipe}$	unpublished survey data, BfR (2018)

		V (1 cm inner layer) $=A_{i.s.} \times 0.01$ m $= 0.1816$ m^3 $= 181.6$ L	$\times L_{pipe}$ $= 2 \pi \times 5$ cm $\times 2000$ cm $= 62832$ cm^2 and (B) 2 vessels (volume 900 L each with inner surface area of $A_{i.svessel.} = 5.94$ m^2, unpublished survey data) Calculation of volume of model machinery $V_{model\ CIP} = V_{pipe} + 2 \times V_{inner\ surface\ vessel}$ $= 50$ L + 2x 900 L $= 1850$ L Calculation of inner surface area of model machinery $\mathbf{A_{i.s.\ CIP}}$ $= A_{\ inner\ surface\ pipe} + 2 \times A_{inner\ surface\ vessel}$ $= 6.2832$ m^2 + 2 $\times$ 5.94 m^2 = 18.16 m^2 The volume of the 1 cm inner layer of food may be considered for solid an semi-solid foods, in which an even distribution in the	

			food cannot be assumed.	
13	**food preparation area in contact with food**	2000 cm^2/ kg food = 0.2 m^2/ kg food	The same default value is applied in the ARTFood Guidance on Non-professional Uses based on the following rationale: In the US EPA model for assessing disinfectant residues, a value of 0.2 m^2 is used for surface area in contact with food. The value is based on a value of 0.4 m^2 which was used by FDA to evaluate food contact sanitizing solutions. The actual basis of this value cannot be documented from FDA sources, but its use is documented. The FDA value reflects surface area of all silverware, dishes and glasses that a person uses in an institutional setting for 3 meals a day. For the purpose of the US EPA model, the FDA value was cut in half, to reflect only counter top	BPR Vol III Parts B+C, section 5.6.1.1

			surfaces. The default value is based on assumptions made for chronic exposure, which were considered conservative enough to also cover the acute situation. (DRAWG Workshop January 2012)	
14	hand surface that can be in contact with food for adults	0.75	75% of hand surface areas is the reasonable worst case for adults. Palms as well as the back of the hands are considered as full hands may be used for preparation steps such as tossing salads or kneading dough. However, food contact with the back of the hands will be less pronounced (less surface area, less friction) than for the inside of the hands. In addition, for other types of food preparation, there will be no contact with the back of the hands. Therefore, in addition to the full	Expert judgement

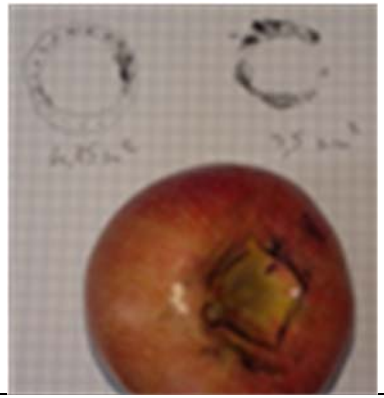
			palms, only half of the back of the hands is considered.	
15	transfer factor from hand to food	50%	Considered as a reasonable worst case of transfer agreed by ArtFood members	Agreed at ARTFood meeting in November 2019
16	retention factor	10%	Refining factor in accordance with SCCNFP/0321/00 Final (p.79 for leave-on products) and agreed by ArtFood 2019	SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation SCCS/1564/15, 9th revision (2015) https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf Agreed at ARTFood meeting in November 2019
		1 %	Refining factor in accordance with SCCNFP/0321/00 Final (p.79 for rinse-off products)	Recommendations no.9 of the BPC Ad hoc Working Group on Human Exposure Based on the SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation, 9th revision (2015): http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf
17	amount of food touched by disinfected hands	1 kg	Based on the 1 kg packaged food used under FCM regulation. The consideration of smaller food items appears too worst case. As an example, one could assume that disinfected hands are used to knead 1 kg dough, which is subsequently formed into rolls. A professional will likely handle more than 1 kg of food in between	Expert judgement.

			disinfections. However, the food batch handled directly following the disinfection step will be the one with maximal exposure to the disinfectant left on treated hands. In subsequent contacts, less substance will be available on the hands to be transferred onto food.	
18	cut-off value for high vapour pressure	> 0.1 Pa		ConsExpo Paint Products Fact Sheet, https://www.rivm.nl/bibliotheek/rapporten/320104008.pdf
5.2. Aseptic packaging				
18	**maximum acceptable concentration of biocide residue in rinsing water**	0.01 mg/L	The trigger values must be adapted to proportionally lower levels for substances with ADIs and/or ARfDs < 0.01 mg/kg bw.	This value is derived from the trigger value of 0.01 mg b.r./kg food, which determines whether an MRL assessment is needed. Therefore, if biocide residues remain at or below this level, no further MRL evaluation is needed. The derivation is explained below this table. The biocide residue refers to the biocide residue definition for DRA
19	**maximum acceptable amount of biocide residue on food contact surface of package after biocide residue removal**	0.17 mg/m^2	The trigger value must be adapted to proportionally lower levels for substances with ADIs and/or ARfDs < 0.01 mg/kg.	This value is derived from the trigger value of 0.01 mg b.r./kg food, which determines whether an MRL assessment is needed. Therefore, if biocide residues remain at or below this level, no further MRL evaluation is needed. The derivation is explained below this table. The biocide residue refers to the biocide residue definition for DRA
20	**water film thickness on external surface of bottle (t)**	20 µm	Biocidal product (in-use solution) left after draining: assumption of 20 µm film thickness on treated surface	BPR Vol III Parts B+C, section 5.6.4.2

21	**migration of biocide residues into the bottle (TF)**	1 %	Migration into closed plastic bottles through bottle material is assumed to be very low. For glass and metal bottles, this value may be set at zero.	expert judgement
5.3. Surface-protective biocides in FCM				
23	**Ratio of food to packaging**	1 kg food / 6.0 dm^2		EFSA Note for Guidance for petitioners presenting an application for the safety assessment of a substance to be used in food contact materials prior to its authorisation http://www.efsa.europa.eu/en/efsajournal/doc/21r.pdf
24	**Portion of the daily food intake in contact with FCM (adult)**	1 kg		EFSA Note for Guidance for petitioners presenting an application for the safety assessment of a substance to be used in food contact materials prior to its authorisation http://www.efsa.europa.eu/en/efsajournal/doc/21r.pdf
5.5. Pest control in the food and drink industry				
no default values				
5.6. Storage protection in the food and drink industry				
no default values				
5.7. Treated wood				
25	**mass transfer efficiency**	10%	Default value derived based on the following assumptions: Fruit boxes are treated every 5 years (new treatment after 5 years). Each box is filled with fruit twice per year. As worst case it is assumed that 100% of surface biocide residues is transferred to fruit in direct contact with treated surface over the timeframe	

			of 5 years. Assuming a linear transfer of the active substance from surface to fruit, this relates to a transfer of 100% of b.r. from wood surface in 5 years corresponding to 20% in 1 year and 10% per filling of the box. A lower value for mass transfer efficiency must be supported by study data.	

1

26	**Contact surface of apple with the wooden box**	5 cm^2	Information provided by ANSES: approx. estimate of apple surface area in contact with box bottom: 5 cm^2 	
27	**Average number of apples per kilo**	6 per kg	The unit weight for an apple is listed in PRIMO rev 3 = 163 g for the RAC (children) and 225 g for the RAC (adults). This means that 1 kg contains 6.13 apples or 4.44 apples; 6.13 apples is worst case.	
28	**Contact surface of food with treated wood**	300 cm^2/kg	A default size for packaged food of 20 cm × 15 cm is considered realistic and still worst case. This results in 300 cm^2 of packaged food (1 kg) that can be in contact with the treated wood. This is in accordance with the default value of 600 cm^2 of food contact material in contact with 1 kg food (as only one side of the packaged food is in contact with the wood).	
29	**Contact duration**	6 months	The longer the contact, the higher the biocide residues in	

			the fruits/vegetables/food in contact with the treated wood. A minimum default duration is assumed. Longer duration should be indicated as fraction of this duration and the equation is multiplied with this fraction.	
30	**Penetration depth**	1 mm	The thickness of the wood and the application method of the wood preservative (brush/paint/direct injection) will have an impact on the amount of biocide residues that is transferred onto food. A larger penetration depth diminishes the biocide residue at the surface, and subsequently fewer biocide residues would be able to enter the fruit. A minimum default depth of 1 mm for contact with fruits/vegetables is proposed. Rationale: For wood preservatives, in the TNsG, there is a secondary exposure scenario (acute, ingestion) for children chewing on timber off-cuts treated with preservative. In the scenario it is assumed that the child chews on a 4 cm × 4 cm wood off-cut of 10 mm thickness that is completely penetrated with preservative and that 10% of the preservative contained in the off-cut is extracted into the saliva. These assumptions are considered equivalent to	TNsG from 2002, part III, page 50. https://echa.europa.eu/documents/10162/16960215/bpd_guid_tnsg+human+exposure+2002_en.pdf

			100% transfer of preservative on the outer 1 mm of treated wooden boxes/pallets in contact with food.	

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Appendix 5-1: (I.1) Derivation of the maximum acceptable biocide residue concentration after rinsing of machinery or packaging materials in the food and drink industry

Table I.1-1: Criteria for rinsing and wiping tests

(see also default values 1, 2, 3 and 4 in Table I-1)

	Value (*)
maximum acceptable concentration of biocide residue in rinsing water	0.01 mg b.r./L
maximum acceptable amount of biocide residue on inner surface of machinery and on flat surfaces	0.06 mg b.r./m^2
maximum acceptable amount of biocide residue on inner surface of food packaging	0.17 mg b.r./m^2

* The biocide residue (b.r.) refers to the biocide residue definition for DRA

The following paragraphs explain how these maximum acceptable concentrations were derived.

Derivation of the maximum acceptable concentration of biocide residues in rinsing water

To determine the maximum acceptable concentration of biocide residue in rinsing water, the trigger value of 0.01 mg b.r./kg food is expressed in biocide residues per liter:

0.01 mg b.r./kg food = **0.01 mg b.r./L**

Use of this value assumes that biocide residue transfer to food is similar to biocide residue transfer to rinsing water. This is in many cases not true, e.g. the transfer of a lipophilic substance into a fatty food will be much higher than that same substance's transfer into rinsing water. For this reason, biocide residue levels must comply to both the maximum acceptable concentration in rinsing water as well as to the maximum acceptable amount on inner surfaces. In addition, there is some inherent uncertainty in this value. Even if biocide residues were to transfer equally well into various foods, biocide residue amounts per kilogram would differ because of the different densities of food.

Derivation of the maximum acceptable concentration of biocide residues on the inner surface of machinery

Conversion of the trigger value expressed per kg food to an area concentration requires a ratio of food to machinery surface:

A model pipeline of 150 mm length with 25 mm diameter, which represents the outlet of the machinery for dispensing small food portions, has a volume of:

$\pi r^2 h = 3.14 * (12.5\ mm)^2 * 150\ mm = 7.36 \times 10^4\ mm^3 = 73.6\ cm^3$

This is equal to 73.6 g food (assuming 1 cm^3 = 1 ml = 1 g) = 73.6×10^{-3} kg food.

The inner surface area of this pipeline is:

$2\pi rh = 2 \times 3.14 \times 12.5\ mm \times 150\ mm = 1.18x10^{4}\ mm^2 = 1.18x10^{-2}\ m^2$

The ratio machinery surface per kg food is equal to:

$(1.18x10^{-2}\ m^2) \div (73.6x10^{-3})$ kg food = 0.16 m^2/kg food

At a maximum biocide residue (b.r.) of 0.01 mg b.r./kg food, this amounts to:

(0.01 mg b.r./kg food) ÷ (0.16 m^2/kg food) = **0.06 mg b.r./m^2**

The same criterion is applied to flat surfaces.

Assumptions regarding the model pipeline:

This model pipeline was chosen based on a combination of factors:

a. Only the first portion that comes out of the machine and food that is dispensed in small portions will contain the highest biocide residues. Therefore we need a single small portion, to represent the realistic worst case. The outlet of a machine generally consists of a small diameter pipe to be able to dispense the food in (small) portions.

b. The ratio of pipe surface: food (m2/kg food) does not change if you change the length of the pipe. Whether you have a length of 5 cm, 10 cm, 15 cm at a diameter of 25 mm, the ratio of the surface to food does not change and the trigger value for surface biocide residues is 0.06 mg b.r./m2 at all pipe lengths. The surface to food ratio only changes if you take another diameter of pipe. So the diameter of the pipe is important.

c. Literature, where a test section of 26 mm × 150 mm was mounted into a pipeline to optically verify the efficacy of cleaning: Schöler et al, 2009, Monitoring of the local cleaning efficiency of pulsed flow cleaning procedures. Proceedings of International Conference on Heat Exchanger Fouling and Cleaning VIII, in Schladming, Austria, 14-19 June 2009. Based on this reference, it was confirmed that a realistic diameter of a pipe system is 25 mm.

d. The fact that sausages often contain high levels of quaternary ammonium compounds and the size of a sausage is approximately 25 mm × 150 mm, confirms the diameter of the pipe as being a realistic size.

e. The volume of this portion is approximately 75 mL, which represents some realistic small size packages for dairy and meat products, e.g. Yakult, Danone desserts, Actimel dairy drinks, liver sausages.

Derivation of the maximum acceptable concentration of biocide residues on the inner surface of food packaging

Conversion of the trigger value expressed per kg food to an area concentration requires a ratio of food to packaging surface.

- Food is packaged at a ratio of 1 kg food per 6.0 dm^2 packaging (EFSA convention for food contact packaging materials; refer to Regulation 10/2011).

- At a maximum biocide residue (b.r.) of 0.01 mg b.r./kg food, this amounts to:

  mg b.r./kg food) ÷ (0.06 m^2/kg food) = **0.17 mg b.r./m^2**

Appendix 5-1: (I.2) Derivation of the default value for "amount of liquid remaining on surface"

Values in the range of 0.4 to 4.0 × 10^{-6} L/cm^2 have been reported for the amount of (aqueous) liquid left on non-rinsed surfaces (see table below). For the sake of consistency (with the ARTFood Guidance on Non-professional Uses) it is proposed to apply the value of 2 × 10-6 L/cm^2 also for the screening model calculations for professional uses (see default value 6 in Table I.1).

Table I.2-1

Amount of liquid left on non-rinsed surfaces [L/cm^2]	Remark	Reference
5.5 × 10^{-7} L/cm^2	Amount of water left on non-rinsed dinnerware from (5.5 × 10^{-4} ml/cm^2 = 5.5 × 10^{-7} l/cm^2)	HERA (2005): Guidance Document Methodology: Human & Environmental Risk Assessment on Ingredients of Household Cleaning Products
2 × 10^{-6} L/cm^2	Assumption: liquid film of 20 µm thickness remains on surface after draining a container This relates to 20 mL liquid per m^2 (≙ 0.002 ml/cm^2 ≙ 2 × 10^{-6} L/cm^2).	Section 5.2
4 - 16 × 10^{-7} L/cm^2	Liquid remain on a surface after draining (20 – 40 ml/m ≙ 0.0004 – 0.0016 L/m^2 ≙ 4 – 16 × 10^{-7} L/cm^2)	Handbook of Hygiene Control in the Food Industry (2016), Chapter 40.4.4

Appendix 5-1: (I.3) Derivation of the default value for Volume (V) and inner surface area ($A^{inner\ surface}$) of bottles, vessels or tanks

The table below contains selected information on volumes and inner surface areas for bottles, vessels and tanks obtained from a non-representative survey in food industry (unpublished data, BfR 2018) as well as from literature. The choice of values chosen for the assessment must be justified where possible. Currently, no final recommendations can be given for specific use areas of biocidal products, e.g. brewery industry, dairy industry, gastronomy, etc.

Table I.3-1

Bottle/vessel/container/ tank	V	$A_{inner\ surface}$	Reference
Beverage bottle	1 L	725 cm^2	Biocidal product authorisation
Beverage bottle	0.5 L	350 cm^2	Wildbrett, 2006
Beer barrel	50 L	0.9 m^2	Wildbrett, 2006
Wine barrel	225 L	0.5 m^2 (calculated considering height: 94.5 cm, Diameter: 55cm)	Size of traditional barrique wine barrel https://de.wikipedia.org/wiki/Barrique https://www.weinkenner.de/ weinlexikon/b/barrique/
Vessel small	1000 L	6.5 m^2	Biocidal product authorisation
Vessel big	400	350 m^2	Biocidal product authorisation

	000 L		
Container	1000 L	6.0 m^2	Wildbrett, 2006
Tank, small	3000 L	12.4 m^2	Wildbrett, 2006
Tank, middle	10 000 L	26.4 m^2	Wildbrett, 2006
Tank, big	25 000 L	49.1 m^2	Wildbrett, 2006
Tank, big	140 000 L	167.2 m^2	Wildbrett, 2006
Fermenting tank	550 000 L	493 m^2	Questionnaire (brewery industry) Data: V = 5500 hL = 550000 L = 550 m^3 H = 30 m Calculations: r^2 = V÷(H × π) = 550 m^3 ÷(30 m × π) = 5,84 m^2 r = 2.42 m $A_{inner\ surface}$ = 2 π r (r+h) = 2 π 2.42 m (30 m + 2.42 m) = 493 m^2
Ice cream machine	1.7 L	102 cm^2	Ice cream machine, gastronomy (market research) Data and technical information: - 1.7 L container (stainless steel) - produces 3 kg ice cream/h - maximum quantity of ingredients: 0.8 kg - external dimensions: 340 × 430 × 320 mm Calculation: $A_{inner\ surface}$ (converted from 1000 L-container of 6 m^2) ➔ 0.0102 m^2 = 102 cm^2
Milk cooling tank	3600 L	16.27 m^2	Milk cooling tank (market research) Data: Volume = 3600 L Ø = 208 cm H = 145 cm Calculation: $A_{inner\ surface}$ = 2 π r (r+h) = 2 π 104 cm (104 cm + 145 cm) = 162709 cm^2 = 16,27 m^2
Milking pail	30 L	0.2941 m^2	Milking pail (market research) Data: Volume = 30 L Ø = 18 cm H = 43 cm Calculation: $A_{inner\ surface}$ = 2 π r (r+h) = 2 π 9 cm (9 cm + 43 cm) = 2941 cm^2 = 0.2941 m^2

Appendix 5-1: (I.4) Derivation of default values for CIP systems

The table below contains selected information on CIP systems obtained from a non-representative survey in food industry (unpublished data, BfR 2018) as well as from

literature. Details on food producing machinery listed below include information on the size of pipes (as part of a machine) and on combinations of containers and pipes (forming one machine). As size and design of food producing machinery is highly variable the values below are not considered representative. Nevertheless, the choice of values considered for the assessment should be justified. Currently, no final recommendations can be given for specific use areas of biocidal products, e.g. brewery industry, dairy industry, etc.

Table I.4-1

CIP system	**L_{pipe}**	**D_{pipe}**	**V_{pipe}**	**$A_{inner\ surface\ pipe}$**	**V_{cont}**	**$A_{inner\ surface\ cont}$**	**Reference**
CIP system (pipes only)	100 m	0.8 m	160000 L	251.33 m^2	-	-	Winkler, P&A Kompendium, 2005 Data: V = 160000 L = 16 m^3 L = 100 m Calculation: $R_{pipe}^2 = V_{pipe} \div L_{pipe}$ = 16 m^3 ÷ 100 m = 0.16 m^2 R_{pipe} = 0.4 m D_{pipe} = 0.8 m
CIP system (pipes only)	100 m	25 mm	40 L	7.85 m^2	-	-	Handbook CIP, Tetra PAK, 2015
CIP system (pipes only)	100 m	38 mm	99 L	11.94 m^2	-	-	Handbook CIP, Tetra PAK, 2015
CIP system (pipes only)	100 m	51 mm	184 L	16.02 m^2	-	-	Handbook CIP, Tetra PAK, 2015
CIP system (pipes only)	100 m	63,5 mm	287 L	19.95 m^2	-	-	Handbook CIP, Tetra PAK, 2015
CIP system (pipes only)	100 m	76 mm	408 L	23.88 m^2	-	-	Handbook CIP, Tetra PAK, 2015
CIP system (pipes only)	100 m	101.6 mm	748 L	31.92 m^2	-	-	Handbook CIP, Tetra PAK, 2015
Kneading machine	2 m	0.8 m	-	5.03 m^2	-	-	Kneading machine (data from questionnaire, one example) Data on kneading machine: W: 1m L: 2.5 m H: 0.75 m
Mixing vessel	6 – 31 m	0.04 – 0.1 m	-	0.75 – 97.39 m^2	-	-	Questionnaire – all (gelatin production)
Flash vessel	20 m	10 cm	50 L	6.28 m^2	900 L	5.94 m^2	Flash vessel for gelatin production (data from questionnaire – one example gelatin production) Data on pipe: L = 20 m Ø = 10 cm

							Calculation: $V_{pipe} = L_{pipe} \times R_{pipe}^2$ = 2000 cm × 25 cm^2 = 50 000 cm^3 = 50 L Data on vessel: W/L → D: 0.7 m H: 2.35 m Food processed: 1800 L/h Calculation: V_{cont} (Volume of flash vessel): V = π r^2 × h = π 0.1225 m^2 × 2.35 m = 0,9 m^3 = 900 L $A_{Inner\ surface\ cont}$ (A of flash vessel): = 2 π r (r + h) = 2 π 0.35 m (0.35 m + 2.35 m) = 5.94 m^2

Abbreviations: CIP: Clean in place, L_{pipe}: pipe length, D_{pipe}: pipe diameter, V_{pipe}: volume of pipe, $A_{innersurface\ pipe}$: inner surface area of pipe, V_{cont}: volume of container, $A_{innersurface\ cont}$: inner surface area of container

Appendix 5-1: (I.5) Derivation of default values for food preparation areas and food contact surface areas

The table below contains selected information on food contact surface areas that had been previously applied in individual cases when assessing biocidal uses. For the sake of consistency (with the ARTFood Guidance on Non-professional Uses) it is proposed to apply the value of 2000 cm^2 also for the screening model calculations for professional uses.

Table I.5-1

Food commodity	Information on food items (e.g. size, dimension, shape)	Food surface area in contact with food preparation area (Food surface area - Weight Ratio)	References and remarks
food (general)	Food prepared on surfaces in the professional context (e.g. rolling out dough, slices of bread placed side by side on food preparation surface) - food density of 1 g/mL	2000 cm^2 per kg food	In the US EPA model for assessing disinfectant residues, a value of 0.2 m^2 is used for surface area in contact with food. Starting point for the derivation of the ARTFood default value was a value of 0.4 m^2 which was used by FDA to evaluate food contact sanitizing solutions. The actual basis of this value cannot be documented from FDA sources, but its use is documented. The FDA value reflects surface area of all silverware, dishes and glasses that a person uses in an institutional setting for 3 meals a day. For the purpose of the US EPA model, the FDA

			value was cut in half to get 0.2 m^2, to reflect only counter top surfaces. The default value is based on assumptions made for chronic exposure, which were considered conservative enough to also cover the acute situation. (DRAWG Workshop January 2012)
food (general)	Assumptions: - food layer (packaged food) of 3.3 cm on the treated surface - food density of 1 g/mL	300 cm^2 per kg food	Educated guess (value has been agreed by ARTFood for wood pallet example 5.7-2)
Meat	No further information available	400 cm^2 /kg	Wildbrett, 2006, Chapter 14, p. 349
Bread (box bread)	Loaf of bread: 1 kg = 21 cm × 9.5 cm	199.5 cm^2/kg	Educated guess (measurement of sliced bread)
Sandwich	Weight of sandwich not reported	2 × 150 cm^2	Reverse reference scenario provided in biocidal product authorization

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Appendix 5-1: (I.6) Density of food

During the ARTfood meeting in Helsinki on 20 November 2019 it was concluded that the 1 g/cm^3 density does not represent the worst case for the PT 4 screening scenario. Therefore worst case default values were derived as described below.

FAO has collected densities for a range of foodstuffs, based on data from several countries: Charrondiere R., Haytowitz D, Stadlmayer (2012) FAO/INFOODS Density Database Version 2.0 (2012). FAO, Rome, Italy E-ISBN 978-92-5-107346-9 (PDF). http://www.fao.org/3/ap815e/ap815e.pdf

Information from this database is summarized in Table I.6-2 and categorized into three groups: liquid, semi-solid (=paste like) and solid foods.

- The densities for **liquids** range from 0.789-1.40 g/mL. The lower densities are associated with pure ethanol, spirits and oils, while the higher densities are associated with syrups.

- The densities for **semi-solids** range from: 0.496 to 1.43 g/mL. The lower densities are associated with whipped cream and ice cream, while the higher densities are associated with syrups, jams and jellies.

- The densities for **solids** range from 0.06-2.2 g/mL. The lower densities are associated with raw leafy vegetables and herbs, potato chips, puffed rice and breakfast cereals, while the higher densities are associated with chemicals such as sodium chloride (table salt) and sodium bicarbonate (baking powder).

Section 5.1.4 "Tier 1 – Screening models for estimating biocide residues in food" includes the following scenarios:

1. PT 4 Disinfection of closed or open containers;
2. PT 4 CIP systems: disinfection of pipes and closed containers
3. PT 4 Disinfection of open food preparation areas
4. PT 1 Hand disinfection

Scenario 1 is applicable to liquids, semi-solids and solids. Scenario 2 is considered to be limited to liquids and semi-solids, while scenarios 3 and 4 are limited to solids.

The density value to be applied should represent the worst-case, i.e. for conversion[94] of the dimension "mg biocide residue per L food" into "mg biocide residue per kg food" the lowest density of the appropriate food type should be chosen. For other calculations the highest value may represent the worst case.

It is noted that in many cases the intended use of a biocidal product does not specify the type of food that may get in contact with treated surfaces, equipment etc. In this case, the worst case default density value listed in Table I.6-1 should be used as a first tier. More specific density values listed in Table I.6-2 can be used when the label for use indicates application for disinfection of specified food machineries.

Table I.6-1: Worst case values for food density (for various food types and

[94] Calculation for Conversion of dimensional units: R_{food} [mg a.s./kg] = R_{food} [mg a.s./L] ÷ ρ_{food} [kg/L]
(with R_{food}: Residues in food [mg a.s./L or mg a.s./kg], ρ_{food}: density of food [g/mL = kg/L])

scenarios)

No.	Screening scenario	Relevant food types	Range of food densities [g/mL = mg/L]	Worst case value for conversion of "mg b.r./ L food" into mg b.r./ kg food" [g/mL = mg/L]
1	General	Liquids	0.789 – 1.40	0.789
2	General	Semi-solids	0.496 – 1.43	0.496
3	General	Solids	0.06 – 2.2	0.06
4	PT 4 Disinfection of closed containers	Liquids, semi-solids, solids	0.06 – 2.2	0.06
5	PT 4 CIP systems: disinfection of pipes and closed containers	Liquids, semi-solids	0.496 – 1.43	0.496
6	PT 4 Disinfection of open food preparation areas	solids	0.06 – 2.2	0.06
7	PT 1 Hand disinfection	solids	0.06 – 2.2	0.06

Table I.6-2: Summary of densities for various foods

No.	Type (assigned by ARTFood)	Foods (as in FAO database)	Density in g/mL (including mass density and bulk density)	Specific density (relative to density of water at 4 °C)
1.	Liquids	water, soft drinks, sports drinks, fruit juices, nectars, brewed coffee, espresso, miso broth, herbal tea infusion, tea liquid, ice tea, beer, wine, cider	0.96-1.06	0.99-1.07
2.	Liquids	Concentrated lemonades	1.17-1.24	1.17-1.24
3.	Liquids	Syrup: blackcurrant, corn, blackberry, grenadine, maple, pancake syrup	1.312-**1.40**	1.18-1.41
4.	Liquids	Ice water at 0 °C	0.916	
5.	Liquids	Ethyl alcohol	**0.789**	
6.	Liquids	Spirits & liqueurs	**0.873** -1.15	0.95-1.18
7.	Liquids	Soy drink, soy milk	1.05-1.08	
8.	Liquids	Cow milk (whole, semi-skimmed, skimmed), chocolate milk, goat milk, buttermilk, milkshake, evaporated milk, sour milk	0.98-1.08	1.02-1.07
9.	Liquids	Vegetable oils, cod oil, whale oil, cooking oil	**0.88**-0.96	0.88-0.93
10.	Liquids	Cream (9-50% fat)	0.98-1.017	0.94-1.00
11.	Liquids	Soya sauce	1.12	
12.	Liquids	Soups: bean soup, cheddar cheese soup, chicken noodle soup, egg drop soup, mushroom soup, meat soup, mixed soup, thick soup (squash, potato), vegetable soup, tomato soup	0.99-1.09	
13.	Semi-solids	Butter, margarine	0.91-0.96	
14.	Semi-solids	Sour cream (18-38% fat)	0.978-1.005	
15.	Semi-solids	Yoghurt	1.031-1.06	1.08
16.	Semi-solids	Whipped cream	**0.496**	0.96
17.	Semi-solids	Ice cream	**0.54**-0.62	0.51-0.61
18.	Semi-solids	Mayonaise	0.91-1.00	
19.	Semi-solids	Maize mash	0.72	
20.	Semi-solids	Porridge flour boiled (with water)	0.73-1.05	

No.	Type (assigned by ARTFood)	Foods (as in FAO database)	Density in g/mL (including mass density and bulk density)	Specific density (relative to density of water at 4 °C)
21.	Semi-solids	Jam, jelly	1.333-**1.43**	
22.	Semi-solids	Nutella	1.26	
23.	Semi solids	Salad dressing	1.1	
24.	Solids	Crushed ice	0.56-0.72	-
25.	Solids	Powders: Coffee powder, chocolate drinking powder, tea powder, powdered milk	0.20-0.56	
26.	Solids	Powdered potatoes	0.77	
27.	Solids	Powdered onions	0.40	
28.	Solids	Garlic powder	0.32	
29.	Solids	Cinnamon powder	0.56	
30.	Solids	Powdered eggs, powdered egg yolks	0.35-0.37	
31.	Solids	Powdered sugar	0.56	
32.	Solids	Powdered mustard	0.26	
33.	Solids	Cereal flours: barley (flour, malted flour, ground, fine ground, malted, rolled, scoured), buckwheat (flour), cake mix, maize (flour, fermented flour, germ flour, gluten flour, grits, ground), donut mix, dough mix, millet (flour, fermented flour), oat (flour, middlings, groats, ground, rolled), raw porridge flour, rye (flour, malted, middlings, shorts), semolina, sorghum (flour, fermented flour), wheat (cracked, flour, malted flour, wholemeal flour, gluten, middlings, shaved)	0.24-0.84	
34.	Solids	Cassava flour	0.55	
35.	Solids	Soya bean flour	0.64-0.74	
36.	Solids	Blood flour	0.48	
37.	Solids	Potato flakes	0.21	
38.	Solids	Soya bean flakes	0.58	
39.	Solids	Garlic flakes	0.35	
40.	Solids	Dried buttermilk, casein, whey	0.50-0.58	
41.	Solids	Cereal brans: barley bran, buckwheat bran, maize bran, rice bran, rye bran, wheat bran	0.18-0.56	
42.	Solids	Oats hulls	0.13	
43.	Solids	Wheat hulls	0.70	
44.	Solids	Soya bean hulls	0.40	
45.	Solids	Bread, cake	0.18-0.45	
46.	Solids	Raw pasta: macaroni style	0.39-	
47.	Solids	Boiled pasta: macaroni style, noodles	0.55-0.59	
48.	Solids	Breakfast cereals	**0.10**-0.37	
49.	Solids	Puffed rice	**0.10**	
50.	Solids	Cereal meals: barley meal, maize meal	0.45-0.64	
51.	Solids	Soya bean meal	0.64	
52.	Solids	Alfalfa meal	0.19-0.35	
53.	Solids	Animal meal: Blood meal, bone meal, meat meal	0.62-0.96	
54.	Solids	Maize starch	0.54-0.67	
55.	Solids	Black tea (dried leaves), herbal tea (dried),	0.23-0.48	
56.	Solids	Animal fat, solid vegetable fat (kimbo, rina)	0.60-0.70	
57.	Solids	Lard	0.919	0.96

No.	Type (assigned by ARTFood)	Foods (as in FAO database)	Density in g/mL (including mass density and bulk density)	Specific density (relative to density of water at 4 °C)
58.	Solids	Cheese (Emmentaler) grated	0.34	
59.	Solids	Coconut chips	0.61	
60.	Solids	Apple slices dried	0.24	
61.	Solids	Frozen cooked spinach	1.046	
62.	Solids	Potato chips, pringles	**0.09**-0.12	
63.	Solids	Snacks puffed low fat	0.11	
64.	Solids	Sugars: sugar, granulated sugar, white sugar	0.70-0.95	
65.	Solids	Sugars: dextrose, glucose glucolin, glucose, sucrose, sucrose octoacetate, maize sugar	0.33-0.85	
66.	Solids	Gelatin	0.72	
67.	Solids	Yeast	0.95	
68.	Solids	Chemicals: Monosodium glutamate, baking powder (sodium bicarbonate), salt (sodium chloride, table salt, granulated salt)	0.90-**2.2**	
69.	Solids	Supplements: Protein supplement, vitamins (additive, compound, enrichment, mix, powder)	0.54-0.70	
70.	Solids	Fruits: loquat, pawpaw, olives with stones	0.56-0.65	
71.	Solids	Brassica vegetables: cauliflower (boiled)	0.45	
72.	Solids	Bulb vegetables: spring onions, onions (chopped, minced, fried cubed, raw cubed)	0.13-0.75	
73.	Solids	Fruiting vegetables: chili pepper (green, red), sweet pepper (raw cubes, raw half rings)	0.39-0.51	
74.	Solids	Sweet corn (green maize) raw or boiled	0.61-0.73	
75.	Solids	Leafy vegetables and fresh herbs: raw 315anage leaves, raw green salad leaves, raw spinach leaves, sage leaves	**0.06**-0.29	
76.	Solids	Legume vegetables: French green beans, fresh green peas, green kidney beans (raw or boiled), green lentils (raw or boiled)	0.53-0.89	
77.	Solids	Pulses: white beans, cowpeas (dry or boiled), green gram (dry or boiled); kidney beans (dry or boiled), pigeon peas (dry or boiled)	0.69-0.96	
78.	Solids	Soya beans (dry or boiled)	0.70-0.79	
79.	Solids	Root vegetables: carrots (chopped, grated)	0.54-0.71	
80.	Solids	Tubers (raw or boiled): arrowroot, cassava, potato, sweet potato, yam,	0.44-0.79	
81.	Solids	Cereal grains: barley, buckwheat, bulrush millet, finger millet, oats, rough rice, rice hulled, white rice, rice boiled, rye, wheat	0.41-0.90	
82.	Solids	Corn/maize: shelled maize, cracked maize, dried cracked maize, dried cracked boiled white maize, White maize boiled or dry, millet, maize boiled, maize ears, maize chops, maize cobs, maize kibbled	0.27-1.07	

No.	Type (assigned by ARTFood)	Foods (as in FAO database)	Density in g/mL (including mass density and bulk density)	Specific density (relative to density of water at 4 °C)
83.	Solids	Nuts: almonds, cashews, peanuts shelled, pistachio with or without shell	0.46-0.69	
84.	Solids	Oilseeds: flaxseed (i.e. linseed); rapeseed, sunflower seed, alfalfa seed, clover seed	0.62-0.77	
85.	Solids	Coffee beans (green), coffee beans (roasted)	0.35-0.62	0.35-0.62
86.	Solids	Flavorings: Dry flavoring mix, barbecue spices, blended spices	0.48-0.70	
87.	Solids	Boiled whole prawns with or without shell	0.58-0.77	
88.	Solids	Boiled/poached chicken eggs	0.60	
89.	Solids	Cow intestines, raw	0.93	
90.	Solids	Cow intestines, boiled	0.56-0.58	
91.	Solids	Cow meat without bones, lean, raw	0.96	
92.	Solids	Pork meat with bones, fatty- medium, raw	0.93-0.97	
93.	Solids	Pork meat with bones, fatty-medium, boiled	0.63-0.70	
94.	Solids	Mixed dishes: various combinations of beef, potatoes, vegetables, banana, pawpaw, pulses, tubers, maize, rice, pasta/noodles, chicken, cheese, tuna	0.60-1.33	

Appendix 5-1 (II) Proposal for Defining Representative Foods

(to be finalised in collaboration with the MRL-setting authority)

Appendix 5-1: (II.1) Introduction

Consideration of representative foods may be helpful in cases when application of biocidal products results in direct contact with food. This may be the case for airspace treatments by fumigation, spraying or fogging applications for protection of stored foods and also for treatments of rooms/facilities used in food production, food trade, food preparation in the presence of food i.e. when food is not removed (or covered) prior to application of the biocidal product.

In order to identify representative foods for an intended use, the information provided by the Applicant on what commodities are likely to be treated should be taken into account. If no particular commodities are specified, foods exhibiting various different properties that may influence the formation of biocide residues should be considered. This covers characteristics such as surface area, texture (e.g. solid, liquid) and composition (e.g. content of water, fat, carbohydrates, protein, fibre; high acid content) of foods. Additionally, other parameters (that are not the focus of this proposal) such as temperature, humidity, storage duration, vapour pressure of substances in the biocidal product etc. may have an impact on the formation of biocide residues. Experiences from storage protection in the field of plant protection products (PPP) indicate that the formulation of the product may only exhibit little impact on biocide residue formation.

Food and feedstuffs may be exposed or contaminated as bulk good or as packaged product (e.g. in paper bags, cardboard boxes, plastic bags/film/containers/bottles, glass containers/bottles, aluminium foil/bags, cans, etc). Different packaging options with commonly used materials should be considered for each food to identify worst case

conditions.

Appendix 5-1: (II.2) Proposal

The following table provides an overview of food groups with various characteristics that may influence biocide residue formation. Please note that only rough ranges of nutrient composition are given in order to assign foods to the respective groups. Food groups were further sub-divided, if applicable. Foods that are considered as potentially relevant for storage protection applications are marked with "#".

The table should help to identify relevant representative foods for a certain intended use. Which food commodities are relevant for evaluation of a specific application of a biocidal product should be decided on a case-by-case basis. The table with its examples is very detailed, but it may be sufficient to pick single representative foods for each food group. The aim should be to cover all types of food with experiments in commodities from different food groups. Grouping of foods for evaluation may be possible. However, currently there is no experience available on extrapolation between commodities as is common practise in the evaluation of PPP.

Table II.2-1 Representative foods

Food characteristics	Food/feed of animal origin[95]	Food of plant origin[95]
High carbohydrate content (mono/oligo-saccharides)	- honey (ca. 70% monosaccharides, ca 10% di- and oligo sacch., <20% water)	# Sugar (96-100% saccharose) *(as a representative for all other sweets, large surface area)* # Chocolate (30-60% sugar, 22-36% fat)
High carbohydrate content (polysaccharides)	/	Low fat # low-fat baked goods # pasta (70% carb, 15% protein, 11% water, 3% fat) # potato flakes (ca 70-80% carb, ca 9% protein, 4-8% water, 0-1% fat) *(large surface area)* # breakfast cereals (cornflakes, muesli etc 67-79% carb, 7-10% protein, 1-2% fat) # flour (ca 80% starch, ca 12% protein, 1% lipids) *(considered a simple processed product from cereal grains and therefore falls within the scope of PPP legislation, but may serve as representative for pasta and low-fat*

[95] Food composition as given in the following references:
Potato flakes: http://www.barryfarm.com/nutri_info/veggies/potatoflakes.html, http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl, http://gesuender-abnehmen.com/abnehmen/naehrwerttabelle-kartoffelerzeugnisse.html
Potato chips: http://nutritiondata.self.com/facts/snacks/5663/2, http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl
Fishmeal: http://www.fao.org/wairdocs/tan/x5926e/x5926e01.htm, http://en.wikipedia.org/wiki/Fish_meal#Nutrient_composition,
Dried meat, milk powder, egg powder: http://gesuender-abnehmen.com/abnehmen/naehrwerttabelle.html
Tofu: http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl
Roasted peanuts: http://www.fitnesswelt.de/kalorien/Erdn%FCsse+ger%F6stet+und+gesalzen, http://gesuender-abnehmen.com/abnehmen/naehrwerte-kalorien-erdnuss-geroestet-und-gesalzen.html
Other foods: H-D.Belitz, W.Grosch, Lehrbuch der Lebensmittelchemie, 3. Auflage, Springer-Verlag, Berlin Heidelberg New York, 1987

Food characteristics	Food/feed of animal origin[95]	Food of plant origin[95]
		baked goods) <u>High fat</u> # high-fat baked goods *(might be comparable to low fat baked goods?)* # potato chips (53-68% carb, 21-34% fat, 7% protein) <u>High fibre</u> # cocoa powder (58% carb (ca 50% of carb is dietary fibre), 18% protein, 10% fat) *(large surface area)* # roasted coffee (30% carb, (ca 80% of carbs non-water soluble polysaccharides), 13% fat, 9% protein, 3% water, 35% unknown components) *(ground product = large surface area)*
High fat content	- butter (81-85% fat, 14-16% water) - marine oils (ca 100% fat)	- vegetable oils (ca 100% fat) *(borderline to PPP applications)* - margarine (80% fat, 18% water) - mayonnaise (50-85% oil, 5-10% egg yolk)
High protein content Combination high fat/high protein	<u>Low water (examples with varying fat content)</u> # Gelatine (85-90% protein, no fat, no carb) *(can possibly be combined with fish meal, dried meat and dried fish)* # Fish meal (60-72% protein, 9% water, 6% fat) *(as a worst-case scenario, fish meal possibly covers dried fish)* # Dried fish *(can possibly be combined with dried meat)* # Dried meat (beef: 64% protein, 27% fat, no carb) # milk powder (25-35% protein, 1-26% fat, 38-51% carb) *(since egg and milk powder differ in fat and carbohydrate content, trials in both categories are necessary, possibly a combination of the two categories will prove possible at a later point)* # Egg powder (46% protein, 42% fat, 2% carb) *(see also comment on milk powder)* <u>High water and varying fat content</u> - fresh meat (15-23% protein, 1-30% fat, 75% water) - fresh fish (edible parts 13-22% protein, 1-26% fat, 60-80% water) <u>High fat</u> # Cheese (large variation between products: e.g. grated Parmesan cheese 39% protein, 29% fat, 4% carb, 21% water, large surface area; mozzarella 22% protein, 22% fat, 2% carb, 50% water) *(grated cheese may represent worst-case, or cheese may possibly be covered by milk powder due to its large surface area)* - dairy products besides cheese *(possibly covered by milk and cheese)*	<u>High water</u> - soya products e.g. tofu (70-88% water, 8-17% protein, 4-10% fat, 1-2% carb) <u>High fat</u> # roasted peanuts (48-53% fat, 25% protein, 9-12% carb, 11% fibre, 2% water)

Food characteristics	Food/feed of animal origin[95]	Food of plant origin[95]
High water content	- milk (cow: 87% water, 4.6% sugar, 3.9% fat, 3.2% protein)	- Beverages (up to 100% water) *(soft drinks, alcoholic drinks etc. may be covered by food simulants used for testing food contact materials:* *A (distilled water or water of equivalent quality),* *B (3% acetic acid (w/v) in aqueous solution),* *C (10% ethanol (v/v) in aqueous solution))*

1

Appendix 5-1 (III) Background Information for Chapters 5.1.1, 5.1.5.1 and 5.8.2

Appendix 5-1 (III.1) Residue monitoring data and occurrence data

- Quaternary ammonium compounds and chlorine compounds are reported below as illustrative examples of possible residues and their by-products that might occur in different product type areas at the same time.

Appendix 5-1 (III.1.1) Quaternary ammonium compounds

Many disinfectants used in the food sector contain quaternary ammonium compounds as the active substance. These substances adhere well to plastics and stainless steel, and rinsing with water does not remove them adequately. They do however dissolve well in milk and other foods rich in protein and fat.

Monitoring data from 2009/2010 of quaternary ammonium compounds in soft ice cream sold by artisans and on fairs showed levels of more than 0.1 mgb.r./kg food of at least one substance in one third of the analysed samples (Knapp 2011).

From 2003 to 2005 the Dutch Food and Consumer Product Safety Authority (VWA) monitored p-toluene sulphonchloramide in various foods. P-toluene sulphonchloramide levels above the Dutch MRL of 0.1 mg/kg were found in 460 out of 23288 samples in 2003/2004 (shrimps, milk shake, whipped cream, ice cream, cream cake, meat, minced meat, sausages). Levels of more than 10 times the MRL were found in each of these foods (Jonker 2005).

Monitoring of quaternary ammonium compounds revealed levels above the Dutch MRL of 0.5 mg/kg in 2.5 % of 472 samples of ice cream and milkshake (2002) and 29 % of 116 samples of whipped cream (2002), 4.0 % of 425 samples of meat and meat products (2002/2003), 647 out of 7851 samples of shrimps, whipped cream, ice cream, cream cake, meat (2003/2004), 14 out of 292 samples of ice cream, milkshake, whipped cream, cream cake, minced meat, sausages, meat (2005). In shrimps, whipped cream and ice cream, levels of more than 10 times the MRL were found (Jonker 2003a, 2003b, 2005a, 2005b).

In 2006 monitoring of quaternary ammonium compounds in raw commodities for whipped cream and ice cream production revealed no significant exceedances. Quaternary ammonium compounds at levels above the Dutch MRL of 0.5 mg/kg were found in only 2 out of 100 samples (raw commodities for whipped cream). The Dutch Food and Consumer Product Safety Authority (VWA) concluded that the raw commodities for whipped cream and ice cream production are not the main source of quaternary ammonium compounds (In't Veld, 2006).

Monitoring data collected in 16 EU member states and Norway during 2012-2013 support the findings reported above (EFSA, 2013):

- Across food groups DDAC and BAC were mostly found in "milk and milk products" (in 12 % of the samples analysed), "leafy vegetables and fresh herbs" (in 6 % of the samples analysed) and "baby food" (in 5 % of the samples analysed). In the groups of "animal feed", "legume vegetables (fresh)", "fungi", "citrus fruit" and "tropical and subtropical fruit" 3-4 % of the samples showed results above the Limit of Quantification (LOQ) of the analytical method.

- In the remaining food groups BAC and DDAC were found with lower frequencies (1-2 %) or were not detected at all. However, the significance of the findings may be limited for several food groups due to the limited number of samples analysed.

- In the food group of animal products highest DDAC and BAC levels were found in ice cream samples (up to 3.64 mg DDAC/kg; 1.74 mg BAC /kg) and in processed milk products (0.01 to 0.03 mg DDAC/kg; up to 1.1 mg BAC/kg [10-16]).

- For vegetables highest values of 0.92 mg DDAC/kg fresh herbs and 0.48 mg BAC10-16 /kg carrots were found.

- In baby food DDAC and BAC levels were found in the range of 0.0033 to 0.12 mg/kg for DDAC and 0.002 to 0.13 mg/kg for BAC [10-16].

Similar to DDAC and BAC, a diamine compound, which is applied as biocidal active substance in the dairy industry, has been quantified in dairy products (Slimani et al, 2018).

Appendix 5-1 (III.1.2) Chlorate

Chlorine compounds, e.g. chlorine, chlorine dioxide or hypochlorite, are commonly applied as biocidal active substances for disinfection of drinking water, water for food processing, surfaces coming into contact with food and food processing equipment. When using chlorine compounds chlorate is formed as a by-product. It is assumed that chlorate residues in food mainly result from the application of chlorine compounds in the food area (EFSA, 2015).

Occurrence data for chlorate residues in food have been summarized in an EFSA CONTAM Panel Scientific Opinion (EFSA, 2015). Within the group of "vegetable and vegetable products" (3752 samples analysed) highest chlorate concentrations were observed for "chilli pepper" (lower bound, LB = 0.164 mg/kg, upper bound, UB = 0.169 mg/kg), "aubergines" (lower bound, LB = 0.157 mg/kg, upper bound, UB = 0.164 mg/kg) and "vegetable products, unspecified (lower bound, LB = 0.216 mg/kg, upper bound, UB = 0.222 mg/kg). In "drinking water (453 samples) chlorate concentrations ranged from 0.028 mg/L (lower bound) and 0.039 mg/L (upper bound), the 99th percentile UB concentration was 0.196 mg/L.

Within each food group food commodities reported as "frozen" showed the highest levels of chlorate. However, as there also were many "frozen" samples with chlorate levels below the LOQ, it may be assumed that chlorate levels may depend on how food is actually processed (chlorine levels in water, rinsing).

For chlorate an MRL of 0.25 mg/l applies for drinking water according to Directive (EU) 2020/2184 on the quality of water intended for human consumption.

Appendix 5-1 (III.2) Commercially available test or indicator strips

Note: Detection levels of test/indicator strips must be sufficient to detect biocide residues in rinsing water or on surfaces at the trigger value (sufficient analytical limit of quantification).

The following test/indicator strips are examples of the possible alternatives on the market and do not constitute a exhaustive list.

- pH indicator strips for pH 1-14 measuring in 1 pH or 0.5 pH increments

- Chlorine measuring strips (i.e. sodium hypochlorite measured as free (available) chlorine (Cl_2)). Strips are available to measure levels of 0-10 mg/L for low-level residuals, 0-200 mg/L for restaurants, 0-1000 mg/L for cruise ships/day care and 0-10000 mg/L for Methicillin-resistant Staphylococcus aureus (MRSA) infection prevention in hospitals. The low-level chlorine strips are meant to detect any residual chlorine present and can detect as little as 0.5 mg/L of free (available) chlorine. The other strips are meant to test whether the chlorine bleach solutions

are at the required strength.

- QAC (quaternary ammonium chloride) strips are available for hyamine- and steramine-type chlorinated amine disinfectants to measure levels of 0-400 mg/L or 200-1000 mg/L. These strips are meant to confirm the required strength of the disinfection solution.

- Hydrogen peroxide strips are available to measure levels of 0-100 ppm for low-level (residual) testing, 0-400 ppm for pools and hot tubs and 10,000-100,000 ppm [1-10 %] for contact lens solutions and food grade H_2O_2. The low-level hydrogen peroxide strips are meant to detect any residual peroxide present down to a level of 0.5 or 1 mg/L. They are suitable for detection of hydroperoxides and ether peroxides. Polymeric peroxides, which can form in diethyl ether, are not detected. Organic peroxides, such as di-tert-butyl peroxide, di-cumyl peroxide or tert-butyl perbenzoate, either do not react or react with significantly reduced sensitivity. The high-level strips are meant to confirm the required strength of the disinfection solution.

Appendix 5-1 (IV) Background Information for Chapter 5.7.4 "Treated Wood"

In the following, information on the training systems for different plants is given.

Grapes

The most representative espalier system is that of vineyards. To support the delicate plants, posts are placed after every third or fourth plant.

Vegetables

Common horticultural varieties that are grown using training systems in greenhouses or in the field are tomatoes, peppers, cucumbers, climbing varieties of peas and beans etc. The plants are grown with the support of ropes, wires or wire netting which is held in place by wooden poles. For indoor cultivation of crops usually plastic training systems are used because of high humidity in the greenhouse, high weight of wooden material and better reusability of plastic materials. For outdoor cultivation of annual plants like tomatoes, usually shorter, bushy varieties are planted so that training systems are not needed.

Fruit trees

Trees grown using espalier practices are trained into flat two-dimensional shapes. The espalier technique is common in cultivations for private consumption where available space is reduced. For intensive crop production, it is still considered experimental. The 5-meter tall posts are mainly made of galvanized steel or concrete, since they are cheaper and longer lasting than wooden posts. Although the technique works best with pome fruits (e.g. apples, pears), other species can be grown with this procedure as well, including citrus and stone fruits (e.g. apricots, peaches, nectarines, cherries and plums). Trained fruit trees start their production between 4 to 5 years after planting. The tree varieties used have reduced roots so the weight of the fruiting tree can only be supported using trellises.

Other fruits

Blackberry bushes and passion fruit plants (a climbing plant) can be grown using trellises. Strawberries and raspberries are grown on wires, but without the use of wooden posts.

Olive trees

Espalier systems are not common for olive trees, but their use is increasing. The juvenile period of the trees is shortened, and production starts in the third year after planting. The trellis system facilitates mechanical harvest, but production costs are increased. Since the roots and trunks of olive trees are sturdy and hardly need support, posts are only placed at the ends of each row.

Hops

Trellis systems are 7 m high, 110 wooden posts per hectare are carrying a wire netting that is fixed to the ground at the sides. Wooden posts (mostly spruce or pine wood) are protected from weather/ environmental effects by treatment with salts (substances not further specified) or creosote for impregnation. For the wire netting steel rope and steel barbwire are used. A complete trellis system lasts up 30 years (some references say 20-25 years).

Depending on the variety, about 1800-2000 hops plants are grown per hectare. For each plant, two wires are connected to the overhead wire superstructure to support the growing vines. These wires are first attached to the overhead wires and then fixed to the

ground.

The lifespan of the wooden posts is about 20-30 years and in the first years after planting (with maximal leaching rate from the treated posts) only a reduced yield of harvest is expected, maximal yield starts in the third year after planting.

Kiwi vines

Similar to grape vines, kiwi vines are trained on trellises, on posts and wires, or on espalier. Since kiwi plants need a strong support system, metal posts are often used. On a single wire the spacing should be 3.0-3.5 m and on a double wire, 4.5-4.8 m vines are spaced 5.5-6.0 m apart in the row. Kiwi vines begin to bear fruit after 4 years; maximum production is not attained until the 8^{th} year.

Appendix 5-1 (V) Information Provided By the Applicant And From Other Regulatory Areas

Table VI.1 Information to be provided by the Applicant

Information relating to the intended use

- target species/organisms
- application method
- frequency of treatments
- application rate
- concentration of active substance in product and in in-use product (e.g. in the spray formulation)
- detailed description of areas to be treated (e.g. countertops, specified equipment, spot treatment)
- product formulation

It should be clearly specified in the intended use description provided by the Applicant whether every treatment is performed with the same application rate or if refresher treatments subsequent to the initial treatment are applied at a different rate.

Information relating to the active substance

- physico-chemical properties
- degradation/volatilisation rate (environmental part of the dossier)

Table VI.2 Information on risk assessment from other regulatory areas

Plant Protection Products	
EU Pesticide database	https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN
Guidelines for pesticide residues	http://ec.europa.eu/food/plant/protection/pesticides/publications_en.htm
EFSA´s conclusions and technical reports	http://www.efsa.europa.eu/en/publications/?f%5B0%5D=im_field_subject%3A62081&f%5B1%5D=sm_field_so_type%3Aconclusion_on_pesticides&f%5B2%5D=sm_field_so_type%3Atechnical_report_post_11
JMPR Reports	http://www.fao.org/agriculture/crops/core-themes/theme/pests/pm/lpe/en/
Veterinary Medicinal Products	
EMA Summary Reports/ Summary Opinions	https://www.ema.europa.eu/en/find-medicine/veterinary-medicines/maximum-residue-limit-assessment-reports
JECFA Reports	https://www.who.int/foodsafety/areas_work/chemical-risks/jecfa/en/
Food and Feed Additives	
EFSA: Evaluations of the Panel on food additives and nutrient sources added to food (ANS)	Available in the EFSA journal: https://www.efsa.europa.eu/de/publications
EFSA: Evaluations of the Panel on food contact materials, enzymes, flavourings and processing aids (CEF)	\\MASNWDATA\GROUP\GROUP\Abteilung-6\2 Projekt Biozide\1 Projektorganisation\Biozid Meetings\EU-Gremien\BPC-WG\ARTFood\TDG_Prof\Überarbeitung 2018-19\200113_RevisionDraftGD\mAvailable in the EFSA journal: https://www.efsa.europa.eu/de/publications
EFSA: Evaluations of the FEEDAP Panel (Additives and products or substances used in animal feed)	Available in the EFSA journal: https://www.efsa.europa.eu/de/publications
Food Contact Materials	
Note for Guidance For the Preparation of an Application for the	http://www.efsa.europa.eu/fr/efsajournal/doc/21r.pdf

Safety Assessment of a Substance to be used in Plastic Food Contact Materials	

1

Appendix 5-1 (VII) Glossary

biocide residue

The residue in rinsing water, on a treated surface or in food, feedstuff or drinking water resulting from the use of a biocidal product, and as defined by the biocide residue definition for DRA (which includes all toxicologically relevant compounds and thus may include the active substance and/or relevant degradation products)

dietary risk assessment

The entire assessment process that leads to the identification of possible consumer risk through biocide residues in food. Dietary risk assessment is further subdivided into:

residue assessment
that part of dietary risk assessment whereby the amount of biocide residue in food is determined quantitatively

dietary exposure assessment
that part of dietary risk assessment whereby consumer dietary exposure through biocide residues in food is determined

dietary risk characterisation
that part of dietary risk assessment whereby dietary risk is determined by comparing dietary exposure with the appropriate toxicological reference values

Appendix 5-1 (VIII) DRAWG Opinion on identifying worst-case uses for PT 6 biocidal products

DRAWG Opinion on identifying worst-case uses for PT 6 biocidal products in order to minimize the number of uses to be assessed for dietary risk Agreed at TMII13[96]

Background:

At TMIII12, a HEEG Opinion on identifying worst-case operator/user exposure scenarios for PT 6 was endorsed; this Opinion aimed at minimising the number of exposure scenarios that have to be assessed for PT 6 products, which comprised a very large number of uses.

DRAWG was asked to propose a similar method for identifying the worst-case dietary exposure scenario for PT 6 products. This request arose during a specific active substance discussion. For this substance, two dietary exposure scenarios were evaluated in the CAR (consumption of food after contact with cleaned or painted surface and exposure from dishes cleaned with preserved dishwashing detergents). The TM asked for an additional scenario covering the use in paper production where the end product may be used as food packaging. While the CAR for this substance remains unaffected by this Opinion and no additional dietary risk assessment was requested for Annex I inclusion, the TM saw the need for a method aimed at focusing the dietary risk assessment for PT 6 products based on worst-case dietary exposure scenarios.

This document was drafted by a working group consisting of DRAWG members from DE, ES, FR, NL, PT, SE, UK, COM, CEFIC and EFSA. The group developed this document by e-mail and telephone conferences.

Summary:

Based on a review of the above mentioned HEEG Opinion and considering the possible dietary exposure scenarios and assessment methods for PT 6 biocidal products, this paper uses an example to demonstrate how the worst-case uses for a PT 6 product might be determined. Since not all of the worst case dietary exposure scenarios identified in this example may be relevant for any active substance used in PT 6, applicants are advised to investigate which of the dietary exposure scenarios need to be addressed for their intended uses within PT 6.

Introduction:

As noted in the HEEG Opinion mentioned before, PT 6 biocidal products are used to preserve a wide range of products. The range of products identified by HEEG from representative uses in the review programme includes water-basedcoatings, polymer dispersions, filler dispersions, pigment slurries, solutions and dispersions of glues and thickeners, concrete additives, construction materials, detergents, cleaners, textile processing chemicals, paper and leather treatment agents and other aqueous formulations.

Considering these uses, the **dietary exposure scenarios** listed in Table 1 have been identified. Methods for assessing these dietary exposure scenarios can be found in the "TNsG on Estimating Livestock Exposure to Active Substances used in Biocidal

[96] https://echa.europa.eu/documents/10162/20733977/drawg_opinion_dietary_exposure-PT6_worst_case_en.pdf/26390f74-49a5-5b3b-512f-eb5e917e5b8f

Products"[97] (in the following referred to as "Livestock TNsG" as well as in the "TNsG on Estimating Transfer of Biocidal Active Substances into Foods – Professional Uses" and the "TNsG on Estimating Transfer of Biocidal Active Substances into Foods – Non-professional Uses"[98] (in the following referred to as "Food TNsGs". The Food TNsGs have not been finalized. If approaches in the Food TNsGs change, these changes will also apply to this document.

Table 1Dietary exposure scenarios

Non-professional dietary exposure scenarios (i.e. use in households):
1. Use of PT 6 products in dishwashing detergents and subsequent dietary exposure via residues on dishes
2. Use of PT 6 products in household cleaners or disinfectants and subsequent dietary exposure via residues on food preparation surfaces
3. Use of PT 6 products for in-can preservation of insecticides and subsequent dietary exposure via residues on food preparation surfaces
Professional dietary exposure scenarios:
4. Use of PT 6 products for in-can preservation of insecticides and subsequent dietary exposure via residues on food storage/processing surfaces
5. Use of PT 6 products in industrial or institutional cleaners or disinfectants and subsequent dietary exposure via residues on food preparation surfaces
6. Use of PT 6 products in the production of food contact materials or components thereof, e.g.: o paper o coatings o polymer dispersions
7. Use of PT 6 products in production of feed packaging (dietary exposure via transfer of residues from feed packaging to feed, subsequent uptake by livestock animals and resulting deposition in edible animal matrixes): o paper o coatings o polymer dispersions

<u>Methodology for identifying the worst-case use for an example product:</u>

Because many of the parameters influencing the status (worst-case, best-case or in between) of a particular use are variable, it is neither useful nor possible to propose a generic method for identifying the worst-case use/uses. Rather an example has been established in order to demonstrate how the worst-case dietary exposure scenario can be identified for a PT 6 biocidal product. In this document, the example PT 6 product from the HEEG Opinion (chapter 3) is considered. The conclusions arrived at in this document are valid for this particular example only and will differ for PT 6 products with different **use categories** and **combinations of uses**.

The example product is applied in the following **use categories**:

[97] https://circabc.europa.eu/faces/jsp/extension/wai/navigation/container.jsp

[98] Under development

Table 2Use categories and potential dietary exposure for the example product

Use Category (i.e. field of use envisaged) for the example product	**Likely concentration at which a.s. will be used**	**Potential dietary exposure**
Paints and Coatings – Used to control the growth of bacteria and fungi in water-based paints and coatings in storage containers before use.	7.5 to 30 ppm total a.s.	**Yes - coatings are components of food packaging and components of other food contact materials (e.g. food contact surfaces such as counter tops)**
Liquid Detergents - Used to control the growth of bacteria and fungi in the preservation products such as liquid fabric softeners, dishwashing detergents, liquid laundry detergents, liquid soaps and hand cleaners, and the surfactants used in formulating such products.	6 to 15 ppm total a.s.	**Yes - dishwashing detergents**
Fuel Preservation – Used to control the growth of fungi and bacteria in liquid hydrocarbon fuels and oils, and any associated water bottom phase, including crude oils, aviations fluids, kerosene, heating oils, residual fuel oils, coal slurries, liquefied petroleum gases, petrochemical feed stocks, and diesel fuels.	1.5 to 6 ppm total a.s.	No dietary relevance
Textiles, Leathers and Inks – Used to control the growth of fungi and bacteria in textile (woven and non-woven, natural and synthetic) processing chemicals, inks (lithographic, photographic, ink-jet fluids), and all chemicals used in the leather process industry.	6 to 30 ppm total a.s.	**Yes - inks are components of food packaging**
Polymer Latex Preservation - Used to control the growth of bacteria and fungi in the manufacture, storage, and transport of synthetic and natural polymer lattices and industrial biopolymers.	7.5 to 50 ppm total a.s.	**Yes - polymers form the basis of many types of food packaging and other food contact materials**
Adhesives and Sealants - Used to control the growth of bacteria and fungi in water soluble and water-dispersed adhesives and tacktifiers in storage containers before use.	7.5 to 30 ppm total a.s.	**Yes - adhesives are components of food packaging**

Mineral Slurries - Used to control the growth of bacteria and fungi in aqueous-based inorganic/mineral slurries and inorganic pigments which are formulated into paints, coatings and paper.	10 to 30 ppm total a.s.	**Yes - formulation into paper used as food or feed packaging; formulation into coatings used in food packaging**
Electro-Deposition Coatings – Used to control the growth of bacteria and fungi in coatings applied by an electro-deposition process and associated rinse systems.	6 to 50 ppm total a.s.	**Yes - coatings are components of food packaging**
Household (HH) and Industrial and Institutional (I&I) – Used to control the growth of bacteria and fungi in products used for car care, floor care, waxes, hard surface cleaners, pre-moistened sponges or mops, and the surfactants used in these types of products.	6 to 25 ppm total a.s.	**Yes - household/industrial/ institutional cleaners (and disinfectants) for food contact surfaces; pre-moistened dish sponges**
Functional Fluids – Used to control the growth of bacteria and fungi in brake and hydraulic fluids, antifreeze, corrosion inhibitors, fuel additives, spinning fluid, and fountain solutions.	6 to 30 ppm total a.s.	No dietary relevance

1

Methodology for an example product: STEP A
Begin by matching the use categories with dietary relevance from Table 2 with the dietary exposure scenarios from Table 1.

Table 3Use categories matched with dietary exposure scenarios

Use category	Dietary exposure scenario
Liquid Detergents	scenario 1 (dishwashing)
Household Cleaners/Disinfectants	scenario 2 (household disinfectants)
additional scenario: Household Insecticides	scenario 3 (household insecticides)
additional scenario: Industrial/Institutional Insecticides	scenario 4 (industrial insecticides)
Industrial/Institutional Cleaners/Disinfectants	scenario 5 (industrial disinfectants)
Coatings	scenario 6 (FCM*****)
Inks	scenario 6 (FCM*****)
Polymer Preservation	scenario 6 (FCM*****)
Adhesives	scenario 6 (FCM*****)
Mineral Slurries	scenarios 6 (FCM*) and 7 (feed packaging)
***** *FCM = food contact materials*	

Methodology for an example product: STEP B
In the next step, predict the worst-case use category within each dietary exposure scenario.

Dietary exposure scenarios 1-3:

As identified in Table 3, the dietary exposure scenarios 1-3 include the following use categories:

- **Liquid detergents** (dietary exposure scenario 1): Use of these products can leave residues on dishes used for serving and eating food. The relevant products are dishwashing detergents and pre-moistened sponges[99].
- **Household Cleaners/Disinfectants** (dietary exposure scenario 2): These products are used to clean food contact surfaces.
- **Household Insecticides** (dietary exposure scenario 3): Aerosols from these products can settle on food contact surfaces.

Food contact with products from these use categories is likely and relatively high because of the large surface area containing residues that is in contact with food, and because use occurs on a daily basis.

For dietary exposure scenarios 1, 2 and 3, dietary exposure is determined via calculation models using default and product-specific values (see Food TNsGs). If the calculations for all three scenarios result in acceptable exposures (below the ADI/ARfD[100]), no further assessment is required.

According to the Food TNsGs, if the ADI/ARfD values are exceeded, further refinement is required where possible. One possibility is to measure the actual amount of biocide residues on the treated surface and use the measured values in the exposure calculations. Particularly for volatile substances, this amount may be very different from the application rate. Another possibility is the experimental determination of the mass

[99] In the HEEG example, these are grouped with hard surface cleaners, but for the purpose of dietary risk assessment, they fit better into the category of liquid detergents

[100] ADI: Acceptable Daily Intake; ARfD: Acute Reference Dose

transfer efficiency rate, which can in turn be applied to the exposure calculations [101]. If the refined calculations result in acceptable exposures (below the ADI/ARfD[102]), no further assessment is required.

If studies measuring residues on the treated surface or transfer efficiency rate are performed, generally, it will suffice to conduct these only for the use with the highest exposure value as determined in the calculations. If the resulting exposure estimate is safe (below ADI/ARfD), the same can be assumed for the remaining uses. However, it must be kept in mind that the formulation of the PT 6 biocidal product and the formulation of the product containing the PT 6 biocidal product (i.e. the cleaner, disinfectant, insecticide or detergent) influence the release of the PT 6 active substance and the residue transfer into food. Particularly in cases where the calculated dietary exposures (before refinement) do not differ much, the formulation might be the discriminating factor. Therefore, if there is an indication that the actual amount of surface residue and/or residue transfer into food will be in different orders of magnitude for the different uses, studies must be performed for all uses. In certain cases, it may be possible to show that the study settings for one use are sufficient to cover the other applications. In such cases, extrapolation of study results might be possible.

Worst case for dietary exposure scenarios 1, 2 and 3: The use with the highest calculated exposure value (only relevant if ADI/ARfD are exceeded)

Dietary exposure scenario 4:

As identified in Table 3, dietary exposure scenario 4 includes the following use category:

- **Industrial/Institutional Insecticides**

According to the Food TNsGs, a dietary risk assessment for industrial/institutional insecticides generally does not have to be performed as long as the insecticide carries appropriate use instructions on its packaging preventing contact with food/food surfaces. Therefore, it is not necessary to determine a worst case. The Food TNsGs further state that there are certain insecticide uses where contact with food surfaces is required. For such uses, the Food TNsGs require residue trials. Therefore, this paper does not apply to such uses.

Worst case for dietary exposure scenario 4: Not applicable.

Dietary exposure scenario 5:

As identified in Table 3, dietary exposure scenario 5 includes the following use category:

- **Industrial/Institutional Cleaners/Disinfectants**

According to the Food TNsGs, for scenario 5, exposure is not calculated using a model calculation. Rather, the results of rinsing/wiping trials are used to determine the need for residue studies[103]. If results are below the threshold levels[104], dietary exposure is considered negligible and no further assessment is needed.

Rinsing/wiping trials will generally only be required for the use with the highest

[101] Please consult the "Guidance on Estimating Transfer of Biocidal Active Substances into Foods – non-professional uses" (under development) for more information on possible refinement options.

[102] ADI: Acceptable Daily Intake; ARfD: Acute Reference Dose

[103] Please note that this is the current proposal in the Food TNsG which has not been finalised. If the approach in the Food TNsG changes (e.g. to include a calculation model prior to rinsing studies), this change will also apply to this document.

[104] The threshold levels can be found in Appendix I, Table I.1, section 5.1 to the "Guidance on Estimating Transfer of Biocidal Active Substances into Foods – Professional Uses" (under development)

calculated residue per unit area. This can be determined from the a.s. concentrations and the applications rates of the different products (surface cleaners and disinfectants) that contain a PT 6 product. If the result of the rinsing trial for the use with the highest calculated residue per unit area is below the threshold levels, the same can be assumed for all other uses in this scenario. However, it must be kept in mind that the formulation of the PT 6 product and the formulation of the product containing the PT 6 product (i.e. the cleaner or disinfectant) influence the residue transfer into food. Particularly in cases where the residues per unit area do not differ much, the formulation might be the discriminating factor. In this case, rinsing studies might be needed for both uses.

Worst case for dietary exposure scenario 5: The product with the highest residue of a.s. per unit area

Dietary exposure scenario 6:

As identified in Table 3, dietary exposure scenario 6 includes the following use categories:

- **Coatings**
- **Inks**
- **Polymer Preservation**
- **Adhesives**
- **Mineral Slurries**

According to the Food TNsGs, for scenario 6, dietary exposure is calculated using default values (for food intake, body weight and area of contact between the food contact material and the food contained within the food container) as well as the migration rate of the a.s from the food contact material which is determined experimentally for each active substance[105]. Food contact materials present a special case in that they can contain residues of PT 6 substances from a variety of sources (e.g. from inks and adhesives; mineral slurries formulated into packaging paper; coatings; polymer dispersions made into plastic packaging). If we assume that the concentrations of the a.s. for the different uses are approximately equal (e.g. the a.s. concentration in an ink is approximately equal to the a.s. concentration in a coating), we can assume that some uses have very limited exposure compared to other uses. As a result, these uses would not be considered worst-case uses. For example, if the a.s. concentration in an ink (used on food packaging) is approximately equal to the a.s. concentration in a coating (used on food packaging), the use in ink could be disregarded because the contact surface of the ink with food is much smaller than the contact surface of the coating with food.
The following use categories can be excluded from the list of worst-case exposure scenarios in dietary exposure scenario 6, because of limited exposure compared to the other uses:

- **Inks and Adhesives** - Inks and adhesives are used in food packaging materials, but the contact surface with the food is low when compared to the contact surface with other PT 6 residues (e.g. in polymer dispersions).

- **Mineral slurries** - Mineral slurries are used in the production of food packaging. However, this results in a high dilution in the finished product. Compared to other food contact materials (e.g. those made from a preserved polymer dispersion or coated with a PT 6 treated coating), the active substance concentration in the paper is assumed to be low. As stated in the TNsG on Human Exposure, "many biocides degrade in paper-making, so in-use concentrations are lower than the

[105] Please note that this is the current proposal in the Food TNsG which has not been finalised. If the approach in the Food TNsG changes (e.g. migration studies may not be required in all cases), this change will also apply to this document.

nominal values"[106]. INERIS further informs that "for in-can preservatives (PT 6), the substance is not designed for fixation onto fibres and it can be assumed that no specific fixation occurs[107]".

- **Coatings** - Coatings are used on food packaging to protect the packaging and the food, may come in direct contact with food.

- **Polymer dispersions** – Polymer dispersions form the basis of food contact materials, and therefore come in direct contact with food.

Based on these assumptions, Coatings and Polymer dispersions can be considered the worst case for scenario 6.

These assumptions can only be made if the concentration of the a.s. in the preserved products is in the same order of magnitude. If the a.s. concentration in the use categories *Inks*, *Adhesives* or *Mineral Slurries* is much higher compared to e.g. a coating or a polymer dispersion, it cannot automatically be assumed that exposure from these categories is negligible. In this case, the amount of a.s. in the finished food contact material (e.g. the amount of a.s. from ink in relation to the packaging on which the ink is printed) should be investigated.

Worst case for dietary exposure scenario 6: Coatings and Polymer Dispersions

Dietary exposure scenario 7:

As identified in Table 3, dietary exposure scenario 6 includes the following use category:

- Mineral Slurries

In addition to food packaging, *Mineral slurries* can also be formulated into packaging paper for animal feed. According to the Livestock TNsG, scenario 7 is assessed by calculating the exposure of the livestock animal, which results in a statement about the relevance of human dietary exposure by consuming food from the exposed animal.

PT 6 residues in mineral slurries formulated into packaging paper for feed have an additional intermediate (the animal) before they reach the consumer. It can therefore be assumed that mineral slurries formulated into packaging paper for feed are covered by the assessment of packaging paper for food. In this example, mineral slurries formulated into packaging paper for food are assumed to be covered by the assessment of coatings. Therefore, mineral slurries formulated into packaging paper for feed are also covered by the assessment of coatings. Whether this assumption also applies in cases where animal-specific metabolites are formed and/or accumulation in the animal occurs, must be decided on a case-by-case basis.

Worst case for scenario 7: None, because it is covered by dietary exposure scenario 6.

Methodology for an example product: STEP C
As a final step, the dietary risk of the worst-case use in each dietary exposure

[106] TNsG on Human Exposure, part 2, 2002, (pp. 98, type 12.01 slimicides for paper pulp)

[107] Institut national de l'environnement industriel et des risques (INERIS). DRC-01-255582-ECOT-CTi/VMi-nº01DR0183.doc. Supplement to the methodology for risk evaluation of biocides: Emission scenario document for biocides used in paper coating and finishing (Product type 6, 7 & 9), May 2001: http://ihcp.jrc.ec.europa.eu/our_activities/public-health/risk_assessment_of_Biocides/doc/ESD/ESD_PT/PT_06/PT_6_PT_7_PT_9_Paper_coating_and_finishing.pdf/view

scenario is assessed.

Based on the assumptions above, the following PT 6-relevant uses were identified as worst-case uses in this particular example taken from the HEEG opinon:

Table 4Worst-case use in each dietary exposure scenario

Non-professional dietary exposure scenario:	**Worst-case use**
Scenarios 1, 2 and 3 Liquid detergents, Household Cleaners/Disinfectants, Household Insecticides	the use with the highest calculated exposure value
Professional dietary exposure scenario:	**Worst-case use**
scenario 4 Industrial/Institutional Insecticides	none; not applicable
scenario 5 Industrial/Institutional Cleaners/Disinfectants	the use with the highest calculated residue per unit area before rinsing
scenarios 6 (FCM) Coatings, Inks, Polymer Preservation, Adhesives, Mineral Slurries	coatings and polymer dispersions
scenario 7 (feed packaging) Mineral slurries	none; covered by scenario 6

An aggregate exposure assessment of the identified worst-case uses should be performed. However, at the moment no harmonised criteria for a quantitative aggregate exposure assessment exist at EU-level. Therefore, until agreed criteria have been established, only a qualitative aggregate assessment should be performed.

Conclusion:

This example illustrates how to identify the worst-case use categories for a biocidal product within PT 6 in order to minimise the number of use categories for which a dietary exposure and risk assessment must be performed. The worst-case use categories presented here are specific to the particular example presented here and may well differ for PT 6 products with other use patterns and use combinations. Therefore, depending on the use patterns of the products in PT 6, applicants need to undertake considerations on the range of their PT 6 uses which might have relevance for dietary exposure. The exposure scenario building process used to arrive at the conclusion here must be undertaken for each individual PT 6 biocidal product and explained in the application for product authorisation. The example in this paper can be used as a guide.

5.2 Estimating Dietary Risk from Transfer of Biocidal Active Substances into Foods – Non-professional Uses

5.2.1 Introduction

The Biocidal Products Regulation (BPR) requires that a risk assessment is performed for biocidal products. Whenever food contamination results from the use of a biocidal product, a dietary risk assessment (DRA) should be performed.

The methods described in this section are to be seen as recommendations for performing assessment of biocide transfer into food. Applicants wishing to propose other methods for assessment may do so as long as these other methods are scientifically justified, robust and well documented, and in line with the general principles of this guidance document. For further information to be provided by the applicant and information on risk assessment from other regulatory areas, see Appendix 5.2-3.

For the purpose of this guidance, the term "biocide residue" is defined as "the residue in food resulting from the use of a biocidal product, which includes all toxicologically relevant compounds and may include the active substance and/or relevant degradation products and metabolites."

The aim of this section is to provide guidance to estimate the dietary risk to humans from

biocidal products that are used in domestic environments (household) and could contaminate food. This document describes methods for estimating dietary exposure for the various non-professional use scenarios without a specific quantification of residues in food and details the reference values to which the exposure estimates are compared to in order to estimate dietary risk.

Non-professional use scenarios cover only biocidal uses in a domestic environment, where biocides may come into contact with food and where this food is consumed within that particular household. Biocidal products are divided into 22 product types (PTs) (Annex V of BPR), some of which are used on objects used to prepare food in domestic kitchens or in kitchens/on kitchen surfaces and other domestic areas where food is stored and/or prepared. In this way, biocidal active substances and/or their degradation products can be transferred into food. The non-professional use of biocides means that the biocidal products may come into contact with food that is consumed within the household: this is highly variable and can be contact with any food in the whole diet and therefore commodity-specific biocide residue estimates are not practicable or enforceable within private residences/households, and for this reason, it is not relevant to propose a maximum residue limit and no need to measure quantitatively biocide residues in food.

Based on representative uses submitted in the course of EU-wide biocidal active substance evaluations, a number of scenarios have been identified for how food can come in contact with biocidal products:

- Disinfectant cleaners in domestic kitchens (PT 4);
- Drinking water disinfection (PT 5);
- In-can preservatives and disinfectants in dishwashing detergents (PTs 4, 6);
- Insecticides in domestic environments (PT 18);
- Repellents and Attractants (PT 19)[108]

Other non-professional use scenarios are less likely to lead to dietary exposure, but this has to be considered on a case-by-case basis.

For each of the scenarios listed above, possible methods for estimation of dietary exposure will be discussed in this section. For these scenarios, the possibility of dietary exposure must be considered and addressed either by an assessment or a waiver in the form of a Justification for Non-Submission of Data detailing the reasons for the waiver.

The methods for assessment of dietary risk from biocides/biocide residue transfer into food described in this section are based on worst-case considerations assuming maximum biocide residue intake. The biocide residue intake is calculated using the area of contact with food, making it unnecessary to include food consumption data in the assessment. The only exception is the scenario for drinking water disinfection which includes water consumption rates in the calculation.

In addition, the methods differentiate between acute and chronic exposure scenarios. An acute or chronic RA is performed depending on the uses and scenario and also on the availability/necessity of the appropriate reference value.

A DRA will only be conducted for two age groups, namely toddlers and adults. Toddlers were identified to be the worst case with regard to dietary assessment and therefore cover the entire population of children (see Appendix 5.2-2 Section 1). Only in cases where another age group also represents the worst case, should exposure also be calculated for this additional age group. Standard body weights and corresponding water intake figures are given in Appendix 5.2-2, Section 2.

Biocidal products may contain formulants that are substances of concern. Substances of concern may be equally or more hazardous to human health than the active substance itself. A risk assessment for all substances of concern must therefore be performed according to CA-Nov14-Doc.5.11.

Particular attention should also be paid to the formation of disinfection by-products

[108] Estimation of residues transfer to food is foreseen only for product applied for airspace treatment. There are ongoing discussions with the MSCAs on whether consumer exposure via transfer of residues from treated skin onto food is required. The Guidance will be updated when this is concluded.

(DBPs). A separate guidance document on how to evaluate DBPs and their formation has been developed (ECHA Guidance Vol V Disinfection By-Products). Currently the guidance document focuses on PTs 2, 11, and 12 and does not specifically address assessment of residues in food. However, the guidance has developed a strategy for risk assessment of DBPs which should be followed for the DRA of active substances intended for drinking water disinfection (PT 5).

Under Article 5(1) of the BPR, active substances that are classified as, or meet the criteria to be classified as, carcinogenic category 1A or 1B, mutagenic category 1A or 1B, reprotox category 1A or 1B (in accordance with the CLP Regulation), and/or meet the criteria for being PBT or vPvB according to Annex III to Regulation (EC) No 1907/2006 and/or have endocrine-disrupting properties should not normally be approved. Such active substances should not be allowed for use in biocidal products unless this would have a negative impact on society compared to the risk to humans and the environment of not using the biocidal product; or the risk is negligible; or the active substance is considered essential (Article 5(2) of the BPR). This section does not apply to active substances with such classifications for health hazard.

5.2.2 Overview of Residue and Dietary Risk Assessment

Biocide residue and DRA follow a stepwise procedure which is outlined in Figure 9. In the first step, the intended uses should be established and it should be assessed whether the use of the biocidal product leads to transfer of biocide residues to food. When transfer of biocide residues into food is foreseen, the dietary exposure is estimated based on modelling and it is compared with toxicological reference values, usually Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD), or other reference values with the same meaning can be used to estimate the risk. If the exposure estimation is above 10% of the ADI or ARfD, the exposure estimate should be refined through the use of additional data. If the refined exposure estimation is still above 10% of the ADI or ARfD, a potential concern is identified and the nature of the residue needs to be defined. Applicants wishing to propose other approaches may do so as long as these are scientifically justified, robust and well documented.

Dietary risk is estimated by comparing the intake of biocide residues via foods with toxicological reference values, provided that these values can address the toxicity of the residues. The applicable toxicological reference values for a DRA are usually the ADI for chronic toxicity and the ARfD for acute toxicity. ADI and ARfD are established as part of the hazard assessment of the active substance. The Human Health Working Group has developed a document on the derivation of ADI and ARfD for biocidal active substance derivation; the criteria outlined in the document should be followed.. If the toxicological information shows that an active substance and/or its toxicologically relevant degradation product(s) do not become systemically available and that primary irritation/corrosion at the site of first contact is the only relevant effect observed, a local risk assessment rather than a systemic DRA is required (see Section 4 of this guidance).

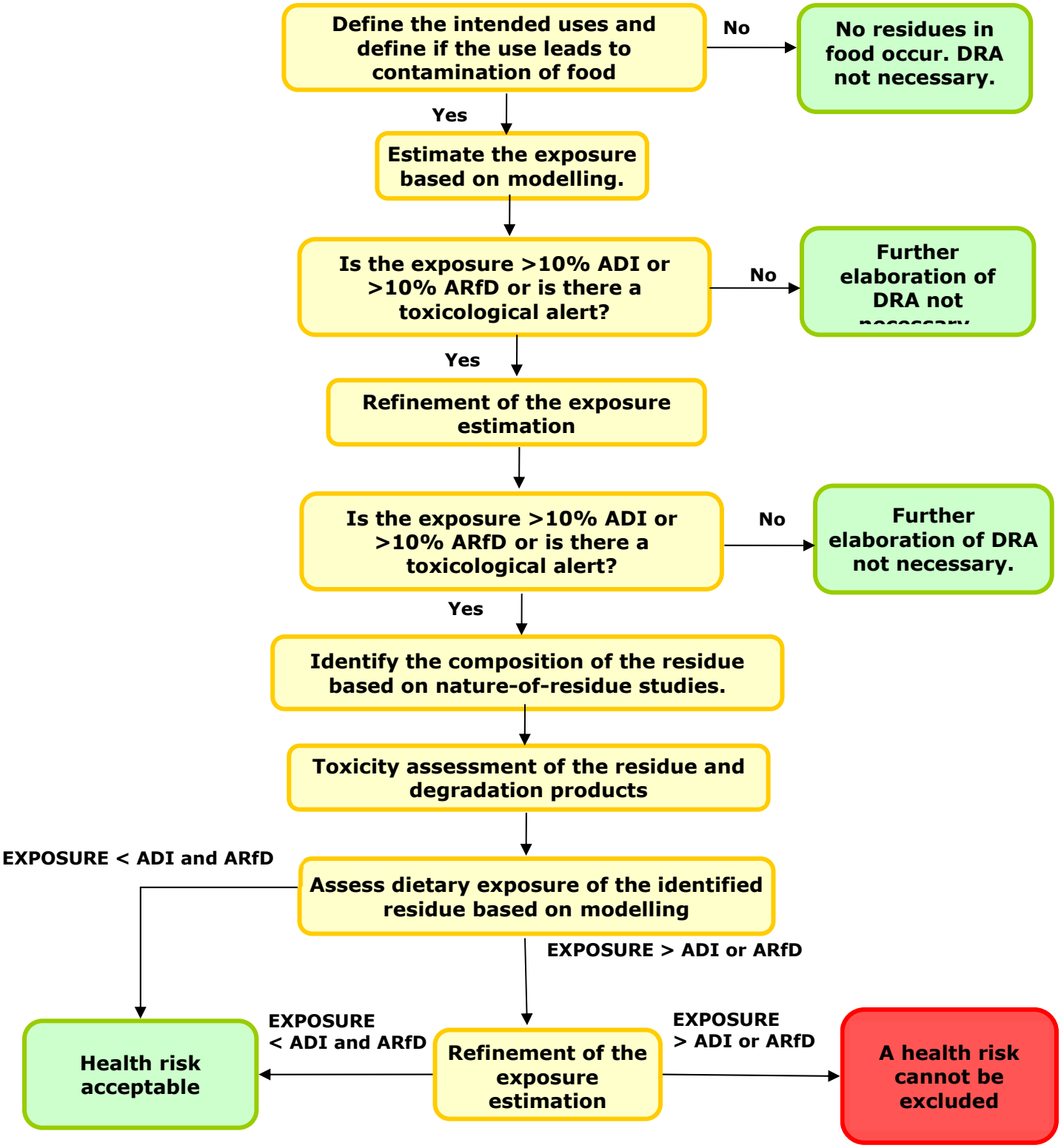

Figure 9: Steps in Dietary Risk Assessment (DRA)

5.2.3 Assessing the possibility of food contamination

In the first step of a DRA, it is assessed whether the use of the biocidal product leads to

contamination of food. Some biocidal products are designed to preclude food contamination. The product may carry on its label instructions to the user, an instruction to avoid food contact (e.g. "Keep away from foodstuff, eating utensils or food contact surfaces.") and/or may be formulated in a way that food contamination is unlikely (e.g. a gel spot application rather than an aqueous formulation, preventing splashes). If the Applicant concludes that food contamination can be excluded due to label instructions and/or special product formulations, the Applicant must submit a Justification for Non-Submission of Data listing the arguments that led to this conclusion. On the basis of the Justification, the Competent Authority evaluates whether the argumentation is valid. If this is the case, dietary risk does not have to be further evaluated.

Label restrictions can generally be accepted as risk management measures, unless the restrictions appear impractical or not plausible. Misuse of any type (e.g. accidental or deliberate) should not be considered in the assessment. Label restrictions can be an appropriate risk management measure for non-professional users, however, this has to be checked on a case-by-case basis. Particular attention should be paid in the evaluation because non-professional users are more likely to ignore or misinterpret unclear label restrictions than professional users.

A general statement regarding acceptable and non-acceptable label restrictions for the non-professional uses cannot be made. Instead it is the combination of label restrictions with specific product characteristics such as, intended use, formulation and product design that will allow decisions on a case-by-case basis. Examples of label restrictions that an assessor may consider unclear or unlikely to be followed, are given in Table 41. The list is not exhaustive and does not constitute a set of rules, but provides examples of how a label restriction may be interpreted: other interpretations are possible depending on the specific product that is being evaluated.

In general, label restrictions on products for non-professional uses should be easy to understand and give clear instructions on what the non-professional user should do (and consider what the non-professional user can be expected to do correctly). They should not be ambiguous, too general or require unrealistic additional efforts by the non-professional user. They should furthermore be clearly visible and legible (i.e. adequate font size, prominent location on the package).

Table 30: Examples of label restrictions unlikely to be followed

Biocidal product	Label restriction	Remarks
Electric vaporiser for insect control in residential homes	"Do not use in kitchens." "Cover food before use."	Non-professional user is likely to ignore or forget. If product works well in rooms it is intended for, non-professional user may also use it in kitchens. For these product formulations (vaporiser), covering food does not prevent food contamination, because vapours diffuse under covers, into cupboards, and into food packaging etc.
Surface disinfectant for domestic kitchen counters	"Do not contaminate food." "Rinse surfaces after disinfection."	Too general. Does not give clear instructions. Non-professional users may e.g. not be aware that food can be contaminated through biocide residues that remain on surfaces. Unrealistic additional effort. Experience shows that non-professional users do not rinse after disinfection.
Biocidal products that	"Do not prepare product	Ready-to-use products may be an

Biocidal product	Label restriction	Remarks
require a preparation step	where food, feed or drinking water could be contaminated."	alternative option to minimise exposure of non-professional users.

Table 42 lists a preliminary set of practical phrases that could be included in the label. This set is neither exhaustive nor finalised and may be changed or expanded in the future. Moreover, additional P statements might be assigned to dangerous substances and preparations in accordance with the CLP Regulation.

Table 31: Example set of practical phrases

Label restriction	Remarks
"Do not use or apply near food, drink and animal feedingstuffs."	This sentence is recommended for acute toxic substances and preparations, which are likely to be used by the general public (non-professional user).
"Do not use or apply near foodstuffs, eating utensils (dishes etc) or food contact surfaces."	• May be acceptable for spray applications on surfaces or gel applications • Not applicable for applications such as evaporation products
"Remove food before application" or "Store food away from the area to be treated"	• Generally acceptable for formulations that are sprayed or applied with a cloth or sponge • Not acceptable for vaporiser formulations
"Do not place product where food, feed or water could become contaminated."	For biocidal products with targeted spot applications (e.g. gel spots applied to cracks and crevices and other hard-to-reach spaces)
"Do not use in larders or food cupboards."	For applications such as evaporation products that may be placed in small closed compartments (e.g. strips/vaporiser)

5.2.4 Estimation of the exposure and comparison with reference values

The initial exposure estimation should be carried out following the principles laid down in section 5.6 below, and according to the correct scenario. This estimation is based on the assumption that the parent substance is not degraded (i.e. the toxicity of the potential degradation products are covered by the toxicological reference value of the parent compound).

The estimated exposure should then be compared to the reference values, ADI for chronic exposure and ARfD for short term exposure to see if the exposure is below or equal to 10% of the ADI or ARfD, moreover, it should also be verified that there is no particular toxicological concerns (e.g. a substance with genotoxic potential based on data from testing (if available) or on chemical structural alert from in silico data. If both of these are true, (i.e. the estimated exposure is 10% or less and there are no toxicological concerns), then there is no need to investigate further the composition of the residue and there is no need to perform a DRA.

However if the exposure is above the 10% of the ADI or ARfD even after the refined estimation, and/or if there is evidence of chemical structural alerts (such as a genotoxic alert), then the composition of the residue should be analysed according to Section 5.5 below, identifying the residue composition.

5.2.5 Identifying the residue composition

Before biocide residues in food or dietary exposure can be estimated, it must be determined which toxicologically relevant compounds the biocide residue consists of. This

may include the active substance, one or more of its degradation products and metabolites or a combination of both. To identify the composition of the relevant biocide residue, nature-of-residue studies that simulate realistic use conditions of the biocidal product should be performed. Applicants may propose other methods for assessment as long as they are substantiated, well documented and in line with the general principles of the guidance.

Generally, nature-of-residue studies should be performed, unless it can be shown that the use of an active substance leads to a consumer exposure (including the parent substance and all degradation products) below a threshold limit of 10% of the ADI (for chronic dietary exposure) or 10% of the ARfD (for acute dietary exposure). This is acceptable providing that the initial exposure estimate is based on the assumption that the parent substance is not degraded. In addition, a justification and/or evidence that structures with a genotoxic alert or any other known toxic alerts with a higher toxicity are not expected to be present, should be provided.

The decision on which degradation products are included in the residue definition for the risk assessment is made based on the toxicological properties of the substances. Degradation products that have been found in sufficient quantities as metabolites in the toxicology studies submitted as part of the core data set, are already considered in setting the ADI/ARfD. It might be that other degradation products will be identified by nature of residue studies and for those products it should be assessed whether the parent reference values cover their toxicity profile. Read-across, QSAR, TTC or other predictive models can be used to conclude on the adequacy of the parent ADI or ARfD with respect to the degradation products.
In some cases, waiving of the residue composition studies is possible on the basis of physical-chemical properties (solubility, log Pow, volatility, biodegradability, light sensibility, pH, pKa) if sufficiently justified or when the reaction products are already known.

In a first step, and if it can be reasonably justified that the active substance will always be at ambient conditions at and after application, the hydrolysis studies that are part of the core set of data submitted for biocidal active substances can be used to define the residue. If degradation is observed in these studies and, if it can be reasonably justified that no new degradation products are likely to be formed at higher temperatures, studies at higher temperatures are not necessary. The relevant residue is then defined on the basis of the hydrolysis studies. Thermal stability data may also be considered. If processing at different conditions (pH, temperature) to ambient ones, then ambient conditions cannot be excluded and this must be considered in the assessment.

If the formation of additional relevant degradation products in significant levels at higher temperatures cannot be ruled out, the assessment of the residue composition moves to the second step. In the second step, the residue composition is assessed on the basis of nature-of-residue studies with radiolabelled compounds designed to reflect the realistic use conditions of the biocidal product. The OECD guideline 507, "Nature of the Pesticide Residues in Processed Commodities-High Temperature Hydrolysis", could be applied for performing studies with radiolabelled compounds. When defining the appropriate study conditions, the following must be kept in mind; degradation of the active substance can occur during (i) the application of the biocidal product, (ii) between application and biocide transfer to food (e.g. when biocide treated equipment is rinsed) and (iii) after biocide transfer to food (e.g. during food processing and/or preparation). To cover degradation that occurs after biocide transfer into food, nature-of-residue studies must be designed to cover common food processing conditions. The parameter which most likely affects the nature of the residue during most processing operations is hydrolysis and three different hydrolysis conditions have been defined to simulate most processing practices (see Table 43, from OECD guideline 507). In addition nature-of-residue studies must cover any other relevant degradation conditions that occur during or after application of the biocidal product. For an example of the application of the biocidal product, biocides contained in machine dishwashing detergents are exposed to elevated temperatures (70°C) and changes in pH (7 and 11) throughout a machine wash cycle of

approximately 215 minutes. These conditions are different from those seen during food processing and must therefore be built into the design of the nature-of-residue studies. On the other hand, single experiments can be waived if a condition does not apply to the use of the biocide under evaluation.

Table 32: Required conditions for nature-of-residue studies (OECD guideline 507)

Temperature (°C)	pH	Time (min)	Process represented
90	4	20	Pasteurisation
100	5	60	Baking, Brewing, Boiling
120	6	20	Sterilisation
Any other relevant conditions occurring during or after application of the biocidal product.			

The presence of the food commodity is not required for the nature-of-residue studies. Where appropriate, these studies should be conducted with exaggerated amounts of radiolabelled active substance. The values of the measured amounts of active substance and degradation products are then adjusted to the actual use conditions of the biocidal product. Regarding the characterisation and identification of degradation products, the principles reported in the OECD Guideline 507 apply.
Degradation products that make up less than 10% of the total residue do not need to be identified and require no additional toxicological information unless there is reason to believe that they are of toxicological concern, such as chemical structure. Based on the nature-of-residue studies and the toxicological data, a decision is made as to which degradation products are included in the biocide residue definition. The OECD guidance on definition of residues (2009) as well as the EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment (2012) and EFSA Guidance on the establishment of the residue definition for dietary risk assessment (2016) may be useful in deciding how to proceed.

5.2.6 Estimating biocide transfer into food

The following sections describe methods for estimation of dietary risk from biocide transfer into food for the different use scenarios. It should be noted that potential transfer into food can be reduced by the introduction of risk management measures and refinement options.
The methods described are to be seen as recommendations for performing assessment of biocide transfer into food. However, it may be noted that applicants wishing to propose alternative methods and/or other refinement options for assessment may do so as long as these are scientifically justified, robust and well documented. Examples are added to illustrate the methods described; please note that the examples are not exhaustive.

5.2.6.1 Disinfectants and Preserved Cleaners in domestic kitchens

NOTE to the Reader

This section is concerned with disinfectants (PT 4) as well as disinfectants/cleaners containing in-can preservatives (PT 6). For more guidance on the assessment of in-can preservatives, please see the "DRAWG Opinion on identifying worst-case exposure scenarios for PT 6 biocidal products in order to minimise the number of scenarios to be assessed for dietary risk[109]". Refer also to section 5.6.4 below for dishwashing.

[109] https://echa.europa.eu/documents/10162/20733977/drawg_opinion_dietary_exposure-PT6_worst_case_en.pdf/26390f74-49a5-5b3b-512f-eb5e917e5b8f

A number of biocidal products marketed for domestic use (e.g. disinfectants and household cleaners containing in-can preservatives) have the potential to come in contact with food. Biocides applied to food contact surfaces such as kitchen counters or dining tables can be transferred to food during preparation and eating. Resulting biocide residues in foods may lead to significant dietary exposure, particularly for children, who consume food in a manner that makes it likely for food to come in contact with contaminated surfaces (Melnyk et al., 2000 and 2011). Estimating the amount of biocide residues in food for these uses would be laborious and not very precise since food preparation in the home is highly variable. Since a biocide-treated surface (e.g. a counter top) can be used to prepare any type of food, not one commodity but the whole diet of the consumer can potentially be exposed to the biocide when it comes into contact with the surface. Commodity-specific biocide residue estimates are therefore not practicable; instead, it is more useful to directly estimate dietary exposure.

5.2.6.1.1 Assessment approach

Assumptions

- 100% of surface biocide residues are transferred to food in contact with the surface. Product specific data on mass transfer efficiency may be considered if available;
- Additional deposition of biocide residues on top of food lying on counter tops is not considered;
- Exposure of adult and toddler age groups (toddlers represent the most sensitive consumer group (see Appendix 5.2-2);
- Default value[110] for contaminated surface area (kitchen counter) in contact with food (that represents daily exposure of consumer) is 0.2 m^2 (acute and chronic exposure) (see Appendix 5.2-1 Table 44);
- The dietary intake fraction is to be considered equal to 1 for acute exposure estimation and to 0.5 for long term exposure to reflect the assumption that in the chronic assessment, half the fraction is consumed per day (on a lifelong basis);
- Accumulation of active substance over time as a result of repeated applications is not considered. In domestic kitchens, daily cleaning of surfaces is assumed, as dirty surfaces would not normally be used in the preparation of food. Attention should be given to active substances with high chelating properties.

Estimation of dietary exposure

$$Exp_{cons} = R_{surface} \times A_{food\ contact} \times TF \times D \div bw$$

Where:

Exp_{cons}	dietary exposure (mg a.s./kg bw/d)
$R_{surface}$	biocide residues on surface (mg a.s./m^2)
$A_{food\ contact}$	area in contact with food (m^2)
TF	mass transfer efficiency factor (fraction of biocide residue transferred from surface to food)
bw	body weight (kg)
D	dietary intake fraction: acute = 1.0/day and chronic = 0.5/day

Refinement options

[110] It should be noted that this default value was derived for adults; flexibility can be applied in regard to the value to be used for toddlers to allow for different or lower food consumption.

- Product specific data on amount of actual surface biocide residues (in particular for volatile substances that partially evaporate before food contact occurs or unstable substances that degrade rapidly following application);
- Product specific data on mass transfer efficiency (fraction of biocide residue transferred from surface to food). Since this parameter depends e.g. on the type of surface, the type of food item, the amount of contact time and the contact pressure (Akland *et al*. 2000), care must be taken when incorporating it in a refined assessment;
- Where fully justified, a dilution factor for PT 6 can be used.

In support of the proposed exposure estimation, the reverse reference scenario might be used to estimate the maximum amount of the exposure that might be acceptable.

Example 1: Disinfectants and preserved cleaners in domestic kitchens

Biocidal product: Liquid disinfectant that is sprayed on counter tops in domestic kitchens

Calculation of surface residues

$R_{surface}$ = concentration of a.s. in biocidal product × application rate (both values are listed in the intended use table of the Applicant's dossier)
= 1 g a.s./L × 0.001 L/m²
= 1 mg a.s./m^2

Estimation of acute and chronic consumer exposure

$Exp_{cons} = R_{surface} \times A_{food\ contact} \times TF \times D \div bw$

$R_{surface}$	1 mg a.s./m^2 (see calculation above)
$A_{food\ contact}$	0.2 m^2 (default value for acute and chronic exposure)
D	dietary intake fraction: acute = 1/day – chronic = 0.5/day
TF	100% (default value in absence of product-specific data)
bw	10 kg / 60 kg (default value for toddler / adult)

Adult (acute) Exp_{cons} = 1 mg a.s./m^2 × 0.2 m^2 × 1/d × 100% ÷ 60 kg = 0.003 mg/kg bw/d

Adult (chronic) Exp_{cons} = 1 mg a.s./m^2 × 0.2 m^2 × 0.5/d × 100% ÷ 60 kg = 0.0016 mg/kg bw/d

Toddler (acute) Exp_{cons} = 1 mg a.s./m2 × 0.2 m2 × 1/d × 100% ÷ 10 kg = 0.02 mg/kg bw/d

Toddler (chronic) Exp_{cons} = 1 mg a.s./m2 × 0.2 m2 × 0.5/d × 100% ÷ 10 kg = 0.01 mg/kg bw/d

5.2.6.2 In-can preservatives and disinfectants in dishwashing detergents

Biocidal active substances can be used as in-can preservatives (PT 6) for a number of materials. Dishwashing detergents may contain in-can preservatives to stabilise or protect the product itself. They may also contain specific ingredients (e.g. silicone based defoamers) that are equipped with an in-can preservative. In addition to in-can preservatives, dishwashing detergents may also contain antibacterial agents (PT 4) intended to kill bacteria on dishes and on hands. Sponges used for hand dishwashing can also be treated with disinfectants before use. Indirect oral consumer exposure can originate from biocide residues present on eating utensils and crockery cleaned with the dishwashing liquid or the disinfected sponge. The amounts of active substance carried over into foods in this way are generally expected to be minimal, nevertheless, a dietary exposure estimate should be carried out. Products are available for dishwashing by hand or with a dishwashing machine. The same default values apply to both cases, except for the concentration of detergent in the dish wash solution, where separate values are given for hand and machine dish washing.

5.2.6.2.1 Assessment approach

Dietary exposure can be estimated using the following calculation according to the HERA

guidance (2005). The default values can be found in Appendix 5.2-1, Table 44. For long-term dietary exposure it can be assumed as a worst case that the scenario takes place daily.

Estimation of dietary exposure

$$Exp_{cons} = [F_1 \times C' \times T_a' \times S_a \times F] \div bw$$

Where:

Exp_{cons} dietary exposure (mg a.s./kg bw/d)

F_1 : percentage of a.s. in dishwashing detergent

C' : concentration of detergent in dish wash solution (mg/L)

T_a': amount of water left on dishes after rinsing (dilution factor for rinsing: 1/10 to be justified) (L/cm^2)

S_a : area of dishes in daily contact with food (cm^2/d)

F : percentage of a.s. transferred from article and ingested

bw : body weight (kg)

Example 2: In-can preservatives and disinfectants in dishwashing detergents

F_1 : 0.04 % (value given by the Applicant)

C' : 1400 mg/L (value given by the Applicant)

T_a': 5.5 × 10^{-8} L/cm^2 (default value)

S_a : 5400 cm^2/d (default value)

F : 100% (default value; refinement possible if based on real data)

BW : 10 kg / 60 kg (default value for toddler / adult)

$Exp_{cons} = [F_1 \times C' \times T_a' \times S_a \times F] \div bw$

$= [(0.0004) \times (1400 \text{ mg/L}) \times (5.5\text{x}10^{-8} \text{ l/cm}^2) \times (5400 \text{ cm}^2) \times (1)] \div 10$ or 60 kg

$Exp_{cons,\ adult}$: 2.77 × 10^{-6} mg/kg bw/d

$Exp_{cons,\ toddler}$: 1.66 × 10^{-5} mg/kg bw/d

5.2.6.3 Insecticides in residential homes

5.2.6.3.1 Airspace treatment

Several insecticide products available for non-professional use are applied into the airspace (i.e. spraying, vaporising, fogging of biocidal product with residues depositing from the air to surfaces). Some insect repellents might also be applied into the airspace. The following assessment model only applies to non-professional uses.

The exposure estimate is performed in two steps:

1. Calculation of biocide residues deposited from air to horizontal surfaces
2. Estimation of transfer from contaminated surfaces to food and calculation of dietary exposure

Assumptions

- a.s. is diffused into air and 100% of a.s. is deposited on horizontal surfaces only. Accumulation of biocide residues over several days is not considered. Biocide residues are assumed to be distributed evenly throughout the airspace. No room ventilation is considered. If refinement options are available and are scientifically justified, they can be proposed;
- Biocidal product is used daily;

- 100% of surface biocide residues are transferred to food in contact with the surface. Product specific data on mass transfer efficiency may be considered if available;
- Exposure of adult and toddler age groups (toddlers represent the most sensitive consumer group, see Appendix 5.2-2);
- Default value for contaminated surface area in contact with food (that represents daily dietary exposure of consumer): 0.53 m^2 ; the dietary intake fraction: acute = 1.0/day and chronic = 0.5/day (see Appendix 5.2-1 Table 44); the default value for chronic exposure reflects the fact that vaporisers are not typically used continuously for 24 hours per day.

Calculation of biocide residues deposited from air to horizontal surfaces

$$R_{surface} = m_{24h} \times h_{room} / V_{room}$$

Where:

$R_{surface}$ biocide residues deposited from air to horizontal surfaces within 24 h (mg a.s./m^2)

m_{24h} mass of active substance released over 24h (should be determined from product information, default values e.g. for common application frequency etc) (mg)

V_{room} room volume treated (m^3)

h_{room} room height (m)

Estimation of dietary exposure

$$Exp_{cons} = R_{surface} \times A_{food\ contact} \times TF \times D / bw$$

Where:

Exp_{cons} dietary exposure (mg a.s./kg bw/d)

$R_{surface}$ biocide residues on surface (mg a.s./m^2), (see calculation above)

$A_{food\ contact}$ area in contact with food (m^2)

TF mass transfer efficiency factor (fraction of biocide residue transferred from surface to food)

bw body weight (kg)

D dietary intake fraction: acute = 1.0/d and chronic = 0.5/d

Refinement options

- Higher tier modelling using product specific data on mass transfer efficiency (fraction of biocide residue transferred from surface to food). Since this parameter depends for example on the type of surface, the type of food item, the amount of contact time and the contact pressure (Akland *et al*. 2000), care must be taken when incorporating it in a second tier assessment;
- Higher tier modelling that includes frequency of use, seasonal use, and removal by ventilation;
- Tests analysing amount of surface biocide residues for the application of the specific biocidal product;

Example 3: Airspace treatment with domestic insecticide
Biocidal product: Liquid used in a heated vaporiser for space treatment against mosquitoes in residential properties by non-professional users
Mass of active substance released in 24h
Product information: 1 bottle containing 240 mg a.s. lasts for 720 h, max use 12 h/d
m_{24h} = (mass of a.s. per bottle/total duration of use per bottle) × duration of

application per day
= (240 mg a.s./720 h) x12h = 4 mg

Calculation of residues deposited from air to horizontal surfaces

Mass of active substance released over 24h = 4 mg (calculation see above)
Room height for domestic homes= 2.5 m (default)
Room volume for domestic kitchen= 15 m^3 (default)
$R_{surface}$ = m_{24h} (mg) × h_{room} (m)/ V_{room} (m^3)
= 4 mg × 2.5 m /15 m^3 = 0.67 mg a.s./ m^2

Estimation of consumer exposure

Exp_{cons}= $R_{surface}$ (mg a.s./m^2) × $A_{food\ contact}$ (m^2) × TF/ bw (kg)
$R_{surface}$ = 0.67 mg/m^2 (calculation see above)
$A_{food\ contact}$ = 0.53 m^2 (default for acute/chronic exposure)
D acute = 1/day – chronic = 0.5/day
TF = 100% (default)
bw = 10 kg / 60 kg (default value for toddler / adult)

Toddler (acute) Exp_{cons} = 0.67 mg/m^2 × 0.53 m^2 × 1/d ÷ 10 kg = 0.036 mg/kg bw/d
Adult (acute) Exp_{cons} = 0.67 mg/m^2 × 0.53 m^2 × 1/d ÷ 60 kg = 0.006 mg/kg bw/d
Toddler (chronic) Exp_{cons} = 0.67 mg/m^2 × 0.53 m^2 × 0.5/d ÷ 10 kg = 0.017 mg/kg bw/d
Adult (chronic) Exp_{cons} = 0.67 mg/m^2 × 0.53 m^2 × 0.5/d ÷ 60 kg = 0.003 mg/kg bw/d

5.2.6.3.2 Direct surface treatment

Insecticides may also be applied directly to surfaces. In this case it should be possible to know or calculate the amount of product used per square meter from the product information given by the Applicant. This value can then be used in the calculation to estimate dietary exposure for airspace applications. Alternatively, the direct surface treatment with insecticides could be compared with the use of disinfectants and cleaners for surface cleaners (section 5.6.1), and the amount of product applied to the surface could be used in the calculation in this scenario.

5.2.6.3.3 Further considerations

Other useful information or default values: (as given in OECD ESD for insecticides, acaricides and products to control other arthropods for household and professional uses):

- Private house: building 17.5 m long and 7.5 m wide, room height 2.5 m, living room 58 m^3, default values for larger buildings available (Chapter 2.6 Building type of OECD ESD)
- More default values for application of insecticides available e.g. number of applications per day, size of treated area/volume (general, targeted spot application, larger building treatment), emission factors (floor, treated surface), spots of gel product per m^2 etc.

5.2.6.3.4 Scenario to estimate the indirect exposure via food by using insect repellents

Insect repellent products (PT 19) available for non-professional use can be applied directly on the skin (i.e. aerosol, pump spray, lotion, wipe). The product is applied using hand palms on different parts of the body (hands, arms, head, legs and feet). The a.s. may be transferred from the treated hands to the food[111].

[111] Food contamination were detected for some active substances. This might be related to the use of PT 19 BP by worker harvesting food crops (EC 2018: Health and Food Safety Directorate

Considering that the exposure to repellent residues via food is not negligible, a scenario to estimate the dietary exposure and risk via food is derived hereafter[112]. The consumption of food in contact with treated skin may occur multiple times a year due to frequent use of skin repellents during mosquito/tick season. Therefore, the scenario is not a strictly acute scenario, but represents a shorter than lifetime exposure. As the ARfD is not appropriate for this duration of exposure, the ADI should be used in the DRA.

Assumptions

•The application rate, expressed as mg of BP per cm^2 of treated skin (mg product/cm^2), is considered to estimate the exposure (one application just before food handling is considered relevant).

•The default values of hand surface that can be in contact with food are expressed as cm^2 and they represent 100 % of hand surface areas for toddler and children (i.e. inside and outside of both hands), and 25 % of hands surface area for adults[113] (i.e. the total inner surface of one hand gets in contact with food) (see Table 44 for default values).

•Transfer factor from hand to food: 50 %

•Exposure of all intended age groups

•The frequency of hand contact with food should not be included in the calculation.

Refinement options

Refinement options for the assessment may be proposed if scientifically justified, robust and well documented.

Hand washing is not considered feasible for a non-professional user in an outdoor setting. Thus, hand washing is not a realistic refinement option for the scenario.

Estimation of indirect exposure via food

EXp_{cons} = ApplRate * C * Hfood contact * TF (*RF)/ bw

Where:

EXp_{cons} Dietary exposure (mg a.s./kg bw/d)

ApplRate Application rate (mg product/cm^2)

C Concentration of a.s. in the BP (% w/w or mg/100 mg)

Hfood contact Hand surface in contact with food (cm^2)

TF % of biocide residue transferred from hands surface to food

RF refinement factor if applicable

General, sante.ddg2.g.5(2018)5591112, Summary Report of the Standing Committee on plants, animals, Food and Feed held in Brussels on 17 September 2018, Section Novel Food and Toxicological Safety of the Food Chain), CIRCABC Link: https://circabc.europa.eu/w/browse/6b693e53-a6ac-42e5-8922-b75d2661b3cb

[112] This scenario was agreed on at ARTFood meetings.

113 in line with Recommendations no. 11 and 14 of the BPC Ad hoc Working Group on Human Exposure

bwBody weight (kg bw)

Example : Insect Repellent with skin application

Biocidal product: a pump spray is used on human skin to repel mosquitos from non-professional users, on all age groups

Estimation of consumer exposure

ApplRateApplication rate in mg of BP/cm^2: 1.5 (value given by the Applicant)

CConcentration of a.s. in BP: 20% (value given by the Applicant)

Hfood contact230.4 cm^2 for toddler (1-2 years old)

330.9 cm^2 for children (2-6 years old)

427.8 cm^2 for children (6-12 years old)

25 % × 820 205 cm^2 for adults

(default values according to Headhoc Recommendation No. 14)

TF50 % (default value)

RF1 (no refinement)

bw10 kg for toddler (1-2 years old)

15.6 kg for children (2-6 years old)

23.9 kg for children (6-12 years old)

60 kg for adults

(default values according to Headhoc Recommendation No. 14)

Toddler Expcons = 1.5 mg/cm^2 × 20% × 230.4 cm^2 × 50% × 1 ÷ 10 kg

= 3.5 mg/kg bw/d

Children 2-6 years old Expcons = 1.5 mg/ cm^2 × 20% × 330.9 cm^2 × 50% × 1 ÷ 15.6 kg

= 3.2 mg/kg bw/d

Children 6-12 year old Expcons = 1.5 mg/ cm^2 × 20% × 427.8 cm^2 × 50% × 1÷ 23.9 kg

= 2.7 mg/kg bw/d

Adult Expcons = 1.5 mg/ cm^2 × 20% × 205 cm^2 × 50% × 1 ÷ 60 kg

= 0.5 mg/kg bw/d

Remark: the following precautionary advices are recommended to be added for all PT 19 biocidal products with skin application, independently from the results of the DRA:

- "Avoid contact of the treated skin or clothes with food."
- "Do not use directly on or near food, feed, drinking water or drinks, or on surfaces or utensils likely to be in direct contact with food, feed, drinking water or drinks." (N-127)
- "Wash or clean hands before handling food."

Additional standard sentences can be found at Frequently used sentences in the SPC.

If the applicant proposed restriction for the biocidal product application, the exposure and risk should be assessed with the intended RMM (i.e. "For children 2 to 12 years: The repellent must be applied by adults", "Do not apply to children's hands", etc).

Table 44: Default values for the DRA of Insect Repellents with skin application

Description	Default values	Background information: Remarks	References
Hand surface that can be in contact with food for children	100% Equivalent to: 230.4 cm² for toddler (1-2 years old) 330.9 cm² for children (2-6 years old) 427.8 cm² for children (6-12 years old)	100% of hand surface areas for toddler and children (i.e. palms and backs of both hands) is the worst case for children.	Recommendations no. 11 and 14 of the BPC Ad hoc Working Group on Human Exposure ARTFood meeting in November 2019
Hand surface that can be in contact with food for adults	25% Equivalent to: 205 cm²	25% of total hand surface area for adult is the reasonable worst case for adult (corresponding to the total inner surface area of one hand). Rationale: Both hands may get in contact with food, however the overall hand surface area in contact with food does not exceed the equivalent of the inner surface area of one hand. (Total hand surface area for adult (inside and outside of both hands):820	Recommendations no. 11 and 14 of the BPC Ad hoc Working Group on Human Exposure ARTFood meeting on 15.05.2024

		cm²)	
Transfer factor from hand to food	50%	Considered as a reasonable worst case of transfer agreed by ArtFood members	ARTFood meeting in November 2019
Body weight	See HEEG opinion 17		Recommendations no. 11 and 14 of the BPC Ad hoc Working Group on Human Exposure

5.2.6.4 Drinking water disinfection

The Drinking Water Directive (Council Directive 98/83/EC on the quality of water intended for human consumption) must be followed for biocides used to disinfect drinking water at all stages before it is drawn from the tap. Drinking water disinfectants that are used at any point after that are within the scope of the BPR. Contamination of drinking water from application of biocidal products may occur for example in dispensers for water for human consumption, storage tanks for animal drinking water, preservation of water softening resins, direct addition to stored drinking water.

The disinfection of water with biocides (e.g. all oxidative biocidal disinfectants) leads to the inevitable formation of disinfection by-products (DBPs). The nature and amount of DBPs is related to the composition of the water, (i.e. the organic matter in the water), and it is not possible to predict beforehand which compounds will be formed and at which concentrations. This hampers a straightforward quantitative risk assessment based on comparisons with toxicological reference values. An approach for risk assessment of DBPs has been developed[114] and should be followed for the DRA of active substances. Currently the DBP guidance document focusses on PTs 2, 11, and 12 and does not specifically address assessment of residues in food. Therefore, the formation of DBPs should be addressed qualitatively in the product assessment report and recommendations to minimize the formation of DBPs should be provided, for example via label instructions.

5.2.6.4.1 Assessment of disinfectants added to drinking water

Residues of disinfectants that are added directly to drinking water are estimated by assuming that they are present in the water in the amount of the application rate given on the label. The application rate is then multiplied by consumer intake rates for water and divided by body weight. Both water consumption data and default body weight should be derived from the WHO 2003database (see Appendix 5.2-2, section 2). Chronic exposures has to be estimated using the following calculation:

$$Exp_{cons} = R_{application} \times I_{water} \div bw$$

Where:

Exp_{cons} dietary exposure (mg a.s./kg bw/d)

$R_{application}$ biocide application rate (mg a.s./L)

I_{water} daily water consumption (L/d)

bw body weight (kg)

5.2.6.4.2 Assessment of disinfectants used to treat water containers

Residues of disinfectants used to treat containers in which water is stored (e.g. water

[114] *ECHA Guidance Vol V Disinfection By-Products*

coolers) can be estimated with a generic approach assuming vessels of small volume with maximal surface area as a worst case (see Appendix 5.2-1 for default values). Chronic exposures has to be estimated using the following calculation:

$$Exp_{cons} = R_{application} \times A_{container} \div V_{water} \times TF \times I_{water} \div bw$$

Where:

Exp_{cons}	dietary exposure (mg a.s./kg bw/d)
$R_{application}$	biocide application rate (mg a.s./m^2)
$A_{container}$	inner surface area of container (m^2)
V_{water}	volume of water in container (L)
TF	mass transfer efficiency factor (fraction of biocide residue transferred from inner container surface to water)
I_{water}	daily water consumption (L/d)
bw	body weight (kg)

Refinement options

- degradation of residues.
- biocidal product (in-use solution) left after draining the container: assumption of film thickness: 20 µm.

5.2.7 Aggregate risk assessment

Within the process of evaluation of dossiers for biocidal products, as specified in Annex VI of the BPR, the possibility of cumulative or synergistic effects must be taken into account (BPR Article 8(3) and Article 19, 2(c)) (see section 4.4).
An aggregate risk assessment[115] should be conducted for a biocidal a.s. when there is exposure:

- through more than one route (e.g. dietary and dermal),
- through more than one use (e.g. professional and non-professional),
- that is used in more than one PT
- through more than one regulatory area (e.g. plant protection products, veterinary medicines, food contact materials or food additives),

However, no EU-wide harmonised guidance exists on how to perform aggregate risk assessments for a biocidal a.s. that is used in more than one PT and/or in more than one regulatory area and therefore, in the absence of such a procedure, no aggregate DRAs needs to be proposed until respective guidance can be developed. Despite this, the concept of aggregate risk assessment is relevant in the evaluation of a single biocidal use. In this case it refers to combining dietary and non-dietary exposures into a single exposure estimate (see section 4.4).

[115] Aggregate risk assessment refers to the assessment of the total exposure to one substance resulting from more than one exposure path (oral, dermal, inhalation and dietary exposure) and/or from more than one use (uses in all relevant product types and uses in other regulatory frameworks).

Appendix 5-2

Appendix 5-2 (1) General default values and derivation of food contact areas

1 General default values for disinfectant and preserved cleaner, insecticides, drinking water disinfection and in-can preservatives in dishwashing detergents

Table 33: General default values

No.	Description	Default Values	Background Information: Remarks	References
5.1. Disinfectants and preserved cleaners in domestic kitchens				
7	Area in contact with food (acute and chronic exposure)	0.2 m^2		In the US EPA model for assessing disinfectant residues, a value of 0.2m^2 is used for surface area in contact with food. The value is based on a value of 0.4 m^2 which was used by FDA to evaluate food contact sanitizing solutions. The actual basis of this value cannot be documented from FDA sources, but its use is documented. The FDA value reflects surface area of all silverware, dishes and glasses that a person uses in an institutional setting for 3 meals a day. For the purpose of the US EPA model, the FDA value was cut in half, to reflect only counter top surfaces. The default value is based on assumptions made for chronic exposure, which were considered conservative enough to also cover the acute situation. (DRAWG Workshop January 2012)
8	Mass transfer efficiency factor	100%	worst case; may be changed based on product specific data on mass transfer efficiency	-
5.2. Insecticides in residential homes				
9	Room height	2.5 m		OECD ESD for insecticides etc for household and professional uses
10	Room volume (kitchen)	15 m^3		General Fact Sheet, RIVM report 320104002/2006
11	Area in contact with food	0.53 m^2	Combination of three surface components: a) area of food contact on kitchen counter b) area of exposed dishes with food contact c) area of exposed food	The derivation of the area in contact with food is explained in Appendix 5.2-1 , section.2. Please note that for chronic exposure calculations a dietary intake fraction of 0.5 may be applied
12	Mass transfer efficiency factor	100%	worst case; may be changed based on product specific data on mass transfer efficiency	-
No.	**Description**	**Default Values**	**Background Information: Remarks**	**References**
5.3. Drinking water disinfection				
13	volume of water container	5 L	A small volume container is considered the worst case. A smaller volume can be used if relevant	-
14	Inner surface	0.18 m^2	Assuming a cylindrical 5-	-

	area of 5-L water cooler		L water cooler with a base diameter of 14 cm, the height is: $V = \pi r^2 h \rightarrow h = V/\pi r^2 = 5000\ cm^3/\pi\ 49 cm^2 = 33$ cm Then the inner surface area is: $A = 2\pi r^2 + 2\pi rh = 1760\ cm^2 = 0.18\ m^2$	
15	Mass transfer efficiency factor	100%	worst case; may be changed based on product specific data on mass transfer efficiency	-
16	Daily water consumption	see Appendix 5.2-2, section 2		
5.4. In-can preservatives in dishwashing detergents				
17	Concentration of detergent in dish wash solution	1400 mg/L		Weegels M.F. (1997), Exposure to chemicals in consumer product use. Faculty of Industrial Design Engineering, Delft University of Technology. The Netherlands.
18	Amount of water left on dishes after rinsing	5.5×10^{-5} mL/cm^2	This value was assigned a quality factor of 2, i.e. it is based on a single data source supplemented with personal judgment. The quality factors range from 1 to 4, where 1 means low quality and 4 means high quality. It is based on the value given in the HERA guidance 5.5×10^{-4} mL/cm^2 taking into account a dilution factor of 1/10 after one rinsing	HERA guidance (2005)
19	Area of dishes in daily contact with food	5400 cm^2		HERA guidance (2005)
20	Mass transfer efficiency factor	100%	worst case; may be changed based on product specific data on mass transfer efficiency	-
No.	**Description**	**Default Values**	**Background Information: Remarks**	**References**
Miscellaneous				
21	Body weight	see HEEG opinion 17		

2. Derivation of the area in contact with food used in the scenario for domestic insecticides (see default value 11 in Table 44)

The use of vaporised insecticides in homes can lead to residues on food that is stored uncovered on counters as well as on food contact surfaces such as kitchen counters and dishes stored in open cupboards and on racks. Dietary exposure to these residues is estimated using the size of the surface in contact with the food consumed daily. For the insecticide scenario, the following default values have been set for the size of these surfaces. Each default value represents a combination of three components as shown in Table 45. The dietary intake fraction (D) is equal to 1/day for acute and 0.5/day for chronic to reflect the assumption that in the chronic assessment half the fraction is consumed per day.

Table 34: Total area of food contact and Dietary intake fraction

Food area	contact area		Dietary intake fraction (D) acute exposure/day	Dietary intake fraction (D) chronic exposure/day
area of food contact on kitchen counter	0.2 m^2		1.0	0.5
area of exposed dishes with food contact	0.27 m^2		1.0	0.5
area of exposed food	0.06 m^2		1.0	0.5
Total area of food contact	**0.53m^2**		1.0	0.5

The **area of food contact for residues on the kitchen counter** is based on the corresponding value from the scenario for disinfectants and preserved cleaners in domestic kitchens (0.2 m^2, see default value 7 in Table 44, Appendix 5.2-1). It should be noted that these default values were derived for adults; flexibility can be applied in regard to the value to be used for toddlers to allow for different food consumption. For the **acute** scenario, this value applies unchanged. For the **chronic** scenario, a dietary intake fraction of 0.5 is applied (i.e. 0.2 m^2 × 0.5/d = 0.1 m^2/d) to reflect the fact that vaporisers are not typically used continuously for 24 hours per day (national specific conditions may apply for certain overseas locations of different climatic conditions).
The **area of exposed dishes with food contact** is based on the corresponding value from the scenario for dishwashing detergents (0.54 m^2, see default value 19 in Table 44, Appendix 5.2-1). For the **acute** scenario, this value is reduced by 50% to reflect the fact that not all dishes will be exposed to the biocidal product (i.e. 0.54 m^2 × 0.5 = 0.27 m^2). Table 46 details why the factor of 50% is justified. For the **chronic** scenario, the value is reduced by an additional 50% to reflect that vaporisers are not used on a daily basis throughout the year (i.e. 0.54 m^2 × 0.5 × 0.5 = 0.135 m^2).

1 **Table 35: Area of exposed dishes with food contact**

	Average size (cm)	Single object area (cm^2)	Objects used per day	Allocated total area (cm^2/day)	Scenario / Exposed food contact surface	Exposed food contact surface (cm^2)
Plates						
Dinner plate	Ø 24	450	3 dinner plates	1350	3 plates piled up = 100% of upper plate	450
					3 plates stored vertically in a rack = max 33% of each object's food contact surface	450
Soup plate (deep part without rim); flat dessert or side plate	Ø 20	300	3 soup, side, dessert plates + 1 dinner plate		3 different piles: soup plates side plates dinner plates = 100% of upper plates	1050
					all stored vertically in a rack = max 33% of each object's food contact surface	450
Subtotal						**450 - 1050**
Cups/mugs & glasses						
Cups/mugs & glasses	Ø 8 h 11	300	3 cups and/or glasses	900	all stored up side down = 0%	0
					3 coffee, tea, beer mugs hanging on a rack = 33% of each object's food contact surface	300
					3 glasses or mugs piled up to single pile =100% of upper object	300
					2 piles for glasses & cups/mugs resp. =100% of upper object	600
Subtotal						**0 - 600**
Cutlery						
Cutlery (set of knife, fork, spoon)		40	3 sets	120	in drawer	0
Pots & pans						
1.5 L Saucepan or small frying pan	Ø 16 h 8	600	any combination of 3 small or	~2200	hanging on a wall or ceiling rack = 33% of each object's	720

			2 small + 1 big, or 2 big saucepans/ pans		food contact surface	
3-4 L saucepan, casserole or frying pan	e.g. Ø22, h 11 or Ø 26, h 7 or Ø28, h 5	1000-1100			piled up on shelf, smallest object on top = 100% of biggest objects surface (to consider uncovered parts of any object)	1000
Subtotal						**720 - 1000**
Cooking Utensils						
Cutting knifes				10	in knife block or drawer = 0%	0
Scoop, spatula, ladle, whisk , wooden spoon etc.				similar to 1 set of cutlery ~ 40	vertically in utensils holder or hanging on rack = max 100% of each object's food contact surface	40
Bowl	Ø 20 h 8		1 mixing bowl	750-800	on shelf, sorted and piled up = 100% of food contact surface of upper object	800
	Ø13 h 6		or 2 small bowls			375
Subtotal						**415 - 840**
TOTAL				~5400		**1585 – 3490 30% - 65%**

If all of the 'highest exposure' scenarios are combined to a very worst case scenario, 65% of the total area in food contact of 5400 cm^2 will be exposed. Creating different scenarios (other than the worst case) out of the possibilities given in the table above, nearly all of the combinations will lead to an exposed area that is smaller than or approx. equal to 50% of the default value of 5400 cm^2.

It should be noted that for the scenarios of piled up objects also the vertical inner surfaces of bowls, saucepans, mugs etc. were considered as fully exposed areas (as if horizontal) which may in reality not be the case - exposure of these areas may be dependent on the design of the object and could be much lower.

In conclusion, a value of 50% of default value of 5400 cm^2/day, i.e. **2700 cm^2/day** is considered a reasonable and conservative estimate for the exposed surface area of dishes stored openly in a domestic kitchen or dining area.

The **area of exposed food** was determined based on the fact that only certain foods are likely to be stored uncovered, e.g. fruits like apples or peaches, tomatoes, cucumbers or bread and other bakery products. Consumption and unit weight data were extracted from the EFSA PRIMo rev.2 and for comparison from the EFSA Comprehensive European Food Consumption Database (CEFCD) in order to determine consumption of fruits and vegetables and estimate the corresponding food surface area. Details are given in Table 47 (chronic consumption) and Table 48 (acute consumption).

Table 36: Area of exposed food (chronic consumption)

i) EFSA PRIMo rev.2 Mean consumption data																
	Unit weight edible portion [g]	Estimated unit surface [~ cm2]	exposed surface default [%]	Food consumption [g/day]			rate p&p [~ %]	Units of fruit/vegetable consumed per day (other than p&p)			Corresponding area 'consumed'					
											absolute [~ cm2 / day]			relative [~ cm2 /kg bw/ day]		
				PT	LT	IE		PT	LT	IE	PT	LT	IE	PT	LT	IE
Fruit																
Apples	131.8	200	75	63.0	130.7	59.7	50	0.239	0.496	0.226	36	74	34	0.60	1.06	0.45
Pears	158.4	230	75	20.3	11.1	17.6		0.128	0.070	0.111	22	12	19	0.37	0.17	0.26
Apricots	40.1	80	75	1.1	0	11.4	50	0.014	0.000	0.142	1	0	9	0.01	0.00	0.11
Cherries	7.0	20	75	2.2	1.9	1.6	30	0.220	0.185	0.160	3	3	2	0.06	0.04	0.03
Peaches	123.5	150	75	21.4	0	42.8	30	0.121	0.000	0.243	14	0	27	0.23	0.00	0.36
Plums	53.3	80	75	0.9	1.9	23.4		0.017	0.036	0.439	1	2	26	0.02	0.03	0.35
Table grapes	581.6	1000	55	16.7	0.9	19.5		0.029	0.002	0.034	16	1	18	0.26	0.01	0.25
Fruiting vegetables																
Tomatoes	102.6	100	75	53.7	43.4	30.2	30	0.366	0.296	0.206	27	22	15	0.46	0.32	0.21
Peppers	160.0	310	75	11.6	1.4	10.3		0.073	0.009	0.064	17	2	15	0.28	0.03	0.20

Cucumbers	411.4	500	75	1.6	27.4	8.0		0.004	0.067	0.019	1	25	7	0.02	0.36	0.10
Total fruit & vegetables				192.5	218.7	224.5					**138**	**142**	**174**	**2.3**	**2.0**	**2.3**

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ii) EFSA Comprehensive European Food Consumption Database

Mean consumption data - chronic

	Unit weight, weighted mean [~ g]	Estimated unit surface, weighted mean [~cm2]	Exposed surface default [%]	Food consumption of 70 kg adult [g/day]			Units of fruit/vegetable consumed per day			Corresponding area 'consumed' absolute [~ cm2 / day]			Corresponding area 'consumed' relative [~ cm2 /kg bw/ day]		
				IT	**ES**	**DK**	**IT**	**ES**	**DK**	**IT**	**ES**	**DK**	**IT**	**ES**	**DK**
Pome fruits	140	210	75	72.6	79.1	70.4	0.52	0.57	0.50	82	89	79	1.2	1.3	1.1
Stone fruits	90	115	75	28.4	24.8	9.3	0.32	0.28	0.10	27	24	9	0.4	0.3	0.1
Fruiting vegetables	170	200	75	129.1	93.3	83.1	0.76	0.55	0.49	114	82	73	1.6	1.2	1.1
Total fruit & vegetable				230.1	197.2	162.8				**223**	**195**	**161**	**3.2**	**2.8**	**2.3**

2 Based on EFSA PRIMo rev.2 the top 3 highest chronic consumption of fruit& vegetables under consideration was obtained for Portugal
3 (General population, bw 60 kg], Lithuania [Adult, bw 70kg] and Ireland [Adult, bw 75.2 kg]. EFSA CEFCD permitted only for extraction
4 of aggregated data for the group of pome fruit, stone fruit and fruiting vegetable, respectively. Highest chronic consumption of adults was
5 found for Italy, Spain and Denmark. A mean bw of 70 kg was applied to these data.
6 Surface area was roughly estimated based on the simplified assumption that all fruits were spheres and that cucumber were a cylinder of
7 30 cm length, using a rounded value for the diameter of the fruits. For the aggregated groups a mean unit weight and area was
8 determined, weighted by the approximate ratio of consumption of individual crops in a group. With the exception of table grapes, where a
9 greater part of the surface area is protected when considering the surface of the single berries, an exposed surface area of 75% is
10 assumed for each fruit (given the bottom side is unlikely to be exposed). Where it is known that a considerable amount (> 25%) of the
11 fruit or vegetable is usually consumed as processed and packaged (p&p) commodities (e.g. juice, jam, sauce, preserve), an additional

factor was introduced for EFSA PRIMo data to account for the amount of food that will not be exposed in the considered scenario. EFSA CEFCD data permit the separate extraction of processed fruit and vegetable data, thus an additional factor was not used.
Result: Based on EFSA PRIMo rev.2, the estimated chronic exposure from openly stored fruits and vegetables will be around **2-2.3 cm²/kg bw daily** (based on data for adults). This is confirmed by EFSA CEFCD data for Pome fruit and Stone fruit. The result is approximately twice as high for Fruiting vegetables due to the aggregation of data and the likely inclusion of additional crops (e.g. melons, aubergines, zucchini) in this group which are not relevant for the scenario considered here.
→ For the '60 kg adult' considered in this draft guidance document, the daily chronic exposure through fruits and vegetables would be around 140 cm². An overall value of 200 cm² provides a sufficiently big margin to also incorporate potentially contaminated bread, cake etc.

Table 37: Area of exposed food (acute consumption)

EFSA Comprehensive European Food Consumption Database 95th percentile acute consumption data															
	Unit weight, weighted mean [~ g]	Estimd. unit surface, weighted mean [~ cm²]	Exposed surface Default [%]	Food consumption of 70 kg adult [g/day]			Units consumed per day			Corresponding area 'consumed'					
										absolute [~ cm² / day]			relative [~ cm² /kg bw/ day]		
				PL	LV	EE	PL	LV	EE						
Total fruit and fruit products				873.9	620.0	572.7									
Pome fruits	140	210	75	759.0	560.9	560.0	5.4	4.0	4.0	854	631	630	12	9.0	9.0
% of total				87	91	98									
				SL	ES	IT	SL	ES	IT						
Total fruit and fruit products				700.0	654.1	589.8									
Stone fruits	90	115	75	700.0	608.2	523.1	7.8	6.8	5.8	671	583	501	9.6	8.3	7.2
% of total				100	93	89									

A higher level of aggregation of consumption data can be useful when considering acute exposure to residues from more than one type of food. In contrary to the Pesticides assessment where it is assumed that different food items consumed within 24 h do not contain residues of the same substance, this must be considered for the food items exposed to residues from the insecticide vaporiser use. Values were calculated as for the chronic data. Fruiting vegetables were not considered for the reasons stated above. If compared to the

Total fruit and fruit products consumption it can be deduced that the estimates for Stone fruits and Pome fruits will indeed be a good approximation of the 'total' acute intake of commodities of interest. Highest acute intake was ~**12 cm^2/kg bw/day** (pome fruits) and **~9.6 cm^2/kg bw/day** (stone fruits)

→ **For the 60 kg adult considered in this draft guidance document, the daily exposure would be 570 to 720 cm^2**, which corresponds well to a default figure of 600 cm^2.

5.2.8 Appendix 5-2 (2) Default age groups, body weights and water consumption

1. Age groups for dietary risk assessment of biocidal products used by non-professionals.

Children in general can be more exposed to residues in food than adults because they have a higher relative food intake (i.e. their food consumption on a bodyweight basis is higher than the adult) and they might be more sensitive to the toxic effects of chemicals. Due to the many developmental stages of children that influence behaviour, diet, and food intake, children are further subdivided into "infants", "toddlers" and "older children". According to the EFSA Scientific Opinion on Default Values, infants of age 3-6 months have the highest food intake on a body weight basis (132.4 g/kg bw/d). However, they consume mainly breast milk and formula milk. Since their diet differs considerably from that of the remaining population, they cannot be regarded as representative for the entire population and most dietary exposure scenarios do not apply to them. The age group with the next highest relative food intake are the 1-3 year old toddlers (114.4 g/kg bw/d). Their diet consists of many of the solid foods which adults eat as well. Toddlers should therefore be regarded the worst case with regard to DRA.

Non-dietary risk assessment of non-professionals currently considers three age groups: infants, older children and adults. Of these age groups, it is the infant who reflects the worst case in most non-dietary exposure situations. Toddlers are not a defined age group in non-dietary risk assessment, but the infant scenarios were in fact built to include typical toddler behaviour (e.g. mouthing of objects). Considering this, toddlers may be regarded to represent the worst case in both dietary and non-dietary risk assessment and therefore cover the entire population of children.

With a view to avoid unnecessarily complex assessment scenarios, risk assessment for children should be limited to one age group, namely toddlers. In addition, exposure should routinely be calculated for the adult.

There may be special circumstances where another age group represents the worst case. In these cases, exposure should additionally be assessed for the most exposed age group.

2. Default body weight and water intake values

The HEEG Opinion 17, "Default human factor values for use in exposure assessments for biocidal products", provides the default body weight according to the age (infant, toddler, child, adult) to be used in the exposure assessment for biocidal product.

In the water drinking scenario, the water consumption values are required and the WHO 2003 default values should be used, including the default values of body weight

2L/d for 60 kg bw adult

1L/d for 10 kg bw toddler

0.75 L/d for a 5 kg bw infant[116]

[116] Additional intake assessments may be required for infants based on higher consumption rates of water that are supported by relevant dietary survey data and/or information.

5.2.9 Appendix 5-2 (3) Information provided by the applicant and from other regulatory areas

Table 38: Information to be provided by the Applicant

Information relating to the intended use
- target species/organisms - application method - frequency of treatments - application rate - concentration of active substance in product and in in-use product (e.g. in the spray formulation) - detailed description of areas to be treated (e.g. countertops, specified equipment, spot treatment) - product formulation It should be clearly specified in the intended use description provided by the Applicant whether every treatment is performed with the same application rate or if refresher treatments subsequent to the initial treatment are applied at a different rate.
Information relating to the active substance
- physico-chemical properties - degradation/volatilisation rate (environmental part of the dossier)

Table 39: Information on risk assessment from other regulatory areas

Plant Protection Products	
EU Pesticide database	http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN
Guidelines for pesticide residues	http://ec.europa.eu/food/plant/protection/pesticides/publications_en.htm
RMS Assessment Reports submitted for the EU peer review of active substances used in plant protection products	http://dar.efsa.europa.eu/dar-web/provision
JMPR Reports	http://www.who.int/foodsafety/publications/jmpr-reports/en/
Veterinary Medicinal Products	
EMA Summary Reports/ Summary Opinions	http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/general/general_content_000433.jsp
JECFA Reports	http://www.who.int/foodsafety/publications/jecfa-reports/en/
Food and Feed Additives	
EFSA: Evaluations of the Panel on food additives and nutrient sources added to food (ANS)	https://www.efsa.europa.eu/en/panels/ans
EFSA: Evaluations of the Panel on food contact materials, enzymes, flavourings and processing aids (CEF)	https://www.efsa.europa.eu/en/panels/cef
EFSA: Evaluations of the FEEDAP Panel (Additives and products or substances used in animal feed)	https://www.efsa.europa.eu/en/panels/feedap
Food Contact Materials	

EFSA Note for Guidance for petitioners presenting an application for the safety assessment of a substance to be used in food contact materials prior to its authorisation	http://www.efsa.europa.eu/fr/efsajournal/doc/21r.pdf

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5.3 Estimating livestock exposure to active substances used in biocidal products

5.3.1 Background

The present guidance focuses on the external exposure assessment of livestock animals. Guidance detailing how to proceed beyond external exposure estimation has been developed by the CVMP-BTM Working Group[117] and it is referenced in this section as "EMA-CVMP guidance"[118]. Maximum residue limits (MRLs) on commodities from livestock origin are set by EMA in line with CA-March17-Doc.7.6.c-final[119].

In the context EU biocidal active substance evaluations, Livestock external exposure estimates have been collected from all EU Member States. These estimates were evaluated in order to compile available tools, identify gaps and define external exposure scenarios. The results of these evaluations are the basis of the following text and provide technical guidance that is intended to be used in the context of a step-wise approach.

The method uses a threshold concept for external exposure of food producing animals to identify those substances for which a more detailed evaluation is needed. If the estimated external exposure of a food producing animal to the pharmacologically active substance and/or its toxic degradation products and/or any substance of concern contained in the biocidal product do not exceeds the trigger value (of 4 µg/kg bw), no significant residues are expected in food of animal origin and evaluation do not proceed further, unless the substance shows toxicological concerns. If the external exposure estimation exceeds that trigger value, the assessment moves to the next tier, which would aim at refining the exposure estimate. If after refinement the trigger value is still exceeded, it can be concluded that a more detailed consideration of the potential for residues in edible products is required. The EMA-CVMP guidance details how to proceed beyond external exposure estimation. According to the EMA-CVMP guidance, an estimation of the worst case consumer exposure (WCCE) is undertaken and compared to the acceptable daily intake (ADI). If the WCCE is lower than 30% of the ADI, and in case where there is no particular concern in relation to the toxicity of the active substance, then an MRL evaluation may not be required. If, on the other hand, it is concluded that WCCE is above 30% of the ADI and in case there is a particular concern in relation to the toxicity of the active substance, then an MRL evaluation may be required.

It should be pointed out that the stepwise approach that serves as a framework for the methodologies presented in this section, is not binding. Applicants and Member States Competent Authorities may choose to skip any of the steps and proceed immediately to the approach detailed in the EMA-CVMP guidance Furthermore, the methods described in this section are to be seen as recommendations for performing assessment of biocide transfer into food. Applicants wishing to propose other methods for assessment may do so as long as these other methods are substantiated, well documented and in line with the general principles of this guidance document and the EMA-CVMP guidance.

[117] CVMP: Committee for Medicinal Products for Veterinary Use; BTM: Biocides Technical Meeting. Guideline on risk characterisation and assessment of maximum residue limits (MRL) for biocides.

[118] Guideline on risk characterisation and assessment of maximum residue limits (MRL) for biocides (EMA/CVMP/SWP/90250/2010). [http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/01/WC500181638.pdf]

[119] CA-March17-Doc.7.6.c-final. [https://www.google.fi/search?rls=com.microsoft%3Aen-GB%3AIE-SearchBox&dcr=0&q=CA-March17-Doc.7.6.c-final&oq=CA-March17-Doc.7.6.c-final&gs_l=psy-ab.12...2798.2798.0.5285.1.1.0.0.0.0.54.54.1.1.0....0...1.1.64.psy-ab..0.0.0....0.Wb8um0se6hw]

5.3.2 Introduction

The principles outlined in the CA-March17-Doc.7.6.c-final should be taken into consideration in order to assess whether the question of residues should be further explored. If it is concluded that the estimation is required, the present guidance document provides the methodology for the estimation of the external exposure of a food producing animal to the biocidal active substance.

Biocidal products are divided into 22 product types (PTs), some of which are used in areas or on objects where food or feed are produced, stored and/or processed. In this way or through direct treatment, biocidal active substances can be carried over into food or feed. In addition, through the use of biocides in animal husbandry, livestock can be exposed leading to residues in the food products obtained from livestock. Five basic groups of intended uses have been identified by way of which livestock animals can be exposed to biocidal active substances:

1. treatment of animal housing (mainly PT 3, 18, 19 and 21);
2. treatment of feedstuff and drinking water or of storage facilities (mainly PT 4, 5 and PT12);
3. treatment of materials that livestock animals may come in contact with (mainly PT 8);
4. direct treatment of livestock animals (mainly PT 3, 18 and 19);
5. treatment of aquaculture (mainly PT3 and PT21).

For each of these groups, possible methods for exposure estimation are discussed in this document.

Other PTs are unlikely to lead to livestock exposure, but this has to be considered on a case-by-case basis. On a general basis, the question on the residue should only be further explored when active substances under the normal conditions of use can lead to livestock exposure.

The possibility of livestock exposure might be considered and be addressed either by an exposure assessment or a waiver in the form of a "Justification for Non-Submission of Data" detailing the reasons for the waiver, which should demonstrate that the transfer of biocidal active substance residues to livestock is unlikely.

For a biocidal a.s. leading to exposure through more than one route (e.g. dietary and dermal), through more than one use (e.g. professional and non-professional), and that is used in more than one PT and/or in more than one regulatory area (e.g. plant protection products, veterinary medicines, food contact materials or food additives), then an aggregate exposure assessment should be conducted. No EU-wide harmonised guidance exists on how to perform aggregate exposure assessment; thus in the absence of such a procedure, no aggregate dietary exposure assessments is proposed in this section until respective guidance has been developed.

5.3.3 Stepwise approach to risk characterisation

A stepwise approach is proposed to performing evaluation of biocidal products where exposure to livestock can be foreseen.

Tier I focuses on the estimation of external exposure arising from contact of animals with the active substance, or its degradation products, in treated areas. Based on the intended use(s) and modelling approaches, realistic worst-case exposure scenarios are developed and a first tier assessment is carried out. In Tier II, experimental data may be requested to refine the external exposure assessment, for example measurements of relevant residues of the active substance or of its degradation products on the walls in stables. Further steps involve the full dietary risk assessment and possible establishment of an MRL (these steps are described in the EMA-CVMP guidance).

5.3.3.1 Tier I: initial external exposure estimation

NOTE to the reader:

It is acknowledge that currently the animal intake triggering the submission of animal studies is 0.1 mg/kg DM for the active substances falling under Reg. (EU) No 544/2011 and 0.004 mg/kg bw under Reg. (EU) No 283/2013 (EFSA, Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin, September 2015. [https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_animal_intake_mrl_2015_en.pdf]. In addition, new figures for feed intake are available (OECD ENV/JM/MONO(2013)8). The section presented here reports outdated figures, which were valid at the time of drafting the document. This section is presented mainly to describe the approach used to derive the external trigger value to be used for the purposes of this guidance document.

A detailed description of the treatment process should identify whether the active substance or its degradation products can be expected to end up in the animal body or food products from these animals. If the estimated external exposure of the animals to the active substance or its degradation products exceeds a pre-defined threshold (trigger value), then this is interpreted as indicating the possible presence of residues in food products from these animals. In this case, Tier II should be followed.

The trigger value to be used is directly derived from the practice of the European Food Safety Agency (EFSA) in the risk assessment of Plant Protection Products (PPP) under Regulation (EC) No 1107/2009.

The rules applied by the European Food Safety Agency (EFSA) to initiate the process of food risk assessment and possible MRL setting in food of animal origin is based on the substance content of the animal feed, which in turn determines the animal's exposure to the substance. The threshold value used is 0.1 mg of substance per kg of feed dry matter (DM). It was decided at TMIII_08 that the threshold value to trigger Tier II and further steps for biocidal livestock exposure assessment should be derived from this value.

Based on standard livestock weights and feed intake, the external exposure values of livestock corresponding to 0.1 mg/kg of feed DM were calculated. The corresponding reference data and calculations have been provided by EFSA. The data on animal weights and feed intake were taken from the DG SANCO Guidelines for the generation of data concerning residues as provided in Annex II part A, section 6 and Annex III, part A, section 8 of Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market (http://ec.europa.eu/food/plant/protection/resources/app-g.pdf , which is available at http://ec.europa.eu/food/plant/protection/resources/publications_en.htm#residues).

The results of the calculations are displayed in the following table[120]:

[120] Substituting the default body weights from the current guidance document (as listed in Appendix II) for the DG SANCO body weights results in a median substance intake of 0.004 mg/kg bw/d.

- **Table 40: External exposure values**

	Chicken	Dairy cattle	Beef cattle	Pig	Model Goat	UK Sheep	UK Turkey
Body weight [kg] -*default**	1.9	550	350	75	70	75	7
Feed (dry matter) intake [kg /day] –*default**	0.12	20	15	3	3	3	0.2
Substance intake [mg/day] at the 0.1 mg/kg trigger value	0.012	2	1.5	0.3	0.3	0.3	0.02
Substance intake [mg/kg bw/ day]	**0.0063**	**0.0036**	**0.0043**	**0.0040**	**0.0043**	**0.0040**	**0.0029**

*please note: the default values have been changed; the current default values are presented in table 1 Appendix 5.3-1.

The first four columns correspond to the four indicator livestock species described in the SANCO guidance (chicken including laying hens, dairy cattle, beef cattle, pig). The additional three columns (Model goat, UK sheep and UK turkey) give values commonly accepted within EFSA.

As was expected, the values obtained differ between species. However, because the variation range is extremely narrow, because the value of 0.1mg / kg feed DM is already conservative, and because there is no need for absolute precision for an indicator of the need for further refinement, it was proposed to use the median value of **0.004 mg / kg livestock bw** of external exposure over 1 day as the threshold for triggering Tier II assessment and further steps across all livestock species.

Under Regulation (EC) No 1107/2009 the trigger value is used for long-term and acute exposure. For the food risk assessment of biocides, the frequency of biocide application may differ from a daily to a monthly basis. In addition it shall be noted that not only the duration of exposure but also the delay between the biocide application and animal slaughter determines the residue in edible tissue. The delay after biocide use could correspond to the withdrawal period, defined in Point 9 of Article 1 of Directive 2001/82/EC, as amended: "*The period necessary between the last administration of the veterinary medicinal product to animals, under normal conditions of use and in accordance with the provisions of this Directive, and the production of foodstuffs from such animals, in order to protect public health by ensuring that such foodstuffs do not contain residues in quantities in excess of the maximum residue limits laid down pursuant to Regulation (EEC) No 2377/90*". In the case of an intermittent application, some products, in particular eggs or milk, can be intermittently but significantly contaminated. This is why, in case of intermittent use, the trigger value should be applied to the most acute exposure pattern (over 24 hours) and not to the averaged exposure over time. Where relevant, a more flexible approach to the exposure pattern may be considered at Tier II.

5.3.3.2 Tier II: refined external exposure estimation

If the estimated external exposure of the animals exceeds the trigger value of 0.004 mg a.s./kg bw/d at Tier I, it is necessary to perform a refined, more realistic external exposure estimation. The need for additional studies for a specific active substance depends to a large extent on the intended use of the biocidal product and is therefore a case-by-case decision involving expert judgement. At this stage, further data should only be related to the refinement of external exposure estimation. Considerations on the bioavailability and distribution of the internal dose, which may be decisive as to the need for setting an MRL, will be made at a later stage. If the estimated external exposure of

the animals at Tier II still exceeds the trigger value of 0.004 mg a.s. / kg bw/d, then it is necessary to proceed further applying the approach reported in the EMA-CVMP guidance.

Figure 10 summarises the overall stepwise approach, and includes steps undertaken by both the national Competent Authorities (CA) and the European Medicines Agency (EMA) as reported in the EMA-CVMP guidance.

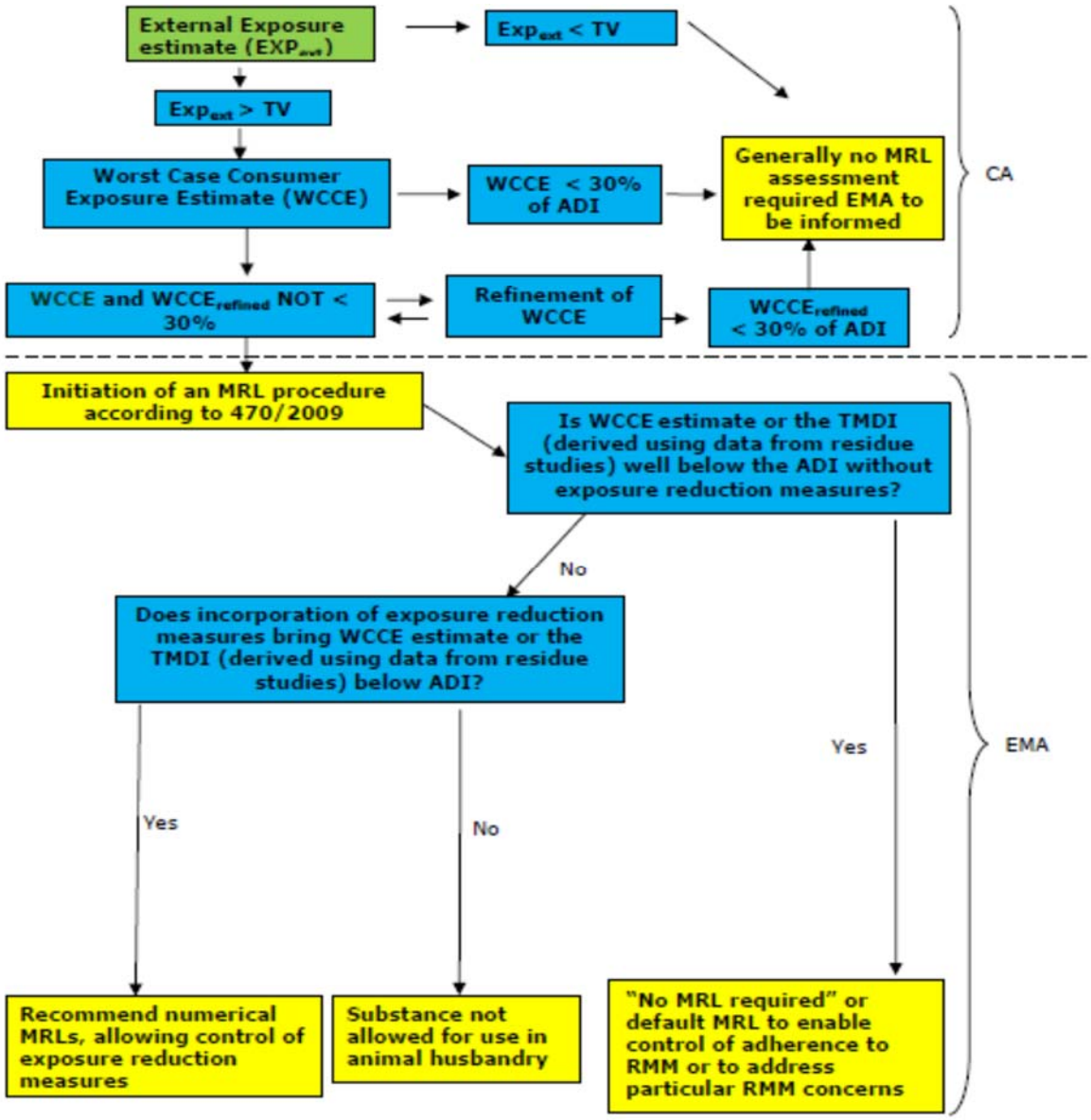

Figure 10: Decision tree summarising the overall approach reported in the EMA-CVMP guidance

Key:

Exp_{ext} = External exposure of the animal
TV = Trigger Value (4 µg/kg/day)
DRA = Dietary Risk Assessment
WCCE = Worst Case Consumer Exposure
TMDI = Theoretical Maximum Daily Intake (based on maximum residue concentrations combined with the standard food basket)
ADI = Acceptable daily intake

WP = Withdrawal period
RMM = risk management measures

5.3.4 General Considerations

5.3.4.1 Substances for which this guidance does not apply

Although it is assumed that the exposure of livestock to active substances below the trigger value of 0.004 mg a.s./kg bw/d would lead to insignificant residues in edible animal matrices, a minute exposure of humans still occurs. Thus, the trigger value is not an appropriate approach for substances that exert a non-threshold toxicity effects (such as genotoxic substances) and substances of particular concern (such as substances with reproductive/developmental/neurotoxic actions). For these substances, the approach of the EMA-CVMP guidance must be followed. In cases where an ADI has not been derived yet, another equal toxicological threshold value (e.g. the $AEL_{long\text{-}term}$, which is in many cases in the same order of magnitude as the ADI) can be used for a preliminary assessment of the toxicity of the active substance.

5.3.4.2 Substances which require particular consideration

Active substances with a potential for accumulation (e.g. substances with a log P_{ow}>3) can also pose a problem even if the estimated exposure is below the trigger value. For active substances that exhibit these characteristics, the Applicant should provide a justification based on absorption, metabolism and elimination data to prove that the active substance and its metabolites are non-accumulating and that the exposure assessment approach described in this guidance can be used for the active substance. Metabolism studies in livestock would be useful as well, if available. If the exposure assessment approach described in this guidance cannot be used, the approach of the EMA-CVMP guidance must be followed. Data provided in the Applicant's dossier may give an indication of the active substance's potential for bioaccumulation.

Biocidal active substances might be essential nutrients; in such cases, consideration should be given to the relevance of the external trigger value and the percentage of the exceedance of the reference values compared to the dietary reference intake.

In case of the possibility of degradation of the active substance before uptake by animals occurs, the degradation products should be assessed. Degradates of the active substance are identified in the physical chemical sections on photolysis and hydrolysis studies in water and air, as well as in stability studies of the formulation or active substance. Degradation products can be more toxic and/or more persistent than the active substance itself. An exposure assessment, based on the same stepwise approach used for the a.s., should be performed for any degradation products if the toxicity of the parent compound does not cover the toxicity of the degradation product.

Biocidal products may contain formulants that are substances of concern (SoC). The guidance of SoC should be applied (see Annex A of this guidance) and a decision on the relevance of these substances in relation to the risk posed through livestock exposure should be assessed case by case in a proportionate manner.

Feed/water is often stored for a period of time after being treated with a biocide. During this time, degradation of the active substance may occur, resulting in the generation of degradation products accompanied by diminishing residues of the active substance itself. When degradation leads to the generation of other toxic substances, it should be assessed whether the parent reference values cover their toxicity profile. Read-across or QSAR, or other predictive models can be used to conclude on the adequacy of the parent ADI with respect to the degradation products. If the toxicity of the degradation products is not covered by the parent compound, these substances must be included as residues in the exposure calculation. Applicant's data on the fate of the active substance provides information on degradation.

5.3.4.3 When to perform an exposure assessment

A livestock exposure assessment must be performed whenever the intended use of a biocidal product is such that livestock animals are exposed to the product. This can be the case for biocidal products used in livestock areas or on materials used in livestock areas. Information concerning the intended use can be found in the Applicant's dossier (see Appendix 5.3-1I). In some cases, however, intended uses in livestock areas are such that livestock exposure is precluded. For products for which this is the case, the biocidal product label must clearly state the restrictions that preclude livestock exposure to the product (including volatilised residues). These restrictions should be practical and feasible. Restrictions which are not practicable should not be considered in the exposure assessment. For example, a requirement for poultry to be removed from their housing prior to biocide application and then to be returned following application is unlikely to be adhered to. This is because such housing often contains thousands of birds and their removal from and return to the housing would require extensive time and space resources.

In case of treatment of animal housing, a label restriction might be feasible for restricting treatment to areas out of reach of animals (including a specific description of where the product may be used) and removing animals before treatment. In the latter case, a re-entry interval needs to be indicated on the label and calculations or studies need to be performed to show that the re-entry interval is sufficient. In case of wood treatment, a label restriction might be feasible to preclude the use of treated wood in livestock areas. In cases where wood is treated industrially, it might be feasible to require a certain time period wherein the wood may not be traded to allow time for volatilization of substances. In this case, calculations or studies need to be performed to show that the non-trading period is sufficient.

In cases where practical and feasible restrictions on the label clearly preclude animal exposure, there will be no need for an exposure assessment. In these cases, a waiver in the form of a Justification for Non-Submission of Data has to be submitted detailing the reasons for the waiver.

5.3.4.4 Choice of Animal

Generally, exposure estimates should be performed for all representative livestock species (beef and dairy cattle, pigs, broiler chickens and laying hens; fish in the case of treatment of aquaculture), unless specific conditions apply, such as the product's intended use is limited in a way that only one species (or age group within a species) is exposed. If additional livestock can be identified as representing the worst-case (e.g. sheep in the case of PT18 products), an exposure assessment for this livestock should be performed as well. The representative species are considered representative because consumption of their edible tissues and products lead to highest human consumer exposure when considering long term and acute dietary patterns.

5.3.4.5 Tier I - Methods of exposure estimation

Tier I of external exposure assessment encompasses a realistic worst-case exposure estimate based on information on the intended use and on a set of default values. The estimation assumes that the entire amount of biocidal product applied is taken up by animals.

Animals can be exposed to the biocidal active substance by different routes of exposure: inhalation, oral uptake and dermal uptake. For screening, route of exposure is irrelevant as uptake of the entire amount of applied product is assumed regardless of the route of

exposure[121]. In subsequent steps, exposure estimates for the different routes of exposure will differ because of the route-specific parameters applied[122]. Therefore, beyond screening, an estimate should be performed for each relevant[123] route taking into account the fraction of applied product available for each route. The results of the individual estimates are then added up to get the total external exposure value.

A number of parameters influence the exposure of animals. For example, some biocidal products have to be applied when the animals are not present in the stables. To calculate the amount of active substance available for animal's exposure, information about the re-entry period and the volatilisation rate are necessary. When animals are present during application, they are exposed directly to the biocidal product. However, since the target of the biocidal product is the animal housing and not the animal, it can be assumed that animals come in contact only with a fraction of the product. Information on the area of the treated surfaces that can be reached by the animals (e.g. the height of the wall that animal reached corresponds to the height of the animal) or information on how often animals lick surfaces can be used to further refine the estimations. Default values have been collected from other guidance documents and publications that can be used to perform a realistic worst-case exposure calculation (see Appendix 5.3-1 for values and references).

Many biocidal products are not used daily, but with longer time intervals between applications (weeks to months). Residues remaining from a single application decline over time due to factors such as degradation, volatilisation or uptake by livestock. As a result, livestock is exposed to ever decreasing amounts of residues in the time interval between applications. The exposure assessment methodology described in this guidance does not however differentiate between the day of application and subsequent days. Instead the worst-case is considered which assumes the presence of the highest possible amount of residue, which is the residue present on the days of the application. This assumption is made to ensure that the case in which edible animal matrices are obtained (through slaughter, milking or laying of eggs) directly following exposure is covered.

The Federal Institute for Risk Assessment (BfR) has developed a tool to facilitate the estimation of the livestock exposure to biocidal active substances as described in this guidance document (BfR calculator for estimating external exposure of livestock animals to biocidal active substances: http://www.bfr.bund.de/en/assessment residue analytics-54528.html).

Five basic groups of intended uses have been identified and methods for tier I exposure estimation will be described for each of them.

5.3.4.6 Treatment of Animal Housing

Animal housing includes the facilities in which livestock are reared and kept as well as the vehicles used to transport animals. Biocides may be used to treat any surface in animal housing facilities (including walls, floors, ceilings, window and door frames, troughs, pen enclosures etc.) as well as bedding and manure. If feed and/or drinking water contained in troughs or in storage areas are not removed from the stable prior to biocidal treatment, they can become contaminated with biocides. Animals can be exposed orally (by licking of and chewing treated surfaces, consumption of dead insects, eating straw

[121] For example, the area available in a stable is multiplied by the application rate of the biocidal product and divided by the number of animals and by body weight to get the total intake per kilogram body weight per day.
[122] For instance, in Example 1.1, the oral uptake of active substance from a wall is calculated using the licking behaviour of a calf. Instead of calculating with the entire amount of active substance available to the animal, only the amount of active substance taken up with the licking scenario is considered. Additional active substance will still be available on the wall for dermal uptake and in the surrounding air for inhalation uptake. The three scenarios have to be added up to arrive at a total exposure estimate.
[123] The assumption that an exposure route is not relevant must always be accompanied by a justification.

from the bedding or the floor), dermally (through contact with treated surfaces) and via inhalation (e.g. for volatile substances), for example from use of PT3 and PT18 biocidal products. For an optional initial screening of exposure, the assumption can be made that all of the active substance applied is taken up by animals. This screening can then be refined by performing a more realistic worst case estimation. For example, instead of assuming a complete carry-over of the residue to the animal, the probable contact of the animal with the treated surface or object is taken into account.

When calculating the total amount of product applied to animal housing, the question arises which areas (roof, walls, floor) should be considered. The assumption that only the floor space is treated is reasonable for scatter applications. But in the case of spray and brush applications – in the absence of specific information provided by the Applicant on the biocidal product label –, it should be assumed that walls and ceilings are treated as well. For fogging applications, the entire housing volume must be taken into account. For the estimation of dermal and oral exposure, only those surfaces which can be reached by the livestock provide a source of exposure. For the estimation of exposure via inhalation, all treated surfaces need to be taken into account since the active substance could be volatilised from all surfaces. Whether inhalation is a relevant pathway of exposure depends on the volatility of the active substance at ambient temperature, on the type of formulation used (e.g. dust formulations can contain inhalable particles) as well as on the application method. An inhalation exposure estimation needs to be performed in order to assess the relevance of this route of exposure. For the collection of manure, some stables are designed with special slatted floors. Manure dropped by livestock collects below these slatted floors preventing contact with the animal. In such cases, it is highly unlikely that livestock would be exposed either dermally or orally to biocidal products used for the treatment of manure since the animals do not come in contact with the stored manure. However, if the active substance is volatile then an inhalation exposure assessment would need to be undertaken. The manure to which the biocidal product is applied would be at a relatively high temperature and therefore the volatility of the residues would need to be ideally assessed at such temperatures. In some countries, livestock is not allowed to be kept on slatted floors, and is hence exposed to the manure collecting in pens.

The exposure of livestock via contact with treated bedding depends on the contact period and surface area of animals in contact with bedding material as well as on the type of bedding material and its ability to release residues. It should be kept in mind that manure may be contaminated with biocide residues and subsequently be spread onto agricultural areas, leading to transfer of residues into cultivated crops. Specific data to address this exposure path is usually not provided in the biocide dossier, however applicants may provide useful information if the scenario is considered relevant for the biocidal product use (this scenario is not further discussed in this guidance document).

5.3.4.6.1 Types of product applications

Fogging applications distribute particles of active substance fairly evenly throughout the air space. The particles settle on the surfaces and are available for oral and dermal uptake. Application with this method does not require an active substance with a high vapour pressure.

Nebulising applications distribute droplets of a liquid that contains solubilised active substance throughout the air space. The particles settle on the surfaces and are available for oral and dermal uptake. Application with this method does not require an active substance with a high vapour pressure.

Spray applications can be used for the treatment of an entire stable or for parts of it. With spraying, aerosols are distributed throughout the air space and settle on surfaces.

Fumigation applications generate gaseous forms of the active substance.

Applications via *vaporizers* allow evaporation of the active substance from impregnated absorbent material at ambient temperature (passive vaporizers) or upon heating (e.g. electric vaporizers).

Brush applications can be used on any surface and are sometimes applied to boards that are subsequently hung up in animal housing.

Granule applications are scattered across the floor. Uptake by livestock animals is mainly oral and possibly dermal.

Dusting powders are applied to horizontal surfaces or in voids. They consist of a low concentration of the active substance mixed with an inert carrier powder and act by contact with the pest.

Bait stations

Some biocidal products are not applied to the animal housing itself, but are contained in bait stations that are put in strategic locations. Examples are products used against termites, flies and rodents. Termite baits are generally installed below ground out in the yard in cylindrical plastic stations or placed indoors over active mud tubes in known areas of termite activity. Considering that the product is enclosed in a container and not exposed to indoor/outdoor conditions, livestock exposure seems to be very limited. However, rodents tend to drag the bait to their nest and may lose bait on their way, providing a source of exposure. Flies may die within reach of animals, providing another source of exposure. To properly address the bait exposure scenario, a detailed explanation on bait placement/frequency/amount of product per bait and robustness of the bait stations to prevent access to the bait by livestock is needed.

5.3.4.6.2 Route of exposure

5.3.4.6.2.1 Oral exposure

Some livestock animals enjoy licking surfaces or objects in their vicinity. Grown ruminants generally prefer the salt licks provided to them, while calves frequently lick other surfaces and objects (e.g. walls). Pigs do not usually lick walls, but prefer metal objects. Poultry and goats do not engage in this type of behaviour. Through grooming an animal can orally take up a substance that has been transferred to its skin by rubbing against treated surfaces or by aerosol dropping or settling after spray treatment in the vicinity of the animal.

Insecticides (PT 18) are used in animal housing to control flies and other insects. Consumption of insects killed by a biocide provides a source of biocidal exposure. Poultry seek out dead insects intentionally. Other animals only accidentally ingest dead insects (e.g. when they have dropped in the feed). It is not necessary to consider the accidental uptake of insects since the amount of residue ingested in this way is minute. For an exposure calculation, the amount of biocidal product consumed by an insect in 24 hours is multiplied by the number of dead insects consumed by livestock.

Feed remaining in troughs may unintentionally be contaminated if it is present in the treated area during application of a biocide. Due to animal behaviour and feeding practices, this exposure scenario varies between species. Cattle are usually fed twice a day and consume all of the feed given to them in a single sitting. Any leftover feed is removed from the trough prior to the next feeding. Some stables are equipped with computerised systems that calculate the nutrition needs of each animal based on monitoring data. When an animal approaches the feeding station, the appropriate amount of feed is released. For each of these feeding practices, direct contamination of feed is unlikely, however, biocidal residues left in troughs may migrate into the next feed batch. Cattle housing is often equipped with a contraption for holding a bale of hay for the animals to nibble on throughout the day. The hay can be contaminated during a biocide application and animals can subsequently take up the residues while nibbling on the hay. To avoid contamination with dirt, water is often provided to cattle via dispenser

bottles, making biocidal contamination with biocides unlikely. However, other dispenser systems work by releasing water into a trough when the animal pushes a lever. With these systems, water may be contaminated directly or through migration of residues from the trough.

Fattening pigs are at a stage in their lifecycle where their feed consumption is large so as to promote the fattening process. Like cattle, they are usually given feed twice a day and consume all of it at one sitting. Direct contamination of feed is therefore unlikely, however, biocidal residues left in troughs may migrate into the next feed batch. Like cattle, fattening pigs are given water in dispenser bottles or in dispenser troughs.

Feeding practices for poultry differ from those for cattle and pigs. Depending on whether poultry is held in battery cages or allowed to roam across the floor, feed is provided to them on conveyor belts or gutters (cages) or in dispenser bowls (ground). Poultry kept free range with access to the outside feed directly from the ground or from dispenser bowls. Dispenser bowls are equipped with a cylinder mounted on the bowl from where stored feed slides into the bowl as it is being emptied. Providing feed in dispenser bowls, on conveyor belts or in gutters allows poultry to feed throughout the day and some portion of the daily feed and water rations is always exposed to the environment, therefore allowing contamination with biocides.

The label of a biocidal product may indicate that feed, water and troughs are to be covered during biocidal treatment. In this case, contaminated feed/water is generally not a source of exposure as long as the cover is put into place properly (i.e. provides a complete cover) and is impermeable to smoke, small particles and droplets.

For an oral exposure calculation, the following parameters may be needed. Default values for these parameters can be found in Appendix 5.3-1:

- Maximum area within reach of animal
- Number of animals per stable
- Available wall and floor area per animal in transport vehicles
- Number of animals per compartment in transport vehicles
- Frequency of surface licking
- Surface area of tongue
- Biocidal product consumption by flies
- Number of dead flies consumed
- Exposed feed surface
- Bodyweight

5.3.4.6.2.2 Dermal exposure

Large slaughter animals, e.g. cattle and pigs, frequently rub against surfaces such as walls and pen enclosures. These surfaces are often treated with biocides, providing a source of exposure. Small animals such as poultry and rabbits do not engage in this type of behaviour. Usually, the biocide label requires that animals be removed from the premises to be treated. But in some cases, animals are present when their housing is treated with a biocide. Animals may be exposed to spray applications during treatment or directly after treatment when aerosols drop and settle on the animals' skin or feathering. Animals prefer not to be close of the treatment area and will try to move away. However, since most animal keeping facilities do not allot much room per animal, moving away from the treatment site may not be possible.

For a dermal exposure calculation, the following parameters may be needed. Default values for these parameters can be found in Appendix 5.3-1:

- Maximum area within reach of animal
- Number of animals per stable
- Available wall and floor area per animal in transport vehicle
- Number of animals per compartment in transport vehicle
- Body surface area in contact with surface
- Bodyweight

5.3.4.6.2.3 Inhalation exposure

Fumigation applications are frequently used to treat animal housing after livestock have been sent to slaughter or been otherwise relocated and before the entry of new livestock. Usually the new livestock are not allowed into the housing until after a specified period of time, when most of the residues have been removed through ventilation. Hence animals are not present during biocidal application. For an exposure calculation, the amount of residue that remains once the new animals are brought into the housing must be determined. Residues from fumigation applications are in the form of small particles and possibly some vapours. Residues from fogging applications in the form of small droplets typically <25µm in diameter are either available for inhalation and/or can settle on surfaces for uptake via the oral and dermal route. Biocidal active substances from aqueous products can also be released into the air and be available for inhalation.

For an inhalation exposure calculation, the following parameters may be needed. Default values for these parameters can be found in Appendix 5.3-1:

- Housing volume per stable
- Number of animals per stable
- Ventilation rate in stable
- Available volume per animal in transport vehicle
- Number of animals in transport vehicle
- Ventilation rate in transport vehicle
- Alveolar ventilation rate
- Bodyweights

In the following, example calculations are given for estimating initial external exposure (screening and realistic worst-case estimate) following treatment of animal housing. The realistic worst-case estimate is an overestimate as it estimates exposure on the first day of application. As the substance is taken up by animals, less substance would be available for exposure on subsequent days.

5.3.4.6.3 Examples of Tier I livestock exposure estimation – treatment of animal housing

5.3.4.6.3.1 Example 1.1: Treatment of Animal Housing – Exposure of calves (special case) to spray treatment

Product: Insecticide spray, VP = $2x10^{-7}$ Pa at 20°C, MW = 449.9 g/mol

Intended Use

Used in and around animal housing. Spray application in areas where flies congregate or settle, such as floors, walls, ceilings and around doors and windows. 1 application every 6 weeks to 4 months at 25 mg as/m^2. No animals are present during treatment.

Exposure Estimation

The calf was chosen as the representative animal. While grown cattle prefer licking salt licks provided in stables, calves are less choosy and like to lick other objects as well. In the following calculations, default values from Appendix 5.3-1 are used.

Screening (route of exposure irrelevant):

Application rate = 25 mg a.s./m^2

Area treated (walls+floor) = 330 m^2

Number of animals per stable = 80

Body weight of calf = 200 kg

25 mg a.s./m^2 x 330 m^2 ÷ 80 ÷ 200 kg

= 0.5156 mg a.s./kg bw/d

Realistic worst-case estimate:

Oral exposure through licking of surface:

For calves, exposure from consumption of dead flies is considered not relevant compared to exposure from licking surfaces.

Emission factor for spraying (fraction emitted to the treated surface during surface treatment by spraying, see Table 54 item #18) = 0.85

Tongue surface area: 0.008 m^2

Licks per day: 10

Body weight: 200 kg

25 mg a.s./m^2 x 0.85 x 0.008 m^2 x 10 ÷ 200 kg

= 0.0085 mg a.s./kg bw/d

Oral exposure through uptake of contaminated feed:

It is assumed as a worst-case that troughs are not covered during biocidal treatment and that all residues contained on the bottom and sides of the trough migrate into the next feed batch that is given after biocidal treatment. It follows that all of the residue contained in the trough is taken up by the animal.

Emission factor for spraying (fraction emitted to the floor during surface treatment by spraying, see Table 54 item #18) = 0.11

Exposed feed surface = 0.5m^2

Body weight: 200 kg

Amount of active substance contained in trough:

25 mg a.s./m^2 x 0.11 x 0.5 m^2 = 1.375 mg a.s.

Exposure of animal:

1.375 mg a.s. ÷ 200 kg

= 0.0069 mg a.s./kg bw/d

Dermal exposure through rubbing against surfaces:

Rubbing against surfaces is considered the relevant path of dermal uptake for calves. It is assumed that all active substance has settled on surfaces and that animals are not exposed to the spray during application. The exposure estimate covers dermal uptake as well as oral intake from grooming.

Emission factor for spraying (fraction emitted to the treated surface during surface treatment by spraying, see Table 54 item#18) = 0.85

Body surface area in contact with surface = 0.87 m^2

Body weight: 200 kg

25 mg a.s./m^2 x 0.85 x 0.87 m^2 ÷ 200 kg

= 0.0924 mg as/kg bw/d

Inhalation exposure:

It is assumed that the animal is exposed to air containing the active substance at its saturated vapour concentration (SVC). This represents a worst-case as the active substance cannot achieve a higher concentration in the air.

SVC =

$$\frac{\text{vapour pressure x molecular weight}}{\text{gas constant x temperature in degrees Kelvin}}$$

$$\frac{2\times10^{-7}\text{ Pa at }20^{o}\text{C x }449.9\text{ g/mol}}{8.31451\text{ J/K mol x }293^{o}\text{K (equivalent to }20^{o}\text{C)}}$$

$$= 3.6935\times10^{-8}\text{ g a.s./m}^3$$

$$= 3.6935\times10^{-5}\text{ mg a.s./m}^3$$

Alveolar ventilation rate = 25 m^3/d

Body weight = 200 kg

3.6935x10^{-5} mg a.s./m^3 x 25 m^3/d ÷ 200 kg

= 4.6169x10^{-6} mg a.s./kg bw/d

Total exposure:

oral exposure (licking) + oral exposure (feed) + dermal exposure + inhalation exposure

= 0.0085 + 0.0069 + 0.0924 + 4.6169x10^{-6}

= 0.1078 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is exceeded. Proceed with a refined exposure assessment based on Tier II data or proceed with the EMA-CVMP guidance.

Possible Tier II refinement option:

- measurement of the amount of residue on surfaces
- measurement of the amount of residue in the air
- measurement of the residue level in feed after contact with the treated trough

For a complete exposure assessment, the calculation needs to be repeated for beef and dairy cattle, pigs, broiler chickens and laying hens.

***Note**: Because this is an example of a spray application, residues were adjusted to account for the fraction emitted to the treated surface during surface treatment by spraying. This adjustment does not apply to other types of applications.*

5.3.4.6.3.2 Example 1.2: Treatment of Animal Housing – Exposure of laying hens from spray treatment

Product: Insecticide, VP = 2.1x10^{-8} Pa at 20°C, MW = 434.3 g/mol

Intended Use

Use in animal housing for combating flies. The product contains 0.8 g a.s./L and is applied with a low pressure sprayer to walls, ceilings and window frames in strips of 1-2 m width with a maximum application rate of 40 mg as/m^2 every 21 days in the months of April to October.

Exposure Estimation

The exposure is estimated for laying hens. In the following calculations, default values from Appendix 5.3-1 are used.

Screening (route of exposure irrelevant):

Wall and roof area per stable = 2030 m^2

Number of animals = 10000

Body weight = 1.9 kg

40 mg a.s./m^2 x 2030 m^2 ÷ 10000 ÷ 1.9 kg

= 4.2737 mg a.s./kg bw/d

Realistic worst-case estimate:

Oral exposure through ingestion of flies:

Chickens do not lick walls, but they seek out dead flies for consumption.

Fly consumption = 10 flies/d

Consumption of biocidal product (spray deposit) by flies = 3.5 mg biocidal product/d

Concentration of a.s. in biocidal product = 0.8 g/L (assuming product density of 1, this is equal to 0.0008 mg a.s./mg biocidal product)

a.s. consumption by flies = 0.0028 mg a.s./fly/d

10 flies/d x 0.0028 mg a.s./fly ÷ 1.9 kg

= 0.0147 mg a.s./kg bw/d

Oral exposure through uptake of contaminated feed:

Body weight: 1.9 kg

Exposed feed surface = 0.01m^2

Emission factor for spraying (fraction of spray product emitted to floor during surface treatment, see Table 54 item #18) = 0.11

Amount of active substance contained in trough:

40 mg a.s./m^2 x 0.11 x 0.01m^2 = 0.0440 mg a.s.

Exposure of animal:

0.0440 mg a.s. ÷ 1.9 kg

= 0.0232 mg a.s./kg bw/d

Dermal exposure through spray treatment:

Poultry does not rub against walls. But dermal exposure can occur from spray hitting poultry during treatment. The exposure estimate includes dermal uptake as well as oral intake from grooming.

Treated area = wall area = 600 m^2

Number of animals = 10000

Body weight of hen = 1.9 kg

% of spray hitting hens = fraction emitted to floor during surface treatment (0.11) (see Table 54 item #18) x 50% (assuming that 50% of the floor is covered by hens) = 0.055 = 5.5%

$$40 \text{ mg a.s./m}^2 \times 600 \text{ m}^2 \times 5.5\% \div 10000 \div 1.9 \text{ kg}$$

$$= 0.0695 \text{ mg a.s./kg bw/d}$$

Inhalation exposure:

It is assumed that the animal is exposed to air containing the active substance at its saturated vapour concentration (SVC). This represents a worst-case as the active substance cannot achieve a higher concentration in the air.

SVC =

$$\frac{\text{vapour pressure x molecular weight}}{\text{gas constant x temperature in degrees Kelvin}}$$

$$\frac{2.1\text{x}10^{-8} \text{ Pa at } 20^{o}\text{C x } 434.3 \text{ g/mol}}{8.31451 \text{ J/K mol x } 293^{o}\text{K (equivalent to } 20^{o}\text{C)}}$$

$$= 3.7437\text{x}10^{-9} \text{ g a.s./m}^3$$

$$= 3.7437\text{x}10^{-6} \text{ mg a.s./m}^3$$

Alveolar ventilation rate = 0.2 m^3/d

Body weight = 1.9 kg

$$3.7437\text{x}10^{-6} \text{ mg a.s./m}^3 \times 0.2 \text{ m}^3\text{/d} \div 1.9 \text{ kg}$$

$$= 3.9408\text{x}10^{-7} \text{ mg a.s./kg bw/d}$$

Total exposure:

oral exposure (flies) + oral exposure (feed) + dermal exposure + inhalation =

$$0.0147 + 0.0232 + 0.0695 + 3.9408\text{x}10^{-7}$$

$$= 0.1074 \text{ mg a.s./kg bw/d}$$

→ The trigger value of 0.004 mg a.s./kg bw/d is exceeded. Proceed with a refined exposure assessment based on Tier II data or proceed with the EMA-CVMP guidance.

Possible Tier II refinement options:

- measurement of amount of residues on surfaces
- measurement of residues on the feathering and skin of poultry
- measurement of concentration of active substance in/on flies
- alternatively, the LD_{50} of the active substance for flies can be used to determine the active substance concentration in/on flies
- measurement of the residue level in feed

For a complete exposure assessment, the calculation needs to be repeated for beef and dairy cattle, pigs and broiler chickens. Exposure from consumption of dead flies should not be included for beef and dairy cattle and pigs.

***Note**: Because this is an example of a spray applications, residues were adjusted to account for the fraction emitted to floor during surface treatment. This adjustment does not apply to other types of applications.*

5.3.4.6.3.3 Example 1.3: Treatment of Animal Housing – Exposure of a dairy cow from a fogging treatment

Product: Disinfectant, VP = 1.58×10^{-4} Pa, MW = 297.18 g/mol

Intended Use

Used indoors by professional users for the disinfection of hatcheries, stables and other infected animal-breeding facilities and materials. Animals are not present during treatment and may not re-enter the premises for 4 hours after treatment. Up to 12 spray, smoke or nebulizer treatments at a rate of 0.005 – 0.1 g a.s./m^3 are intended over the course of a year.

Exposure Estimation

The exposure is estimated for dairy cattle. In the following calculations, default values from Appendix 5.3-1 are used.

Screening (route of exposure irrelevant):

Housing volume per stable = 9630 m^3

Number of animals per stable = 100

Body weight of dairy cow = 650 kg

$$9630\ m^3 \times 100\ mg\ a.s./m^3 \div 100 \div 650\ kg$$

$$= 14.8154\ mg\ a.s./kg\ bw/d$$

Realistic worst-case estimate:

***NOTE**: For the calculation of oral and dermal uptake, the fraction of residue that has not volatilised, but has settled on surfaces must be calculated. A calculation method has not been agreed, so that the residue amount in the following exposure calculations was set to an arbitrary value of 0.01 mg/m^2 for illustrative purposes only. A value for the fraction of residue that has settled on surfaces must be provided by the Applicant.*

Oral exposure through ingestion of residues:

Exposure from consumption of dead flies is considered not relevant compared to exposure from uptake via food.

Exposure from oral uptake from surfaces is considered not relevant, because grown cattle do not have a habit of licking surfaces.

Oral exposure through uptake of contaminated feed:

It is assumed as a worst case that troughs are not covered during biocide treatment and that all residues contained on the bottom and sides of the trough migrate into the next feed batch that is given after biocide treatment. It follows that all of the residue contained in the trough is taken up by the animal.

Body weight: 650 kg

Exposed feed surface = 2.9 m^2

Amount of active substance contained in trough:

$$0.01\ mg\ a.s./m^2 \times 2.9\ m^2 = 0.029\ mg\ a.s.$$

Exposure of animal:

$$0.029\ mg\ a.s. \div 650\ kg$$

$$= 0.00004 mg\ a.s./kg\ bw/d$$

Dermal exposure through rubbing on surfaces:

Rubbing against surfaces is considered the relevant path of dermal uptake for cows. The exposure estimate includes dermal uptake as well as oral intake from grooming.

Body weight: 650 kg

Body surface area in contact with surface = 1.68 m^2

Total area rubbed = Surface area of skin in contact with surfaces

0.01 mg a.s./m^2 x 1.68 m^2 ÷ 650 kg

= 0.00003 mg a.s./kg bw/d

Inhalation exposure of dairy cow from a fogging treatment

Due to the waiting period of 4 hours, the air concentration at the time of re-entry was calculated with ConsExpo using the following values:

Emission duration: 1 min (This is the time during which application occurs. It is set at the arbitrary value of 1 minute, since it is not relevant for the purpose of this calculation.)

Treated area = housing volume = 9630 m^3

Product amount: housing volume x application rate (100 mg a.s./m^3) = 963 g

Vapour pressure: 1.58x10^{-4} Pa

Molecular Weight: 297.18 g/mol

Temperature: 25 °C

Ventilation rate: 0.9/h

Air concentration at the time of re-entry = 0.0190 mg a.s./m^3

Body weight of dairy cow = 650 kg

Alveolar ventilation rate of dairy cow = 62 m^3/d

0.0190 mg a.s./m^3 x 62 m^3/d ÷ 650 kg

= 0.0018 mg a.s./kg bw/d

Total exposure:

oral exposure + dermal exposure + inhalation exposure

= 0.00004+ 0.00003 + 0.00181

= 0.0019 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is not exceeded. No significant residues are expected in food from dairy cattle. Dietary risk assessment can be stopped for dairy cattle.

Possible Tier II refinement options (in case the trigger value would have been exceeded)

- measurement of amount of residues on surfaces
- measurement of amount of residues in the air
- measurement of amount of residues in feed

For a complete exposure assessment, the calculation needs to be repeated for beef cattle, pigs, broiler chickens and laying hens.

5.3.4.6.3.4 Example 1.4: Treatment of Transport Vehicles – Exposure of pigs from a liquid treatment

Product: Disinfectant

Intended Use

The product is used for the disinfection of transport vehicles. Surfaces and materials need to be cleaned thoroughly with water and detergent, and any detergent needs to be rinsed of with clean water. Excess water needs to be removed before disinfection. For disinfection, 390 mg a.s./m^2 are applied and enough liquid is used so that surfaces (floors, walls) stay wet during the treatment period. The minimum treatment period is 5 minutes.

Exposure Estimation

The pig was chosen as the representative animal. In the following calculations, default values from Appendix 5.3-1 are used.

Screening (route of exposure irrelevant):

Body weight: 100 kg

Available wall+floor area per animal = 1 m^2

390 mg a.s./m^2 x 1 m^2 ÷ 100 kg

= 3.9 mg a.s./kg bw/d

Realistic worst-case estimate:

Oral exposure:

Exposure from oral uptake from walls is considered not relevant, because pigs do not have a habit of licking walls. They do however enjoy licking metal bars such as the ones separating compartments in a transport vehicle.

Body weight: 100 kg

Tongue surface area: 0.008 m^2

Licks per transport period: 10

390 mg a.s./m^2 x 0.008 m^2 x 10 ÷ 100 kg

= 0.3120 mg a.s./kg bw/d

Dermal exposure through rubbing on surfaces:

Rubbing against surfaces is considered the relevant path of dermal uptake for pigs. The exposure estimate includes dermal uptake as well as oral intake from grooming.

Body weight: 100 kg

Body surface area in contact with surface = 0.45 m^2

Total area rubbed = Surface area of skin in contact with surfaces

390 mg a.s./m^2 x 0.45 m^2 ÷ 100 kg

= 1.7550 mg a.s./kg bw/d

Inhalation exposure:

Exposure to vapours is not considered relevant since the active substance does not volatilise.

Total exposure:

oral exposure + dermal exposure + inhalation exposure

= 0.3120 + 1.7550 + 0

= 2.0670 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is exceeded. Proceed with a refined exposure assessment based on Tier II data or proceed with the EMA-CVMP guidance.

Possible Tier II refinement options:

- measurement of amount of residue remaining on surfaces
- data on the efficiency of the rinsing

For a complete exposure assessment, the calculation needs to be repeated for beef and dairy cattle, broiler chickens and laying hens.

5.3.4.7 6.5.2 Treatment of Drinking Water or of Storage Facilities for Feed and Drinking Water

Biocidal products of PT 5 are used for the direct treatment of drinking water. Other types of biocidal products are used for the treatment of feed/water storage facilities, piping systems for the transport of feed/water, feed/water troughs (PT4) or packaging materials for feedstuff (PT12). When feed or water is treated through direct application, the assumption can be made that all of the active substance applied is carried over into the feed/water. When storage facilities, piping systems, troughs and packaging materials are treated, a realistic worst case estimate must factor in the amount of residue that migrates from the treated surface into the feed/water (e.g. based on the fat solubility of the active substance compared to the type of feed). In such a scenario, the outer layers of a feed batch will contain the bulk of the biocide residue while the core will be residue-free. Feed will be mixed during release from storage silos and during filling of troughs. Animals might not be exposed to residues from exposed feed on a daily basis and residue burden will be higher on some days than on others. An exposure assessment involving exposed feed/water should therefore be based on the assumption that residues migrating from treated surfaces to feed are evenly distributed throughout the feed batch.

Feed/water is often stored for a period of time after being treated with a biocide. During this time, degradation of the active substance may occur, resulting in the generation of degradation products accompanied by diminishing residues of the active substance itself. In the case of non-toxic degradation products, a degradation factor can be included in the Tier II exposure calculation. But when degradation leads to the generation of other toxic substances, it should be assessed whether the parent reference values cover their toxicity profile. Read-across or QSAR, or other predictive models can be used to conclude on the adequacy of the parent ADI with respect to the degradation products. If the toxicity of the degradation products is not covered by the parent compound, these substances must be included as residues in the exposure calculation. Applicant's data on the fate of the active substance provides information on degradation

For an oral exposure calculation, the following parameters may be needed. Default values for these parameters can be found in Appendix 5.3-1:

- Feed/drinking water intake
- Size and holding capacity of feed silos
- Size of packaging material
- Volume of feed/water contained in storage tank, trough or packaging material or moving through piping system
- Exposed feed surface
- Bodyweight

5.3.4.7.1 Examples of tier I livestock exposure estimation – treatment of drinking water or storage facilities

5.3.4.7.1.1 Example 2.1: Treatment of Drinking Water

Product: Disinfectant

Intended Use

The product is added to drinking water for livestock animals at a rate of 5 mg a.s./L.

Exposure Estimation

The exposure is estimated for a broiler chicken. In the following calculations, default values from Appendix 5.3-1 are used.

Water consumption = 0.25 L/d

Body weight = 1.7 kg

Screening:

0.25 L/d x 5 mg a.s./L ÷ 1.7 kg

= 0.7353 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is exceeded. Proceed with a refined exposure assessment based on Tier II data or proceed with the EMA-CVMP guidance .

Possible Tier II refinement option:

- measurement of amount of residues in water

For a complete exposure assessment, the calculation needs to be repeated for beef and dairy cattle, pigs and laying hens.

5.3.4.7.1.2 Example 2.2: Treatment of a Feed Storage Facility

Product: Disinfectant

Intended Use

The product is used for the disinfection of feed storage tanks. Tanks are treated once a day with an application rate of 100 mg a.s./m^3. Tanks are filled completely with the disinfectant solution and are later drained.

***NOTE**: Due to the variety of available sizes of feed silos, a default value cannot be established. Instead, a range of sizes is provided in Appendix 5.3-1. Exposure calculations must be performed for all sizes. In case of exceedance of the trigger value for only a few smaller sizes, expert judgement is used to decide whether Tier II estimates are necessary.*

Exposure Estimation

In the following calculations, default values from Appendix 5.3-1 are used.

First, the concentration of the active substance in the feed is calculated. Disinfectants are designed to have short-term efficacy, so the desired effect will have been achieved by the time the tank is filled again with feed. It can be assumed then that the migration rate of the active substance into the feed is large, e.g. 100%. Taking a tank with a volume of 13.56 m^3 and a holding capacity of 5.7 tons, we have:

100 mg a.s./m^3 x 13.56 m^3 ÷ 5700 kg feed = 0.2379 mg a.s./kg feed

To calculate the exposure of the animal, in this case a fattening pig:

Feed consumption = 3 kg/d

Body weight = 100 kg

Screening:

3 kg feed/d x 0.2379 mg a.s./kg feed ÷ 100 kg

= 0.0071 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is exceeded. Proceed with a refined exposure assessment based on Tier II data or proceed with the EMA-CVMP guidance.

Possible Tier II refinement options:

- measurement of amount of residues on silo surface
- measurement of amount of residues in feed.
- biocidal product (in-use solution) left after draining the container:assumption of film thickness: 20 µm (default value based on expert judgement)

For a complete exposure assessment, the calculation needs to be repeated for beef and dairy cattle, broiler chickens and laying hens for the range of silo sizes given in Appendix 5.3-1.

5.3.4.7.1.3 Example 2.3 Treatment of Paper/Cardboard used for Packaging Feed

Product: slimicide for paperpulp

Intended use

The active substance is used as slimicide in process water and for equipment in and on which slimes may be formed (e.g. during paperpulp processing). The continuous background concentration is 2.5 mg a.s./L. The principal residue will not decompose and may migrate into the food with which the treated paper comes into contact.

Exposure estimation

In the following calculations, default values from Appendix 5.3-1 are used.

Feedstuffs which are packaged in paper or cardboard which was treated during manufacture with a slimicide may contain biocidal residues as a result of migration from the packaging material into feed.

The amount of active substance present in the paper or cardboard is calculated as follows:

The ESD recommends to perform risk assessment in the papermaking industry with the RIVM/FEI-scenario.

The ESD assumes that 90% of the a.s. is lost in waste water and 10% remains in the paper.

A paper mill produces 5000 m^3 waste water per day.

Active concentration in water = 2.5 mg/L = 2.5 g/m^3 (see intended uses)

Active substance lost by waste water is 5000 m^3 x 2.5 g/m^3 = 12500 g = 12.5 kg/day

The amount of active substance remaining in dry paper is 12.5 kg/day x 0.1 ÷ 0.9 = 1.3889 kg/day

A paper mill produces 200 t/d.

Dry paper contains 1.3889 ÷ 200000 = 6.94 10^{-6} kg as/kg paper = 6.94 mg as/kg paper.

The amount of active substance present in feedstuffs is calculated as follows:

A worst case estimate of the quantity of active substance which may migrate onto packaged feed is made based on the assumption that all of the active substance which

remain in the paper from the processing will migrate into feed. According to the EU Notes for Guidance for Food Contact Materials prepared by the European Food Safety Authority (updated June 2006), the migration of a substance from a packaging material to food with which it is in contact can be estimated with the assumption that 1 kg of feed is in contact with 600 cm^2 of food packaging (i.e. 1670 mg feed/cm^2).

Dry paper weighs 600 g/m^2 (= 60 mg/cm^2)

Dry paper contains 6.94 mg as/kg paper (= 6.940×10^{-6} mg a.s./mg paper).

1 cm^2 of paper contains: $6.940 \times 10^{-6} \times 60 = 4.17 \times 10^{-4}$ mg as/cm^2.

1 kg feed is wrapped in 600 cm^2 paper = 1670 mg feed/cm^2.

Therefore, the amount of active substance per kg of feed is: $4.17 \times 10^{-4} \div 1670 = 2.5 \times 10^{-7}$ mg as/ mg feed = 0.25 mg as/kg feed.

Livestock exposure:

The exposure is calculated for beef cattle.

Feed consumption = 20 kg

Body weight = 500 kg

Screening:

The screening is based on the assumption that all of the feed the animal consumes comes packaged in treated paper/cardboard.

20 kg feed/d x 0.25 mg a.s./kg feed ÷ 500 kg =

0.01 mg a.s./kg bw/d

Realistic worst-case estimate:

Instead of assuming that 100% of the livestock feed is packaged in treated paper/cardboard, a more realistic assumption is made, e.g. 10% of feed is packaged in treated paper/cardboard.

10% x 20 kg feed/d x 0.25 mg/kg feed ÷ 500 kg

= 0.001 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is not exceeded. No significant residues of the active substance in food of animal origin occur. Risk assessment can be stopped.

Possible Step 2 refinement options (in case the trigger value would have been exceeded):

- measurement of the actual active substance concentration in the packaging material
- determination of the active substance migration from paper into feed
- measurement of the actual active substance concentration in feed

For a complete exposure assessment, the calculation needs to be repeated for dairy cattle, pigs, broiler chickens and laying hens.

5.3.4.8 6.5.3 Treatment of materials that livestock animals may come into contact with.

Materials are treated with biocidal products to protect them from decay. Treated materials can be formed into structures that livestock animals have access to (e.g. wooden fence posts around paddocks), and may become part of animal housing and transport vehicles. In addition, existing structures may be treated with biocides. By chewing on (e.g. horses, rabbits, goats), rubbing against (large slaughter animals) or licking (e.g. ruminants) the treated materials, animals can take up residues of the biocidal product. In addition, volatile substances being released from the treated material may be inhaled. Only a fraction of the application amount will be available to animals and

can be quantified by the amount of material an animal comes into contact with and the amount of residue that can be extracted from the material.

For an exposure calculation, the following parameters may be needed. Default values for these parameters can be found in Appendix 5.3-1:

- Frequency of surface licking
- Amount of wood consumed
- Residue extraction from wood
- Body surface in contact with surface
- Alveolar ventilation rate
- Bodyweight

5.3.4.8.1 Examples of livestock exposure estimation –treatment of materials that livestock animals may come into contact with.

5.3.4.8.1.1 Example 3.1: Treatment of Materials – Exposure of horses to treated wood

Product: Wood protection product, VP = $1x10^{-4}$ Pa at 20°C, MW = 349.9 g/mol

Intended Use

Wood (used for edgings of stall in a horse stable) is treated with the biocidal product by vacuum pressure impregnation. The active substance concentration in the biocidal product is 0.5% w/w. Following treatment, the maximal concentration of active substance in the wood is 250 g/m^3.

Exposure Estimation

The treated wood is incorporated into edgings of the horse stall. Livestock animals can be exposed orally by chewing on the wood. Here the exposure is estimated for a horse. In the following calculations, default values from Appendix 5.3-1 are used.

Maximum absorption of biocidal product into treated wood = 50 L/m^3

Amount of active substance in the outer 1 cm layer of wood = 50 L/m^3 x 0.5% = 250 g a.s./m^3

Wood consumption: $1.9x10^{-5}$ m^3/d (value based on one study, not a confirmed default value)

Body weight: 400 kg

Realistic worst-case estimate:

Oral exposure:

250 g a.s./m^3 x $1.9x10^{-5}$ m^3/d ÷ 400 kg

= $1.1875x10^{-5}$ g a.s./kg bw/d

Dermal exposure:

Thickness of surface layer of the wooden wall representing the amount of substance per square meter = 0.05 mm

Amount of active substance per square meter: 250 g a.s./m^3 x $0.05x10^{-3}$ m = 12.5 mg a.s./m^2

Body surface area in contact with surface = 1.62 m^2

12.5 mg a.s./m^2 x 1.62 m^2 ÷ 400 kg

= 0.0506 mg a.s./kg bw/d

Inhalation exposure:

It is assumed that the animal is exposed to air containing the active substance at its saturated vapour concentration (SVC). This represents a worst-case as the active substance cannot achieve a higher concentration in the air.

SVC =

vapour pressure x molecular weight

gas constant x temperature in degrees Kelvin

$1x10^{-4}$ Pa at 20°C x 349.9 g/mol

8.31451 J/K mol x 293°K (equivalent to 20°C)

= $1.44x10^{-5}$ g a.s./m^3

= 0.0144 mg a.s./m^3

Alveolar ventilation rate = 43 m^3/d

Body weight = 400 kg

0.0144 mg a.s./m^3 x 43 m^3/d ÷ 400 kg

= 0.0015 mg a.s./kg bw/d

Total exposure:

oral exposure + dermal exposure + inhalation exposure

$1.1875x10^{-2}$ + 0.0506 + 0.0015

= 0.0639 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is exceeded. Proceed with a refined exposure assessment based on Tier II data or proceed with the EMA-CVMP guidance.

Possible Tier II refinement options:

- measurement of the amount of wood chewed by animals.
- measurement of the release rate of active substance from wood (if applicable, consideration of the period between wood treatment and the actual use of wood)
- information on evaporation of substance from treated wood
- transfer coefficient from a treated surface from Biocides Human Health Exposure Methodology[124] (page 171) might be applicable

For a complete exposure assessment, the calculation needs to be repeated for beef and dairy cattle, pigs, and goats.

5.3.4.9 Direct Treatment of Animals

Biocidal products used for the direct treatment of livestock are intended for general disinfection purposes or for repelling insects (flies, mosquitos, midges, ticks etc). They are to be distinguished from veterinary medicinal products, which are intended to prevent or treat disease. For example, the disinfection of teats is considered a biocidal use while treatment of teats for the prevention on mastitis is a veterinary medicinal use.

[124] Available on ECHA BPR ad hoc Working Group – Human Exposure webpage https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/human-exposure .

The use classification of products containing active substances with lethal effects on external parasites to be used on animals will depend on the intended use and/or demonstrated claims for the product

(https://circabc.europa.eu/sd/a/51ca9945-167d-411f-9763-92e634af9e1c/Biocides-2002-01%20-%20Borderline%20with%20%28veterinary%29%20medicinal%20products.pdf).

5.3.4.9.1 Teat disinfection

In the following example, the external exposure of dairy cows is estimated following treatment with a biocidal teat dip product. Teat dips can contaminate milk in two ways, indirectly via dermal uptake of the product by the dairy cow and subsequent partitioning of residues into milk, and directly by being washed into the milk during milking. A teat dip is a local treatment restricted to the udder of the dairy cow, and teat dip residues absorbed by the skin of the udder may potentially mainly be deposited in the tissue where the milk collects. In view of this, it has been considered to set a local trigger value for teat dips. However, residues taken up dermally by the animal can also enter the systemic circulation and be distributed throughout the animal. In addition, no numerical data are currently available on which to base a local trigger value. Hence residues from teat dips that are dermally taken up by the animal are compared to the trigger value of 0.004 mg as/kg bw in livestock (see calculation A in the example below). For residues that go directly into the milk (no dermal uptake assumed), a worst case consumer exposure (WCCE) should be calculated and compared to the ADI (see calculation B in the example below). It should be noted that the WCCE is exceptionally provided here, as normally the evaluation of the WCCE is described in the EMA-CVMP guidance.

The following parameters are needed (default values for these parameters can be found in Appendix 5.3-1, otherwise use data provided by the applicant):

- Number of daily milkings: default value is 2/day;
- Volume of product applied to teats per cow and milking: default values are 10 mL for dipping, 20 mL for spraying, 2.5 mL for foams;
- Fraction of applied product remaining on teats: The Emission Scenario Document for PT3 products highlights that the amount of the disinfectant remaining on teats depends on the viscosity of the solution and indicates to use 0.5 of the fraction of disinfectant remaining on teats as a worst case. The value is presented as a conservative value;
- Bodyweight of the dairy cow: default value: 650 kg bw;
- Daily milk yield of the dairy cow: default value: 20 L/day.

Three different cases can be distinguished depending on the intended use:

1. Pre-milking teat disinfection: Perform calculation A and B
2. Post-milking teat disinfection: Perform calculation A and B
3. Both pre- and post-milking teat disinfection: Perform calculations A and B twice (i.e. once for pre-milking teat dip and once for post-milking teat dip).

When no information on dermal absorption through teat skin is available, the WCCE for calculation A and B, is the maximum WCCE from either A or B. When information on dermal absorption through teat skin is available, the WCCE for A and B is the sum of WCCEs.

Calculation A assumes that the fraction of the biocidal product that remains on the teats is carried over into the animal (i.e. no residues will directly enter the milk because of contamination). With this assumption, residues can be expected in milk and/or tissues after some hours or days after application depending on the ADME rate of the animal for this compound.

Calculation B assumes that the fraction of the biocidal product that remains on the teats is carried over directly into the milk (i.e. all residues appear in the milk after milking). With this assumption, no biocidal product is taken up by the animal (i.e. the route of dermal uptake can be ignored) and residues in tissues are not expected; no biocidal product is lost in the milking process because of wiping or other handling procedures.

NOTE to the reader:

It should be highlighted that for the aim of this guidance document, (i.e. to estimate whether further information is needed and an MRL procedure should be started), the EMA food basket should be applied. The daily milk consumption in the EMA food basket is 1.5L/day. The food basket is mainly reflecting the dietary pattern of adults, which differs from the children's pattern. This difference is not fully covered by the food basket, but the EMA considered that the system in place for the establishment of MRLs for milk is adequate also for children (EMEA/CVMP/391/02-FINAL-corrigendum November 2002). In case consumer exposure to an active substance is performed **only** with the aim of the estimation of the dietary risk assessment and the MRL status of this active substance is not to be established, other EU agreed consumption figures might be applied to consider the different daily milk intake of the toddler and children, as milk is a relevant commodity for both toddler and children. Data from EFSA food consumption database or EFSA PRIMo model can be used for this purpose.

5.3.4.9.1.1 Example 4.1: Direct Treatment of Animals –Teat disinfection through dipping

Product: Disinfectant

Intended Use

The product is used for the disinfection of teats on dairy cows and is used twice daily before and after each milking. Prior to the next milking, teats are cleaned with a detergent. For each teat disinfection, 10 mL product with an active substance concentration of 2000 ppm (C_prod = 2 mg a.s./mL) are used per animal per treatment. The fraction of product remaining on teats is 0.5 of the fraction applied on the teats (according to ESD for PT3).

Exposure estimation

In the following calculations, default values from Appendix 5.3-1 are used.

Screening:

n= Number of milkings per day = 2 milkings/day

V_prod = Product volume on teats per milking: 10 mL/milking for 4 teats (default value only applies in case no volume is specified on the product label);

f_prod = The fraction of product remaining on teats is 0.5 of the fraction applied on the teats.

bw = Body weight of the dairy cow = 650 kg bw

V_milk = daily milk yield of the dairy cow = 20 L/day

Screening calculation A

Dermal exposure via teat dips (assuming 100% dermal absorption, a product concentration of 2 mg a.s./mL and 0% degradation of the active substance):

n x (V_prod x f_prod x C_prod) / bw

2 milkings/day x (10 mL/milking x 0.5 x 2 mg a.s./mL) ÷ 650 kg bw

= 0.031 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d for livestock is exceeded. Proceed with a refined exposure assessment based on Tier II data.

In case of pre- and post-milking teat disinfection, this calculation needs to be performed twice (i.e. once for pre-milking teat disinfection and once for post-milking teat disinfection). V_prod, f_prod and C_prod could be different.

Screening calculation B

Estimated residues in milk through contamination during milking (assuming 0% dermal absorption, an product concentration of 2 mg a.s./mL, and assuming 0% degradation of the active substance):

n x (V_prod x f_prod x C_prod) / V_milk

2 milkings/day x (10 mL/milking x 0.5 x 2 mg a.s./mL) ÷ 20 L/day

= 1 mg a.s./L

The estimated residues in milk cannot be compared to the trigger value of 0.004 mg a.s./kg bw/d for livestock, because such trigger value is related to the external exposure of the livestock (see the section "Tier I: initial external exposure estimation" for further information).

The worst case consumer exposure (WWCE) should be calculated applying EMA standard food basket:

WCCE= amount a.s. transferred into milk * I_{milk}÷ bw human

Amount a.s. transferred into milk = amount of the active substance transferred into milk as estimated in the first step of the calculation B.

I_{milk} = daily milk consumption (from EMA food basket: 1.5 L/day).

Bw_human = default body weight for adult (60 kg bw).

WCCE= (1 mg a.s./L*1.5 L)/60 kg bw

WCCE=0.025 mg a.s./kg bw/d

→ If WCCE is above 30% of the ADI, proceed with a refined exposure assessment based on Tier II data.

In case of pre- and post-milking teat disinfection, this calculation needs to be performed twice (i.e. once for pre-milking teat dip and once for post-milking teat dip). V_prod, and C_prod could be different.

Combining calculations A and B

WCCE calculation for calculation A:

I_{milk} = daily milk consumption (from EMA food basket: 1.5 L/day = 1.5 kg/day)

$I_{tissues}$ = daily edible tissue consumption (from EMA food basket: 0.5 kg tissues made up of 0.300 kg of muscle, 0.100 kg of liver, 0.050 kg of kidney and 0.050 kg of fat)

WCCE= amount a.s. transferred into milk and edible tissues * ($I_{tissues}$ + I_{milk}) ÷ bw human

WCCE= 0.031 mg a.s./kg bw/d * (0.5 kg + 1.5 kg) ÷ 60 kg bw= 0.001 mg a.s./kg bw/d

WCCE calculation for A= 0.001 mg a.s./kg bw/d

WCCE calculation for B= 0.025 mg a.s./kg bw/d

When no information on dermal absorption through teat skin is available, the WCCE for calculation A and B, is the maximum WCCE from either A or B. So in this case the WCCE = 0.025 mg/kg as/day, based on calculation B (0% dermal absorption).

For pre- and post-milking disinfections, it means the maximum contribution from pre-milking (A or B) needs to be added to the maximum contribution from post-milking (A or B).

If the overall WCCE is above 30% of the ADI, proceed with a refined exposure assessment based on Tier II data or proceed with the approach described in the EMA-CVMP guidance.

Both calculation (A and B) need to be conducted. Ideally calculation A and B should be corrected for % dermal absorption (see Tier II refinements below) i.e. the portion of the residue absorbed in the animal cannot be found in the milk through direct contamination.

Dermal absorption:

When information on dermal absorption through teat skin is available, the WCCE for A and B is the sum of WCCEs based on the formula D x WCCE (calc A) + (1-D) x WCCE (calc B), where D is dermally absorbed fraction. For example if a dermal absorption (D) of 20% was found for teat skin, the sum of WCCE would be calculated as:

Sum of WCCEs from calculation A and B

$WCCE_{calculation\ A}$ + $WCCE_{calculation\ B}$

= 0.2 x 0.001 mg a.s./kg bw/d + (1-0.2) x 0.025 mg a.s./kg bw/d = 0.020 mg a.s./kg bw/d

For pre- and post-milking applications, calculation A consists of two contributions and calculation B consist of two contributions.

Possible Tier II refinement option:

Dermal absorption is likely to be between 0 and 100% and part of the residue may evaporate or be wiped off in the milking process and therefore Tier II refinement options are encouraged:

- Pre milking products are normally less viscous compared to the post-milking products and the teat is cleaned before milking. Therefore, if information is available, consideration could be given in reducing the fraction of the product (f_prod) that remains on the teat (for calculation A and B).

- Measurement of the amount of residues in the milk at various time-points after application, to determine the likely residue levels in milk (to get an indication whether both calculations A and B are needed and to refine the WCCE from milk). Measurement of residues in the milk just after the treatment shows the direct contamination of the milk. With a continuous teat treatment over the days, the active substance might be absorbed and absorption may reach a plateau. After some days of the treatment, the measured residues correspond to the amount from direct milk contamination and the plateau of the absorption. The measurement of the amount of residues in milk at the plateau of the absorption can be used directly in the WCCE.

- Measurement of the amount of residue remaining on teats in the time period between cleaning after teat-dip application and milking. Ideally, measurement of residues on the teats should be performed just after the application and after the cleaning to estimate the fraction of the product wiped off, which is not available for absorption or direct milk contamination.

- Dermal absorption of the residue through teat skin to determine the amount of residue available for systemic circulation within the animal (this refinement option is relevant for calculation A and B). Calculation A needs to be multiplied by D and calculation B needs to be multiplied by (1-D), where D is a fraction between 0-1 representing the amount available for dermal absorption.

Conclusion:

If one result (from calculation A or B or A+B) exceeds the trigger value or the 30% of the ADI respectively, further refinement can be performed based on additional data. In case after refinement the 30% for the ADI is still exceeded, further evaluation of the substance by the CVMP is required.

5.3.4.9.2 Foot/Hoof Disinfection

Animals walk through disinfection baths at least twice daily when they exit and enter the stable/milking parlour. Dairy cows walk through six times because they are milked twice a day and let out to graze. The bath is set up at the entrance of the stable or the milking parlour. Although the disinfectant is meant for hooves only, contact with the skin should always be assumed. The depth of the level of disinfectant in the bath will often be above the hoof and splashing will occur as the animals walk through the bath. Some hoof disinfectant baths consist of foam rather than liquid formulations. Foam formulations contain volatile components available for inhalation and exposure to foam formulations lasts longer as foam adheres to legs.

5.3.4.9.2.1 Example 4.1: Direct treatment of Animals – Exposure via hoof disinfectant baths

***NOTE**: An example product for this use has not been submitted at EU-level. The following calculations are based on a hypothetical product with a hypothetical application scenario.*

Product: Disinfectant

Intended Use

The formulation is filled into shallow tubs which animals walk through as they enter or exit their stable /milking parlour. Each tub contains 375 L foam with an active substance concentration of 100 mg/L. A single tub is sufficient for 100 walk-through events.

Exposure estimation

The exposure is calculated for a dairy cow. In the following calculations, default values from Appendix 5.3-1 are used.

Screening:

(calculated for 1 walk-through event of a single cow)

Number of animals per stable: 100; in case the hoof disinfection is performed on dairy cows from or to the milking parlour, a number of 82 cows should be considered unless a different information is provided by the applicant (See footnote of the Table 2, Animal housing, for further information). In this specific example, it is indicated that a single tub is sufficient for 100 walk-through events bath, therefore 100 cows are considered.

Bodyweight: 650 kg

(375 L product x 100 mg a.s./L product) ÷ 100 animals/stable ÷ 650 kg bw/animal

= 0.5769 mg a.s./kg bw

Realistic worst-case estimate:

Oral exposure:

Oral exposure is not considered relevant, since cattle do not lick or groom their hoofs

(calculated for 2 daily walk-through events of a single cow).

Dermal exposure from walking through the bath:

Daily passes through the tub = 2

Exposed skin/hoof area = 1590 cm^2

Layer of product absorbed = 0.01 cm

Body weight = 650 kg

To calculate the product amount in contact with one hoof/skin:

0.01 cm x 1590 cm^2 = 15.9 cm^3 = 0.0159 L

If 1 L product contains 100 mg a.s., then 0.0159 L product contains 1.59 mg a.s.

Assuming each hoof steps into the hoof bath once at each pass through the bath, then the amount of a.s. each animal comes into contact with during one pass equals -2 x 1.59 mg a.s. = 3.18 mg a.s.

3.18 mg a.s. x 2 daily passes ÷ 650 kg

= 0.0098 mg as/kg bw/d

Inhalation exposure from breathing in vapours released from the formulation:

Inhalation exposure is considered to be negligible. Exposure is transient as livestock traverses the hoof disinfection bath within a matter of seconds, and vapours do not diffuse in significant amounts beyond the entrance/exit area.

Total exposure:

dermal exposure

= 0.0098 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is exceeded. Proceed with a refined exposure assessment based on Tier II data or proceed with the EMA-CVMP guidance.

Possible Tier II refinement option:

- measurement of the amount of residue on hoofs and legs

5.3.4.9.3 Insecticides and Repellents

The products included in this category are products with repellent and/or insecticidal activity (PT 18 and 19) that are not classified as veterinary drugs. Examples of such products are collars, neckties, ear tags, dips, skin and bath treatments and products used to control fish parasites.

5.3.4.9.3.1 Example 4.2: Direct Treatment of Animals – Exposure via fly ear tags

Product: Fly treatment

Intended Use

The product is supplied as ear tags for cattle and has a biocidal effect against flies. Up to two ear tags are attached to each animal, and tags are effective for one whole fly season. Each ear tag contains 935 mg active substance, which is released gradually onto the surface of the tag throughout the season. Through body movements, the lipophilic active substance is transferred onto the hairs of the animal's coat. From there it is dispersed all over the animal, giving protection to the entire body. The release rate of the active substance to the surface of the tag depends on the amount that is removed from the tag. For the purpose of this exposure calculation, an instant release rate is assumed.

Exposure estimation

In the following calculations, default values from Appendix 5.3-1 are used.

Residues can be taken up by the animal through dermal absorption and through grooming. Calculation of dermal uptake assuming 100% absorption covers all paths of exposure.

Body weight = 500 kg

Dose rate 935 mg a.s. x 2 ear tags/animal = 1870 mg as/d

Screening:

1870 mg a.s./d ÷ 500 kg

= 3.7400 mg a.s./kg bw/d

→ The trigger value of 0.004 mg a.s./kg bw/d is exceeded. Proceed with a refined exposure assessment based on Tier II data or proceed with the EMA-CVMP guidance.

Possible Tier II refinement options:

- measurement of the amount of residue on the animal's skin
- release rate of the ear tags

For a complete exposure assessment, the calculation needs to be repeated for dairy cattle.

5.3.4.10 Treatment of Aquaculture

The available literature on parameters needed for the exposure assessment of fish is scarce, and reliable default values cannot be established. Consequently, for fish, Step 1 exposure assessment must be skipped unless the Applicant can provide a well justified exposure calculation model. Future development of an assessment model for fish would be useful. The following paragraphs provide some general information on the exposure of fish.

Biocidal products such as disinfectants and antifoulants are used for the protection of structures (e.g. control of growth and settlement of fouling organisms in fish tanks, on fishnets etc.) and for water hygiene in aquaculture. Fish can be exposed orally, dermally and through respiration via the gills. In the case of water treatment in fish enclosures, residues are evenly distributed throughout the water and fish are exposed via all pathways.

The treatment of structures usually occurs on dry land. After the treated objects have been put into the water the active substance of the biocidal product is normally only slowly released in order to maintain its desired effect of the biocidal product. The released substances are diluted in the surrounding water and are available for uptake by fish. Exposure to the fraction remaining on the treated structure can also occur, in particular when fish come into frequent contact with the treated structure.

5.3.5 Tier II - Principles for exposure estimation

NOTE to the reader:

In this section principles for exposure estimation are laid down. Due to the complexity of Tier II exposure estimations, a comprehensive description of methods for all possible scenarios is not feasible. It should be noted that a Tier II refinement does not necessarily involve performing new studies. Any reliable existing data and/or information that is suitable for refinement purposes can be used. The principles outlined below can be used to help design Tier II trials and build suitable models to estimate exposure from the obtained data on a case-by-case basis.

When the first step of external exposure assessment results in the exceedance of the trigger value of 0.004 mg/kg bw/day, the exposure estimate can be refined in a second tier assessment.

Within Tier I, a realistic worst-case estimate of exposure is given. In Tier II, a further refinement of the estimation of external exposure is performed based on specific data provided by the Applicant related to the active substance and its actual intended use. This may include data already provided by the Applicant, such as information on

substance degradation. The Applicant may also submit additional studies providing data for refinement.

Examples for Tier II studies include:

- Studies to allow the identification and quantification of the available active substance or of its degradation products in the treated area (treated surfaces, materials, objects, air, water or feed, the animal itself) at the time animals are exposed (e.g. if animals are not present during treatment, degradation or volatilisation of the active substance may occur before animals have the opportunity to take it up). When taking into account the degradation rate of an active substance, it has to be considered that degradation products may be more toxic and more persistent than the active substance itself, and an exposure assessment based on the residues of the active substance as well as the toxic degradation products has to be performed using the same step-wise approach as for the a.s. Data on abiotic degradation (hydrolysis, photolysis) can be found in the environmental part of the dossier. Measurement of the concentration of active substance on insects or determination of the LD_{50} for insects can be used in place of the active substance concentration in/on insects.
- Studies to allow the quantification of the dislodgeable fraction, (i.e. the amount of active substance that can be removed from the treated surface), of the active substance or of its degradation products from the treated area (e.g. wiping tests mimicking licking/rubbing behaviour of animals). The biocidal product must remain available at the application site for being effective. It can therefore be assumed that only a fraction of the residue on treated surfaces (the dislodgeable fraction) is available to the animal. Experimental values of the dislodgeable fraction can be used in the calculation. When the product is applied as granules, dislodgeability is not an issue, because granules do not stick to surfaces. For ear tags, the release rate can be determined.
- Studies characterising the effectiveness of a required rinsing step or a justification proving the effectiveness of rinsing based on scientific data or information (e.g. water solubility of the active substance);
- Measurement of the release rate of active substance from treated wood to allow determination of residues remaining after a certain time period (e.g. after a withdrawal period);
- Measurement of the release rate of active substance from e.g. ear tags;
- Studies of exposure patterns linked, for instance, to the behaviour of the exposed animals (e.g. amount of wood chewed).

Tier II can be omitted in favour of proceeding directly to the next phase of risk assessment as detailed in the EMA-CVMP guidance.

5.3.5.1 Principles for design of Tier II trials

The following section outline some principles that should be taken into consideration when performing tier II trials:

- **Relevant residue**: Before obtaining data, the composition of the relevant residue has to be defined. The relevant residue consists of all toxicologically relevant substances (active substance and possibly degradation products) that remain on treated areas as a result of the use of the biocide in question. Radiolabelled studies on the fate of the active substance (i.e. degradation into toxicologically relevant compounds, formation of reaction products) as well as data on the reactivity of the active substance would provide the necessary information;
- **Analytical method:** A valid analytical method is needed in order to perform measurements. All compounds that comprise the relevant residue (this may include

the active substance and toxicologically relevant metabolites, degradation products, by-products and excipients) have to be accounted for;

- **Time frame:** To define a time frame for the trial, the degradation rate/reaction rate as well as the label instructions can be taken into account. When degradation/reaction occurs, a minimum time frame of 2x the half-life might be appropriate. The conditions of degradation/reaction compared to the conditions in the treated area must be considered. If no degradation/reaction occurs, the frequency of application according to label instructions can serve as a guide;
- **Number of trials:** Measurements should be performed at various time points to adequately capture the degradation of the active substance throughout the treatment period;
- **Site selection, site requirements:** Trials should be performed under realistic circumstances (e.g. in an actual stable) or under conditions reflecting realistic circumstances. The material treated and the application rate must reflect the intended use of the biocidal product;
- **Application of biocidal product:** Trials should be performed using the highest proposed rate of application and using the formulation in question. In cases where multiple applications are intended, this should be reflected in the residue trial;
- **Sampling:** Sampling should occur under as realistic circumstances as possible. Since residue levels will vary within the treated area or in the treated feed/water, several samples have to be obtained. Conditions and time period of storage should be considered as well. For example, for feed stored in treated tanks, samples from the feed layer in direct contact with the tank surface and samples from the inner layers of feed would be obtained and the results averaged. Where no single type of feed is specified, several types of feed need to be tested in order to identify the critical case. For example, for water stored in treated tanks, samples should be taken at various time points to account for the maximum period the water is stored within the treated tank.

Data obtained from the studies are used to make refined exposure estimate(s) for an appropriate time period (e.g. day 1, day 2 etc.) and subsequently each exposure estimate is compared to the trigger value. In cases where the trigger value is exceeded only for the initial exposure period (e.g. only day 1 and 2) management options may be considered. Where the trigger value is exceeded for a longer time period then dietary risk assessment has to proceed to follow the approach detailed in the EMA-CVMP guidance.

Appendix 5-3

Appendix 5-3 (1) Default Value Working Tables

- **Table 41: Animal Size and Physiology** (for references and explanations see Table 56)

Animal Species	**Body weight (kg)**	**Animal height (cm)** Height to withers or shoulder/ height to top of head/ maximum reaching height	**Body surface area (m^2)** calculated from default bw	**Body surface area in contact with surface (m^2)** (30% of total body surface area)	**Alveolar ventilation rate (l/h)** resting AVR calculated from default bw, (to account for activity use a correction factor of 3)	**Alveolar ventilation rate (m^3/d)** resting AVR calculated from default bw, (to account for activity use a correction factor of 3)	**Feed intake (kg dry matter/day)** based on default bw	**Drinking water intake (l/d)** based on default bw
Beef cattle	500	145/161/177	4.8	1.44	2110	51	12	50
Dairy cattle	650	145/161/177	5.6	1.68	2589	62	25	115
Calf	200	116/129/142	2.9	0.87	1032	25	8	20
Fattening pig	100	77/-/92	1.5	0.45	601	14	3	10
Breeding pig	260	110/-/125	2.8	0.84	1267	30	6	15
Sheep	75	65/72/79	1.5	0.45	480	12	2.5	10
Lamb	40	61/67/73	1.0	0.30	294	7	1.7	5
Slaughter goat (=goat kids)	13	43/57/200	0.5	0.15	122	3	0.5	1.3
Lactating goat	70	76/100/200	1.5	0.45	455	11	2.8	7
Broiler chickens	1.7	-/25/-	0.05	0.015	8.2	0.2	0.12	0.25
Laying hen	1.9	-/25/-	0.05	0.015	8.9	0.2	0.13	0.25
Turkey	7	-/34/-	0.3	0.090	23	0.6	0.5	1.0
Horse	400	158/196/234	5.4	1.62	1773	43	16	40

Rabbit	2.5	-/0.3/-	0.20	0.060	34	0.9	0.25	0.5

- **Table 42: Animal Housing** (for references and explanations see Table 56)

Animal Species	**Number of animals per stable**	**Floor area per stable (m^2)**	**Wall and floor area per stable (m^2)**	**Housing volume per stable (m^3)**	**Floor area per animal (m^2)**	**Maximum area (wall) within reach of animal (m^2)** considering max reaching height (No of compartment walls considered)	**Maximum area within reach of animal (wall+floor) (m^2)** considering max reaching height (No of compartment walls considered)	**Exposed feed surface per animal (m^2)** in case of direct treatment of troughs	**Exposed feed surface per animal (m^2)** in case of treatment of surfaces surrounding troughs	**Ventilation rate housing (m^3/h)** per 500 kg live weight	**Ventilation rate housing (m^3/h)** per animal	**Ventilation rate housing (1/h)** air exchanges per hour
Beef cattle	125	370	1000	3063	2.96	10.8 (3)	13.7 (3)	2.6	0.7	Winter min 50 Summer max 333	Winter min 50 Summer max 333	Winter min 2 Summer max 13.6
Dairy cattle	100*	1170	1670	9630	11.7	21.4 (3)	33.1 (3)	6.6	2.9	Winter min 67 Summer max 417	Winter min 87 Summer max 542	Winter min 0.9 Summer max 5.6
Calf	80	160	330	590	2.0	8.5 (4)	10.5 (4)	2.0	0.5	Winter min 75 Summer max 500	Winter min 30 Summer max 200	Winter min 4.1 Summer max 27.1
Fattening pig	400	600	970	2110	1.5	4.0 (3)	5.5 (3)	1.2	0.4	Winter min 50 Summer max 500	Winter min 10 Summer max 100	Winter min 1.9 Summer max 19.0

Breeding pig **- individual housing** **- group housing**	132 132	560 710	910 1160	1960 2480	4.2 5.4	9.1 (3) 10.3 (3)	13.4 (3) 15.7 (3)	2.4 2.8	1.1 1.3	Winter min 100 Summer max 1000	Winter min 52 Summer max 520	Winter min 3.5 Summer max 35,0 Winter min 2.8 Summer max 27.7
Broiler chickens **- free range, litter floor** **- parent broiler chickens, free range (grating floor)** **- parent broiler chickens in rearing, free range (grating floor)**	20000 7000 9000	1110 390 500	1600 600 750	4170 1458 1880	0.056 0.056 0.056	- - -	- - -	- - -		Winter min 278 Summer max 1853	Winter min 0.9 Summer max 6.3	Winter min 4.3 Summer max 30.2 Winter min 4.3 Summer max 30.2 Winter min 4.3 Summer max 30.2

Laying hen **- battery** **- free range (litter floor)** **- Free range (grating floor)**	21000 10000 20000	750 1430 1270	1100 2030 1822	2810 5360 4780	0.036 0.14 0.064	- - -	- - -	- - -	0.01	Winter min 175 Summer max 2000	Winter min 0.7 Summer max 7.6	 Winter min 5.2 Summer max 56.8 Winter min 1.3 Summer max 14.2 Winter min 2.9 Summer max 31.8
Rabbit	5 per cage	0.24 per cage	0.84 per cage	0.072 per cage	0.048	0.27 (4)	0.32 (4)					

* **Please, note** that for the purposes of the human exposure estimation, the number of the dairy cows that are milked daily corresponds to 82. According the ESD for PT3, the default value for a dairy cow herd side is 100 animals. Dairy cows are regularly milked twice per day. The lactation period for dairy cows is normally 270 lactating period of 300 days, 82 milk producing cows are milked per day, from a herd of a 100 dairy cows.

From Recommendation number 13 of the ad hoc WG Human exposure [https://echa.europa.eu/documents/10162/21664016/recommendation_13_teat_disinfection_en.pdf/fbeb394b-e74b-685d-c231-5e3a530e311c].

Table 43: Animal Transport

(for references and explanations see Table 56)

Animal Species	**Time spent in transport vehicles (h)** transport + resting period + transport	**TRUCK** **No of floors/No of compartments per floor/No of animals per compartment** Default truck of 7.0m x 2.5 m	**COMPARTMENT** **Length (m)/ Width (m)/ relevant height (m)**	**Required floor area per animal during transport (m^2)**	**Available wall+floor area per animal (m^2)** within a compartment	**Available volume per animal (m^3)** within a truck of 7.0m x 2.5 m	**Ventilation rate**
Beef cattle	14+1+14	1/2/6	3.5/2.5/1.8	1.35	5.1	2.6	Forced ventilation systems 60 m^3/h/kN loading capacity (with 1000 kg = 9.80665 kN) and a temperature between 5-30°C
Dairy cattle	14+1+14	1/2/5	3.5/2.5/1.8	1.61	6.1	3.2	
Calf	14+1+14	2/2/11	3.5/2.5/1.5	0.73	2.4	1.2	
Fattening pig	24	3/2/20	3.5/2.5/1.0	0.43	1.0	0.4	
Breeding pig	24	2/2/10	3.5/2.5/1.3	0.80	2.4	1.1	
Sheep (with wool)	14+1+14	2/2/18	3.5/2.5/0.8	0.47	1.0	0.4	
Lamb	9+1+9	3/2/35	3.5/2.5/0.8	0.25	0.5	0.2	
Slaughter goat (=goat kids)	9+1+9	3/2/62	3.5/2.5/1.0	0.14	0.3	0.1	
Lactating goat	14+1+14	2/2/16	3.5/2.5/1.5	0.53	1.7	0.8	
Broiler chickens	24	8/12/53	1.17/1.25/0.27	0.0272	0.052	0.0074	
Laying hen	24	7/40/14	0.88/0.5/0.27	0.0304	0.085	0.0084	
Turkey	24	6/6/39	1.17/2.5/0.40	0.0735	0.15	0.030	
Horse	24	1/2/5	3.5/2.5/2.4	1.75	7.5	4.2	

Default values for transport crates for rabbits can be found in an EFSA document at http://www.efsa.europa.eu/en/efsajournal/doc/1966.pdf .

- **Table 44: Miscellaneous Values and Calculations**

	Animal Species	Description	Default	Background Information Remarks	References
1	Dairy cattle	Daily milkings	• 2 milkings/day	• Number of milkings per day may be more frequent, e.g. 3 times per day for high production cows. • For reasons of consistency EMA prefers the number of 2 milkings a day in their evaluations.	EMA Guidance Document: Note for Guidance for the Determination of Withdrawal Periods for Milk; EMEA/CVMP/473/98-FINAL http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004496.pdf • Information given by MS
2	Dairy cattle	Volume of teat dip	• For dipping 10ml/cow/milking • For spraying 20ml/cow/milking • For foams 2-2.5 ml/cow/milking	• In most cases the volume to be applied will be given by Applicant (instruction for use). In all other cases the default value based on information from ES and FR will be applied.	Information provided by ES and FR Pauline Brightling, Graeme A. Mein, Jakob Malmo, Diane P. Ryan. TN07 Lactation, pp. 43. Countdown Downunder: Farm Guidelines for Mastitis Control, ISBN 0 642 37362 0
3	Calf	Surface area of tongue	0.008 m^2		• Information provided by SE
4	Calf	Frequency of surface licking	10 licks per day	• Pen licking frequencies in the studies provided were 2-30 per day and are highly dependent on the calf`s environment • In the studies, licking frequency was not defined. Thus, the question arose whether a licking frequency is a single lick or a distinct period of time during which an animal engages in licking behaviour. When a calf engages in a licking incident, it might not lick widely across a large surface, but basically lick repeatedly at the same general spot on a surface.	• Verga M, Pavesi M, Cerutti F, Behaviour and performance of veal calves under different stabling conditions. Ann. Zootech., 1984, 34 (3), 247 – 256 • Boe K.E., Andersen I.L., Early weaning of calves – how does it affect the behaviour?, pp 604 – 610, in livestock environment VI: Proceedings of the 6th international symposium. 2001 ASAE Number 701P0201. ISBN: 1892769212 • Phillips C.J.C., The effects of Forage Provision and Group Size on the Behaviour of Calves. J. Dairy Sci. 2004, 87: 1380 – 1388. • Margerison J.K., Preston T.R., Berry N., Phillips C.J.C, Cross-sucking and other oral behaviours in calves, and their relation to cow suckling and food provision. Applied

				So, for our calculation it would not make a difference, whether the calf licks once at the same spot or several times during one licking incidence. In the calculation we assume anyway that the entire amount of a.s. on the licked spot is taken up by the animal, so whether this happens with one lick or several is inconsequential.	Animal Behaviour Science, 2003, 80 (4), 277-286. doi:10.1016/S0168-1591(02)00231-9. ISSN: 0168-1591
5	Cattle	Volume of tub for hoof disinfection	375 l	Defaults as given in the ESD: For the disinfection of animals' feet, basins filled with biocides are used. The volume of the bathing device can vary between 375 l and 675 l. In order to cover a worst case, a tub content of 375 l is assumed, which is replaced after 100 walk-through events. For a stable with 100 dairy cows which are milked twice a day, four tub fillings per day are needed.	ESD for Product Type 3: Emission scenarios for veterinary hygiene biocidal products (JRC Scientific and Technical Reports, 2011); EUR 25116 EN – 2011; JRC 67706; doi:10.2788/29747. http://echa.europa.eu/es/guidance-documents/guidance-on-biocides-legislation/emission-scenario-documents
6	Cattle	Daily passes through hoof disinfection tub	Dairy cow: 2	Hooves of dairy cows are regularly disinfected. Cows walk through tubs containing the disinfection solution on their way from or to the milking parlour. As the default number of daily milking event is 2, the daily passes through the hood disinfection tub is set at 2 accordingly.	ESD for Product Type 3: Emission scenarios for veterinary hygiene biocidal products (JRC Scientific and Technical Reports, 2011); EUR 25116 EN – 2011; JRC 67706; doi:10.2788/29747.http://echa.europa.eu/es/guidance-documents/guidance-on-biocides-legislation/emission-scenario-documents
7	Cattle	Number of ear tags per animal	2		

8	Pig	Surface area of tongue	0.008 m^2		Information provided by DE
9	Pig	Frequency of surface licking	10 licks per day	Due to unavailability of literature, the value was adopted from the information on calves.	
10	Chicken	Number of dead flies consumed by chicken	• 10 dead flies per chicken per day	• Educated guess by DRAWG • For evaluation it should be calculated how many flies a chicken must eat in order to reach the trigger value of 0.004 mg/kg. To evaluate the result of this calculation a default value of 10 flies per chicken and day was considered reasonable based on expert judgement. Based on information on stable dimensions in the ESD for veterinary hygiene biocidal products this would refer to about 70 flies/m^2 (10000 laying hen on litter floor, total floor area 1430 m^2) or 180 flies/m^2 (20000 broiler chickens on litter floor, total floor area 1110 m^2).	
11	Chicken	Biocidal product consumption by flies	• Average body weight of fly: 10-12 mg • Sucrose intake 2.5-3.5 mg per fly per day	• Flies cover all other insects that may possibly be the target of biocidal products. • It appears that biocidal product uptake for 24 hours seems a realistic scenario. • It is reasonable to assume that daily biocidal product intake by the fly does not exceed daily sucrose intake.	• T. Michael Cooper, Robin J. Mockett, Barbara H. Sohal, Rajindar S. Sohal, and William C. Orr, Effect of caloric restriction on life span of the housefly, Musca domestica. The FASEB Journal express article10.1096/fj.03-1464fje. Published online August 19, 2004. http://www.fasebj.org/content/early/2004/10/02/fj.03-1464fje.full.pdf

12	Chicken	Floor area covered by animals	• 50%	• See also Example 1.2	
13	Horse, goat, rabbit	Amount of wood consumed	• Horse: no default set • Rabbit <1.25 g/d • Goat: no default set	• Horses: Stereotypic behaviour of wood chewing develops at a higher rate in horses kept in barns and stables, however horses generally do not swallow the wood.. • Rabbit: <0.5% of the total feed intake (considering default feed intake this is < 1.25 g per day) Normal browsing behaviour of goats includes oral investigation of everything in their environment. Goats chew on pen partitions or other structures made of wood; they will chew on almost everything if the goat considers it palatable.	• Horse Broom D.M. and Fraser, A.F., Domestic animal behaviour and welfare, 4th Edition, CAB International, Cambridge, UK, 2007; ISBN-13: 978-1845932879; p. 236 mentions 'wood consumption by wood chewer (horse) of 0.5 kg of wood per day from edges of stalls' but this figure is not supported by experimental data. Wood chewing by stabled horses: diurnal pattern and effects of exercise. W.E. Krak, H.W. Gonyou and L.M.Lawrence; J. Anim. Sci.; 1991, 69, p. 1053-1058. Highest reported values in the study are $1.9x10^{-5}$ m^3 and 9.8 g per day (the results are not consistent). • Rabbit: Jordan, D; Gorjanc, G; Kermauner, A; Stuhec, I., Wooden Sticks as Environmental Enrichment: Effect on Fattening and Carcass Traits of Individually Housed Growing Rabbits; World Rabbit Science, 2008,16 (4):237-243, • Goat Papachristou, T.G.; Dziba, L.E.; Provenza, F.D. ,Foraging ecology of goats and sheep on wooded rangelands, Small Ruminant Research 59 (2005) , n.2-3, 141–156 Mary C. Smith & David M. Sherman, Goat medicine, 2nd Ed., 2009 Blackwell Publishing, USA. ISBN:978-0-781-79643-9
14	/	Extraction from wood	• 100%	• Option for refinement if sufficiently justified	
15	/	Maximum absorption of biocidal	• Treatment with double vacuum pressure: 50L/m^3		Biocides Human Health Exposure Methodology, Wood preservatives, Page 47: "In vacuum-pressure processes, wood absorbs 150 litres of preservative solution per m^3. In double vacuum processes, wood absorbs 10 to 50 litres of

		product into treated wood	(amount in outer 1 cm layer of wood) • Treatment by dipping: 0.05 L/m^2 (amount in outer 1 cm layer of wood)		preservative solution per m^3. In pressure processes, wood absorbs around 300 litres per m^3. For dipping etc., wood appears to absorb 0.2 litres per 4 m^2 fence panel." https://echa.europa.eu/es/about-us/who-we-are/biocidal-products-committee/working-groups/human-exposure
16	/	Density of wood	0.4 g/cm^3		Technical Agreements for Biocides (TAB) version 1.2 (Dec 2016)
17	/	Conversion of amount of active substance per cubic meter to a.s. per square meter	• Thickness of layer "representing" one square meter: 0.05 mm	• rough conversion calculation based on the assumption that a layer of 0.05 mm thickness is negligible and represents the amount of substance per square meter $c_{square} = c_{cubic} \times Th_{layer}$ C_{cubic}: Amount of substance per cubic meter of wood (mg/m^3) c_{square} Amount of substance per square meter of wooden wall (mg/m^2) Th_{layer} Thickness of layer "representing" one square meter (m)	
18	/	Emission factors for spraying	• Fraction emitted to floor during air space spray treatment: 0.96 • Fraction emitted to floor during surface treatment by spraying: 0.11		• OECD Series on Emission Scenario Documents Number 18; Emission Scenario Document for insecticides, acaricides and products to control other arthropods for household and professional uses, ENV/JM/MONO(2008)14, 17th July 2008 Table 3.3.-5 Review of the different emission factors for unspecified mode of spraying,

			• Fraction emitted to the treated surface during surface treatment by spraying: 0.85		
19	/	Dislodgeable residue	• 100%	• Option for refinement if sufficiently justified	
20	/	Amount of product hitting animals during treatment	Values to be applied in the formulas: • Available from description of intended use • Available from Tables 1, 2 and 3 • Thickness of layer of product in contact with skin (default 0.01 cm in TNsG on Human Exposure)	• For inhalation exposure apply equations given in the TNsG on human exposure: $C_{inh} = \frac{Q_{prod} \times Fc_{prod}}{V_{room}}$ $A_{inh} = \frac{F_{resp} \times C_{inh} \times Q_{inh} \times T_{contact}}{BW} \times N$ C_{inh} Average concentration in inhaled air (mg/m^3) Q_{prod} Amount of undiluted product used (mg) Fc_{prod} Weight fraction of active substance in the product V_{room} Volume of the room (m^3) A_{inh}Amount of active substance inhaled/respired (mg/kg bw/d) F_{resp}Inhalable or respirable fraction of product (default 1) Q_{inh}Ventilation rate of –animal (m^3/hour) $T_{contact}$Duration of exposure (hours) BWbody weight (kg)	Biocides Human Health Exposure Methodology https://echa.europa.eu/es/about-us/who-we-are/biocidal-products-committee/working-groups/human-exposure • OECD Emission Scenario Document for insecticides, acaricides and products to control other arthropods for household and professional uses, Table 3.3.-5, ENV/JM/MONO(2008)14, 17th July 2008

				N_{event}Number of events (usually per day) • For dermal exposure also equations are available in the TNsG on Human Exposure: $C_{der} = \frac{C_{\Pr od}}{D} = \frac{Q_{\Pr od} \times Fc_{\Pr od}}{V_{\Pr od} \times D}$ $A_{der} = C_{der} \times V_{appl} = C_{der} \times TH_{der} \times Al$ C_{der}Average skin concentration of active substance in product on skin (mg/cm^3) C_{Prod}Average concentration of substance in undiluted product DDilution factor (if dilution results in 1% dilution the D is 1/0.01 = 100, default is 1) Q_{Prod}Amount of undiluted product used (mg) Fc_{Prod}Weight fraction of active substance in the product V_{Prod}Volume of undiluted product (cm^3) A_{der}Amount of active substance on skin (mg, mg/event, mg/d, mg/kg) V_{appl}Applied volume of product in contact with skin (cm^3) TH_{der}Thickness of layer of product in contact with skin (cm)	

				$AREA_{der}$Surface area of exposed skin • During fumigations the applicator and presumably also livestock animals will not be present during application. (see OECD ESD for insecticides, acaricides …)	
21	/	Volatilisation rate	Values to be applied in the formulas: • vp and mw available from dossier • Gas constant R=8.31451 J/K*mol • Ambient temperature 298 K (=25°C)	• Saturated vapour concentration model calculates exposure to an active substance volatilised from the treated surfaces. It includes the worst-case assumption that the livestock would be exposed to air containing the active substance at the active substance`s saturated vapour concentration at a specific ambient temperature for 24 hours. Further assumptions: no air changes, absorption via inhalation 100%: $SVC = \frac{mw[\mathrm{g/mol}] \cdot vp[\mathrm{Pa}]}{R[\mathrm{J\ mol^{-1}\ K^{-1}}] \cdot T[\mathrm{K}]} = 0.4$ *SVC*Saturated vapour concentration (mg as/m³) *vp*Vapour pressure of active substance (Pa) *mw*Molecular weight (g/mol) *R*Gas constant (J/K*mol) *T*Ambient temperature (K)	• Saturated vapour concentration HEEG Opinion 13: Assessment of inhalation exposure of volatilised biocide active substance http://echa.europa.eu/documents/10162/19680902/heeg_opinion_13_volatilised_inhalation_exposure_en.pdf • Additional formulas for more refined calculations of air concentrations of an active substance can be found in ConsExpo RIVM report 320104004/2005. ConsExpo 4.0 Consumer Exposure and Uptake Models Program Manual J.E. Delmaar, M.V.D.Z. Park, J.G.M. van Engelen (http://www.rivm.nl/en/healthanddisease/productsafety/ConsExpo.jsp.)
22	/	Skin area exposed to hoof bath		Dairy cow: 1590 cm² The exposed skin area is estimated from the depth of the hoof bath, the height to which splashing	Diameter of hoof confirmed by DE veterinary expert

				occurs and the diameter of the hoof. Height to which splashing occurs = 30 cm Diameter of hoof = 15 cm To calculate the area of exposed hoof/skin, we assume hoof and leg to be of cylindrical shape: $2\pi rh + \pi r^2 = (2\pi \times 7.5cm \times 30\ cm) + \pi \times (7.5\ cm)^2 = 1413 + 177 = 1590\ cm^2$	
23	/	Thickness of the layer of disinfectant on hoof/skin that could be absorbed		0.01 cm, this values is the estimated thickness of the layer of the product for calculation of the human dermal exposure.	ConsExpo 4.1 Consumer Exposure and Uptake Model s and related Cleaning products Fact Sheet (RIVM report 320104003/2006) HEAdhoc recommendation no.13, Exposure Assessmenr of Teat Disinfection Products for Veterinary Hygiene (PT3)https://echa.europa.eu/documents/10162/21664016/recommendation_13_teat_disinfection_en.pdf/fbeb394b-e74b-685d-c231-5e3a530e311c
24	/	Feed silo sizes and holding capacities		Volume Diameter Height Holding capacity 13.56 m^3 2.55 m 4.30 m 5.7 tons 26.62 m^3 2.55 m 7.80 m 16.0 tons 18.00 m^3 2.30 m 6.95 m 10.8 tons 7.3 m^3 2.00 m 4.85 m 8.3 tons	Information obtained from feed silo suppliers.
25	/	Migration rate to feed	100%	Option for refinement if sufficiently justified	

26	/	Slimicides: loss of a.s. with waste water during paper production	90%	• Default value taken from RIVM/FEI scenario • See also Example 2.4 • As a worst case is it is considered that 10% of the a.s. remains in the paper	Supplement to the methodology for risk evaluation of biocides, Harmonisation of Environmental Emission Scenarios for Slimicides (product type 12), European Commision DG ENV / RIVM, September 2003 Reference 4L1784.A0/R0009/FBA/TL/Nijm http://echa.europa.eu/documents/10162/16908203/pt12_slimicides_en.pdf
27	/	paper mill waste water	5000 m^3	See also Example 2.4	Supplement to the methodology for risk evaluation of biocides, Harmonisation of Environmental Emission Scenarios for Slimicides (product type 12), European Commision DG ENV / RIVM, September 2003 Reference 4L1784.A0/R0009/FBA/TL/Nijm pp. 27, Table 4.1 http://echa.europa.eu/documents/10162/16908203/pt12_slimicides_en.pdf
28					
29	/	daily paper production per mill	200 t/d	See also Example 2.4	Supplement to the methodology for risk evaluation of biocides, Harmonisation of Environmental Emission Scenarios for Slimicides (product type 12), European Commision DG ENV / RIVM, September 2003, Reference 4L1784.A0/R0009/FBA/TL/Nijm pp. 51 average is 200 tonnes of paper per day http://echa.europa.eu/documents/10162/16908203/pt12_slimicides_en.pdf
30	/	dry paper weight	600 g/m^2	See also Example 2.4	Supplement to the methodology for risk evaluation of biocides. Emission scenario document for biocides used in paper coating and finishing (Product type 6, 7 & 9). INERIS –DRC-01-25582-ECOT-CTi/VMi-nº01DR0183.doc pp. 3: grammage (i.e. the weight in grams of one square meter of paper) is 25-300 $g.m^{-2}$ for papers 170 – 600 $g.m^{-2}$ for paperboards

					https://echa.europa.eu/documents/10162/16908203/pt6_pt7_pt9_paper_coating_and_finishing_en.pdf
31	/	packaging surface in contact with 1 kg feed	600 cm^2	See also Example 2.4	EU Notes for Guidance for Food Contact Materials prepared by the European Food Safety Authority Updated on 30/07/2008 http://www.efsa.europa.eu/de/search/doc/21r.pdf A = is area of the food contact material in cm^2, conventionally set at 600 cm^2.(pp. 91)
32	/	Fraction of feed (that was packaged in treated cardboard/paper) consumed by animals	10%	See also Example 2.4	Expert judgement

1

2

- **Table 45: References and Explanations**

No.	Description	Default Values	Background Information Remarks	References
1	**Body weight**	See Table 1	• Relevant body weights are those at slaughter for meat-producing animals and those during milk and egg production. • In EU only young goats are slaughtered. Information on slaughter weights for goats were available from MS: 8-10 kg and 13 kg (NL), 8-12 kg (IT), 13-18 kg (EL). • For lactating goat the value of 70 kg is commonly accepted by EFSA. • For horses the age of slaughter exhibits a range as horses are slaughtered at young and older ages. To account for this, an average slaughter weight for horses was chosen. • For rabbits the slaughter weight in the EU ranges from 1.8 to 3.2 kg, an average value was chosen as default value.	• Beef and dairy cattle, sheep, lamb, breeding and fattening pig, broiler chicken, laying hen, turkey: OECD guidance document on overview of residue chemistry studies, Annex 4, ENV/JM/MONO(2009)31, July 28th 2009 • Calf: Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directive~~s~~ 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97 • Goat: Information provided by MS • Goat kids: Information provided by MS • Horse: Revised guideline on environmental impact assessment for VMPs in support of VICH guidelines GL6 and GL 38, EMEA/CVMP/ERA/418282/2005-Rev.1 • Rabbit: Opinion of the EFSA AHAW Panel, The Impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits, Annex to the EFSA Journal (2005) 267, 1-31
2	**Animal height**	See Table 1	• The height of animals is highly variable between breeds of one species. The default values for animal height were estimated based on species commonly kept as food producing species. • Height to withers: The withers is the ridge between the shoulder blades of a four-legged animal. In many species it is the tallest point of the body, and in horses and dogs it is the standard place to measure the animal's height. • For the height to top of head the distance head to withers was estimated and added to the height to the	• Cattle, pig, sheep, goat, horse: http://www.ansi.okstate.edu/breeds/cattle/ (visited April 30, 2015) • Pig: Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97 • Goat: British goat society www.allgoats.com and Information provided by MS • Poultry: Code of Recommendations and Minimum Standards for the Welfare of Animals Transported within New Zealand. Animal Welfare Advisory Committee, Ministry of Agriculture and

			withers. This was not done for pigs as their head is lower than their shoulders or back. • The maximum reaching height considers stretching of animals. For cattle, sheep and horses this has been calculated as the height to the withers plus twice the distance head to withers. For pigs this was calculated as the height to the back plus the extra head allowance of 15 cm given in Council Regulation (EC) No 1/2005. For goat the maximum reaching height includes standing on its hind legs, based on information provided by MS this was estimated to be 2m. • For poultry animal height is not needed, for calculations for transport vehicles values from New Zealand reference were applied.	Fisheries, Wellington, New Zealand. Code of Animal Welfare No. 15. ISBN 0-478-07372-0, ISSN 1171-090X, November 1994 and Amendments to this document from June 1996 • Rabbit: Opinion of the EFSA AHAW Panel, The Impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits, Annex to the EFSA Journal (2005) 267, 1-31
3	**Body surface area (BSA)**	See Table 1	Mathematical formulas relating external surface area BSA to total body weight (W) or eviscerated body weight (E): • Pig: BSA (cm^2)= 734 x $W^{0.656}$ • Cattle: BSA (m^2)= 0.14 x $W^{0.57}$ • Sheep: BSA (m^2)= 0.085 x $W^{0.67}$ • Chicken: BSA (cm^2)= 0.67 x E + 536 • Duck: BSA (cm^2)= 0.66 x E + 583 • • Turkey >7 kg: BSA (cm^2)= 0.10 x E + 3025 (applied for default BSA) • All mammals: BSA (m^2)= 0.11 x $W^{0.65}$	Pig: Grommers F.J. et al (1970), Swine-Floor Contact Area as a Function of Body Weight and Posture, J. Anim Sci 31: 1232-1234. https://www.animalsciencepublications.org/publications/jas/pdfs/31/6/JAN0310061232 (visited April 30, 2015) • Cattle, sheep, : Berman, A. (2003), Effects of Body Surface Area Estimates on Predicted Energy requirements and heat Stress, J. Dairy Sci. 86: 3605-3610, http://jds.fass.org/cgi/reprint/86/11/3605 • Chicken, duck, turkey: Thomas (1978), Observations of the relationship between the surface area and weight of eviscerated carcases of chicken, ducks and turkeys, J. Fd.Technol 13:81-86, http://www3.interscience.wiley.com/cgi-bin/fulltext/120060846/PDFSTART (visited April 30, 2015) • All mammals (applied for horse, rabbit): US EPA USEPA (US Environmental Protection Agency). 1993. Wildlife Exposure Factors Handbook. EPA/600/R-93/187. Office of Research and Development, Washington, DC, USA
4	**Body surface area in contact with surface**	30% of total body	For a fully relaxed pig lying flat on the side 6-16% of total body surface area is in contact with the floor (Grommers et al.). For all animal species a default	• Grommers F.J. et al (1970), Swine-Floor Contact Area as a Function of Body Weight and Posture, J. Anim Sci 31: 1232-1234.

		surface area See Table 1 for values considering the default body weight.	value of 30% of total body surface area was estimated from the available pig data. This should comprise the fact that animals may lie on both sides.	• EFSA Scientific Report Q-2006-028 (2007), Scientific Report on animal health and welfare aspects of different housing and husbandry systems for adult breeding boars, pregnant, farrowing sows and unweaned piglets, http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178655708740.htm (visited April 30, 2015)
5	**Alveolar ventilation rate (AVR)**	See Table 1 for values considering the default body weight.	A scaling approach for calculation of alveolar ventilation rates in farm animals is proposed. From the listed references the following formulae have been deduced: Resting AVR • Mammals: AVR (ml/mn) = 276 x $bw^{0.78}$ • Birds: AVR (ml/mn) = 92.3 x $bw^{0.735}$ To account for activity, a correction factor of 3 is suggested to arrive at the non-resting alveolar ventilation rate.	• Calder, W. A. (1984). Size, Function and Life History. Harvard University Press, Cambridge, Mass. • Stahl, W. R. (1967). Scaling of respiratory variables in mammals. Am. J. Physiol. 22:453–460. • Lasiewski, R.C., and W.A. Calder. 1971. A preliminary allometric analysis of respiratory variables in resting birds. Resp. Phys. 11:152-166. • Bech C, Johansen K, Maloiy GMO. 1979. Ventilation and expired gas composition in the flamingo (Phoenicopterus ruber) during normal respiration and panting. Physiological Zoology 52(3):313-328. • Dawson, T. J. and Needham, A. D. (1981). Cardiovascular characteristics of two resting marsupials: an insight into the cardio-respiratory allometry of marsupials. J. Comp. Physiol. 145, 95-100. • Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., and Beliles, R. P. (1997). Physiological parameter values for physiologically based pharmacokinetic models. Toxicol. Ind. Health 13:407–484. • National Greenhouse Gas Inventory Committee (2007). Australian Methodology for the Estimation of Greenhouse Gas Emissions and Sinks 2006: Agriculture. Department of Climate Change, Australia. ISBN: 978-1-921297-91-5. Glazier DS (2008). Effects of metabolic level on the body size scaling of

				metabolic rate in birds and mammals. Proc. R. Soc. B 275: 1405–1410. • Weibel ER, Bacigalupe LD, Schmitt B, Hoppeler H (2004). Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. Respiratory Physiology & Neurobiology 140:115–132
6	**Feed intake**	See Table 1 for values considering the default body weight.	Various sources for feed intake of livestock animals are available. The feed intake relates to body weight (and age) of the animals. The ratio dry matter feed intake/body weight gives a stable value and these values are applied as default values: Ruminants and horses: 4% of body weight Pigs: 3% of body weight Poultry (except turkey): 7% of body weight Turkey: 5% of body weight Rabbit: 10% of body weight These values were confirmed by study data available to MS from evaluations of various substances. (Please note that defaults given in the OECD and EU Commission guidance documents (see references) may deviate from the proposed default values agreed by DRAWG for this document.)	• OECD GUIDANCE DOCUMENT ON RESIDUES IN LIVESTOCK, Series on Pesticides No. 73 ENV/JM/MONO(2013)8 • EU Commission guidance document 7031/VI/95 rev. 4, July 22nd 1996, page 4 • Turkey: Nutrient Requirements of Poultry, Subcommittee on Poultry Nutrition, National Research Council, 8th and 9th revised edition, 1984 and 1994, National Academy Press, Washington, DC • Rabbit: Opinion of the EFSA AHAW Panel, The Impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits, Annex to the EFSA Journal (2005) 267, 1-31
7	**Drinking water intake**	See Table 1 for values considering the default body weight.	• For beef cattle, calf, fattening pig, horses and goat default drinking water intake corresponding to 10% of body weight. According to Regulation (EC) No. 1/2005 the minimal water supply during transport should be 10% of animal live weight. • For dairy cattle, breeding pigs, sheep and lamb values as reported in the references were chosen. • For poultry consumption data for animals at age of common slaughter time were chosen	• Dairy cattle, breeding pig, sheep, lamb: Ontario Ministry of Agriculture Food & Rural Affairs, http://www.omafra.gov.on.ca/english/engineer/facts/07-023.htm (visited April 30, 2015) • Chicken, turkey USDA National Agricultural Library http://www.nal.usda.gov/ (visited April 30, 2015)

			• For rabbits the ratio between feed intake and water consumption is about 1:2.	Ontario Ministry of Agriculture Food & Rural Affairs http://www.omafra.gov.on.ca/english/engineer/facts/07-023.htm (visited April 30, 2015) • Beef cattle, calf, slaughter goat, lactating goat, horse: Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97 • Rabbit: Opinion of the EFSA AHAW Panel, The Impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits, Annex to the EFSA Journal (2005) 267, 1-31
8	**Number of animals per stable**	See Table 2	• For rabbits information for cages not for complete stable: 5 rabbits per cage of0.6 m length, 0.4 m width and 0.3 m height.	• Beef and dairy cattle, calf, breeding and fattening pig, broiler chicken, laying hen: OECD Emission Scenario Document for Insecticides for Stables and Manure Storage Systems, ENV/JM/MONO(2006)4, January 25th 2006, table 5.2 • Rabbit: Opinion of the EFSA AHAW Panel, The Impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits, Annex to the EFSA Journal (2005) 267, 1-31
9	**Floor area per stable**	See Table 2	See Table 5, line 8	See Table 5, line 8
10	**Wall and roof area per stable**	See Table 2	See Table 5, line 8	See Table 5, line 8
11	**Housing volume per stable**	See Table 2	See Table 5, line 8	See Table 5, line 8
12	**Floor area per animal**	See Table 2	Calculated from default values: "floor area per stable" divided by "number of animals per stable"	/

13	**Maximum area within reach of animal**	See Table 2	Calculated from floor area A per animal and maximum reaching height H of animal: • Assuming each animal is kept in a rectangular pen of area A with one side x and another side 2x, pen side x is calculated as $x = \frac{\sqrt{A}}{\sqrt{2}}$ • For the maximum wall area W within reach of an animal it was considered that the animal is standing in a pen with solid walls. The relevant height of the wall is the maximal reaching height H of the animal. For pigs and cattle the wall in the back was not included: $W = 5x \times H$ For horses and calves all four walls were included: $W = 6x \times H$ For poultry and sheep this parameter is not given as default value. • The overall maximum area within reach of animal (wall+floor) is the sum of floor area plus wall area per animal.	/
14	**Exposed feed surface in a trough**	see Table 2	**For cattle and pigs**, the exposed feed surface in a trough equals the inner surface area of a trough. Troughs are empty and uncovered during biocidal treatment. It is assumed that all residues contained on the bottom and the sides of the trough migrate into the next feed batch placed into the troughs after biocidal treatment. In case of direct treatment of troughs, the entire inner surface area of the trough contains residues in the amount of the application rate. In case of treatment of surrounding surfaces, residues equal the amount that drops to the floor (= bottom of	

			trough). Therefore, the exposed feed surface equals the surface area of the bottom of the trough. **For poultry**, the exposed feed surface equals the surface area of the bottom (=top) of the trough. Troughs are filled during biocidal treatment, and the top layer of the feed batch is contaminated directly. To calculate the surface areas, the following assumptions are made: All animals: • Troughs are designed to stretch across the entire width (w) of an animal's pen enclosure. • The depth of a trough is assumed to equal ¼ of the length (¼ l) of an animal's pen enclosure. Cattle and pigs: • Each pen enclosure is assumed to have short sides of length x (width w of animal pen) and long sides of length 2x (length l of animal pen). x can be calculated using the value for the available floor area per animal (A) (for values see Table 2) • The height (h) of a trough is assumed to be 50 cm for cattle and 30 cm for pigs. Poultry: • Each battery cage is assumed to be square-shaped with sides x and to house one chicken. x can be calculated using the value for the available floor area per animal (see Table 2) <u>Calculation of Exposed feed surface FS_{exp} for direct treatment of trough</u>	

			$FS_{exp} = 0.25A + 2(w \times h + 0.25l \times h)$ $= 0.25A + 3h\frac{\sqrt{A}}{\sqrt{2}}$ Calculation of Exposed feed surface FS_{exp} for treatment of surrounding surfaces: FS_{exp} = w*¼l = ¼ A	
15	**Ventilation of animal housing**	see Table 2 for values considering default body weights or default dimensions of animal housing	• Default values are based on the publication Seedorf et al. that reports recommendations and actual measurements for livestock buildings in Northern Europe. This reflects the worst-case scenario compared to Southern Europe where ventilation rates would be higher due to hot climate. • The ventilation rate per 500 kg live weight as reported in the publication. • The ventilation rate per animal was calculated based on default body weights. • The air exchanges per hour were calculated based on default dimensions of animal housing.	• SEEDORF, J., ET AL. (1998): A survey of ventilation rates in livestock buildings in northern Europe. J. agric. Engng Res. 70, 39 – 47
16	**Time spent in transport vehicles**	See Table 3	• EC transport requirements are different for short (< 8 hrs) and long (> 8 hrs) journeys. Since the maximum time is spent in a vehicle during long distance transports (> 8 hrs), these seem most relevant for worst case biocide exposure assessment.	• Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97
17	**Model truck for animal transport**	See Table 3	• Assumed size of model truck for animal transport: 7.0m x 2.5m	• Information obtained from various livestock transport companies
18	**Compartments for animal transport**	See Table 3	• Length and width of compartments were calculated for a model truck of 7.0 m x 2.5 m. • Relevant compartment height was estimated based on information obtained from livestock transporters and recommendations for minimal compartment heights	• Information obtained from various livestock transport companies • SCAH report on "The welfare of animals during transport (details for horses, pigs, sheep and cattle)", March 11th 2002,

			during transport by SCAH, EFSA Panel AHAW and New Zealand Animal Welfare Advisory Committee. • No of animals per compartment was calculated as $n = \frac{l \times b}{f}$ and rounded down to the nearest integer linternal length of a compartment (m) binternal width of a compartment (m) frequired floor area per animal during transport (m^2) nnumber of animals in a compartment	https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out71_en.pdf • EFSA Panel AHAW Scientific Opinion related to the Welfare of Animals during Transport, EFSA Journal 2004; 44, 1-36 • EFSA Panel AHAW Scientific Opinion Concerning the Welfare of Animals during Transport, EFSA Journal 2011; 9(1): 1966 • Code of Recommendations and Minimum Standards for the Welfare of Animals Transported within New Zealand. Animal Welfare Advisory Committee, Ministry of Agriculture and Fisheries, Wellington, New Zealand. Code of Animal Welfare No. 15. ISBN 0-478-07372-0, ISSN 1171-090X, November 1994 and Amendments to this document from June 1996
19	**Required floor area per animal during transport**	See Table 3	• Default values (A) as given in Regulation (EC) No 1/2005 or calculated based on default body weights (bw) applying formulas given in the SCAH report. Cattle, calf, lamb A = 0.021 $bw^{0.67}$ Pigs A = 0.0192 $bw^{0.67}$ Lactating goat A = 0.031 $bw^{0.67}$ Sheep, slaughter goat A = 0.026 $bw^{0.67}$ Chicken A = 0.016 bw Turkey A = 0.0105 bw Horse	• Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97 • SCAH report on "The welfare of animals during transport (details for horses, pigs, sheep and cattle)", March 11th 2002, https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scah_out71_en.pdf (visited April 30, 2015) • EFSA Panel AHAW Scientific Opinion related to the Welfare of Animals during Transport, EFSA Journal 2004; 44, 1-36 • EFSA Panel AHAW Scientific Opinion Concerning the Welfare of Animals during Transport, EFSA Journal 2011; 9(1): 1966

			See Council Regulation No 1/2005	
20	**Available wall+floor area per animal during transport**	See Table 3	• Default values calculated from length, width and relevant compartment height. $wf = \frac{F + W}{n}$ $= \frac{(l \times b) + 2(l \times r) + 2(b \times r)}{n}$ wf available wall+floor area in a compartment (m^2/animal) linternal length of a compartment (m) binternal width of a compartment (m) rrelevant compartment height (m) Favailable floor area in a compartment (m^2) Wavailable wall area in a compartment (m^2) nnumber of animals in a compartment Animals have only access to the walls and floors of their compartment. Available wall areas are calculated based on the assumption that the surface area is solid. This is generally not the case. Walls for larger livestock have metal bars. Therefore surface areas for walls are overestimated. However, since floors have ribbed surfaces, surfaces areas for floors are underestimated. Poultry are kept in cages. Surface areas (wall, floor) are overestimated.	/
21	**Available volume per animal during transport**	See Table 3	• Default values calculated as	/

			$v = \frac{V}{N}$ $= \frac{L \times B \times H}{N}$ $= \frac{7.0m \times 2.5m \times (c \times h)}{c \times d \times n}$ v available volume per animal (m^3) V available volume in a truck (m^3) n number of animals in a compartment (default see table 3) N total number of animals in a truck c number of floors in a truck (default see table 3) d number of compartments per floor (default see table 3) Linternal truck length (m) (default 7.0 m) Binternal truck width (m) (default 2.5 m) R internal truck height (m) linternal compartment length (m) binternal compartment width (m) h internal compartment height (m) • Very worst-case calculation Division of the truck floor in compartments does not influence the available volume in a truck, but may influence the maximum number of animals within a truck.	

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22	**Ventilation during transport**	See Table 3	Forced ventilation systems are required for very long transport duration (e.g. 14 hrs transport – 1hr rest – 14 hrs transport -24 hrs rest).	• Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97

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Appendix 5-3 (2) Information provided by the Applicant and from other Regulatory Areas

- **Table 46: Information to be provided by the Applicant**

Information relating to the intended use
- target animals - application method - frequency of treatments - application rate - re-entry period if animals are not present during treatment - concentration of active substance in product and in in-use product (e.g. in the spray formulation) - detailed description of areas to be treated (e.g. floors, walls, specified equipment, spot treatment) - product formulation It should be clearly specified in the intended use description provided by the Applicant whether every treatment is performed with the same application rate or if refresher treatments subsequent to the initial treatment are applied at a different rate.
Information relating to the active substance
- physico-chemical properties - degradation/volatilisation rate (environmental part of the dossier)

Table 47: Information on risk assessment from other regulatory areas

PPP	
EU Pesticide database	http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN
RMS Assessment Reports submitted for the EU peer review of active substances used in plant protection products	http://dar.efsa.europa.eu/dar-web/provision
JMPR Reports	http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmpr/jmpr-rep/en/
VMP	
EMEA Summary Reports/ Summary Opinions	http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/ vet_mrl_search.jsp&mid=WC0b01ac058006488e
JECFA Reports	http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx
Food and feed additives	
EFSA: Evaluations of the Panel on food additives and nutrient sources added to food (ANS)	http://www.efsa.europa.eu/en/applications/foodingredients/regulationsandguidance

EFSA: Evaluations of the Panel on food contact materials, enzymes, flavourings and processing aids (CEF)	http://www.efsa.europa.eu/en/applications/foodcontactmaterials/regulationsandguidance
EFSA: Evaluations of the FEEDAP Panel (Additives and products or substances used in animal feed)	http://www.efsa.europa.eu/en/applications/feedadditives/regulationsandguidance
JECFA Reports	http://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx

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