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GUIDANCE

Guidance on the Biocidal Products Regulation

Volume III Human Health - Assessment & Evaluation (Parts B+C)

Version 2.1
February 2017

LEGAL NOTICE

This document aims to assist users in complying with their obligations under the Biocidal Products Regulation (BPR). However, users are reminded that the text of the BPR is the only authentic legal reference and that the information in this document does not constitute legal advice. Usage of the information remains under the sole responsibility of the user. The European Chemicals Agency does not accept any liability with regard to the use that may be made of the information contained in this document.

Guidance on the BPR: Volume III Human Health Assessment & Evaluation (Parts B+C)

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European Chemicals Agency

Mailing address: P.O. Box 400, FI-00121 Helsinki, Finland
Visiting address: Annankatu 18, Helsinki, Finland

DOCUMENT HISTORY

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Version 1.1	Corrigendum covering the following: (i) Added Annex A, a Commission document on Substances of Concern (ii) Reformatting into ECHA corporate style (iii) Editorial revisions such as punctuation, spelling, etc. (iv) Correcting broken hyperlinks (v) Adding hyperlinks to list of abbreviations and section cross references	April 2015
Version 2.0	Update to section 3 Exposure Assessment The section has been fully revised as follows: • updated text on Exposure Assessment • alignment of the guidance with REACH principles/guidance on exposure • editorial revisions such as punctuation, spelling, etc. • removal of the "technical aspects" into a separate document on Biocides Human Health Exposure Estimation Methodology (available on Biocides webpages). • improvement of workflow diagrams	October 2015
Version 2.1	Corrigendum to update the guidance to address Part C Evaluation and to add text and links on "Applicability of Guidance" The text has been revised as follows: • Preface: updated to be in line with the general information in the Part A. • General Introduction: a new paragraph to explain the association of the evaluation and assessment processes. • Preface: to add text and links on "Applicability of Guidance"	February 2017

PREFACE

The Guidance on the Biocidal Products Regulation (BPR) is to be applied to applications for active substance approval and product authorisation as submitted from 1 September 2013, the date of application (DoA) of the Biocidal Product Regulation (the BPR).

This document describes the BPR obligations and how to fulfil them.

The scientific guidance provides technical scientific advice on how to fulfil the information requirements set by the BPR (Part A), how to perform the risk assessment and the exposure assessment for the evaluation of the human health and environmental aspects and how to asses and evaluate the efficacy to establish the benefit arising from the use of biocidal products and that it is sufficiently effective (Parts B & C).

In addition to the BPR guidance, the Biocidal Products Directive (BPD) guidance and other related documents are still considered applicable for new submissions under the BPR in the areas where the BPR guidance is under preparation. Furthermore these documents are still valid in relation to the evaluation of applications for active substance approval or applications for product authorization submitted for the purposes of Directive 98/8/EC (BPD) which may be still under evaluation under the Biocidal Products Regulation (BPR)), . Also the Commission has addressed some of the obligations in further detail in the Biocides competent authorities meetings documents which applicants are advised to consult. Please see ECHA Biocides Guidance website for links to these documents: [https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation].

Applicability of Guidance

Guidance on applicability of new guidance or guidance related documents for active substance approval is given in the published document "*Applicability time of new guidance and guidance-related documents in active substance approval*" available on the BPC Webpage[1] [https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee] and for applicability of guidance for product authorisation, please see the CA-document CA-july2012-doc6.2d (final), available on the ECHA Guidance page [CA-July2012-doc6.2d(final)].

[1] Link available under Working Procedures (right column) [https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee]

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NOTES to the reader

1. References: The references in this document have (in the majority) been carried over from former BPD documents and some of the details are missing. Many of the details have been traced and the references updated but there are some that are still incomplete: this is on-going work and will be further updated at a future update.

2. Hyperlinks to Abbreviations: Hyperlinks have been added to abbreviations throughout the document and not only on first use; this is because readers may not necessarily read the complete document and may only reference to sections they require at that time.

How to move to the abbreviations list and then back to the text: if you Ctrl+click on a hyperlink to jump to the target location, you can go back to your previous location by pressing **Alt+left arrow** key. For Mac PCs: the equivalent is either **Command+left arrow** in Adobe Reader or **Command+[** (open square bracket) in Preview.

4. Hyperlinks to Sections: Hyperlinks have been added to text that cross refers to another section of this Guidance document; this is on-going work because of the current update to section 3 and will be completed for a future update.

List of Abbreviations

NOTES to the reader

How to move to the abbreviations list and then back to the text:

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For Mac PCs: the equivalent is either **Command+left arrow** in Adobe Reader or **Command+[** (open square bracket) in Preview.

Standard term / Abbreviation	Explanation
ADI	Acceptable daily intake
ADME	Absorption, distribution, metabolism, and excretion
AEC	Acceptable Exposure Concentration
AEL	Accepted exposure level
AF	Assessment factor
AMPeakMet	Peak rate of hepatic metabolism
AOEL	Acceptable Operator Exposure Level
APF	Assigned Protection Factors
ARfD	Acute Reference Dose
a.s.	Active substance
ASTM	American Society for Testing and Materials
ATP	Adenosine-tri-phosphate
AUC	Area under the curve
BEAT	Bayesian Exposure Assessment Tool (computerised database of exposure data)
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
bw	Body weight

Standard term / Abbreviation	Explanation
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
CEFIC	European Chemical Industry Council
CEM	Consumer Exposure Module
C.I.	Confidence interval
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
CSA	Chemical safety assessment
CSAF	Chemical specific adjustment factors
CYP	Cytochrome P isoforms
d	Day(s)
DEREK	Deductive Estimation of Risk from Existing Knowledge
DG	European Commission Directorate General
DG SANCO	European Commission Directorate-General for Health and Consumers
DIN (TTC, INT)	Deutsches Institut für Normung e.V. (German Institute for Standardisation)
DMEL	Derived Minimal Effect Level
DNA	Deoxyribonucleic acid
DNEL	Derived No Effect Level
DPD	Dangerous Preparations Directive (1999/45/EC)
DSD	Dangerous Substance Directive (67/548/EEC)
EBPF	European Biocidal Product Forum

Standard term / Abbreviation	Explanation
EC	European Communities or European Commission
EC_{50}	Median effective concentration
ECB	European Chemicals Bureau
ECD	Electron Capture Detector
ECETOC (TRA)	European Centre for Ecotoxicology (and Toxicology of Chemicals) (Targeted Risk Assessment)
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Community
EFSA	European Food Safety Agency
ELISA	Enzyme-linked Immunosorbent Assay
EN	European norm
EPA (DK)	Environmental Protection Agency of Denmark
EPA (USA)	Environmental Protection Agency of the United States of America
EU	European Union + Norway, Iceland and Lichtenstein Please note the BPR applies to the European Economic Area (EEA) and thus all references to the EU in the text should be understood as EEA (EU + Norway, Iceland and Lichtenstein)
EUROPOEM	European Predictive Operator Exposure Model Database Project
FAO	Food and Agriculture Organization
FCA	Freund's Complete Adjuvant
FDA	U.S. Food and Drug Administration
FQPA	Food Quality Protection Act
GI(T)	Gastrointestinal (tract)
GEV	Generic Exposure Value
GLEV	Generic Lowest Exposure Value
GLP	Good laboratory practice
GPMT	Guinea Pig Maximisation Test
GSD	Geometric standard deviation
h	Hour(s)
HEEG	Human Exposure Expert Group (under BPD)[2]
HI	Hazard index

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

Standard term / Abbreviation	Explanation
HPT	Human Patch Test
HQ	Hazard quotient
HRIPT	Human Repeat-Insult Patch Test
IC_{50}	Median immobilisation concentration or median inhibitory concentration 1 (explained by a footnote if necessary)
ICD	Irritant contact dermatitis
ICRP	International Commission on Radiological Protection
IHCP	Institute for Health and Consumer Protection (DG Joint Research Centre)
ILSI	International Life Sciences Institute
INT	2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazoliumchloride testing method (please refer to DIN)
IOEL	Indicative occupational exposure level
IPCS	International Programme on Chemical Safety of the World Health Organisation
IR	Infrared
ISO (TC, SC, WG)	International Organisation for Standardisation (Technical Committee, Scientific Committee, Working Group)
ITS	Integrated testing strategy
JECFA	Joint FAO/WHO Expert Committee on Food Additives and Contaminants
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
JRC	Joint Research Centre
k	Rate constant for biodegradation
K	Kelvin
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
Kst	Dust explosion constant
LC	Langerhans cells
$LD(C)_0$	Lethal dose for 0% of the group of tested animals
$LD(C)_{50}$	Lethal dose for 50% of the group of tested animals
LEL	Lower explosive limit
LEV	Local exhaust ventilation

Standard term / Abbreviation	Explanation
LLNA	Local lymph node assay
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOC	Limiting oxygen concentration
log P	Octanol/water partition coefficient
LOQ	Limit of quantification
LVET	Low volume eye test
M	Molarity
MAC	Maximum admissible concentration
MCCEM	Multi-Chamber Concentration and Exposure Model
MIT	Minimum ignition temperature
MITI	Ministry of International Trade and Industry (Japan)
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)
MOS	Margin of Safety
MOTA	Manual of Technical Agreements (of the Biocides Technical Meeting)
MRL	Maximum residue level
MS	Mass spectrometry
MSCA	Member State Competent Authority
MTD	Maximum tolerated dose
M&K	The guinea pig maximization test of MAGNUSSON and KLIGMAN
NAEL	No Adverse Effect Level
NESIL	Non Expected Sensitisation Induction Level
N(L)OAEL	NOAEL and/or LOAEL
nm	Nanometre(s)
No	Number
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration

Standard term / Abbreviation	Explanation
NOEL	No observed effect level
OC	Operational condition
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational exposure limit
OPPT	Office for Pollution Prevention and Toxics (U.S. Environmental Protection Agency)
OSHA	Occupational Safety and Health Administration (European Agency for Safety and Health at Work)
Pa	Pascal(s)
para.	Paragraph
PBPK	Physiologically based Pharmacokinetic
PEC	Predicted environmental concentration
PHED	Pesticide handler exposure database
pKa	Negative decadic logarithm of the acid dissociation constant (describes how acidic (or not) a given hydrogen atom in a molecule is)
PKPD	Pharmacokinetic/pharmacodynamic
PNEC	Predicted no effect concentration
PPE	Personal Protective Equipment
PPP	Plant Protection Product
PT	Product type
(Q)SAR	(Quantitative) structure activity relationship
QSPR	Quantitative structure-property relationships
r	Correlation coefficient
RA	Risk Assessment
RAC	Committee for Risk Assessment (ECHA body)
$rate_{a.s.}$	Use rate of active substance [kg/ha]
$rate_{metabolite}$	Application rate at which metabolite should be tested [kg/ha]
RC	Risk Characterisation
REACH	Regulation (EC) No 1907/2006 on Registration, Evaluation, Authorisation and Restriction of Chemicals
RDT	Repeated dose toxicity
RD_{50}	Respiratory Depression expressed as decrease of respiratory rate by 50%
RD_{10}	Respiratory Depression expressed as decrease of respiratory rate by 10%

Standard term / Abbreviation	Explanation
rLLNA	Reduced LLNA
RMM	Risk Management Measures
RMS	Rapporteur Member State
RPE	Respiratory Protective Equipment
RT	Respiratory tract
s	Second(s)
SAF	Safety Assessment Factor
SCIES	Screening-Level Consumer Inhalation Exposure Software
SDS	Safety data sheet
SD	Standard deviation
SETAC	Society of Environmental Toxicology and Chemistry
SHEDS	Stochastic Human Exposure and Dose Simulation model
SME	Small and medium-sized enterprise
SMILES	Simplified molecular-input line-entry system
SoC	Substances 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)
STP	Sewage treatment plant
TD	Toxicodynamic
TKTD	Toxicokinetic/toxicodynamic
TLV	Threshold limit value
TMDI	Theoretical maximum daily intake
Test Methods Regulation	Regulation (EC) No 440/2008 laying down test methods pursuant to the REACH Regulation
TK	Toxicokinetic
TG	Technical guideline(s), technical group(s)
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
UDS	Unscheduled DNA synthesis

Standard term / Abbreviation	Explanation
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
WPEM	Wall Paint Exposure Assessment Model

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.
active substance (a.s.)	is the substance (or microorganism) that has an action on or against harmful organisms (Article 3(1)(c) BPR)..
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.
application	refers to using the in-use biocide(biocidal product).
biocidal product	is a substance or mixture that consists of, contains or generates one or more active substances and which has a biocidal intention (see full definition at Article 3(1)(a) BPR).
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.
bulk samples	are samples of the biocide in use (and where necessary, the concentrate).
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
clothing	can range from minimal (e.g. T-shirt and shorts) through to leisure wear, work clothing and coveralls, to impermeable suits. It includes PPE.
Degradation of PPE	a damaging change in one or more physical properties of the protective glove as a result of exposure to a chemical substance
deterministic estimates	are single-value, including worst-case estimates.
dislodgeable residues	are post-application residues that are available for uptake through human contact with substances on surfaces.
empirical (database) model	is a data distribution of exposures derived from site surveys or laboratory simulations, strongly associated with the biocide application task(s). The only inputs are new exposure data to reinforce the model. The outputs are "indicative exposure values" which when modified by pattern of use data, are compared with toxicological endpoint data. This is used in Tier 1 and Tier 2 assessments.
exposure reduction	measures are techniques to reduce risk through substitution of products, controlling the product, its sectors for use, specifying in-use control measures.
exposure data (experimental)	are personal samples (for inhalation and dermal exposure) and each is a data-point. It is unlikely that a sufficiently powerful data set would exist for meaningful statistics to apply to most scenarios.
exposure information	includes the frequency and duration of exposure, the selection of products in preference to others on the market, and the patterns of use.
exposure models	are used to predict exposure from databases, from statistical relationships and through mechanistic calculations. They provide information which, in conjunction with other data, leads to a quantitative estimate of exposure.
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 of plumes drifting off-site and ingesting contaminated food or water.
field blank samples	are sampling media that are treated in the same way as monitoring media, without being exposed to the biocide in use.
foreseeable non-proper (incorrect) use	is the use of biocidal products not in line with the instructions for use or without the consideration of some or all common and specific technical, operational and personal protective measures (e.g. the over-application or inadequate dilution of a biocide, common spillage scenarios, use without or with non-proper RPE and PPE). Accidents, malfunctions or deliberate misuse are not addressed.

Standard term / Abbreviation	Explanation
likelihood of exposure	is the expression of probability that exposure will occur at all. It can be quoted to reflect "none detected" values in exposure surveys and studies. See also LoD, LoQ.
in-use biocide	is the product as it is being applied, whether or not diluted by the user, as a paint, a dust, a spray, a solid, a solution, or as a component of a fluid.
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 detected, and cannot be measured accurately, respectively.
mathematical model	is a tool whereby inputs by the user result in a prediction of exposure through calculation. This is used in Tier 1 and Tier 2 assessments.
mixing & loading	handling biocide concentrates, diluting them and where necessary, putting the in-use formulation into the application apparatus.
NOAEL	the no observed adverse effect level.
none-detected	values from exposure studies - see likelihood of exposure, limits of detection.
non-professional applications	where products are for non-professional user (consumer) application, and include examples where people in a workplace are not employed to use biocides (e.g. fly sprays in an office).
non-professional users	are the general public - consumers - .There is an expectation – but little guarantee, that non-professionals will comply with instructions for use of a product. They have no access to controls or formal PPE.
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.

Standard term / Abbreviation	Explanation
permeation of PPE	the migration of biocide through the PPE barrier, e.g. solvent-based product through latex-based gloves.
personal monitoring	is the sampling of a biocide during its application or mixing and loading, using samplers deployed on the person. See also static monitoring.
personal protective equipment (PPE)	includes head, eye, respiratory (RPE), body, hand and foot protection that is designed to protect the wearer. The basic safety requirements that PPE must satisfy, in order to ensure the health protection and safety of users, are laid down in the Council Directive 89/686/EEC.
phases of activity	are mixing & loading, application, post-application and removal of the biocide.
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)	will handle biocidal products within the framework of statutory requirements. They 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. Not all professional users will have the knowledge and skills to handle hazardous biocidal products (e.g. incidental use of slimicides, insecticides, irregular disinfections and use of products containing preservatives).
protocols	are detailed descriptions of the work to be undertaken in surveys or studies and the objectives to be achieved.
removal and disposal phase	includes removing exhausted antifoulant coatings, disposing of used preservative fluids and burning treated timber.
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.

Standard term / Abbreviation	Explanation
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).
risk assessment	is the comparison of a predicted human dose from undertaking a task or tasks with appropriate toxicological endpoint values or NOAELs.
scenario	is one or a number of well defined tasks for which exposure can be characterised.
secondary exposure	is that which is not primary. It is characterised through the exposed person having little or no control over their exposure, which may be acute or prolonged. It includes re-entry to treated zones (contact with treated surfaces, inhalation of residual vapours, ingestion of residues).
static monitoring	is sampling of background atmospheric concentrations or deposition.
studies	are short laboratory simulations of limited tasks, or workplace based small surveys to indicate a likely exposure pattern.
surrogates or tracers	- e.g. strontium salts, dyes, fluorescent agents - are used in surveys and studies to enable analysts to trace the exposure pattern.
surveys	are extensive measurement of exposure resulting from real biocide application tasks.
task	covers the phases of use of a biocide. It is a unit of operation within one or several scenarios.
Tier 1	is a screening level risk assessment.
Tier 2	is a detailed risk assessment, taking into account patterns of work and risk management measures.
Tier 3	is the output of an individual exposure study, possibly generated as a result of a data requirement for product registration.
trained professional users	probably have specialised knowledge and skill in handling hazardous chemicals. Protective measures as foreseen in the European Communities regulations on safety and health at work (instruction, training, exposure control, PPE) should be observed. Qualification might be documented by the endorsement of management systems for occupational safety and health, by certification to branch-specific standards or by approval through competent authorities. The term specialised professional user has the same definition as trained professional user.
TWA	time weighted average exposure by inhalation.
user sectors	industrial, professional, non-professional and secondary.

Standard term / Abbreviation	Explanation
ventilation	has several meanings. It may be a control measure in the workplace; it may refer to passive air changes within a building; and it may refer to the human breathing rate. The context should be clear from the text.
visualisation	involves the introduction of a coloured or fluorescent tracer to the biocide in-use formulation for post-exposure quantitation.
work clothing	- work uniform or work wear is a set of clothes worn at work. They are not designed to protect the health and safety of the worker and do not constitute PPE. However, they do protect the wearer to some extent from dermal exposure.

General introduction

Evaluation

The process of evaluation of active substance applications is given in Article 8 (BPR) 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 Annex VI (BPR).

The evaluating or receiving 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 general principles of assessment are given in Annex VI (BPR) and the evaluation is carried out according to these general principles. 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) and/or whether the active substance may be approved.

Thus the risk assessment is the principle part of the evaluation process and this guidance explains how to perform the risk assessment and the exposure assessments for the evaluation of the human health aspects.

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 level of exposure to a substance, and the incidence and severity of an effect, where appropriate.

(2) **Exposure assessment:** estimation of the concentrations/doses to which human populations (i.e. workers, consumers and human exposed indirectly via the environment) or environmental compartments (aquatic environment, terrestrial environment and air) are or may be exposed.

(3) **Risk characterisation:** estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance, and may include "risk estimation", i.e. the quantification of that likelihood. Combined exposure to multiple chemicals and dietary risk assessment should also be considered where relevant.

Risk assessment containing all steps must be carried out for all biocidal active substances.

Possible results of the risk assessment for active biocidal substances:

- Recommendation for the approval of an active substance for use in biocidal products (the approval shall, where appropriate, be subject to certain requirements).
- Recommendation for the non-approval of an active substance for use in biocidal products.

The risk assessment for human health shall address the following potential toxic effects and human populations, considering each population's exposure by the inhalation, oral and dermal routes:

Effects

- acute toxicity;
- irritation;
- corrosivity;
- sensitisation;
- repeated dose toxicity;
- mutagenicity;
- carcinogenicity;
- toxicity for reproduction.

Human population

- 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. The availability of representative and reliable monitoring data and/or the amount and detail of the information necessary to derive realistic exposure levels by modelling, in particular at later stages in the life cycle of a substance (e.g. during and after use in preparations and articles), will also vary. Again, expert judgement is needed.

The risk assessment should be carried out on the basis of all data available, applying the methods described in the following sections of the document. As a general rule for the risk assessment the best and most realistic information available should be given preference.

However, 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 that the substance is of "no concern", the risk assessment for that human population can be stopped.

If, in contrast, the outcome is that a substance is "of concern", the assessment must, if possible, be refined.

General Principles

In essence, the procedure for the risk assessment for human health of a substance consists of comparing the exposure level(s) to which the population(s) are exposed or are likely to be exposed with the exposure level(s) at which no toxic effects are expected to occur.

Where possible, a risk assessment is conducted by comparing the exposure level, the outcome of the exposure assessment, with the relevant AEL or AEC (derived on the basis of threshold levels such as NOAEL, LOAEL, NOAEC, BMD, etc. with the use of assessment factors), the outcome of the hazard characterisation. The exposure levels can be derived based on available monitoring data and/or model calculations. The N(L)OAEL values are determined on the basis of results from animal testing, or on the basis of available human data. For some effects N(L)OAEL and the corresponding AEL values are not usually available. For genotoxic substances it is considered prudent to assume that a threshold exposure level cannot be identified.

Also, for substances which are corrosive or skin/eye irritants, or skin sensitisers N(L)OAEL and the corresponding AEL values are often not available.

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

For both the exposure assessment and the effects assessment, data on physico-chemical properties including chemical reactivity may be needed. The data on physico-chemical properties are required, for example, to estimate emissions and the human exposure scenarios, to assess the design of toxicity tests, and may also provide indications about the absorption of the substance for various routes of exposure. The chemical reactivity may also be of importance, e.g. in the estimation of the exposure of the substance, and also has an impact on its TK and metabolism.

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

The comparison of the exposure with the potential effects is done separately for each human population exposed, or likely to be exposed, to the substance, and for the critical effect. It should be noted that, 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 during risk characterisation. Thus, exposure levels are derived separately for each relevant population/sub-population, and different AELs or AECs (derived on the basis of threshold levels such as NOAEL, LOAEL, BMD), where appropriate, are identified for the critical endpoints, and respective ratios of exposure level/AEL or AEC values are established.

The risk assessment process depends heavily upon expert judgement in the interpretation of exposure and effects. The risk assessor should focus the assessment on those effects of toxicological relevance to humans which may be expected at the predicted levels of 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 the effects and all the expected human exposure patterns are considered, there may be indications for several tests, possibly using more than one route of exposure. Particularly when early and/or extensive further testing is being considered, it is important to ensure that either high quality and relevant measured exposure levels, or the best possible estimates of human exposure, are obtained so that the decision to test or not to test can be justified. In addition, it should be considered whether toxicokinetic, metabolic, or mechanistic data/information, if obtainable, may be useful for defining which tests and which routes of exposure should be used, or such data may be useful in themselves in the assessment of the risks to human health. At any particular stage, integrated requirements for further testing must be developed, using professional judgement, so that the necessary information is obtained using the least amount of testing in animals.

1 Effects 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 determined or review classification.
- **hazard characterization: dose (concentration) - response (effect) assessment**, which 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, if this is not possible, the LOAEL, or BMD shall, where possible and appropriate, be determined for the observed effects. If appropriate, the shape of the dose-response curve should also be considered (see Section 2).

During both steps of the effects assessment it is of high importance to evaluate the data with regard to their adequacy and completeness. The evaluation of adequacy shall address the reliability and relevance of the data.

For the effects for which it is not possible to determine a N(L)OAEL, it is generally sufficient to evaluate whether the substance has an inherent capacity to cause such an effect. Where for such an effect 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 given endpoint is to be given preference for the risk assessment. Exemptions from this general rule are studies conducted with human volunteers. These studies are strongly discouraged as they are problematic from an ethical point of view. Results from such studies should be used only in justified cases (e.g. tests which were conducted for the authorisation of a medical product or when effects in already available human volunteer studies with existing substances have been observed to be more severe than deduced from prior animal testing). However, the potential differences in sensitivity of human studies and studies in animals should be taken into account in the risk assessment, on a case-by-case basis. In relation to 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.

The structure of the section on **hazard identification** for each endpoint is as follows:

- definition of the effect;
- data to be used in the effects assessment;
- remaining uncertainty;
- concluding on classification and labelling;
- concluding for risk assessment.

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 shown in Figure 1, the first two steps in hazard assessment include the collection of all available information and its assessment before deciding if additional testing needs to be performed. Once new test results become available, as part of step 3 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).

For **Step 1** of the process, various sources exist for gathering all available information on chemicals. The eChemPortal (http://www.echemportal.org) and the QSAR Toolbox (http://www.qsartoolbox.org) 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. Additional list of sources to be considered during step 1 is available in the *Guidance on information requirements and chemical safety assessment,Chapter R.3 (Information Gathering)*.

Step 2 in the process of hazard identification, is described in this Guidance under the sections "Data to be used for effects assessment" for each endpoint.

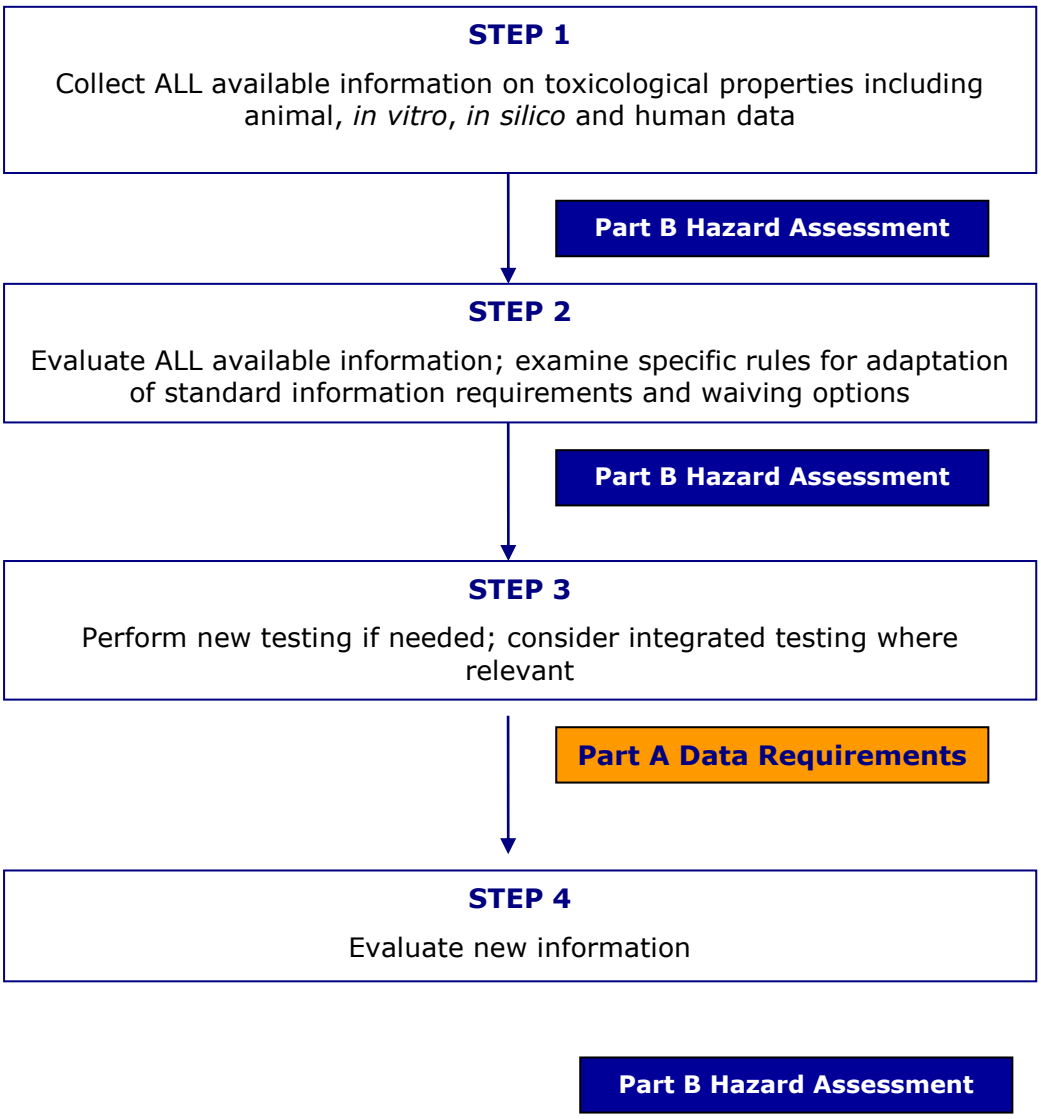


Figure 1: **Schematic representation of stepwise approach for hazard assessment under the BPR and interlink to the Data requirement**

1.2 Evaluation of data

During both steps of the effects assessment it is very important to evaluate the data with regard to their adequacy and completeness. This is particularly important for well studied existing substances where there may be a number of test results available for each effect but where some or all of them have not 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 active biocidal substances and products, the BPR gives the dispositions on data requirements for authorisation. In Annexes II and III of the BPR detailed core data requirements common to all active substances and biocidal products, respectively, are specified whereas Annex IV of the BPR specifies the general rules for the adaptation of the data requirements.

1.2.2 Adequacy of data

The adequacy of a 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 each effect, the greatest weight is attached to the most reliable and relevant.

The evaluation of animal test data with respect to reliability is outlined below. Additional sections consider issues specific to the reliability of human and *in vitro* data, relevance to humans and QSAR.

1.2.3 Reliability of data

For active biocidal 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 available have been generated prior to the requirements of GLP and the standardisation of testing methods. That data may still be used for risk assessment but the data and the methodology used must be evaluated in order to determine their reliability for assessment purposes. The evaluation needs 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. That is, studies carried out according to current methods (e.g. EC EU Test Methods Regulation, OECD Test Guidelines Programme - http://www.oecd.org/env/ehs/ or U.S. EPA Test Guidelines - http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm) appropriately reported, should be considered the most reliable for risk assessment. Klimisch *et al*. (1997) developed a scoring system to assess the reliability of data, particularly from toxicological and

ecotoxicological studies, that may be extended to physico-chemical and environmental fate and behavioural studies.

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

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

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

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

The use of such scoring tools e.g. the mentioned Klimisch codes, allows ranking the information and organising it for further review. This implies focusing on the most relevant ones, taking into account 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, e.g. according to Klimisch codes, should not exclude all unreliable data from further consideration by expert judgment because of possible pertinence of these data related to the evaluated endpoints. In general, some types of data that are not reliable (i.e. those where insufficient documentation exist for making an assessment) and data from which it is not possible to assign reliability, may only be used as supporting data.

When looking at 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 described is in accordance with generally accepted scientific standards. The information in such a report should be considered to be reliable and should be used for risk assessment;
- the reliability of the data cannot be fully established or the test procedure described differs in some respects from the test guidelines and/or generally accepted scientific standards. The assessor must decide in that case whether the data will be taken into consideration in the risk assessment and how they will be used (e.g. as supporting information where a reliable study has already been identified) or whether they should be regarded as invalid;
- the following factors, among others, can be used to support the view that these data may be acceptable for use in a risk assessment:
    - there are other studies or calculations available on the substance, and the data under consideration are consistent with them;

- other studies, for example on isomers with similar structure activity profile, homologues, relevant precursors, breakdown products or other chemical analogues, are available 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 supporting information is not reported (e.g. species tested, substance identity, dosing procedure) the test data should be considered to be 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.

General principles for data evaluation were discussed at the IPCS meeting on International Co-ordination of Criteria Document Production (the outcomes of the meeting are summarised in Annex 5 of the meeting report (IPCS, 1993) and have also been described in relation to occupational exposure (EEC, 1992).

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 (WHO, 1983). Epidemiological studies with negative results cannot prove the absence of an intrinsic hazardous property of a substance but well documented "negative" studies of good quality may be useful in the 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 and 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 the proper selection and characterisation of the exposed and control groups, adequate characterisation of exposure, sufficient length of follow-up for disease occurrence, valid ascertainment of effect, proper consideration of bias and confounding factors, and a 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) When they are already available, well-conducted controlled human exposure studies (4) in volunteers, including low exposure TK studies, can also be used in risk assessment in some rare cases. 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 authorization of a 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).

Experimental human toxicity studies must not be conducted specifically for the purpose of inclusion in the Union List of the Biocidal Products Regulation.

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, low dose levels resulting in poor sensitivity in detecting effects.

It is emphasised that testing with human volunteers is strongly discouraged but when there are good quality data already available they should be used as appropriate, 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 basic (and perhaps additional) studies on genotoxicity, skin or eye irritation/corrosion studies, for example. There may also be data from *in vitro* studies on, for instance, metabolism and/or mechanisms of action (including studies in cell cultures from different species), dermal absorption (which may also be for different species) and various aspects of toxicity (e.g. tests for cytotoxicity in different types of cells, macromolecule binding studies, tests using embryo culture systems, sperm motility tests). For any of these studies, their usefulness will be influenced 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.

However, there are also some criteria which need 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 replicates (within the tests and of the tests) ;
- use of the appropriate test system (e.g. appropriate cell lines).

Relevance of data

In order 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 be able to assess the latter it is necessary that the substance is properly identified and any significant impurities described.

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 in order to reach a conclusion about the relevance to humans of effects observed in studies 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, even when they are relatively limited. Clear, well-documented evidence for a species-specific effect/response (e.g. light hydrocarbon-induced nephthropathy in the kidney of male rats) should 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, if it can be scientifically justified, on a close structural analogue), "threshold" adverse effects observed in studies in animals will normally be assumed to be likely to occur also in humans exposed to the substance above a certain level of exposure.

In any case, 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.

Interpretation of the relevance of data derived from tests conducted *in vitro* 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*. According to the validation procedures established by ECVAM, 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 (Worth and Balls, 2001).

In general, the results of *in vitro* tests (with the exception of those that are used as standard test guideline protocols for the assessment of specific endpoints like skin irritation/corrosion and mutagenicity) 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.

Although *in vitro* data alone are not often of direct relevance for humans, highly electrophilic substances which give positive results in genotoxicity tests conducted *in vitro* may be of concern with regard to their potential to be mutagenic to humans at the initial site of the contact (e.g. the skin, the respiratory tract or the GI tract). The special case of interpretation of data from *in vitro* tests for genotoxicity is addressed in Section 1.8 of this Section.

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

When 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.

SARs may be of value in indicating a potential hazard, toxicokinetic properties or the need for further testing. Additional guidance is provided in the *Guidance on information requirements and chemical safety assessment Chapter R.6 (QSARs and Grouping of Chemicals)*.

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 extrapolation factors. In addition, this may also enable sensitive sub-populations who may be at particular risk to be taken into account in the risk assessment by evaluating inter-individual differences. In conjunction with information on the relationship between concentration/dose at the target site and the toxic effect, TK information may be an important tool for extrapolation from high to low dose effects. Toxicokinetic data can be used to make informed decisions on further testing. In specific circumstances, valid toxicokinetic data may be used to support derogation statements. For example, proof that a substance is not systemically available may be considered as part of a justification for non-conduct of further testing, e.g. reproductive toxicity tests.

In addition, when there is a need for higher tier refinement in risk characterisation (see Section 4.6), TK can be essential in refining hazard characterisation (e.g. derivation of chemical specific adjustment factors, elaboration of mode of action).

Information on TK can be derived either from *in vitro* and *in vivo* experiments, or from the use of PBPK modelling.

Section 8.8 on TK within the ECHA Biocides Guidance, Volume III Human health Part A (Information requirements) should be considered together with the elements described in this section for the assessment of TK.

1.3.1 Definitions

The term toxicokinetics (TK) is used to describe the time-dependent fate of a substance within the body. This includes absorption, distribution, metabolism, and/or excretion. The term toxicodynamics 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).

Toxicokinetic 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). From these data the toxicokinetic parameters can be derived by appropriate techniques. The information which can be taken from *in vivo*/ex-vivo toxicokinetic studies is:

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, if appropriate;
- protein binding and binding to erythrocytes, if relevant (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 systemic availability after oral administration may be greater than after non-oral administration. This will result in an AUC (which reflects 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 toxicokinetic information for the (expected) exposure route(s) in humans (oral, inhalation, dermal) at appropriate dosing level(s). 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. Hence, if information on the accumulative potential of a substance is important for the risk assessment, it will be necessary to gather data from studies with repeated dosing regimes. Information on TK from more than one species can enable the assessment of interspecies differences. In the absence of *in vivo* data some of the toxicokinetic 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 arising from exposure to a substance 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 to 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 (ECETOC, 2006; WHO/IPCS, 2005; Boobis *et al*., 2008).

Prior to any animal study, it is crucial to identify the benefits that will be gained from conducting such a study. 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. Overall the formation of data that are unlikely to be used and that constitute an unnecessary use of animals, time, and resources shall be avoided using any supporting data. TK information can provide important information for the design of (subsequent) toxicity studies, for the application of read-across and building of categories. For the generation of new toxicokinetic data this part of the Guidance should be used together with the ECHA Biocides Guidance, Volume III Human health Part A (Information requirements). The aim of this document 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 testing strategies to become more intelligent (ITS).

The TK phase begins with exposure and results in a certain concentration of the ultimate toxicant at the target site (tissue dose). This concentration is dependent on the ADME of the substance (ECETOC, 2006). ADME describes the uptake of a substance into the body and its lifecycle within the body, including excretion (EU B.36; 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 of interest into a structurally different chemical (metabolite);
- **excretion**: the physical loss of the parent substance and/or its metabolite(s); the principal routes of excretion are via the urine, bile (faeces), and exhaled air.[3]

Metabolism and excretion are the two components of elimination, which describe the loss of substance by the organism, either by physical departure or by chemical transformation. For consistency, and unless otherwise specified, metabolism does not include largely reversible chemical transformations resulting in an observable equilibrium between two chemical species. This latter phenomenon is termed inter-conversion.

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

1.3.2.1 Absorption

The 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 lipidic 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 that are more actively directed and therefore require energy.

1.3.2.2 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 blood flow through the organ, the ability of the substance to cross membranes and capillaries, and its relative affinity for the various tissues determine the rate of distribution and the target tissues. Regarding the cross-membrane transfer, not only the passive but also the active transport mechanisms by transport proteins (e.g. p-glycoprotein) shall be taken into consideration, as this is of particular importance for crossing the blood-brain-barrier but also elsewhere (e.g. in the intestine).

[3] Breast milk is a minor but potentially important route of excretion.

Distribution is in fact a dynamic process involving multiple equilibria. 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, often compounds distribute so rapidly into the highly perfused tissues, such as liver, kidney, and lungs, that kinetics cannot be distinguished from events in the blood. At that point, such organs are classed as being part of the initial, central compartment, and peripheral compartment is reserved for slowly equilibrating tissues, e.g. muscle, skin, and adipose. There is 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 (rapid or central compartment).

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

1.3.2.3 Metabolism or biotransformation

Biotransformation is one of the main factors, which influence the fate of chemicals in the body, its toxicity, and its rate and route of elimination. Traditionally, biotransformation is divided into two main phases: phase I and phase II. **Phase I**, the so-called functionalisation phase, has a major impact on lipophilic molecules, rendering them more polar and more readily excreted. In **phase II**, often referred to as detoxification, such functionalised moieties are subsequently conjugated with highly polar molecules before they are excreted. Specific enzymes, which are either membrane-bound (microsomal proteins) or present in the cytosol (cytosolic or soluble enzymes), catalyse both phases . Furthermore, 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 of some sort, and all cells and tissues are potentially capable of biotransforming compounds. However, 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 to be considered. Notably, variations occur in the presence of metabolising enzymes in different tissues, and also between different cells in the same organ. Another aspect is the existence of marked differences between and within various animal species and humans in the expression and catalytic activities of many biotransforming enzymes. Any knowledge concerning metabolic differences may provide crucial insight in characterising the potential risk of chemicals to humans.

1.3.2.4 Excretion

As chemicals are absorbed at different entry portals, they can also be excreted via various routes and mechanisms. The relative importance of the excretion processes depends on the physical and chemical properties of the compound and its various metabolites.

Besides passive transportation (diffusion or filtration), there are carrier-mediated mechanisms to shuttle a substance through a biological membrane. It is well known that there is a variety of pumps responsible for transportation of specific types of substances (e.g. 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 (e.g. in the removal of particulate matter from the alveoli by alveolar phagocytes, and the removal of some large molecules (Pritchard, 1981) from the body by the reticulo-endothelial system in the liver and spleen (Klaassen, 1986).

1.3.2.5 Bioavailability, saturation vs. 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.

1.3.2.5.1 Bioavailability

Bioavailability usually describes the passage of a substance from the site of absorption into the blood of the general (systemic) circulation, thus meaning systemic bioavailability (Nordberg *et al*., 2004). The fact that at least some of the substances considered are systemically bioavailable is often referred to as systemic exposure.

Systemic bioavailability is not necessarily equivalent to the amount of substance absorbed, because in many cases parts of that amount may be excreted or metabolised before reaching the systemic circulation. This may, for instance, occur for substances metabolised in the gut after oral exposure before any absorption has taken place. Conversely, substances absorbed from the intestine can be partly eliminated by the liver at their first passage through that organ (so-called first-pass effect).

1.3.2.5.2 Linearity vs. non-linearity and saturation

When all transfer rates between the different compartments of the body are proportional to the amounts or concentrations present (this is also called a process of first order), the process is called linear. This implies that the amounts of a substance cleared and distributed, as well as half-lives are constant and the concentrations are proportional to the dosing rate (exposure). Such linear kinetics displays the respective dose-toxicity-relationships.

Once a kinetic process is saturated (e.g. by high level dosing/exposure) a process might become non-linear, as the enzymes involved in biotransformation processes, or transporters involved in distribution or elimination, or binding proteins (i.e. receptors) are inhibited or reaching their maximum activity,. This may result in concentration or dose-dependency, or time-dependency of some of the kinetic characteristics. In some cases this can lead to a change in biotransformation products or the metabolic capacity. It is advised to consider systematically the possible sources for non-linear kinetics, especially for repeated dose testing.

1.3.2.5.3 Accumulation (Kroes *et al*., 2004)

Everything in a biological system has a biological half-life, that is, a measure of how long it will stay in that system until it is lost by mainly excretion, degradation, or metabolism. To put it in different words, the amount of a substance eliminated from the blood in a unit of time, is the product of clearance (the volume of blood cleared per unit of time) and concentration (the amount of a compound per unit of volume). For the first order reactions, clearance is a constant value that is a characteristic of a substance. If the input of a substance to an organism is greater than the rate at which the substance is lost, the organism is said to be accumulating the substance. When the concentration has increased so that the amount eliminated equals the amount of substance-input there will be a constant concentration, a steady-state. The extent of accumulation reflects the relationship between the body-burden compared with the steady-state condition. Species differences in clearance will determine the difference in steady-state body-burden between experimental animals and humans.

1.3.2.6 Toxicokinetics in practice: prediction, derivation and generation of information

A tiered approach has been proposed by DG SANCO (EC, 2007) for the derivation and generation of TK information. In addition, for the purpose of BPR the ECHA Biocides Guidance, Volume III Human health Part A Information requirements describes the type of strategies to be considered for the generation of TK information. In alignment with this, a strategy can be derived on how much effort on TK evaluation for different levels of importance of a substance is appropriate. Considerations on the possible activity profile of a substance derived from physico-chemical and other data, as well as structurally related substances should be taken into account as a minimum request. This might help in the argumentation on waiving or triggering further testing and could provide a first impression of the mode of action of a substance. Subsequent TK data need to focus on the studies which interpret and direct any additional toxicity tests that were conducted.

Prediction of toxicokinetics

1.3.2.6.1 Absorption

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 provides information on the relative solubility of the substance in water and in the hydrophobic solvent octanol (used as a surrogate for lipid) 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. Nevertheless, a substance with such log P value can be poorly soluble in lipids and hence not readily absorbed when its water solubility is very low. It is therefore important to consider both, the water solubility of a substance and its log P value when assessing the potential of that substance to be absorbed.

(a) Oral/GI absorption

When assessing the potential of a substance to be absorbed in the GI tract it should be noted that substances could undergo chemical changes in the GI fluids as a result of metabolism by GI flora, by enzymes released into the GI tract, or by hydrolysis. These changes will alter the physico-chemical characteristics of the substance and hence predictions based upon the physico-chemical characteristics of the parent substance may no longer apply (see Appendix 1-1)for a detailed listing of physiological factors, data on stomach and intestine pH, data on transit time in the intestine).

One consideration that could influence the absorption of ionic substances (i.e. acids and bases) is the varying pH of the GI tract. It is generally thought that ionized substances do not readily diffuse across biological membranes. Therefore, when assessing the absorption potential of an acid or a base, knowledge of its pKa (pH at which 50% of the substance is in ionized and 50% in non-ionised form) is advantageous. Absorption of acids is favoured at pH <pKa whereas absorption of bases is favoured at pH >pKa.

Other mechanisms by which substances can be absorbed in the GI tract include the passage of small water-soluble molecules (molecular weight up to around 200) through aqueous pores or carriage of such molecules across membranes with the bulk passage of water (Renwick, 1994). The absorption of highly lipophilic substances (log P ≥4) may be limited by the inability of such substances to dissolve into GI fluids and hence make contact with the mucosal surface. However, the bile salts micellular solubilisation enhances the absorption of such substances (Aungst and Shen, 1986). 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 (passage through gaps in membranes left when the tips of villi are sloughed off) (Aungst and Shen, 1986). 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 absorption is minimal and occurs by passive diffusion, if at all. Therefore, substances enter directly the systemic circulation; however, some enzymatic degradation may occur. Like in the mouth, absorption in the stomach is minimal and occurs only by passive diffusion - the acidic environment favours uptake of weak acids. There is a 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 - 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. However, lipophilic compounds may form micelles and be absorbed into the lymphatic system and larger molecules/particles may be taken up by pinocytosis. Gut microflora or enzymes in the GI mucosa may metabolise the compounds 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 present too. Compared to the small intestine, the rate and extent of absorption within the large intestine is 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 (SO3H), hydroxyl (OH-), carboxyl (COOH) or amine (NH2).
Molecular weight	Generally the smaller the molecule the more easily it may be taken up.

Data source	What it tells us
	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 gastrointestinal fluids. Absorption of very hydrophilic substances via passive diffusion may be limited by the rate at which the substance partitions out of the GI fluid. However, if the molecular weight is low (<200) the substance may pass through aqueous pores or be carried through the epithelial barrier by the bulk passage of water.
Log P	Moderate log P values (between -1 and 4) are favourable for absorption by passive diffusion. Any lipophilic compound may be taken up by micellular solubilisation but this mechanism may be of particular importance for highly lipophilic compounds (log P >4), particularly those that are poorly soluble in water (≤1 mg/L) and would otherwise be poorly absorbed.
Dosing vehicle	If the substance has been dosed using a vehicle, the water solubility of the vehicle and the vehicle/water partition coefficient of the substance may affect the rate of uptake. Compounds delivered in aqueous media are likely 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) (D'Souza, 1990) or in soil, the latter being an important vehicle for children.
Oral toxicity data	If signs of systemic toxicity are present then absorption has occurred[4]. Also coloured urine and/or internal organs 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. Also some clinical signs such as hunched posture could be due to discomfort caused by irritation 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 effects.
Hydrolysis test	The hydrolysis test (EU C.7; 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, toxicokinetic predictions based on the characteristics of the parent

[4] Ensure that systemic effects do not occur secondary to local effects.

Data source	What it tells us
	compound may be of limited relevance.

(b) 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 (both liquid substances and solid substances in solution) and finely divided powders/dusts. Substances may be absorbed directly from the respiratory tract or through the action of clearance mechanisms, may be transported out of the respiratory tract and 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. The increasing water solubility increases the amount absorbed per breath. However, the gas or vapour must also be sufficiently lipophilic to cross the alveolar and capillary membranes. Therefore, a moderate log P value (between -1 and 4) would be favourable for absorption. The deposition pattern of vapours in the form of readily soluble substances (i.e. hydrophilic) differs from the lipophilic substances. The hydrophilic substances are effectively removed from the air in the upper respiratory tract, whereas the lipophilic 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, thereby reducing the amount available for absorption. Besides the physico-chemical properties of the compound, physical activity (such as exercise, heavy work, etc.) has a great impact on absorption rate and must also be addressed (Csanady and Filser, 2001).

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. As a rough guide, particles with aerodynamic diameters <100 µm have the potential to be inspired. Particles with aerodynamic diameters <50 µm may reach the thoracic region and those <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 (Velasquez, 2006). Thus, the quantitative deposition pattern of particles in the respiratory tract varies. Nonetheless, general deposition patterns may be derived (Snipes, 1989). Several models exist to predict the particle size deposition patterns in the respiratory tract (U.S. EPA, 1994).

Generally, liquids, solids in solution, and water-soluble dusts would readily diffuse/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. Some evidence suggests that substances with higher log P values may have a longer half-life within the lungs but this has not been extensively studied (Cuddihy and Yeh, 1988). Very hydrophilic substances might be absorbed through aqueous pores (for substances with molecular weights <ca. 200) 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 (Schlesinger, 1995). Such dusts depositing in the tracheo-bronchial region would mainly be cleared from the lungs by the mucocilliary mechanism 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). This value has been used within the ECETOC TRA model; however, for biocidal active substances and products the HEEG Opinion on Inhalatory exposure and Section 3 (Exposure Assessment) should be followed regarding the consideration of vapour pressure in assessing respiratory absorption.
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 diameters 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. Thus the quantitative deposition pattern of particles in the respiratory tract varies with the particle size distribution of the inspired aerosol and may further depend on physical and physico-chemical properties of the particles (e.g. shape, electrostatic charge). Nonetheless general deposition patterns may be derived (Snipes, 1989; U.S. EPA, 1994)
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 signs of systemic toxicity are present then absorption has occurred. This is not a quantitative measure of absorption.
Oral toxicity data	If signs of systemic toxicity are 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 (EU C.7; 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, toxicokinetic predictions based on the characteristics of the parent compound may be of limited relevance.

(c) Dermal absorption

The skin is a dynamic, living multilayered biomembrane and thus, 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 (Flynn, 1985).

Dermal absorption is influenced by many factors, e.g. physico-chemical properties of the substance, its vehicles and concentration, and the exposure pattern (e.g. occlusion of the application site) as well as the skin site of the body (for review see ECETOC, 1993; Howes *et al*., 1996; Schaefer and Redelmeier, 1996). Substances that can potentially be taken up across the skin include gases and vapours, liquids, and particulates. As it is not always mandatory to submit experimental data for the assessment of dermal absorption, as a first step default values (depending on physico-chemical properties of the active substance) can be used. A tiered approach for the estimation of skin absorption has been proposed within a risk assessment framework (EC, 2007). According to this initially, basic physico-chemical information should be taken into account, i.e. molecular mass and lipophilicity (log P). Following, a default value of 100% skin absorption is generally used unless molecular mass is above 500 and log P is outside the range [-1, 4], in which case a value of 10%[5] skin absorption is chosen (De Heer *et al*., 1999). However, for the purpose of estimating dermal absorption for biocidal active substance and products, using default values on the basis of physico-chemical properties, the principles described in the OECD Guidance on Dermal Absorption (OECD, 2004; OECD, 2011) as well as the approach and default values described in the EFSA Guidance Document for dermal absorption (EFSA, 2012) should be considered.

The assessment of dermal absorption data (experimental data) should follow the principles according to the OECD Guidance on Dermal Absorption (OECD, 2004; OECD, 2011) as well as the EFSA Guidance Document (EFSA, 2012).

In addition, 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. A flow diagram outlining the principles within the tiered approach is presented in Figure 2; this diagram should be considered in line with the Tiering schema for refinement of risk characterisation as

[5] The lower limit of 10% was chosen because there is evidence in the literature that substances with molecular weight and/or log P values at these extremes can to a limited extent cross the skin. This alternative value can be used if there are available data indicating that the use of an alternative dermal absorption percentage value is appropriate (e.g. data on water solubility, ionogenic state, 'molecular volume', oral absorption, and dermal area dose in exposure situations in practice). Scientific justification for the use of alternative values should be provided.

described in Section 4.6 especially regarding the use of PPE in exposure assessment. If an initial assessment ends up with a risk, more refinement could be obtained in the next tier if more information is provided on the dermal absorption. In the first tier of risk assessment a worst-case value for dermal absorption of 100% could be used for external dermal exposure in case no relevant information is available (Benford *et al*., 1999). As the second tier, an estimate of dermal absorption could be made by considering other relevant data on the substance (e.g. molecular weight, log P and oral absorption data) or by considering experimental *in vitro* and *in vivo* dermal absorption data. If at the end of the third tier still a risk is calculated, the risk assessment could be refined by means of actual exposure data. This approach provides a tool for risk assessment, and in general it errs on the safe side.

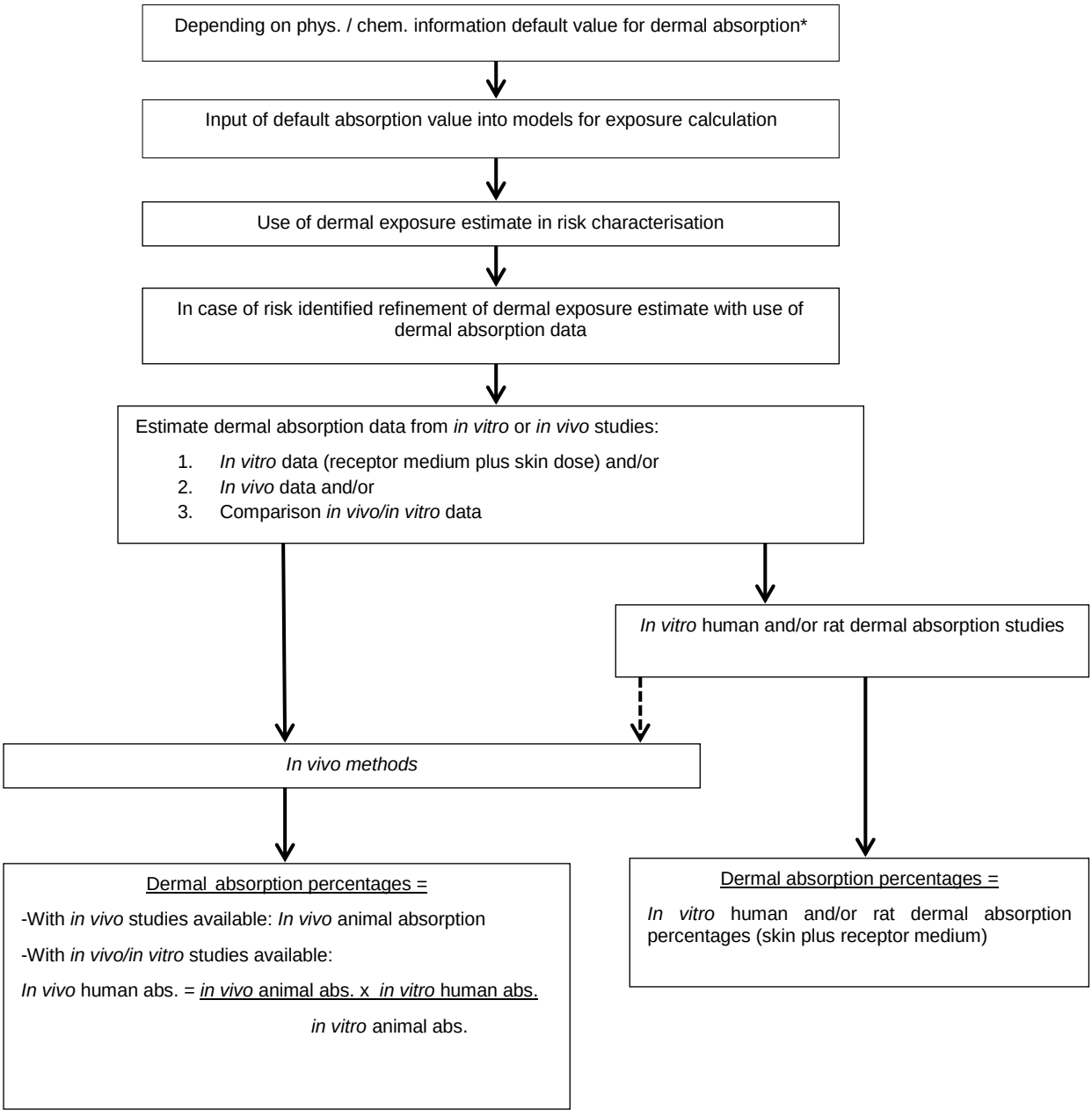
In addition to the default values for dermal absorption estimation, *in vivo* and or *in vitro* studies can be used as standalone or in combination for estimation of dermal absorption percentage (Benford *et al*., 1999).

If appropriate, dermal penetration data are available for rats *in vivo* and for rats and humans *in vitro.* 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 (e.g. Howes *et al*., 1996). A generally applicable correction factor for extrapolation to human can not, however, be derived because the extent of overestimation appears to be dose, substance, and animal specific (Howes *et al*., 1996; Bronaugh and Maibach, 1987). For the correction factor based on *in vitro* data, preferably maximum flux values should be used. Alternatively, the dermal absorption percentage (receptor medium plus skin dose) may 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.

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

Similar adjustments can be made for differences between formulants (e.g. *in vivo* active substance in rat and *in vitro* rat data on formulants and active substance).

Figure 2: Flow diagram on the use of dermal absorption information in calculating exposure estimates



*Default dermal absorption values can be calculated following the approach described in the EFSA Guidance Document for dermal absorption (EFSA, 2012) where applicable and the principles described within the OECD Guidance Document on Dermal Absorption (OECD, 2004; OECD, 2011) .

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 evaporates off the skin surface (Pryde and Payne, 1999).
Molecular weight	<100 favours dermal uptake but when >500 the molecule 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. However, if water solubility is above 10,000 mg/L and the mg/L value <0 the substance may be too hydrophilic to cross the lipid rich environment of the *stratum corneum*. Dermal uptake for these substances will be low.
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 likely to be sufficiently lipophilic to cross the *stratum corneum*, therefore 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. > 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. > 6, the rate of transfer between the *stratum corneum* and the epidermis will be slow and will limit absorption across the skin. Uptake into the *stratum corneum* itself may be slow.
Vapour pressure	The evaporation rate will offset the rate at which gases and vapours partition from the air into the *stratum corneum*. Therefore, although a substance may readily partition into the *stratum corneum*, it may be too volatile to penetrate further. This can be the case for substances with vapour pressures above 100-10,000 Pa (ca. 0.76-76 mmHg) at 25°C, though the extent of uptake would also depend on the degree of occlusion, ambient air currents, and the rate at which it is able to transfer across the skin. Vapours of substances with vapour pressures below 100 Pa are likely to be well absorbed and the amount absorbed dermally may be more than 10% of the amount that would be absorbed by inhalation.
Surface tension	If the surface tension of an aqueous solution is <10 mN/m, the substance is a surfactant and this will enhance the potential dermal uptake. Surfactants can also substantially enhance the absorption of

Data source	What it tells us
	other compounds, even 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.
Dermal toxicity data	Signs of systemic toxicity indicate that absorption has occurred. However, if steps have not been taken to prevent grooming, the substance may have been ingested and therefore signs of systemic toxicity could be due to oral rather than dermal absorption.
Skin sensitization data	If the substance has been identified as a skin sensitizer then, provided the challenge application was to intact skin, 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.

Even though many factors (Table 3) are linked to the chemical itself, one should bear in mind that the final preparation or the production conditions, or the use can influence both rate and extent of dermal absorption. For biocidal products, the bridging approach given in the EFSA Guidance Document on Dermal Absorption (EFSA, 2012), Chapter 6.2 Use of data on similar formulations) should be followed when considering estimation of dermal absorption.

1.3.2.6.2 Distribution

The concentration of a chemical in blood or plasma (blood level) is dependent on the dose, the rates of absorption, distribution, and elimination, and on the affinity of the tissues for the compound. Tissue affinity is usually described using a parameter known as the volume of distribution which is a proportionality factor between the amount of compound present in the body and the measured plasma or blood concentration. The larger the volume of distribution is, the lower the blood level will be for a given amount of compound in the body. A particularly useful volume term is the volume of distribution at steady-state (Vdss). At steady-state, all distribution phenomena are completed, the various compartments of the body are in equilibrium, and the rate of elimination is exactly 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. In theory, steady-state can be physically reached only in the case of a constant zero-order input rate and stable first-order distribution and elimination rates. However, many real situations are reasonably close to steady-state, and reasoning at steady-state is a useful method in kinetics.

The rate at which highly water-soluble molecules distribute may be limited by the rate at which they cross cell membranes and access of such substances to the CNS or testes is likely to be restricted by the blood-brain and blood-testes barriers (Rozman and Klaassen, 1996). It is not clear what barrier properties the placenta may have. However, species differences in trans-placental transfer may occur due to differing placental structure and also differing metabolic capacity of the placenta and placental transporters in different species.

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 they will bind. Furthermore, if a substance undergoes

extensive first-pass metabolism, predictions made on the basis of the physico-chemical characteristics of the parent substance may not be applicable.

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 the toxicologically active species, it may be possible to draw some conclusions about the distribution of that substance from its target tissues. If the substance is a dye, coloration of internal organs can give evidence of distribution. This will not provide any information on the amount of substance that has distributed to any particular site. 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 sensitization data	If the substance has been identified as a skin sensitizer then, provided the challenge application was to intact skin, 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.

1.3.2.6.3 Accumulative potential

It is important to consider the potential of a substance to accumulate or to be retained within the body because due to the gradual build up with successive exposures, the body burden can be maintained for long periods of time.

Lipophilic substances have the potential to accumulate within the body if the dosing interval is shorter than four times the whole body half-life. 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 (e.g. daily at work). 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 that 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 (Rozman and Klaassen, 1996).

Table 5 provides an overview of data that can be considered for the estimation of accumulation.

Table 5: 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. Here particles 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 and 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, which leads to the possibility of long-term retention (Snipes, 1995).
Adipose tissue	Lipophilic substances will tend to concentrate in adipose tissue and depending on the conditions of exposure may accumulate. If the interval between exposures is less than 4 times the whole body half-life of the substance then there is the potential for the substance to accumulate. Generally, substances with high log P values have long biological half-lives. On this basis, daily exposure to a substance with a log P value of around 4 or higher could result in a 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. Once exposure to the substance stops, the substance will be gradually eliminated at a rate dependent on the half-life of the substance. If fat reserves are mobilized more rapidly than normal, e.g. if an individual or an animal is under stress or during lactation, there is the potential for large quantities of the parent compound to be released into the blood.
Bone	Certain metals e.g. lead and small ions, such as fluoride, can 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.

1.3.2.6.4 Metabolism

The main reason for species and route specific toxicity are the differences in the metabolism of substances among the different species and tissues. The liver has the greatest capacity for metabolism and is commonly causing route specific pre-systemic effects (first pass) especially following oral intake. However, route specific toxicity may result from several phenomena, such as hydrolysis within the GI or respiratory tract, metabolism by GI flora or within the GI tract epithelia (mainly in the small intestine) (for

review see Noonan and Wester, 1989), respiratory tract epithelia (sites include the nasal cavity, tracheo-bronchial mucosa [Clara cells] and alveoli [type 2 cells]), and skin.

Prediction of the changes that a substance may undergo is difficult to make only on the basis of the physico-chemical information alone. Although it is possible to look at the structure of a molecule and identify potential metabolites, it is not certain that these reactions will 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 to which it will be metabolised along different pathways and what species differences may exist. Consequently, experimental data shall help in the assessment of potential metabolic pathways.

1.3.2.6.5 Excretion

The major routes of excretion for substances from the systemic circulation are the urine and/or the faeces, via bile and directly from the GI mucosa (see Rozman, 1986).

The excretion processes involved in the kidney are 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 compounds) and generally, they are conjugated metabolites (e.g. glucuronides, sulphates, glycine conjugates) from Phase II biotransformation. Kidneys will have filtered out of the blood most of them, 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 (Smith, 1973) involves active secretion rather than passive diffusion. Substances that are excreted in the bile tend to have higher molecular weights or may be conjugated as glucuronides or glutathione derivatives. It has been found that in rats substances with molecular weights < ca. 300 do not tend to be excreted into the bile (Renwick, 1994). There are species differences and the exact nature of the substance also plays a role (Hirom *et al*., 1972; Hirom *et al*., 1976; Hughes *et al*., 1973). Hepatic function highly influences the excretion of compounds via bile, as the metabolites formed in the liver may be excreted directly into the bile without entering the bloodstream. Additionally, blood flow as such is a determining factor.

Substances in the bile pass through the intestines before they are excreted in the faeces. As a result the substances may undergo enterohepatic recycling (i.e. circulation of bile from the liver, where it is produced, to the small intestine, where it aids in digestion of fats and other substances, back to the liver) which will prolong their biological half-life. This is a particularly 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 in the 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. However, depending on the possible metabolic changes, the compound that is finally excreted may have few or none of the physico-chemical characteristics of the parent compound.

Table 6 provides an overview of the data that can be used for estimation of excretion.

Table 6: 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 ionization of the molecule at the pH of urine.

Exhaled air	Vapours and gases are likely to be excreted in exhaled air. Also volatile liquids and volatile metabolites may be excreted as vapours in 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 general, it is unlikely for rats 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 (Smith, 1973). 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 generally also may be found in breast milk. Lipid soluble substances may be present at higher concentrations in milk than in blood/plasma. Although lactation is minor route of excretion, exposure of neonates via nursing to mother's milk may have toxicological significance for some chemicals.
Saliva/sweat	Non-ionized and lipid soluble molecules may be excreted in saliva or in 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 skin cells.

1.3.2.7 Generating and integrating toxicokinetic information

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, a TK *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 those that cause adverse effects in toxicity studies. Ideally there should be also a dose without toxic effect, 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 and thus, essential for extrapolation and risk assessment.

In an *in vivo* study the systemic bioavailability is usually estimated by the comparison of either dose-corrected amounts excreted, or of 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 a bioavailability of 100%. 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 experimental strategy has to be revised on a case-by-case basis, depending on the type of non-linearity involved (e.g. saturated protein binding, saturated metabolism, etc.).

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 to facilitate interspecies comparisons (e.g., metabolite profile, metabolic rate constants). In recent years methods using the appropriate physiologically based kinetic models to integrate a number of *in vitro* results into a prediction of ADME *in vivo* have been developed. 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 resulting information on ADME can be used to inform development decisions, as well as part of the risk assessment process. The uncertainty associated with the prediction depends largely on the amount of available data.

1.3.2.7.1 Important methods for generation of ADME data

In addition to the predictive approaches described earlier and to the test methods described in the *Guidance on the BPR: Volume III Human Health, Part A Information Requirements*, Section 8.8. 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. Additional guidance has been developed by WHO/IPCS on Characterisation and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment (WHO/IPCS, 2010).

1.3.2.7.1.1 *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 of chemicals in the body. These methods include, for example, QSAR models, compartmental models, or allometric equations (Ings, 1990; Bachmann, 1996). Their main advantages compared to classical (*in vitro*, *in vivo*) methods are that they estimate the TK of a given agent in a quicker and cheaper manner, and reduce the number of experimental animals. A detailed discussion of the approaches that integrate information generated *in silico* and *in vitro* is presented in, Appendix 1-2 of this guidance.

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

- Either the fitting situation, where values of some or all parameters are unknown and the model is adjusted (fitted) to data in order to extract from the dataset these parameter values;
- or the 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 or scientific teams can perform the model fitting as well as the simulation operations, because both aspects have specific technical problems and pitfalls. Simulation is an extremely useful tool because it is the only way to predict situations for which it is not, and often will never be, possible to generate or collect real data. The results of carefully designed simulations with attached uncertainty estimations are then the only available tools for quantitative risk assessment. The better the model-building steps are performed, the better defined the predictions are.

In order to identify the TK relationship in a risk assessment context as well as possible, , the TK information collected from *in vitro* and *in vivo* experiments could 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 of 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 called pharmacokinetic/pharmacodynamic modelling, or PKPD modelling. The term toxicokinetic/toxicodynamic modelling, or TKTD, covers the same concept.

TK models typically describe the body as a set of compartments through which chemicals are transformed or travel. They fall into two main classes: empirical models and physiologically-based kinetic models (Andersen, 1995; Balant and Gex-Fabry, 1990; Clewell and Andersen, 1996; Gerlowski and Jain, 1983). All these models subdivide the body into compartments within which the toxic agent is assumed to be homogeneously distributed (Gibaldi, 1982) and thus simplify 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 (with a low number of parameters), allow describing many kinds of kinetics, and can be easily 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, exceptionally more than 3 compartments per compound or metabolite) where ADME phenomena occur. 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 are often 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
- 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 such parameters are measured (see e.g. Brown *et al*., 1997; Clewell and Andersen, 1996; Dedrick and Bischoff, 1980) and used to construct an a priori model that qualitatively describes a dataset, then 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 (Theil *et al*., 2003). 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 below) of these models (Gueorguieva *et al*., 2006; Nestorov, 1999) may be performed for identifying which parameters are important within a model. It helps prioritizing and focusing on only those parameters which have a significant impact on the risk assessment process and to identify sensitive populations. A discussion on the applicability of physiologically-based kinetic modelling for the development of assessment factors in risk assessment is presented in Appendix 1-3 of this document.

The potential of physiologically-based kinetic models to generate predictions from *in vitro* or *in vivo* information is one of their attractive features 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, as well as on the feasibility of generating additional data. When new information becomes available, the physiologically-based kinetic model should be calibrated. Bayesian techniques, for example, can be easily used for that purpose.

Physiologically-based kinetic models are very useful when the kinetic process of interest cannot be directly observed and also when extrapolations are needed. Indeed, inter-species, inter-individual, inter-dose or inter-route extrapolations are more robust when they are based on physiologically-based kinetic rather than on empirical models. The intrinsic capacity for extrapolation makes physiologically-based kinetic models particularly attractive 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. More specifically, physiologically-based kinetic models also allow evaluating TK in reprotoxicity, developmental and multi-generational toxicological studies. Physiologically-based kinetic model can be developed to depict internal disposition of chemical during pregnancy in the mother and in the embryo/foetus (Corley *et al*., 2003; Gargas *et al*., 2000; Lee *et al*., 2002; Luecke *et al*., 1994; Young *et al*., 2001). Lactation transfer of toxicant from mother to newborn can also be quantified using physiologically-based kinetic models (Byczkowski and Lipscomb, 2001; Faqi *et al*., 1998; You *et al*., 1999). One of the main benefits of physiologically-based kinetics is also the ability to check complex hypothesis (for example, the existence of an unknown metabolism pathway or site) and to give predictions on the internal doses (which are not always observable in human). Finally, 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.

1.3.2.7.1.2 Sensitivity analysis

As biological insight increases, more complex mathematical models of physiological systems that exhibit more complex non-linear behaviour appear. 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 those predictions were produced. That is; how do the hypotheses that fit together to make the model interact with each other? Which of the assumptions or mechanisms are the most important in determining the output? How sensitive is the model output to changes of the input parameters or the model structure? Sensitivity analysis techniques that give a measure of the effects on model output caused by

variation in its input can address these questions. Sensitivity analysis 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 (Saltelli *et al*., 2000).

Predictions from a complex mathematical model require a detailed sensitivity analysis in order to assess the limitations of the model predictions provided. 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.

1.3.2.8 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 minimize uncertainty in order to assess the variability that may exist between individuals so that there is confidence in the TK results such that they can be useful for risk analysts and decision-makers.

1.3.2.8.1 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, lifestyles, physiological status, age, sex, etc. Variability is characteristic for animal and human populations. It can be observed and registered as information about the population but it cannot be 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 then 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 the variability into physiologically-based kinetic models. The propagation of the variability to model predictions may be evaluated using Monte Carlo simulation methods.[6]

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

Uncertainty may be related to:

[6] 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.

- **The experimental nature of the data**. Indeed, uncertainty comes from errors in experimental data. Experimental data are typically known with finite precision dependent of the apparatus used. However, such uncertainties may be easily assessed with quality measurement data. They 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. In this case, the world is not fully understood and therefore not modelled exactly. Summing up, a massive amount of information in a model can 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 not feasible to integrate all the interactions between its components (most of them are not even fully known and quantified) in the development of a model. Therefore modelers have to simplify reality. Such assumptions will however introduce uncertainty. A general statistical 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[7]) 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 it is not fully understood and attributed to randomness.

1.3.2.9 Include human data when available to refine the assessment

Human biological monitoring and biological marker 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 (Woollen, 1993). The results from these studies, e.g. temporal situational biological monitoring, provide a realistic description of human exposure.

Biomonitoring (the routine analysis of human tissues or excreta for direct or indirect evidence of human exposures to substances) can provide unique insights into the relationship between dose and putative toxicity thresholds established in experimental animals, usually rats. Pioneering research by Elkins *et al*. (1954) on the relationship between concentrations of chemicals in the workplace and their concentrations in body fluids helped to establish the Biological Exposure Index (ACGIH, 2002). Urine is the most frequently used biological specimen, due to its non-invasive nature, ease of 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, the biological relevance, and feasibility considerations, in order to maximise the relevance of the information obtained.

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

1.3.2.10 Illustration of the benefit of using toxicokinetic information

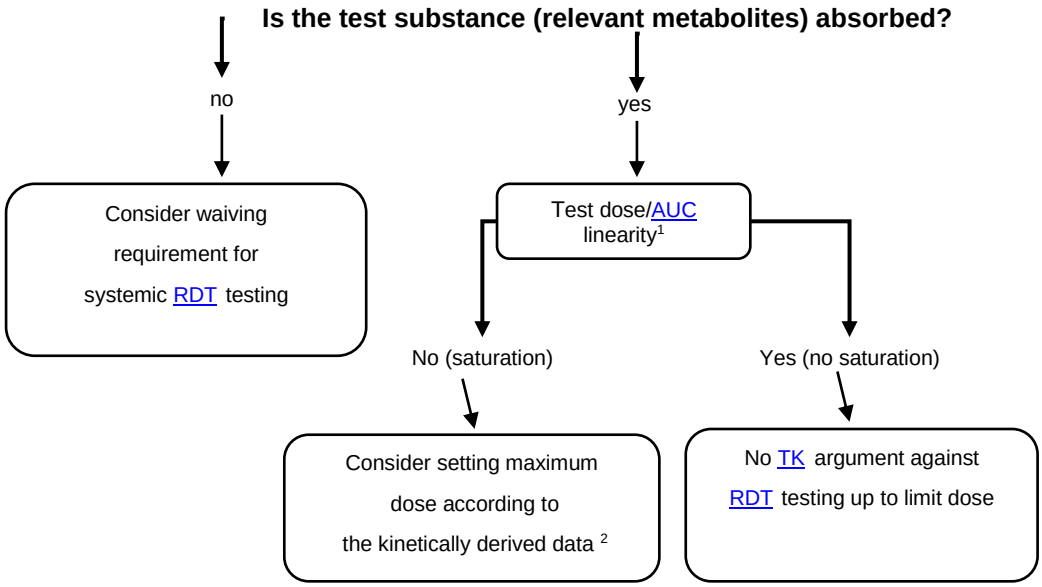
The following diagrams shall illustrate the way of thinking that can be applied regarding making use of TK information when this is available. It should be acknowledged that just in very rare cases a *yes-no* answer could be applied. Often a complex pattern of different information creates specific situations that deviate from the simplified standard procedures given below. The answer *no* can be understood with regard to *no significant* effect based on substance dependent expert judgment and detection limits of sensitive test method. Therefore, experts need to be consulted on use of TK data for designing tests individually, on interpretation of results for clarifying the mode of action, on grouping or read-across approach and also on the use of computational physiologically-based kinetic model systems.

1.3.2.10.1 Use of toxicokinetic information to support dose setting decisions for repeated dose studies

TK data, especially information on absorption, metabolism, and elimination, are highly useful in the process of the design of RDT studies. RDT studies should be performed according to the respective OECD/EU guidelines. The highest dose level in such studies should be chosen with the aim to induce toxicity but not death or severe suffering in the test animals. To do this, the OECD/EU guidelines suggest to test up to the standardised limit dose level called 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 be readily metabolised and cleared from the body. Consequently, when designing repeated dose toxicity studies, it is convenient to select the appropriate dose levels on the basis of results from metabolic and toxicokinetic investigation.

Figure 3 illustrates how TK data could assist in dose setting decisions for repeated dose toxicity studies.

Figure 3: Use of TK data in the design of RDT studies



[1] In the dose-range under consideration for RDT testing.

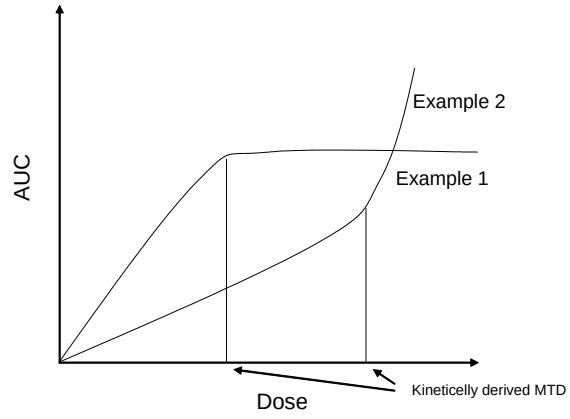
[2] Meaning that the highest dose-level should not exceed into the range of non-linear kinetics.

The question which needs to be addressed initially is whether the substance is absorbed. If it can be demonstrated that a substance is not absorbed, it cannot induce direct systemic effects. In such a case, there is no need for further repeated dose testing, from the kinetic point of view.[8] However, if the substance is absorbed the question, whether there is a linear relationship between the administered dose and the AUC in the blood, arises. If this is the case, and the substance is not metabolised then there is no kinetic argument against testing at the standardised MTD suggested by the OECD/EU guidelines.

Often the dose/AUC relationship deviates from linearity above a certain dose. This is illustrated in Figure 4. In both cases described 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 4: 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 beyond this dose.



1.3.2.10.2 Use of kinetic information in the design and validation of chemical categories/grouping

Information on kinetics *in vivo* will assist the design of 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 or membership of a certain substance to a category can be verified using kinetics information.

1.3.2.10.3 Metabolism Studies as basis for internal dose considerations

Biotransformation of a substance produces metabolites that may have different toxicological properties than the substrate from which they are formed. Although metabolism is generally referred to have a detoxification purpose, there are also many examples where metabolites have higher intrinsic toxicity than the parent compound

[8] Secondary effects misinterpreted as primary toxic effects need to be excluded.

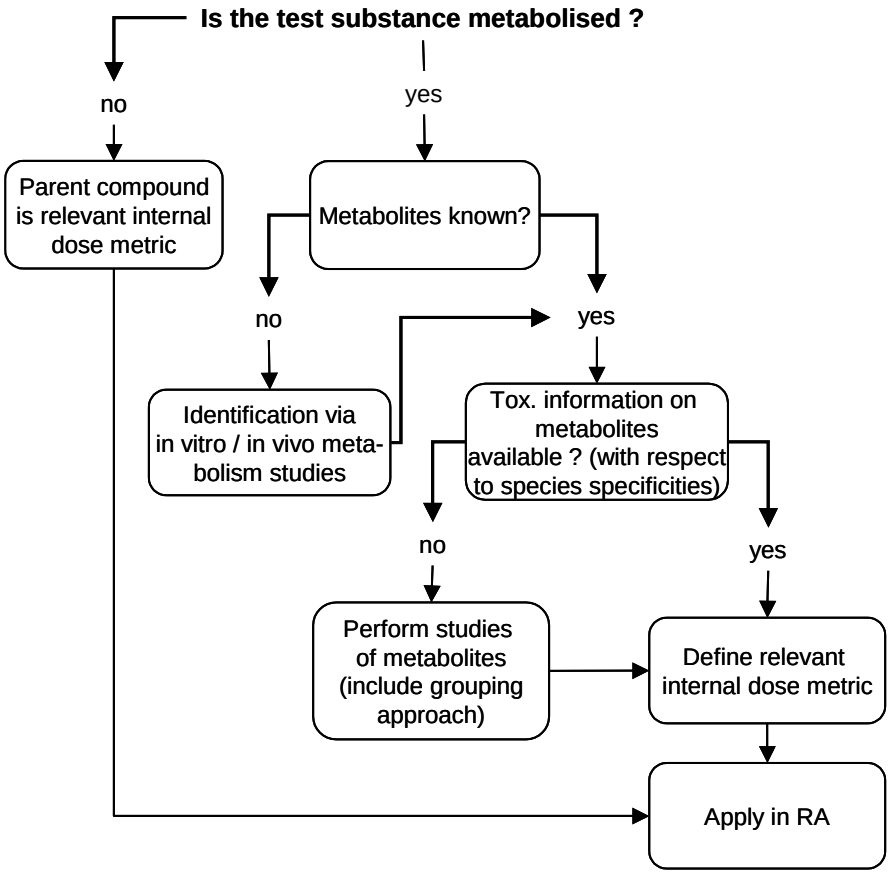
itself (metabolic activation). Therefore, it is necessary to know if the test substance is metabolised and to which metabolites. This enables the assessment of the results from toxicity studies in respect to waiving and grouping approaches, and defines the internal dose (see Figure 5).

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 which metabolites are formed is essential for any further step in an assessment. When this information is not available, it can be investigated by appropriate *in vitro* and/or *in vivo* metabolism studies. In special cases metabolites may show a high degree of isomeric specificity and this should be kept in mind when designing and interpreting mixtures of isomers, including racemates. If the metabolites are known and the toxicity studies are available for these metabolites, the risk assessment may be carried out based on these data and an assessment based on the definition of the internal dose can be made. If the toxicity profile of the metabolites is unknown, studies that address the metabolites toxicity may be performed under special considerations of potential group approaches. Especially, if a chemical substance is the metabolite of different compounds, e.g. carboxylic acid as a metabolite of different esters.

TK information can be very helpful in bridging various gaps as encountered in the whole risk assessment, from toxicity study design and biomonitoring[9] setup to the derivation of the threshold levels and various extrapolations as usually needed (cross-dose, cross-species including human, cross-exposure regimens, cross-routes, and cross-substances). The internal dose is the central output parameter of TK studies and therefore the *external exposure – internal dose – concept* is broadly applicable in the various extrapolations mentioned. If, for that purpose, route-to-route extrapolation is necessary and in case assessment of combined exposure (via different routes) is needed, for systemic effects, internal exposure may have to be estimated.

[9] Biological monitoring information should be seen as equivalent (i.e. as having neither greater nor lesser importance) to other forms of exposure data. It should also be remembered that biological monitoring results reflect an individual's total exposure to a substance from any relevant route, i.e. from consumer products, and/or from the environment and not just occupational exposure. Data from controlled human exposure studies are even more unlikely available. This is due to the practical and ethical considerations involved in deliberate exposure of individuals.

Figure 5: Use of increasing knowledge on substance metabolism



Exposure should normally be understood as an 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 in combined with the exposure duration, as appropriate. In cases, where a comparison needs to be made with systemic effects data (e.g. when inhalation or dermal toxicity values are lacking, or when exposures need to be combined due to more than one route) the total body burden has to be estimated and expressed as an internal dose.

Determination of the level of systemic exposure is considered synonymous to determination of the substance bioavailability to the general circulation. Depending on the problem considered and other related information (e.g. exposure scenarios), this could be expressed as a fraction bioavailable (F), a mass bioavailable, a concentration profile, an average concentration, or AUC. It should be emphasised, that it is usually not possible to show that the amount of a substance bioavailable is zero, apart from favourable 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 are 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, the estimated variability in the target population, etc.

The compound's tissue distribution characteristics can be an important determinant of its potential to cause toxicity in specific tissues. In addition, tissue distribution may be an important determinant of the ability of a compound to accumulate upon repeated exposure. However, the accumulation is substantially modified by the rate at which the compound is cleared. Correlation of a tissue distribution with target tissues in toxicity

studies should be accomplished while substantial amounts of the chemical remain present in the body (e.g. once or more times around the peak blood concentration following oral absorption. Such data should quantify the parent compound and the metabolites to the feasible extent. If the metabolites are unknown or difficult to quantify, subtracting parent compound from total radioactivity will estimate the behaviour of the total metabolites formed.

1.3.2.10.4 Extrapolation

For ethical reasons, if data allowing model parameters to be estimated are poor, sparse, and do not often concern human populations; the recourse to extrapolation is needed. TK data are mostly gathered for few concentrations (usually <5 different concentrations) and limited number of different exposure times. However, risk evaluation should assess the different doses (exposure concentrations and times). Inter-dose/inter-exposure time extrapolation is a common way to satisfy this demand, using mathematical methods (e.g. linear regression). The non-linear kinetic behaviour of chemicals in a biological organism is the result of a number of mechanisms, e.g. saturable metabolism, enzyme induction, enzyme inactivation and depletion of glutathione, and other cofactor reserves. High-dose-low-dose extrapolation of tissue dose is accomplished via physiologically-based kinetic modelling by accounting for such mechanisms (Clewell and Andersen, 1996).

In the rare case where data on human volunteers are available, they only concern a very limited number of subjects. Extrapolation to other populations can be done (inter-individual extrapolation). The problem of sensitive populations also arises and hence TK study should assess other populations, such as gender, age and ethnic groups, etc. As it is nearly impossible to control the internal dose in humans in practice, alternative animal study is often proposed. Since risk assessment aims at protecting human population, inter-species extrapolation (Davidson at al., 1986; Watanabe and Bois, 1996) should be done. For practical reasons, the administration route in experimental study can be different from the most likely exposure route. IN this case, the risk assessment suggests to conclude on another route than the one experimentally studied. Inter-route extrapolation should be performed.

Default values have been derived to match the extrapolation idea in a general way. The incorporation of quantitative data on interspecies differences or human variability in TK and TD into the dose/concentration-response dose assessment through the development of CSAFs might improve risk assessment of single substances. Currently, relevant data for consideration are often restricted to the component of uncertainty related to interspecies differences in TK. At the present time, there are commonly fewer data to address interspecies differences in TD and inter-individual variability in TK and TD. It is anticipated that the availability of such information will increase with a better common understanding of its appropriate nature (IPCS, 2001). The type of TK information that could be used 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, information on the reactive metabolites formation and possible species differences, and knowledge of the half-life and potential for accumulation under repeated exposure.

The need for these extrapolations can lead to more frequently use of the physiological TK models rather than the empirical models (Davidson at al., 1986; Watanabe and Bois, 1996; Young *et al*., 2001). Indeed, physiologically-based kinetic models facilitate the required extrapolations (inter-species, inter-subjects, etc.). For example, by changing anatomical parameters, such as organ volumes or blood flows, a physiologically-based kinetic model can be transposed from rat to human.

Interspecies extrapolation

The use of animal data for toxicological risk assessment brings the question of how to extrapolate experimentally observed kinetics to human subjects or populations. The ability to compare data from animals and from humans will enable defining chemical-specific interspecies extrapolation factors to replace the default values. One option is the extrapolation based on different body sizes, which calculates the allometric factors. The most complex procedure for inter-species extrapolation is collecting different data and using 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 (Schneider *et al*., 2004). 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:

$$Y = a\ BM^b$$

Y … quantity of interest
a … species-independent scaling coefficient[10],
BM … body mass
b … allometric exponent

Values of b depend upon whether the quantity of interest scales approximately with body mass (b=1), metabolic rate[11] (b=0.75), or body surface area (b=0.6712) (Davidson at al., 1986; Fiserova-Bergerova and Hugues, 1983; West *et al*., 1997). As it is easy to apply the allometric scaling, it is probably the most convenient approach to interspecies extrapolation. However, it is very approximate and may not hold for the chemical of interest. As such, it can be conceived as default approach to be used only in the absence of specific data in the species 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 have been applied in recognition of the uncertainty involved. Whereas the metabolic rate constant estimated in this way 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, where possible.

Consequently, to better estimate tissue exposure across species, physiologically-based kinetic models may be used for the considered toxicant (Watanabe and Bois, 1996). These models account for transport mechanisms and metabolism within the body. The same equation set then models the processes for all species considered.. Differences between species are assumed to be due to different (physiological, chemical and metabolic) parameter values. Extrapolation of physiologically-based kinetic models then relies on replacing the model parameter values of one species with the parameter values of the species of interest. For physiological parameters, numerous references (Arms and Travis, 1988; Brown *et al*., 1997; ICRP, 2002) give standard parameter values for many species. Chemical (partitioning coefficient) and metabolic parameter values are usually less easily found. When parameter values of physiologically-based kinetic model are not known for the considered species, the option of *in vitro* data, QSPR predictions or allometric scaling of those parameters is still possible. To take into account population

[10] Fits single data points together to form an appropriate curve.

[11] In this context it is not metabolism of compounds! The factor adapts different levels of oxygen consumption.

[12] This scaling factor is generally justified on the basis of the studies by Freireich et al. (1966), who examined the interspecies differences in toxicity of a variety of antineoplastic drugs.

variability in the extrapolation process, probability distributions of parameters may be used rather than single parameter values. Physiologically-based kinetic models can be particularly useful where data are being extrapolated to population subgroups for which only little information is available, e.g. pregnant women or infants (Luecke *et al*., 1994; Young *et al*., 2001).

Inter-route extrapolation

Route-to-route extrapolation is defined as the prediction of 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 of a substance administered by another route.

In general, route-to-route extrapolation is considered to be a poor substitute for toxicity data obtained using the appropriate route of exposure. Uncertainties in extrapolation increase when performing risk assessment with toxicity data obtained by an administration route which does not correspond to the human route of exposure. Insight into the reliability of the current methodologies for route-to-route extrapolation has not been obtained yet (Wilschut *et al*., 1998).

When route-to-route extrapolation is to be used, the following aspects should be carefully considered:

- nature of the effect: the route-to-route extrapolation is only applicable for the evaluation of systemic effects. For the evaluation of local effects after repeated exposure can be used only results from toxicity studies performed with the route under consideration;
- toxicokinetic data (ADME): the major factors responsible for differences in toxicity due to route of exposure include:
- differences in bioavailability or absorption;
- differences in metabolism (first pass effects);
- differences in internal exposure pattern (i.e. internal dose).

In the absence of relevant kinetic data, route-to-route extrapolation is only possible if the following assumptions are reasonably valid:

- Absorption can be quantified;
- Toxicity is a systemic effect not a local one (compound is relatively soluble in body fluids, therefore systemically bioavailable) and internal dose can be estimated;
- First-pass effects are minimal.

Provided that the listed criteria are met, the only possibility for the route-to-route extrapolation is to use default values. If route-to-route extrapolation is required or if an internal N(O)AEL/starting point needs are to be derived in order to assess combined exposure from different routes, information on the extent of absorption for the different routes of exposure should be used to modify the starting point. On a case-by-case basis a judgement has to be made, whether the extent of absorption for the different exposure routes determined from the experimental absorption data is applicable to the starting point of interest. Special attention should be given to the dose ranges employed in the absorption studies (e.g. very high dose levels), compared to those used to determine the starting point (e.g. much lower dose levels, especially in the case of human data). Consideration should also be given to the age of the animals employed in the absorption studies (e.g. adult animals), 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 lead to an additional modification of the starting point.

In practice, in the absence of dermal toxicity factors, the U.S. EPA (2004) has developed a simplified paradigm for making route-to-route (oral-to-dermal) extrapolations for systemic effects. This approach is subject to a number of factors that might compromise the applicability of an oral toxicity factor for dermal exposure assessment. The estimation of oral absorption efficiency, in order to adjust the toxicity 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 is defined as the rate and amount of chemical that reaches the systemic circulation unchanged. That is, bioavailability accounts for both absorption and pre-systemic metabolism. Although pre-systemic metabolism includes both gut wall and liver metabolism, it is liver first pass effect that plays the major role for the most parts.

In the absence of metabolic activation or detoxification, toxicity adjustment should be based on bioavailability rather than absorption because the dermal pathway appears to estimate the amount of parent compound entering the systemic circulation. Simple adjustment of the oral toxicity factor, based on the oral absorption efficiency, 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. The adjusted dermal toxicity factor may overestimate the true dose-response relationship because it would be based upon the amount of parent compound in the systemic circulation rather than on the toxic metabolite. Additionally, percutaneous absorption may not generate the toxic metabolite in the same rate and extent as the GI route.

In practice, an adjustment in oral toxicity factor (to account for absorbed dose in the dermal exposure pathway) is recommended when the following conditions are met: (1) the toxicity value derived from the critical study is based on an administered dose (e.g. dose delivered in diet or by gavage) in its study design; (2) a scientifically defensible database demonstrates that the GI absorption of the chemical in question, from a medium (e.g. water, feed) similar to the one employed in the critical study, is significantly less than 100% (i.e. <50%). If these conditions are not met, a default value of complete (i.e. 100%) oral absorption may be assumed, thereby eliminating the need for oral toxicity-value adjustment. In addition, when the oral absorption rate exceeds 80%, the default value of 100% should be applied for the derivation of AELs and internal exposure levels. The Uncertainty Analysis could note that employing the oral absorption default value may result in underestimating risk, the magnitude of which being inversely proportional to the true oral absorption of the chemical in question.

The chemical extrapolation of the kinetic behaviour from one exposure route to another can also be performed using physiologically-based kinetic models. Inclusion of appropriate model equations to represent the exposure pathways of interest is the basis of the extrapolation procedure. Once the chemical has reached the systemic circulation, its biodistribution is assumed to be independent of the exposure route. To represent each exposure pathway, different equations (or models) are typically used. The oral exposure of a chemical may be modelled by introducing a first order or a zero order uptake rate constant. To simulate the 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. After the equations describing the route-specific entry of chemicals into systemic circulation are included in the model, it is possible to conduct extrapolations of TK and dose metrics.

In conclusion, route-to-route extrapolation can follow the application of assessment factors, as long as the mentioned pre-conditions are met. Any specific TK information may refine the assessment factor in order to meet the precautionary function of the application of the factors as such.

1.4 Acute toxicity

The section on Acute Toxicity, Section 11.8.7 of the ECHA Biocides Guidance, Volume III Human health Part A (Information requirements) 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 the adverse effects, which may result from a single exposure (i.e. a single exposure or multiple exposures within 24 hours) 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 (for animals, refer to OECD Guidance Document 19 (OECD, 2000), 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 by this document, 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 effect, (i) general basal cytotoxicity (ii) selective cytotoxicity and (iii) cell-specific function toxicity. Acute toxicity may also result from chemicals interfering with extracellular processes (Seibert *et al*., 1996). 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; and/or
- in studies in animals, the lethal potency of the substance based on the LD50, the LC50, the discriminating dose and/or the acute toxic class; and/or
- 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); and
- when possible, the slope of the dose-response curve; and
- when possible, whether there are marked sex differences in response; and
- 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. Indeed, information on lethality is not an essential requirement for the classification decision or risk assessment. Other parameters and observations and their type of dose response may yield valuable information. The potential to avoid acute toxicity testing should be carefully explored by application of read-across or other non-testing means. Furthermore, there is an overriding obligation to minimize the use of animals in any assessment of acute toxicity. 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 which cause toxic signs, as well as the relationship of the severity of the toxic signs with the dose and the dose level at which the toxicity is not observed (i.e. the acute NOAEL). Although it is possible to use information from substance 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. Please note that 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 in case of the inhalation route (vapour pressure, MMAD, log K_{ow}), determining the technical feasibility of the testing and acting upon the distribution in the airways in particular for 'local-acting substances'. Indeed, 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 x 10^{-5} kPa (7.5 x 10^{-5} mmHg) for indoor uses, and <1 x 10^{-4} kPa (7.5 x 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. In that way, particular attention should be driven on results of aerosol particle size determination.

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 be useful for:

- 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 approach) data can be associated with results from particle size distribution analyses (see the *Guidance on information requirements and chemical safety assessment R.6 (QSARs and Grouping of Chemicals).*

Physico-chemical properties are also important to determine the potential of exposure through the skin, for example: log K_{ow}, molecular weight and volume, molar refraction, degree of hydrogen bonding, melting point (Hostýnek, 1998).

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

Generic guidance on the application of grouping approaches is provided in the *Guidance on information requirements and chemical safety assessment R.6 (QSARs and Grouping of Chemicals)*.

(c) (Q)SAR systems

Several (Q)SAR systems are available that can be used to make predictions about, for example, dermal penetration or metabolic pathways (see cross-cutting QSAR guidance for list of models). 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 complexity of the acute toxicity endpoint (possibility of multiple mechanisms) 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 ECHA Guidance IR+CSA Chapter R.7a.

In the case of grouping approaches, adequacy should be assessed and documented according to guidance described in the *Guidance on information requirements and chemical safety assessment Chapter R.6 (QSARs and Grouping of Chemicals).*

1.4.2.1.2 Testing Data for acute toxicity

(a) *In vitro* data

The *in vitro* tests that are currently available provide supplementary information which may be used to determine starting doses for *in vivo* studies, assist evaluation of data from animal studies, especially in identification of species differences, or to increase understanding of the toxicological mechanism of action of the substance. They cannot be used to replace testing on animals completely, although this may be possible in the future.

The outcome of the EU-US (ECVAM-ICCVAM) validation study on the Use of *In vitro* Basal Cytotoxicity Test Methods For Estimating Starting Doses For Acute Oral Systemic Toxicity (http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_brd.htm) was that the peer review panel agreed that the applicable validation criteria have been adequately addressed for using these *in vitro* test methods in a WoE approach to determine the starting dose for acute oral *in vivo* toxicity protocols. Moreover, on the basis of a preliminary analysis of data, there is the indication that the cytotoxicity tests might be useful in predicting low toxicity substances (LD_{50} ≥2g/kg body weight) and that they might, therefore, be used to filter these out in the future. This application needs to be validated with a wider range of compounds.

In vitro data may be useful for predicting acute toxicity in humans, providing 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 IC_{50} (inhibitory concentration 50%) value have been investigated. Indeed, on the basis of a preliminary comparison of data, there is the indication that the results of *in vitro* cytotoxicity tests may be more predictive of acute oral toxicity in humans than rat or mouse data. This aspect needs to be further investigated.

Generic guidance is given in the *Guidance on information requirements and chemical safety assessment Chapter 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 should be considered. These may be available from a wide variety of animal studies and give different amounts of direct or indirect information on the acute toxicity of a substance. Such studies can be for example:

- OECD TG 420 (EU B.1 bis) Acute oral toxicity – Fixed dose procedure;
- OECD TG 423 (EU B.1tris) Acute oral toxicity – Acute toxic class method;
- OECD TG 425 Acute oral toxicity – Up-and-down procedure;

- OECD TG 401 (EU B.1) Acute Oral Toxicity (method deleted from the OECD TGs for testing of chemicals and from Annex V to Directive 67/548/EEC; see below);
- OECD TG 402 (EU B.3) Acute dermal toxicity;
- OECD TG 403 (EU B.2) Acute inhalation toxicity;
- 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 mutagenicity (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 regarded as the key information for hazard assessment and supportive information for risk assessment. However, derivation of a precise LD_{50} or LC_{50} value is no longer considered essential. Indeed, some of the current standard acute toxicity test guidelines, such as the fixed dose procedures (OECD TG 420, EU B.1 bis and draft OECD TG 433), use signs of non-lethal toxicity and have animal welfare advantages over the other guidelines.

Existing OECD TG 401 (EU B.1) data would normally be acceptable but testing using this obsolete method must no longer be performed.
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 *Guidance on information requirements and chemical safety assessment Chapter 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 choice of optimum starting dose will minimize 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 (pathological findings have to be documented) 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. It is important to identify those dose levels which produce signs of toxicity, 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 (i.e. the acute NOAEL).

In addition to current available OECD or EU test methods, alternative new *in vivo* test methods for assessment of acute dermal and inhalation toxicity may be developed in future for regulatory purposes. Whichever test is used to evaluate an acute toxicity on animals, the evaluation of studies takes into account the reliability based on the approach of Klimisch *et al*., (1997) (standardised methods, GLP, detailed description of the publication), the relevance and the adequacy of the data for the purposes of evaluating the given hazard from acute exposure. The best studies are those that give a precise description of the nature and reversibility of the toxic effect, 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). The relevance of the data should be determined in describing the lethal or non-lethal endpoint being measured or estimated.

In addition, when several studies results are available for one substance, the most relevant one should be selected; data from others studies that have been evaluated should be considered as supportive data for the full evaluation of the substance.

The classification criteria for acute inhalation toxicity relate to a 4-hour experimental exposure period. If data for a 4-hour period are not available then extrapolation of the results to 4 hours are often achieved using Haber's Law (C.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 order to determine the 4-hour LC_{50} for C&L purposes.

Nowadays, a modification of Haber's Law is used ($C^n.t = k$), as for many substances it has been shown that *n* value is not equal to 1 (Haber's Law). 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 may be used. It is recommended to set n = 3 for extrapolation to shorter duration than the duration for which the LC_{50} or EC_{50} was observed and to set n = 1 for extrapolation to longer duration (ACUTEX TGD, 2006), 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 (EU B.2) or the CxT approach could be taken into consideration. The OECD TG 403/(EU B.2) will result in a concentration-response curve at a single exposure duration, the CxT approach will result in a concentration-time-response curve, taking different exposure durations into account. The CxT approach (under consideration for the revision of OECD TG 403) uses two animals per CxT 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). Nevertheless, the conduct of human studies for the purpose of the Biocides Regulation implementation is prohibited.

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 poisoning centres.

Available human data could also be useful to identify particular sensitive sub-populations like new born, children, patients with diseases (in particular with chronic respiratory conditions, such as asthma, COPD).

Additional guidance on the reliability and the relevance of human studies is provided in the *Guidance on information requirements and chemical safety assessment Chapter R.4*, as there are no standardised guidelines for such studies (except for odour threshold determination). Moreover, these studies are normally not conduced according to GLP. Poor quality of reporting often adversely affects the usefulness of reports about the effects arising from accidents or abuse, and may also be a problem in reports of the effects of short-term exposures in the workplace. Suspected subjective reporting of symptoms by the exposed people may complicate evaluation of a study. However, if there are several reports listing similar effects, this can be useful. 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 will need to be taken into account. It is sometimes 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 have the capacity to 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 available for classification and labelling. If the data available is not sufficient, additional testing will be required as described in the ECHA Biocides Guidance, Vol. III, Part A (Information Requirements).

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. It is more usual that the only numerical value derived is the $LD(C)_{50}$ value. When reviewing classification, care should be taken when using $LD(C)_{50}$ values from dermal or inhalation acute toxicity tests in which the duration's of exposure were different from those specified in Commission Regulation (EC) No 440/2008.

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, both standard OECD/EU test guideline data and all applicable data 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 the severity of the specific acute toxic effects can be used to make specific recommendations with respect to handling and use of the substance.

Information on acute toxicity is not normally limited to availability of a LD_{50} or LC_{50} value. Additional information which is important for the risk assessment will be both qualitative and quantitative and will 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 (adapted from ECETOC Monograph No 6, 1985).

If a NOAEL can be identified this can be used in determination of a threshold level. However, depending upon the nature of the acute toxicity information available, this may not always be possible. For instance, data from an OECD/EU test 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, but may not provide information on the dose level at which no adverse effects on health are observed. If the data permits construction of a dose-response curve, then derivation of the NOAEL may be possible. 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, then 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 certainly 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 process to be able to determine threshold levels for particular sensitive sub-populations like new-borns, children or those in poor health (patients).

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

The section on skin/eye irritation/corrosion within ECHA Biocides Guidance, Vol. III, Part A (Information Requirements) as well as Section 4.3.2. of this guidance should be considered together with the elements described in this section for the assessment of irritation/corrosivity.

1.5.1. Definitions

Irrespective of whether a substance can become systemically available, changes at the site of first contact (skin, eye, mucous membrane/GI tract, or mucous membrane/respiratory tract) can be caused. These changes are considered local effects. A distinction in local effects can be made between those observed after single and after repeated exposure. For local effects after repeated exposure reference is made to Section 4.3.2 of this guidance. Local effects after single ocular, dermal or inhalation

exposure are only dealt with in this section. Substances causing local effects after single exposure can be further distinguished in irritant or corrosive substances, depending on the (ir) reversibility of the effects observed.

Irritant substances are non-corrosive substances which, through immediate contact with the tissue under consideration, may cause inflammation. Corrosive substances are those which may destroy living tissues with which they come into contact.

Criteria for classification of irritant and corrosive substances are given in Annex I to Regulation (EC) No 1272/2008.

The general objectives are to find out:

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

Taking into account the severity of the effect, as far as it can be judged from the test data, the likelihood of an acute corrosive or irritant response of humans using or otherwise exposed to the substance is assessed in a pragmatic manner in relation to the route, pattern and extent of the expected human exposure.

Definitions of skin- and eye irritation/corrosion/respiratory irritation:

- **Dermal irritation**: Defined in the *Guidance on the Application of the CLP Criteria* as "…the production of reversible damage of the skin following the application of a test substance for up to 4 hours".
- **Dermal irritation after repeated exposure**: Substances which may cause skin dryness, flaking or cracking upon repeated exposure but which can not be considered a skin irritant.
- Substances may also cause irritant effects only after repeated exposure, for example organic solvents. This type of chemicals may have defatting properties (Ad-hoc Working group on Defatting substances, 1997). Chemicals that have a similar mechanism need to be considered for labelling with the risk phrase 'repeated exposure may cause skin dryness or cracking'.
- **Dermal corrosion**: Defined in the *Guidance on the Application of the CLP Criteria* as "…the production of irreversible damage to skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to four 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…".
- **Eye irritation**: Defined in the *Guidance on the Application of the CLP Criteria* as "…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".
- **Eye corrosion**: Defined in the *Guidance on the Application of the CLP Criteria* as "…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**: There is no EU or OECD TG for respiratory irritation and testing for respiratory irritation is not required under BPR. Respiratory irritation is often used to describe either or both of the two different toxicological effects, 'sensory irritation' and 'local cytotoxic effects'.

1.5.2. Mechanisms of skin corrosion and irritation

Clinically different types of ICD exist, and have been classified on the basis of differences in morphology and mode of onset, as: (a) acute irritant dermatitis (primary irritation); (b) irritant reaction; (c) delayed, acute irritant contact dermatitis; (d) cumulative irritant dermatitis; (e) traumatic irritant dermatitis, pustular and acneiform irritant dermatitis; (f) non-erythematous irritant dermatitis; and (g) subjective irritation (Lammintausta and Maibach, 1990).

Two different pathogenetic pathways may be involved in ICD. Acute ICD is characterised by an inflammatory reaction which mimics allergic contact dermatitis, with the release of inflammatory mediators and cytokines. Chronic ICD, on the other hand, is characterised by disturbed barrier function, associated with an increased epidermal turnover which leads clinically to lichenification (Berardesca and Distante, 1994).

The clinically relevant elements of skin irritation are disturbance of the desquamation process, resulting in scaling or hyperkeratosis (chronic effects), i.e. epidermal events, and an inflammatory response with vasodilation and redness in combination with extravasation of water, which may be observed as papules, vesicles and/or bullae and oedema (acute effects), i.e. events essentially taking place in the dermis (Serup, 1995). The onset of irritation takes place at the *stratum corneum* level and later in the dermis , whereas early events in sensitisation occur in the dermis. Variations in the skin reactions are dependent on the degree of injury induced, as well as on the effects of an irritant substance on different cell populations. For example, pigmentary alterations are due to effects on melanocytes, whereas ulcerations are due to extensive keratinocyte necrosis (skin corrosion). The release of cytokines and mediators can be initiated by a number of cells, including living keratinocytes and those of the *stratum corneum*, which thus modulate inflammation and repair (Sondergard *et al*., 1974; Hawk *et al*., 1983; Barker *et al*., 1991; Baadsgaard and Wang, 1991; Hunziker *et al*., 1992; Berardesca and Distante, 1994).

The physico-chemical properties, concentration, volume and contact time of the irritant give rise to variations in the skin response. Furthermore, inter-individual differences exist, based on age, gender, race, skin colour and history of any previous skin disease. In the same individual, reactivity differs according to differences in skin thickness and skin sensitivity to irritation of the different body regions. Finally, a greater sensitivity to some irritants (Dimethyl sulfoxide (DMSO), propylene glycol, Sodium Lauryl Sulfate (SLS) and soap) has been reported during winter, because of the reduced hydration state of the skin (Frosch and Pilz, 1995). Although clinically different types of irritant reactions can be observed, they are all based on cellular and biochemical mechanisms which induce the irritant response. It is not yet possible to conclude whether the observed clinical differences are actually due to differences in biochemical mechanisms, and further investigations are needed.

According to Barratt (1995) and further elaborated by Walker *et al*. (2004), for organic chemicals, the mechanisms leading to skin irritation are normally described by a two-stage process where a chemical first has to penetrate the *stratum corneum* and then trigger a biological response in deeper epidermal or dermal layers.

For strong inorganic acids and bases, no *stratum corneum* penetration is needed because they erode the *stratum corneum*. According to the past TGD supporting Commission Directive 93/67/EEC on risk assessment for new notified and existing substances (EC, 2003), the percutaneous absorption of acrylates, quaternary ammonium ions, heterocyclic ammonium ions and sulphonium salts is slow, since these chemicals

are binding to macromolecules in skin. As a result of binding, corrosion can occur as the stratum corneum is eroded. Reactivity can be caused by electrophiles and/or pro-electrophiles. Electrophiles contain atoms, such as N, O or halogens attached to a C-atom, which makes that specific C-atom positively charged and therefore reactive with electron-rich regions of peptides and proteins. This causes irritation via covalent binding to the skin.

Currently (since 2007), the following mechanisms are proposed for inducing skin irritation or skin corrosion by affecting the structure and function of the stratum corneum:

1) Mechanisms of skin irritation:
   - Reaction with skin proteins and interference with lipids in the stratum corneum by surface-active agents (denaturation of proteins, disruption of plasma membrane lipids)
   - Dissolving of plasma membrane lipids and thus defatting and disintegration of skin by low molecular weight organic chemicals.
2) Mechanisms of skin corrosion:
   - Erosion of the stratum corneum by most inorganic acids and bases and by strong organic acids with pH <2.0 and bases with pH >11.5 and
   - Binding to skin components in the stratum corneum by cationic surfactants and percutaneous absorption of acrylates, quaternary ammonium ions, heterocyclic ammonium ions and sulphonium salts.
3) Mechanisms that may lead to both skin irritation and corrosion:
   - Penetration of the stratum corneum by anionic or non-surfactant organic chemicals with sufficient hydrophobic and hydrophilic properties, and
   - elicitation of a inflammatory and/or cytotoxic response in the epidermis or dermis.

     The severity of these responses may determine whether irritation or corrosion occurs.

1.5.3. Mechanisms of eye irritation

Eye injury can be caused by many insults. These can be physical such puncture by sharp objects. Eye injury can be caused by chemicals, such as systemic drugs, that can enter into the eye through the blood stream (e.g. cyclosporine, vaccines, intravenous immunoglobulins, and intravenous streptokinase). Various degrees of eye injury can also be caused by direct (topical) contact with chemicals or chemical mixtures such as acids, alkalis, solvents or surfactants. These materials may contact the eye intentionally e.g. through the use of eye drops, medications, products intended for use around the eyes but also unintentionally e.g. accidental spills and splashes of consumer products or accidental exposures in the workplace.

In general, chemicals or chemical mixtures which contact the eye directly may cause local effects on the frontal tissues and substructures of the eye, e.g. cornea, conjunctiva, iris, lachrymal system and eye lids. There are several modes of action by which topical chemicals and chemical mixtures cause eye injury (see Table 7).

Table 7: Categories of irritant chemicals and their typical mode of action in eye irritation

Chemical/chemical mixtures	Mode of Action
Inert chemicals	May cause effect due to large size. Protrusions may cause direct puncture of the eye.
Acids	May react directly with eye proteins and cause coagulation or precipitation resulting in relatively localised injury.
Bases (Alkalis)	May actively dissolve cell membranes. May penetrate to the deeper layers of the eye tissue.
Solvents	May dissolve lipids in plasma membranes of epithelial and underlying cells resulting in loss of the cells affected and, as a result, tissue degradation that might be – depending on the repair mechanisms (cell proliferation, tissue restoration) transient.
Lachrymators	May stimulate the sensory nerve endings in the corneal epithelium causing an increase in tearing.

The degree of eye injury is usually dependent on the characteristics (chemical category/class) and concentration of the chemical or chemical mixture. Acids and alkalis usually cause immediate irritation to the eyes. Other substances may cause eye injuries that start as mild but progress to be more severe at a later period.

Upon exposure of the ocular surface to eye irritants, inflammation of the conjunctiva can be induced. This includes dilation of the blood vessels causing redness, increased effusion of water causing swelling (oedema/chemosis) and an increase in the secretion of mucous leading to an increase in discharge. Visual acuity can be impaired. Irritants may also produce an increase in tear production and changes to the tear film integrity such as increased wetness. Iritis may result from direct irritation or become a secondary reaction to the corneal injury. Once the iris is inflamed, infiltration of fluids can follow which affects the ability to adjust the size of the pupil and decreases the reaction to light leading to decreased visual acuity. Due to the richness of nerves in the iris, irritation also causes subjective symptoms such as itching, burning and stinging.

Eye injury can be reversible or irreversible depending on the degree of damage and degree of repair. Damage to the corneal epithelium alone can repair quickly, often with no permanent eye damage. The cornea may still repair fairly well if the damage goes beyond the basement membrane into the superficial part of the stroma but the repair process may take days or even weeks to occur. Once the damage extends significantly into the stroma, corneal ulceration can occur due to the subsequent series of inflammatory processes. If damage extends to and beyond the endothelium, corneal perforation may occur which is irreversible and may cause permanent loss of vision. Eye injury can cause different degrees of functional loss e.g. increase of tear production, opacification of the cornea, oedema and so decrease visual acuity.

The body has its own defence mechanisms e.g. sensing the pain, stinging and burning and the eyelids will blink to avoid full exposure to the chemical. Increased tear production and blinking of the eyes with the help of the drainage apparatus help to dilute or clear the causative agent. Such defence mechanisms are highly developed in human with rapid blinking and profuse tear production resulting from exposure of the eye to a foreign material that is irritating. It is well reported in the literature that species differences occur in the rate of blinking and tear production mechanism that can influence how effectively foreign materials are removed from the eye.

1.5.4. Mechanisms of respiratory irritation

The term "respiratory tract irritation" is often used to indicate either or both of two different toxicological effects. These are i) cytotoxic effects in the affected tissue, and ii) sensory irritation. The first type of irritation is comparable to dermal and eye irritation.

Cytotoxic irritant effects are characterised by inflammation (increased blood flow (hyperaemia), local infiltration with white blood cells, swelling, and oedema) and there may also be haemorrhage, and eventual necrosis and other pathological changes. The effects are in principle reversible.

Chronic irritation can lead to repeated episodes of cell proliferation in the affected tissues, and this may increase the risk of tumour development. The nature of effects depends on the chemical compound and its primarily targeted region, the severity of effects depends on the concentration and duration of exposure. In general, repeated exposure studies in animals tend to focus on observing (histo) pathological evidence for tissue damage rather than for sensory irritant effects. In case overt tissue damage (mucosal erosion and ulceration) occurs, a non-specific cytotoxic action at the site of contact along the respiration route can be assumed. Depending on the concentration and duration of exposure a severity gradient of lesions from anterior to posterior regions can be observed (in contrast to effects in certain mucosa types depending on the metabolic activation of the test substance) and, depending on the severity and the extent of the lesions, adjacent submucosal tissues can also be affected (e.g. by cartilage destruction). Such lesions are not fully reversible due to scar formation or replacement of the original mucosa, or may induce other serious health effects as marked bleeding or persistent airway obstruction.

According to the *Guidance on the Application of CLP Criteria*, "sensory irritation" refers to the local and central reflex interaction of a substance with the autonomic nerve receptors, which are widely distributed in the mucosal tissues of the eyes and upper respiratory tract. Compound or compound-group specific target sites of sensory irritation generating different responses can be identified: i) nasal (and eye) irritation, i.e. interaction with the trigeminal nerve, ii) pharyngeal irritation, i.e. interaction with the glossopharyngeal nerve, and iii) larynx and lower respiratory tract, i.e. interaction with the vagus nerve.

Sensory irritation leads to unpleasant sensations such as pain, burning, pungency, and tingling. The severity depends on the airborne concentration of the irritant rather than on the duration of exposure. Sensory irritation is a receptor-mediated effect, and usually occurs almost immediately upon exposure to the inhaled irritant. It leads to reflex involuntary responses such as sneezing, lacrimation, rhinorrhoea, coughing, vasodilatation of blood vessels in the nasal passages, and changes in the rate and depth of respiration. In humans, protective behavioural responses such as covering the nose and mouth can also occur. Sensory irritation is distinct from odour sensation, which is mediated via different nerve pathways (olfactory). However, there is evidence that odour perception and other cognitive influences can affect the perception of sensory irritation in humans.

In rodents, sensory irritation leads to a reflex reduction in the respiratory rate (breath-holding); this reflex effect on respiration can be measured experimentally (determination of the RD_{50} value in the Alarie assay) although results may vary considerably depending on the species and strain of rodents, on the exposure duration (time should be long enough to induce changes), and results also show inter-laboratory variability. Investigations into the correlation of the results of the Alarie test with human data are difficult since the parameters examined in humans and mice are different and adequate human data to determine a human equivalent to the RD_{50} is not available at the moment. The results of a study by Cometto-Muniz and Cain (1994) indicate that RD_{50} values in animals are not easily comparable with 'nasal pungency thresholds' in humans.

As indicated, human data are mostly based on subjective experiences and need to be carefully controlled in order to prevent confounding by odour perception (Dalton, 2003; Doty *et al*., 2004). Validated questionnaires have been developed for the investigation of sensory irritation responses in human volunteers. During recent years, emphasis was given to develop a spectrum of objective measurements (see review by Arts *et al*., 2006).

There is a view in the occupational health literature that sensory irritation may be a more sensitive effect than overt tissue-damaging irritation (which is a non-receptor mediated unspecific mode inducing cell death at the site of contact). Sensory irritation-related effects are fully reversible given that its biological function is to serve as a warning against inhaled substances that could damage the airways, and that it triggers physiological reflexes that limit inhalation volumes and protect the airways. However, there is a lack of documented evidence to indicate that this is a generic position that would necessarily apply to all inhaled irritants. It should be noted that no clear relationship between the RD_{50} value and the onset of histologically observable lesions in animals has been observed.

1.5.5. Data to be used in the effects assessment

The integrated testing strategies described within the *Guidance on the Application of CLP Criteria*, should be considered together with the following elements on data to be used in the effects assessment.

1.5.5.1 Non-Human Data for irritation/corrosion (skin and eye)

1.5.5.1.1 Non-testing data for irritation/corrosion (skin and eye)

(a) Physico-chemical properties

According to the current EU and OECD guidelines, substances should not be tested in animals for irritation/corrosion if they can be predicted to be corrosive from their physico-chemical properties. In particular, substances exhibiting strong acidity (pH ≤2) or alkalinity (pH ≥11.5) in solution are predicted to be corrosive, and should not be tested. However, no conclusion can be made regarding corrosivity when the pH has an intermediate value (when 2< pH <11.5).

(b) Physico-chemical properties for skin corrosion/irritation:

Chemicals that have other pH values will need to be considered further for their potential for skin and eye irritation/corrosion.

The Decision Logic for classification of substances as described within the Guidance of the implementation of CLP Regulation should be followed with regard to physico-chemical properties and decision rules for skin corrosion/irritation.

Several studies have investigated and confirmed the usefulness of pH as a predictor of corrosion (Worth and Cronin, 2001) and as an element in tiered testing strategies (Worth, 2004).

However, where extreme pH is the only basis of classification as corrosive, it may also be important to take into consideration the acid/alkaline reserve, a measure of the buffering capacity of a chemical substance (Young *et al*., 1988.; Botham *et al*., 1998; Young and How 1994), as mentioned in the OECD TG 404. However, the buffering capacity should not be used alone to exonerate from classification as corrosive. Indeed, when the acid/alkaline reserve suggests that the substance might be non-corrosive, further *in vitro* testing should be considered.

(c) Physico-chemical properties for eye irritation:

A chemical known or predicted to be corrosive to the skin is automatically considered to be severely irritating to the eye (Eye Damage 1)). However, no conclusion can be made

regarding eye irritation potential when the pH has an intermediate value (when 2< pH <11.5).

The Decision Logic for classification of substances as described within the Guidance for the implementation of CLP Regulation should be followed with regard to physico-chemical properties and decision rules for skin corrosion/irritation.

To predict the eye irritation potential of non-corrosive chemicals, the distribution of pH values for irritants and non-irritants in a data set of 165 chemicals has been analysed (Worth, 2000). The irritants spanned a wide range of pH values from 0 to about 12, whereas the non-irritants spanned a much narrower range from about 3 to 9. Using the cut off values generated by classification tree analysis, the following model was formulated:

If pH <3.2 or if pH >8.6, then consider the chemical for eye irritation classification; otherwise make no prediction.

According to the way the model was developed, irritant can either be Eye Damage 1 or Eye Irritation 2. Further information and/or reasoning are needed to conclude on the risk phrases. The more severe classification (Eye Damage 1) should be assumed if no further information is available.

This model had a sensitivity of 53% (and therefore a false negative rate of 47%), a specificity of 97% (and therefore a false positive rate of 3%), and a concordance of 76%. A QSAR Model Reporting Format (QMRF) has been developed (see also the *Guidance on information requirements and chemical safety assessment Chapter R.6, Section R.6.1* and JRC QSAR Model Database: http://qsardb.jrc.it).

Based on these statistics, this model is not recommended for the stand-alone discrimination between eye irritants and non-irritants. However, could be used in the context of a tiered testing strategy to identify eye irritants (due to its very low false positive rate) but not non-irritants (due to its relatively high false negative rate).

(d) Read-across to structurally or mechanistically similar substances (SAR)

Generic guidance on the application of grouping approaches is provided in the *Guidance on information requirements and chemical safety assessment Chapter R.6.*

(e) (Q)SARs systems

The mechanism of irritation and corrosion has toxicodynamic and toxicokinetic parameters. Models that solely predict irritation and corrosion on toxicodynamics properties such as acidity or basicity, electrophilicity, other reactivity, surfactant activity, solving membranes, have to be additionally evaluated for their toxicokinetic parameters. These parameters can be physical chemical parameters or others and indicate the potential to cross the skin (stratum corneum) and be active in the living tissue underneath the stratum corneum. Also models that solely predict (the absence of) activity, irritation and corrosion, e.g. by physical chemical properties that illustrate the toxicokinetic behaviour of chemicals, have to be evaluated for their activity (toxicodynamics).

For example, the BfR physico-chemical rule base predicts the absence of skin and eye irritation. Evaluations of the BfR rule bases for the prediction of no skin irritation/corrosion (Rorije and Hulzebos, 2005; Gallegos Saliner at al., 2007) and for the prediction of no eye irritation (Tsakovska *et al*., 2005) have been carried out independently. However, when the absence of irritation cannot be excluded, further information on the structure of the chemical is needed to predict presence of irritation/corrosion.

The absence of skin and eye irritation and corrosion is well predicted with the BfR rule base and therefore these rules can be applied.

There is no other model yet available which sufficiently describes the absence of effects. Neutral organics are expected not to be irritants; however, their defatting potential should be discussed. The definition of a neutral organic is a chemical which do not have potential reaction centres, even after skin metabolism. The absence of reactivity needs to be described in sufficient detail or be substantiated with other information.

The presence of effects has been well established using the pH cut off values for high acidity and basicity and can be applied. Structural alerts for the presence of effects can be used, however further characterisation of the effect needs to be described in sufficient detail or be substantiated with other information. For instance, the BfR structural rule bases for the prediction of skin irritation/corrosion (Rorije *et al*., 2007; Gallegos Saliner at al., 2007) and for the prediction of eye irritation (Tsakovska *et al*., 2007) have been validated.

Examples of available QSAR systems for skin and eye irritation are available in the *Guidance on information requirements and chemical safety assessment Chapter R.7a (Appendix R.7.2-2 and R.7.2-3)*.

1.5.5.1.2 Testing data for irritation/corrosion (skin and eye)

(a) *In vitro* data

Skin irritation/corrosion

As described in the Section 8.1 of the ECHA Biocides Guidance, Vol. III, Part A Information Requirements, *in vitro* assays are the first choice if testing is needed to assess skin irritation and corrosion potential.

Other validated assays designed to examine skin irritation potential can also provide evidence for irritant or non irritant properties and can be considered in the assessment especially if the information is sufficient to classify for skin irritation.

Eye irritation

As described in the Section 8.2 of the ECHA Biocides Guidance, Vol. III, Part A (Information Requirements), *in vitro* assays are the first choice if testing is needed to assess eye irritation potential taking into account limitations with currently validated assays in predicting non ocular corrosive and irritating properties. When data is available from *in vitro* assays (non validated, pre-validation status) they should be taken into account in a WoE approach to consider if the information is sufficient for classification and labelling and risk assessment.

(b) Animal data

Well-reported studies particularly if conducted in accordance with principles of GLP, can be used to identify substances which would be considered to be, or not to be, corrosive or irritant to the skin or eye. There may be a number of skin or eye irritation studies already available for a substance, none of which are fully equivalent to a EU test method such as those in the Test Methods Regulation (Regulation (EC) No 440/2008). If the results from such a batch of studies are consistent, they may, together, provide sufficient information on the skin and/or eye irritation potential of the substance.

If the results from a variety of studies are unclear, based on the criteria given below for evaluation of the data, the evaluator will need to decide which of the studies are most reliable, relevant for the endpoint in question and will be adequate for classification purposes.

Particular attention should be given to the persistence of irritating effects, even those which do not lead to classification. Effects such as erythema, oedema, fissuring, scaling, desquamation, hyperplasia and opacity which do not reverse within the test period may indicate that a substance will cause persistent damage to the human skin and eye.

Data from studies other than skin or eye irritation studies (e.g. other toxicological studies on the substance in which local responses of skin, eye mucous membranes and/or respiratory system have been reported) may provide useful information though they may not be well reported in relation to, for example, the basic requirements for information on skin and eye irritation. However, information from studies in animals on mucous membrane and/or respiratory system irritation can be very useful for risk assessment provided the irritation is clearly substance-induced, and particularly if it can be related to exposure levels.

(c) Quality Aspects

Data from existing irritation studies in animals must be taken into account before further testing is considered. A quality assessment of any such reports should be done using, for example, the system developed by Klimisch *et al*. (1997), and a judgement will need to be made as to whether any further testing is required. Some examples to note are:

- Was the animal species the rabbit or was it another, such as rat or mouse? Rat and mouse, as species, are not as sensitive as the rabbit for irritation testing.
- How many animals were used? Current methodology requires 3 but 6 was frequently used in the past.
- How many dose levels were used? If dilutions were included, what solvent was used (as this may have influenced absorption)? Which dose volume was used?
- For skin, which exposure period was used? Single or repeated exposure?
- The method used to apply the chemical substance to the skin should be noted i.e. whether occluded or semi-occluded, whether the application site was washed after treatment.
- Check the observation period used post exposure. Shorter periods than in the current guideline may be adequate for non-irritants but may require a more severe classification for irritants when the observation period is too short to measure full recovery.
- For eye irritation, was initial pain noted after instillation of the test substance, was the substance washed out of the eye, was fluorescent staining used?
- For eye irritation, how was the test material applied into the eye?

Irritation scores from old reports, reports produced for regulatory submission in the USA or in publications may be expressed as a Primary Irritation Score. Without the original data it is not always possible to convert these scores accurately into the scoring system used in the EU. For extremes i.e. where there is either no irritation or severe irritation, it may not be necessary to look further, but average irritation scores pose a problem and judgement may be required to avoid repeat testing.

Observations such as the above can all be used to assess whether the existing animal test report available can be used reliably to predict the irritation potential of a substance, thus avoiding further testing.

(d) Specific considerations for eye irritation

A refinement of the classical Draize test is the rabbit LVET. The LVET (Griffith *et al*., 1980) is a modification of the standard OECD TG 405 test method, the differences being:

- the test material is placed directly on the cornea instead of introducing it in the conjunctival sac inside the lower lid;
- a reduction in the volume of test material applied (0.01 ml, or corresponding weight for solids, compared with the standard 0.1 ml).

Data from the LVET should be considered but must be carefully evaluated. The applicability domain up to now is limited to detergent and cleaning products. It is stated that positive data are a trigger for appropriate classification, but that negative data are

not conclusive for a non- classification. However, they should be considered in a WoE determination in line with the *Guidance on the Application of CLP Criteria*.

(e) Specific considerations for respiratory irritation

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

Human data:

- Experience from occupational exposure
- Published data on volunteers (objective measurements, psychophysical methods, and subjective reporting)
- Other data (e.g. from nasal lavage)
- Animal data:
- Alarie assay
- 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 indicating the cytotoxic type of respiratory irritation, which were mainly gained from histopathological examinations of tissues, are considered in the reference value derivation for the acute toxic effects or for the repeated dose toxic effects (see also Section 4, section on risk characterisation for local effects) of this guidance.

With respect to the sensory irritation response, the evidence from all sources has to be considered for the quantitative risk assessment procedure.

Although the Alarie test for various reasons has never become an OECD TG, results of the Alarie assay can be used for hazard identification of sensory irritation as the Alarie test detects the potential of a substance to stimulate the trigeminal nerve. Like in acute inhalation toxicity testing, results from Alarie tests may show high inter-laboratory variability. Therefore, the use of Alarie data for deriving quantitative information for instance to establish short-term reference values (e.g. AECs) for irritation should be done with caution (i.e. taking into account the actual breathing pattern, whether a response plateau is being reached; see the review by Bos *et al*., 1991). In that review it was shown that data of the Alarie test could not be used to establish TLV values for lifetime exposure. It can be expected that a substance that is capable to stimulate the trigeminal nerve in mice will also have this potential in humans. However, because the human response at an exposure concentration equal to the RD_{50} cannot quantitatively be determined and because responses in the Alarie-test of less than 10-12% are considered to be within the expected normal variation (Boylstein *et al*., 1996; Doty *et al*., 2004; ASTM, 2004), use of the Alarie-bioassay in a quantitative risk assessment, if any, is suggested to start from an RD_{10} rather than from an RD_{50}.

Although anatomical differences in rodents and humans do exist (e.g. rodents are obligate nose breathers and humans not), sensory irritation will be present in both but the location and the type of effect may differ, i.e. in rodents a decrease in breathing frequency may be observed, whereas in humans this may result in coughing.

Sensory irritation does not necessarily lead to tissue damage. Effects characterising overt tissue damage are covered by inhalation studies for acute or repeated exposure toxicity. In this sense the Alarie assay is not designed to predict such pathological changes (Bos *et al*., 2002). If available from other studies with the inhalation route (acute and repeated exposure) the characterisation of histomorphological lesions at the respiratory tract could be used as supplemental information.

Although both the Alarie test and for instance human nasal pungency threshold determinations are aimed to test for sensory irritation, correlation of the results of the Alarie test with such human data is difficult as the first is looking at rather strong effects upon exposure for at least 20 min (a 50% decrease in breathing frequency may be experienced by humans as unbearable) whereas human data are based on, for instance, very short exposure durations (sniffing for a few seconds). The results of a study by Cometto-Muniz and Cain (1994) indicated that RD_{50} values in animals are not easily comparable with 'nasal pungency thresholds' in humans (see also Bos *et al*., 2002).

1.5.5.2 Human data for irritation/corrosion (skin and eye)

Well-documented existing human data of different sources can often provide very useful information on skin and/or respiratory irritation, sometimes for a range of exposure levels. Often the only useful information on respiratory irritation is obtained from human experience (occupational settings). The usefulness of all human data on irritation will depend on the extent to which the effect, and its magnitude, can be reliably attributed to the substance of interest. Experience has shown that it is difficult to obtain useful data on substance-induced eye irritation, but data may be available on human ocular responses to certain types of preparations (e.g. Freeberg *et al*., 1986).

The quality and relevance of existing human data for hazard assessment should be critically reviewed. For example, in occupational studies with mixed exposure it is important that the substance causing the irritation or corrosion has been accurately identified. There may also be a significant level of uncertainty in human data due to poor reporting and lack of specific information on exposure.

Examples of how existing human data can be used in hazard classification for irritancy are provided in an ECETOC monograph (ECETOC, 2002a).

Human data on local skin effects may be obtained from existing data on single or repeated exposure. The exposure could be of accidental nature or prolonged, for example in occupational settings. The exposure is usually difficult to quantify. When looking at the effects, corrosivity is characterised by destruction of skin tissue, namely visible necrosis through the epidermis and into the dermis. Corrosive reactions are typified by ulcers, bleeding and bloody scabs. After recovery the skin will be discoloured due to blanching of the skin, complete areas of alopecia and scars (see Section 3.2 of the *Guidance on the Application of the CLP Criteria*), i.e. corrosivity is an irreversible damage. With this characterisation it should be possible to discern corrosive properties in humans. Discrimination between corrosives and skin irritants in rabbits is made on the effects caused after 4 hours' exposure. Irritants to the skin cause a significant inflammation which is reversible.

Severe eye irritants (Eye Damage 1, H318) give more severe corneal opacity and iritis than eye irritants (Eye Irritation 2, H319). Severe eye irritant compounds induce considerable tissue damage which can result in serious physical decay of vision. The effects normally do not reverse within 21 days (relates to animals); see Section 3.3 of the *Guidance on the Application of the CLP Criteria*. In contrast, the effects of eye irritant compounds are reversible within 21 days. In humans, a sight control by a physician would reveal a decay of vision. If it is not transient but persistent it implies classification with Eye Damage 1.

1.5.5.2.1 Human data for respiratory irritation

Consideration should be given to real-life human observational experience, if this is properly collected and documented (Arts *et al*., 2006), e.g. data from well-designed workplace surveys, worker health monitoring programmes. For substances with an array of industrial uses and with abundant human evidence, the symptoms of respiratory irritation can sometimes be associated with certain concentrations of the irritants in the workplace air and might thus allow derivation of AECs. However, the exposure details

need to be well documented and due consideration should be given to possible confounding factors.

Data on sensory irritation of the airways may be available from volunteer studies including objective measurements of respiratory tract irritation such as electrophysiological responses, data from lateralization threshold testing, biomarkers of inflammation in nasal or bronchoalveolar lavage fluids. Including anosmics as subjects could exclude odour as a bias.

1.5.6. Remaining uncertainty on irritation/corrosion

Usually it is possible unequivocally to identify (or accept) 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 (because of poor reporting, lack of specific information on exposure, subjective or anecdotal reporting of effects, small numbers of subjects, etc.).

Data from studies in animals according to internationally accepted test methods will usually give very good information on the skin or eye irritancy of a substance in the test species, and, in general, it is assumed that substances which are irritant in studies in animals performed with internationally accepted test methods will be skin and/or eye irritants in humans, and those which are not irritant studies performed with internationally accepted test methods will not be irritant in humans. Good data, often clearly related to exposure levels, can be obtained on respiratory and mucous membrane irritation, from well-designed and well-reported inhalation studies in animals. However, inconsistent results from a number of similar studies increases the uncertainty in deriving 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 suffice for defined hazard identification purposes.

1.5.7. 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, and consideration should be given also to the Guidance for the implementation of the CLP Regulation.

1.5.8. Concluding on suitability for risk assessment

A dose-response assessment is difficult to make for irritation and corrosion simply because up to the present time most data have been produced with undiluted chemicals in accordance with test guidelines and traditional practice (which continues today). From a risk characterisation perspective it is therefore advisable to use the outcome of the classification procedure, i.e. a substance that is classified is assumed to be sufficiently characterised. However, a complete risk assessment requires both hazard, as well as dose-response data. Consequently, if the latter are available, they must be taken into account. For instance, dose-response information might be available from sub-acute dermal, repeated dose dermal and inhalation toxicity studies as well as from human experience.

However, with specific regard to respiratory irritation, special attention needs to be given to as to whether extrapolation of the dose-response assessments from animal tests to the human situation is possible.

1.6. Sensitisation

The section on Sensitisation of the Biocides Guidance, Vol. III, Part A (Information Requirements) should be considered together with the elements described in this section

for the assessment of skin and respiratory sensitisation as well as the element described in Section 4 within the section of risk characterisation for local effects, in this guidance.

1.6.1. Definitions 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 its mechanism of action is poorly understood, so it has been considered but not discussed in detail.

A sensitiser is an agent that is able to cause an allergic response in susceptible individuals. The consequence of this is that 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), respectively, may be provoked. Asthma and rhinitis are generally thought to be a result of an allergic reaction; however, other non-immunological mechanisms may occur, makes it more appropriate to use a term based on disease rather than mechanism.

This wider understanding is reflected in the criteria for the classification of skin and respiratory sensitisers, which provide a useful tool against which the hazardous properties of a substance can be judged.

Respiratory hypersensitivity is a term that is used to describe asthma and other related respiratory conditions, irrespective of the mechanism by which they are caused. When directly considering human data in this document, the clinical diagnostic terms asthma, rhinitis and alveolitis have been retained.

In summary, in this guidance, the term skin sensitisation specifies an allergic mechanism of action, while respiratory hypersensitivity does not. For this reason, the two health hazards have on occasion been approached differently in this guidance.

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;
- 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 who are using or who are otherwise exposed to this agent is determined by several factors including the route, duration and magnitude of exposure and the potency of the substance.

1.6.2. Mechanisms of immunologically-mediated hypersensitivity

Among the key steps required for a chemical to induce sensitisation via skin contact are gaining access to the viable epidermis, protein binding, metabolic activation (if required), internalization and processing by LC and further specialised dermal dendritic cells, transport of antigen by LC to draining lymph nodes, and presentation to and recognition by T-lymphocytes. For chemicals that sensitise via the respiratory tract, the relevant mechanisms are believed to be essentially similar, although gaining access to the respiratory epithelium may be somewhat easier than at skin surfaces due to the lack of a *stratum corneum*. Moreover, because the lining of the respiratory tract, the professional antigen presenting cells, and regulatory mechanisms in the respiratory tract differ from those in the skin, they all may have an impact on the type of immune response evoked. Although the site of induction of an adaptive immune response to a chemical allergen may be influenced by local conditions and local immune-regulatory mechanisms, the fact remains that the inherent properties of the chemical itself play a major role in determining whether an immune responses is induced and the qualitative characteristics of that response. Though it is commonly assumed that a further inflammatory signal in

addition to the antigenic signal is delivered by the same allergen and is a prerequisite for all allergic reactions, only few signalling pathways such as in case of nickel and Toll-like receptor (TLR)4 are elucidated. Although it is sometimes assumed that immune responses induced following encounter with antigen in or on the skin are often of selective Th_1-type, this is not necessarily the case. It is clear that cutaneous immune responses can be of either Th_1-,Th_2- or Th_{17} type according to the nature of the antigen (Peiser *et al*., 2012). In the respiratory tract, chemical respiratory allergens appear to preferentially elicit Th_2-immune responses (Maestrelli *et al*., 1997); observations that are consistent with experimental experience in mice (Dearman *et al*., 2002; Herrick *et al*., 2003; Farraj *et al*., 2004), and possibly also rats (Arts *et al*., 1998). Th_2 type immune responses are characterised by the production of cytokines such as IL4 and IL5 and by the production of IgE antibodies. However, the mechanisms through which chemicals are able to induce sensitisation of the respiratory tract are not fully understood and there remains controversy about the roles played by IgE antibody-mediated mechanisms, and whether IgE represents a mandatory universal requirement for the induction by chemicals of allergic sensitisation of the respiratory tract. The area is complicated because although for all chemical respiratory allergens there are patients who display serum IgE antibodies of the appropriate specificity, in other instances (and particularly with respect to the diisocyanates) there are symptomatic subjects in whom it is not possible to detect IgE antibody. There are two, non-mutually exclusive, possibilities. The first is that IgE does play a central role but that for one or more of various reasons it is not being detected accurately in the serum of patients with occupational asthma. The second is that allergic sensitisation of the respiratory tract by chemicals can be effected through IgE antibody-independent immunological mechanisms (Kimber and Dearman, 2005; Kimber and Dearman, 2002). These may also include Th_1-type immune responses. In this context it has been reported, for instance, that inhalation challenge of sensitised rodents with contact allergens may elicit respiratory allergic reactions (Garssen *et al*., 1991; Garcia *et al*., 1992; Buckley *et al*., 1994; Zwart *et al*., 1994; Satoh *et al*., 1995; Arts *et al*., 1998). This comes as no surprise because it is clear that contact sensitisation is systemic in nature and that there is no reason to suppose that encounter of sensitised animals with the relevant contact allergen at respiratory epithelial surfaces will not cause an adverse immunologic reaction. However, it is important to note that in reality only a very few precedents for the elicitation of pulmonary reactions by skin sensitising chemicals in humans have been observed, and in practice it may not represent a significant health issue.

In addition, there is a growing body of evidence that effective sensitisation of the respiratory tract by chemicals defined as respirator y allergens (such as for instance the acid anhydrides, diisocyanates and others) can and does occur in response to dermal contact (reviewed by Kimber and Dearman, 2002). There are also experimental animal data and human evidence for sensitisation by inhalation and skin effects following dermal challenge (Kimber and Dearman, 2002; Baur *et al*., 1984; Ebino *et al*., 2001; Stadler and Karol, 1984). Therefore, it is not necessarily the case that chemicals that cause allergic dermal reactions require sensitisation via the skin, or that chemicals that cause allergic airway reactions require sensitisation via the respiratory tract.

1.6.3. Data to be used in the effects assessment

1.6.3.1 Skin Sensitisation

1.6.3.1 1 Non-Human Data for skin sensitisation

1.6.3.1.1.1 Non-testing data for skin sensitisation

(a) Read-across to structurally or mechanistically similar substances (SAR)

Generic guidance on the application of grouping approaches is provided in the *Guidance on information requirements and chemical safety assessment Chapter R.6.*

(b) (Q)SAR Systems

The evaluation and assessment of a chemical using (Q)SARs is dependent on both the chemical of interest and the (Q)SAR model(s) used to make a prediction.

A prediction needs to be evaluated in the context of the likely chemistry and the available like chemicals available within the training set. i.e. is the compound of interest within the scope of the model and are similar chemicals in the training set of the model well predicted. This type of information provides additional weight to whether the estimate derived is meaningful and relevant. For global models available in the literature, the training sets and the algorithm(s) are usually available to allow such comparisons to be made.

Although the main factors driving skin sensitisation (and therefore the (Q)SARs) is the underlying premise of the electrophilicity of a chemical, other factors such as hydrophobicity encoded in the octanol/water partition coefficient (log P) may also be considered as playing a role in the modifying the sensitisation response observed.

Additional information on available QSAR systems for use in the assessment of sensitisation is available in the *Guidance on information requirements and chemical safety assessment Chapter R.7a, Section R.7.3.*

1.6.3.1.1.2 Testing data for skin sensitisation

(a) *In vitro* data

In vitro data obtained with non-validated methods can only be used in a WoE approach. If such data are considered for the evaluation, expert judgement is needed to assess their reliability. In particular, attention should be paid to the level of optimisation of the method that should meet at least the ECVAM criteria for entering pre-validation (Curren *et al*., 1995), including evidence of the reproducibility of the method, its mechanistic relevance and predictive capacity (Balls *et al*., 1995, Hartung *et al*., 2004, Worth and Balls, 2001).

Currently *in vitro* assays only cover a (specific) part of the process of sensitisation that occurs *in vivo*.

(b) Animal data

Well reported studies using internationally acceptable protocols, particularly if conducted in accordance with the principles of GLP, can be used for hazard identification. Other studies not fully equivalent to OECD test protocols can, in some circumstances, provide useful information. Particular attention should be paid to the quality of these tests and the use of appropriate positive and negative controls. The specificity and sensitivity of all animal tests should be monitored through the inclusion of appropriate positive and negative controls. In this context, positive controls are the 6-monthly sensitivity checks with an appropriate positive control substance, and negative controls are the vehicle-treated control animals included as part of each test.

Guideline-compliant tests

For new *in vivo* testing of skin sensitisation potential, the LLNA is the preferred method. This assay has been validated internationally and has been shown to have clear animal welfare benefits and scientific advantages compared with the guinea pig tests described below. The LLNA is designed to detect the potential of chemicals to induce sensitisation as a function of lymphocyte proliferative responses induced in regional lymph nodes. This method is described in OECD TG 429/EU B.42.

Two further animal test methods for skin sensitisation are described in OECD TG 406/EU B.6: GPMT and the Buehler test. The GPMT is an adjuvant-type test in which the acquisition of sensitisation is potentiated by the use of FCA and in which both intradermal and topical exposure are used during the induction phase. The Buehler test is a non-adjuvant method involving for the induction phase topical application only.

Both the GPMT and the Buehler test are able to detect chemicals with moderate to strong sensitisation potential, as well as those with relatively weak sensitisation potential. In such methods activity is measured as a function of challenge-induced dermal hypersensitivity reactions elicited in test animals compared with controls. Since the LLNA is the preferred method for new *in vivo* testing, the use of the standard guinea pig tests to obtain new data on skin sensitisation potential will be acceptable only in exceptional circumstances and will require scientific justification. However, existing data of good quality deriving from such tests will be acceptable and will, if providing clear results, preclude the need for further *in vivo* testing.

ECETOC Monograph 29 (2000) contains a useful discussion of these tests.

For the conduct and interpretation of the LLNA the following points should be considered:

- the vehicle in which the test material and controls have been applied;
- the concentrations of test material that have been used;
- any evidence for local or systemic toxicity, or skin inflammation resulting from application of the test material;
- whether the data are consistent with a biological dose response;
- the submitting laboratory should be able to demonstrate its competency to conduct the LLNA.

OECD TG 429/EU B.42 provides guidance on the recommended vehicles, number of animals per group, concentrations of test chemical to be applied and substances to be used as a positive control. A preliminary study or evaluation of existing acute toxicity/dermal irritation data is normally conducted to determine the highest concentration of test substance that is soluble in the vehicle but does not cause unacceptable local or systemic toxicity. The submission of historical control data will demonstrate the ability of the test laboratory to produce consistent responses. Based on the use of radioactive labelling, chemicals that result in a stimulation index (SI) of ≥3 at one or more test concentrations are considered to be positive for skin sensitisation. Both positive and negative responses in the LLNA conducted as described in OECD TG 429/EU B.42 meet the requirements for classification of a substance as a skin sensitizer: no further testing is required.

In addition to radioactive labelling, two further methods were accepted to detect lymph node cell proliferation. Laboratories that do not have the possibility to work with radioactive substances can detect ATP content by bioluminescence as an indicator of proliferation (LLNA: DA, OECD TG 442A) or measure 5-bromo-2-deoxyuridine (BrdU) content, an analogue of thymidine (LLNA: BrdU-ELISA, OECD TG 442B). Further Guidance on interpretation of results from these assays is provided within the OECD Test Guideline protocol and in the *Guidance on the Application of CLP Criteria*. The guinea pig test methods described in OECD TG 406/EU B.6, the GPMT (Magnusson and Kligman,

1969; Schlede and Eppler, 1995) and the Buehler, can also be used for hazard identification. Recommendations on conducting and analysing these methods are provided by Steiling *et al*., 2001. Particular attention should be paid to the quality of these tests with consideration given to the following points:

- numbers of test and control guinea pigs;
- number or percentage of test and control animals displaying skin reactions;
- whether skin irritation was observed at the induction phase;
- whether the maximal non-irritating concentration was used at the challenge phase;
- the choice of an appropriate vehicle (ideally, one that solubilises or gives a stable suspension or emulsion of the test material, is free of allergenic potential, is non-irritating, enhances delivery across the stratum corneum, and is relevant to the usage conditions of the test material, although it is recognised that it will not always be possible to mee*t al*l these conditions);
- whether there are signs of systemic toxicity (a sighting study should be performed to determine an appropriate induction dose that causes irritation but not systemic toxicity);
- staining of the skin by the test material that may obscure any skin reactions (other procedures, such as chemical depilation of the reaction site, histopathological examination or the measurement of skin fold thickness may be carried out in such cases);
- results of re-challenge treatments if performed;
- checking of strain sensitivity at regular intervals by using an appropriate control substance (as specified in OECD guidelines and EU Test Methods). Currently (2007), the recommended interval is 6 months.

The investigation of doubtful reactions in guinea pig tests, particularly those associated with evidence of skin irritation following first challenge, may benefit from re-challenge of the test animals. In cases where reactions may have been masked by staining of the skin, other reliable procedures may be used to assist with interpretation; where such methods are used, the submitting laboratory should provide evidence of their value.

Non-guideline compliant tests and refinements to the standard assays

Existing data may be available from tests that do not have an OECD guideline, for example:

- other guinea pig skin sensitisation test methods (such as the Draize test, optimisation test, split adjuvant test, open epicutaneous test);
- additional tests (such as the mouse ear swelling test);

Information may also be available from other endpoints, for example, repeated dose dermal studies that show effects indicative of an allergic response, such as persistent erythema and/or oedema.

The submitted dossier should include scientific justification for conducting any new test that is a modification or deviation from guideline methods. In such cases, it would be advisable to seek appropriate expert advice on the suitability of the assay before testing is begun.

The rLLNA assay should not be performed for the identification of sensitisation potential for biocidal active substances as it is less scientifically rigorous than the standard LLNA, with an associate increased level of uncertainty.

The rLLNA assay (described in OECD TG 429, 2010) reduces the use of animals by requiring only a single (high) dose group (≥10%) and a concurrent negative control group. A preliminary study or evaluation of existing acute toxicity/dermal irritation data

is normally conducted to determine the highest concentration of test substance that is soluble in the vehicle, but that does not cause unacceptable local or systemic toxicity. As with the full LLNA, although a concurrent positive control group is not required, it would be required to submit historical positive control data supportive of their competence. The rLLNA can be used only in appropriate circumstances:

- where hazard identification is the primary objective and
- where potency data are not required

As in the standard (OECD guideline-compliant) LLNA, group sizes should comprise four or five animals. A positive result in a rLLNA will suffice in circumstances where risk assessment and/or risk management is NOT required.

Historically, guinea pig studies that are not fully equivalent to OECD test protocols have been conducted and can provide useful hazard information. These studies include, but are not limited to, the following: Draize test, optimisation test, split adjuvant test, open epicutaneous test and the cumulative contact enhancement test. In the case of positive results the substance may be considered as a potential skin sensitizer. If, taking into account the above quality criteria, especially the positive and negative control data, there is a clear negative result, i.e. no animals displaying any signs of sensitisation reactions, then no further animal testing is required. Where there is a low level of response, the quality of the study is questionable, or where unacceptably low concentrations of the test material have been used for induction and/or challenge, further testing may be required.

1.6.3.1 2 Human data for skin sensitisation

When reliable and relevant human data are available, they can be useful for hazard identification and even preferable over animal data. However, lack of positive findings in humans does not necessarily overrule positive and good quality animal data. Studies that report on cutaneous (allergic contact dermatitis, eczema) or respiratory (asthma, rhinitis, alveolitis) reactions should be of particular significance. Studies indicating negative results should also be evaluated.

Well conducted human studies can provide very valuable information on skin sensitisation. However, in some instances (due to lack of information on exposure, such as: a small number of subjects; the test group is patients in dermatology/allergology and not the general population; concomitant exposure to other substances; local or regional differences in patient referral) there may be a significant level of uncertainty associated with human data. Moreover, diagnostic tests are carried out to see if an individual is sensitised to a specific agent, and not to determine whether the agent can cause sensitisation.

For evaluation purposes, existing human experience data for skin sensitisation should contain sufficient information about:

- the test protocol used (study design, controls)
- the substance or preparation studied (should be the main, and ideally, the only substance or preparation present which may possess the hazard under investigation)
- the extent of exposure (magnitude, frequency and duration)
- the frequency of effects (versus number of persons exposed)
- the persistence or absence of health effects (objective description and evaluation)
- the presence of confounding factors (e.g. pre-existing dermal health effects, medication; presence of other skin sensitizers)
- the relevance with respect to the group size, statistics, documentation
- the healthy worker effect

Evidence of skin sensitising activity derived from diagnostic testing may reflect the induction of skin sensitisation to that substance or cross-reaction with a chemically very similar substance. In both situations, the normal conclusion would be that this provides positive evidence of the skin sensitising activity of the chemical used in the diagnostic test.

Human experimental studies on skin sensitisation are not normally conducted and are generally discouraged. Where human data are available, then quality criteria and ethical considerations are presented in ECETOC monograph No 32 (2002a).

Ultimately, where a very large number of individuals (e.g.10^5) have frequent (daily) skin exposure for at least two years and there is an active system in place to pick up complaints and adverse reaction reports (including via dermatology clinics), and where no or only a very few isolated cases of allergic contact dermatitis are observed then the substance is unlikely to be a significant skin sensitizer. However, information from other sources should also be considered in making a judgement on the substance's ability to induce skin sensitisation.

It is emphasised that testing with human volunteers is strongly discouraged, but when there are good quality data already available they should be used as appropriate in well justified cases.

1.6.3.2 Respiratory sensitisation

1.6.3.2.1 Non-human data for respiratory sensitisation

1.6.3.2.1.1 Non-testing data for respiratory sensitisation

(a) Read-across to structurally or mechanistically similar substances (SAR)

Generic guidance on the application of grouping approaches is provided in the *Guidance on information requirements and chemical safety assessment Chapter R.6.*

(b) (Q)SAR Systems

Given the current lack of available (Q)SARs for respiratory sensitisation no further guidance can be provided.

1.6.3.2.1.2 Testing data for respiratory sensitisation

(a) *In vitro* data

There are no *in vitro* tests available to assess respiratory sensitisation. If such a method were to become available then it would need to be assessed for its relevance and reliability.

(b) Animal data

Although the LLNA does not represent a method for the specific identification of chemical respiratory allergens, there is evidence that chemical respiratory allergens will also elicit positive responses in this assay (Kimber, 1995). The interpretation is, therefore, that a chemical which fails to induce a positive response in the LLNA (at an appropriate test concentration) most probably lacks the potential for respiratory allergy. Conversely, it cannot be wholly excluded that a chemical that induces a positive response in the LLNA, might sensitise the respiratory tract upon inhalation or via dermal exposure. Any potential hazard for respiratory sensitisation could only be positively identified by further testing, although such testing is neither validated nor widely accepted.

One further approach to the identification of chemicals that have the potential to induce allergic sensitisation of the respiratory tract is *cytokine fingerprinting* (Dearman *et al*., 2002). This method is predicated on an understanding that allergic sensitisation of the respiratory tract is favoured by selective Th_2-type immune responses and that in many

instances chemical respiratory allergy and occupational asthma are associated with IgE antibody.

In addition, there are other approaches that have been proposed and these have been reviewed recently (Arts and Kuper, 2007) - although again it is important to emphasise that there are currently available no fully evaluated or validated animal models for the predictive identification of chemical respiratory allergens.

As indicated previously, some chemicals may have the potential to induce pulmonary reactions via Th1-type immune responses. Studies with typical skin allergens such as DNCB, DNFB and picryl chloride (trinitrochlorobenzene) in BALB/c mice, guinea pigs or Wistar rats have shown the potential of these chemicals to induce allergic reactions in the lungs that are independent of IgE (Garssen *et al*., 1991; Garcia *et al*., 1992; Buckley *et al*., 1994; Zwart *et al*., 1994; Satoh *et al*., 1995; and see for a review Arts and Kuper, 2007). Sensitisation and challenge with DNCB resulted in laryngitis in low IgE-responding Wistar rats (Arts *et al*., 1998). [In addition, cellular immune responses to these sensitizers were shown to be associated with hyperreactivity of the airways to non-specific stimuli (Garssen *et al*., 1991).] For these reasons, it might be the case that people who are sensitised via the skin might suffer adverse pulmonary reactions if they were to inhale sufficient amounts of the contact allergen to which they were sensitised. As indicated previously, very few precedents for the elicitation of pulmonary reactions by skin sensitising chemicals in humans have been observed. In practice it appears not to represent a health issue.

1.6.3.1 1 Human data for respiratory sensitisation

Although human studies may provide some information on respiratory hypersensitivity, the data are frequently limited and subject to the same constraints as human skin sensitisation data.

For evaluation purposes, existing human experience data for respiratory sensitisation should contain sufficient information about:

- the test protocol used (study design, controls)
- the substance or preparation studied (should be the main, and ideally, the only substance or preparation present which may possess the hazard under investigation)
- the extent of exposure (magnitude, frequency and duration)
- the frequency of effects (versus number of persons exposed)
- the persistence or absence of health effects (objective description and evaluation)
- the presence of confounding factors (e.g. pre-existing respiratory health effects, medication; presence of other respiratory sensitizers)
- the relevance with respect to the group size, statistics, documentation
- the healthy worker effect

Evidence of respiratory sensitising activity derived from diagnostic testing may reflect the induction of respiratory sensitisation to that substance or cross-reaction with a chemically very similar substance. In both situations, the normal conclusion would be that this provides positive evidence for the respiratory sensitising activity of the chemical used in the diagnostic test.

For respiratory sensitisation, no clinical test protocols for experimental studies exist but tests may have been conducted for diagnostic purposes, e.g. bronchial provocation test. The test should meet the above general criteria, e.g. be conducted according to a relevant design including appropriate controls, address confounding factors such as medication, smoking or exposure to other substances, etc. Furthermore, the differentiation between the symptoms of respiratory irritancy and allergy can be very

difficult. Thus, expert judgment is required to determine the usefulness of such data for the evaluation on a case-by-case basis.

Although predictive models are under validation, there is as yet no internationally recognized animal method for identification of respiratory sensitisation. Thus, human data are usually evidence for hazard identification.

Where there is evidence that significant occupational inhalation exposure to a chemical has not resulted in the development of respiratory allergy, or related symptoms, then it may be possible to draw the conclusion that the chemical lacks the potential for sensitisation of the respiratory tract. Thus, for instance, where there is evidence that a large cohort of subjects have had opportunity for regular inhalation exposure to a chemical for a sustained period of time in the absence of respiratory symptoms, or related health complaints, then this will provide reassurance regarding the absence of a respiratory sensitisation hazard.

1.6.4. Remaining uncertainty on sensitisation

Reliable data can be generated on skin sensitisation from well designed and well conducted studies in animals. The use of adjuvant in the GPMT may lower the threshold for irritation and so lead to false positive reactions, which can therefore complicate interpretation (running a pre-test with FCA treated animals can provide helpful information). In international trials, the LLNA has been shown to be reliable, but like the guinea pig tests is dependent on the vehicle used, and it can occasionally give false positive results with irritants. Careful consideration should be given to circumstances where exposure may be sub-optimal due to difficulties in achieving a good solution and/or a solution of sufficient concentration. In some circumstances inconsistent results from guinea pig studies, or between guinea pig and LLNA studies, might increase the uncertainty of making a correct interpretation. Finally, for existing human data consideration must be given to whether inter-individual variability is such that it is not scientifically sound to generalize from a limited test panel.

When considering whether or not a substance is a respiratory sensitizer, observations of idiosyncratic reactions in only a few individuals with hyper-reactive airways are not sufficient to indicate the need for classification.

Major uncertainties remain in our understanding of the factors that determine whether or not a substance is an allergen, and if so, what makes it a skin or a respiratory sensitizer.

1.6.5. 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, and consideration should be given also to the Guidance for the implementation of the CLP Regulation.

1.6.6. Concluding on suitability for risk Assessment

There is evidence that for both skin sensitisation and respiratory hypersensitivity dose-response relationships exist (although these are frequently less well defined in the case of respiratory hypersensitivity). The dose of agent required to induce sensitisation in a previously naïve subject or animal is usually greater than that required to elicit a reaction in a previously sensitised subject or animal; therefore the dose-response relationship for the two phases will differ. Little or nothing is known about dose-response relationships in the development of respiratory hypersensitivity by non-immunological mechanisms.

It is frequently difficult to obtain dose-response information from either existing human or guinea pig data where only a single concentration of the test material has been examined. With human data, exposure measurements may not have been taken at the same time as the disease was evaluated, adding to the difficulty of determining a dose response.

Dose-response data, however, can be generated from local lymph node assays or, in exceptional cases, using specially designed guinea pig test methods. Such types of data can give data on induction and elicitation thresholds in these models, but it must be remembered these cannot be translated directly to human thresholds.

Measurement of potency: Appropriate dose-response data can provide important information on the potency of the material being tested. This can facilitate the development of more accurate risk assessments. This section refers to potency in the induction phase of sensitisation.

Neither the standard LLNA not the GPMT/Buehler is specifically designed to evaluate the skin sensitising potency of test compounds, instead they are used to identify sensitisation potential for classification purposes. However, all could be used for some estimate of potency. The relative potency of compounds may be indicated by the percentage of positive animals in the guinea pig studies in relation to the concentrations tested. Likewise, in the LLNA, the EC3 value (the dose estimated to cause a 3-fold increase in local lymph node proliferative activity) can be used as a measure of relative potency (ECETOC, 2000). Often linear interpolation of a critical effects dose from the EC3 is proposed (ECETOC, 2000), but more advanced statistical approaches basing conclusions on the characteristic of the dose response curve and variability of the results is also used (Basketter *et al*., 1999; Van Och *et al*., 2000). The dose-response data generated by the LLNA makes this test more informative than guinea pig assays for the assessment of skin sensitising potency. EC3 data correlate well with human skin sensitisation induction thresholds derived from historical predictive testing (Schneider *et al*., 2004; Griem *et al*., 2003; Basketter *et al*., 2005a). Accordingly, there are proposals for how this information may be used in a regulatory sense (Basketter *et al*., 2005a) and for risk assessment.

1.6.7. 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 which indicate that effective sensitisation of the respiratory tract can result from dermal contact with a chemical respiratory allergen. Thus, in this case, it appears that the quality of immune response necessary for acquisition of sensitisation of the respiratory tract can be skin contact with chemical respiratory allergens (Kimber and Dearman, 2002). Such considerations have important implications for risk management. Thus, for instance, there is a growing view that effective prevention of respiratory sensitisation 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. The generic advice is that appropriate strategies to minimise the risk of sensitisation to chemical allergens will require consideration of providing protection of all relevant routes of exposure.

1.7. Repeated dose toxicity

The Section on Repeated Dose Toxicity, Neurotoxicity and Immunotoxicity of the ECHA Biocides Guidance, Vol. III, Part A (Information Requirements) 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 4.6).

1.7.1. Definition of repeated dose toxicity

Repeated dose toxicity comprises the adverse general (i.e. excluding reproductive, genotoxic or carcinogenic effects) toxicological effects occurring as a result of repeated daily dosing with, or 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, in the case of chronic exposure.

The term general toxicological effects (in this report often referred to as *general toxicity*) includes effects on, e.g. 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 the nervous system as well as in organs and tissues in general, and 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 e.g., 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, 2003).

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 defined as 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, however, 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 process for substances, data on the adverse effects which a substance may cause, and the dose levels at which the effects occur, are evaluated in the light of the likely extent of human exposure to the substance so that the potential risk(s) to health may be ascertained.

The objectives of assessing repeated dose toxicity are to evaluate:

- whether exposure of humans to a substance has been associated with adverse toxicological effects occurring as a result of repeated daily exposure for a part of the expected lifetime or for the major part of the lifetime; 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 for a part of the

expected lifespan or for the major part of the lifespan; 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. 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 organ(s) and tissue(s).

The physico-chemical properties are also important in order to judge whether testing is technically possible. Testing for repeated dose toxicity may, be omitted if it is technically not possible to conduct the study as a consequence of the properties of the substance, e.g. very volatile, highly reactive or unstable substances cannot be used, or mixing of the substance with water may cause danger of fire or explosion.

Additional generic guidance on the use of physico-chemical properties is provided in the TK part of this guidance.

(b) Read-across

The potential toxicity of a substance for which no 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 on this endpoint.

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 organ(s) and tissue(s).

(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. Therefore, a negative result from current (Q)SAR models without other supporting evidence cannot be interpreted as demonstrating a lack of a toxicological hazard or a need for hazard classification. Another limitation of QSAR modelling is that dose-response information, including the N(L)OAEL, is not provided. Similarly, a validated QSAR model might identify a potential toxicological hazard, but because of limited confidence in this approach, 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, QSAR's 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, at present, is not useful on its own 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. Since, at present, there are not validated and regulatory accepted *in vitro* methods, the quality of each of these studies and the adequacy of the data provided should be carefully evaluated.

Generic guidance is given in the *Guidance on information requirements and chemical safety assessment Chapter R.4 and Chapter 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 primarily 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 EU/OECD test guideline studies for repeated dose toxicity is briefly summarised below. Table 8 (below) summarises the parameters examined in these OECD test guideline studies in more detail to facilitate overview of the similarities and differences between the various studies.

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), or inhalation (EU B.8/OECD TG 412). The principle of these study protocols is identical although the OECD TG 407 protocol includes additional parameters compared to those for dermal and inhalation administration, enabling the identification of a neurotoxic potential, immunological effects or reproductive organ toxicity.

The 28-day studies provide information on the toxicological effects arising from exposure to the substance during a relatively limited period of the animal's life span.

Repeated dose 90-day toxicity studies:

Separate guidelines are available for studies using oral administration (OECD TG 408/409/EU B.26/B.27 in rodent/non-rodent species, respectively), dermal application (OECD TG 411/EU B.28), or inhalation (OECD TG 413/EU B.29). The principle of these study protocols is identical although the revised OECD TG 408 protocol includes additional parameters compared to those for dermal and inhalation administration, enabling the identification of a neurotoxic potential, immunological effects or reproductive organ toxicity.

The 90-day studies provide information on the general toxicological effects arising from sub-chronic exposure (a prolonged period of the animal's life span) 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 in this study type. In addition, the combined study will allow for detection of neoplastic effects and a determination of a carcinogenic potential and the life-shortening effects.

- The 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 the repeated dose toxicity part, 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. The neurotoxicity study in rodents 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 the 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. These studies are summarised in Table 9 (below).

It should be noted that the repeated dose toxicity studies, if carefully evaluated, may provide information on potential reproductive toxicity and on carcinogenicity (e.g., pre-neoplastic lesions).

The one- , two-generation or the extended one generation reproductive toxicity studies (OECD TG 415/416/443EU 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 some 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, also 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.

The basic concept of repeated dose toxicity studies to generate data on target organ toxicity following sub-acute to chronic exposure is to treat experimental animals for 4 weeks, 13 weeks or longer. In addition, other studies performed in experimental animals may provide useful information on repeated dose toxicity. While at this time most alternative methods remain in the research and development stage and are not ready as surrogates for sub-chronic/chronic animal studies there are opportunities to improve data collection for risk assessment providing greater efficiency and use of fewer animals and better use of resources.

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 assessment factors in deriving threshold levels and in the design of new tests if the data is not sufficient for classification and labelling and risk assessment.

The following general guidance is provided for the evaluation of repeated dose toxicity data and the development of the WoE; in this respect all other information, including non test methods shall be taken into account in the WoE building.

- Studies on the most sensitive animal species should be selected as the significant ones, 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 route(s), 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 should be given greater weight than a repeated dose toxicity study of a shorter duration in the determination of the most relevant NOAEL.

If sufficient evidence is available to identify the critical effect(s) (with regard to the dose-response relationship(s) and to the relevance for humans), and the target organ(s) and/or tissue(s), greater weight should be given to specific studies investigating this effect in the identification of the NOAEL. The critical effect can be a local as well as a systemic effect.

While data available from repeated dose toxicity studies not performed according to conventional guidelines and/or GLP may still provide information of relevance for risk assessment and classification and labelling such data require extra careful evaluation.

Data from non-guideline studies shall be considered to be equivalent to data generated by corresponding test methods if 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 test methods referred to in REACH Article 13(3);
- exposure duration comparable to or longer than the corresponding test guideline method if exposure duration is a relevant parameter; and
- adequate and reliable documentation of the study is provided.

In all other situations, non-guideline studies may contribute to the overall weight of the evidence but cannot stand alone for a hazard and risk assessment of a substance and thus, cannot serve as the sole basis for an assessment of repeated dose toxicity as well as for exempting from the standard information requirements for repeated dose toxicity, i.e. cannot be used to identify a substance as being adequately controlled in relation to repeated dose toxicity.

The existing information is considered sufficient when, based on a WoE analysis, the critical effect(s) and target organ(s) and tissue(s) can be identified, the dose-response relationship(s) and NOAEL (s) and/or LOAEL(s) for the critical effect(s) can be established, and the relevance for human beings can be assessed.

It should be noted that potential effects in certain target organs (e.g. thyroid) following repeated exposure may not be observed within the span of the 28-day study. Attention is also drawn to the fact that 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 it is considered that the existing data as a whole is inadequate to provide a clear assessment of this endpoint, the need for further testing should be considered in view of all available relevant information on the substance, including use pattern, the potential for human exposure, physico-chemical properties, and structural alerts.

Specific investigations such as studies for neurotoxicity or immunotoxicity are also elements in the testing strategy that should be taken into account.

Regarding neurotoxicity and immunotoxicity, standard oral 28-day and 90-day toxicity studies include endpoints capable of detecting such effects. 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 standard 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.

More focus has also been put on endocrine disrupters during the latest decade. In relation to hazard and risk assessment, there are currently no test strategies or methods available, which specifically detect all effects, which have been linked to the endocrine disruption mechanisms.

In general, results from toxicological studies requiring repeated administration of a test substance such as reproduction and developmental toxicity studies as well as carcinogenicity studies can contribute to the assessment of repeated dose toxicity. However, such toxicological studies rarely provide the information obtained from a standard repeated dose toxicity study and therefore, cannot stand alone as the sole basis for the assessment of repeated dose toxicity or for exempting from the standard information requirements for repeated dose toxicity.

Studies such as acute toxicity and irritation studies as well as *in vivo* genotoxicity studies contribute limited information to the overall assessment of the repeated dose toxicity. However, such studies may be useful in deciding on the dose levels for use in repeated dose toxicity.

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 treatment-related toxicity is not associated with the administration of excessive high doses causing saturation of absorption and detoxification mechanisms. The results obtained from studies using 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 8: Overview of *in vivo* repeated dose toxicity test guideline studies

Test	Design	Endpoints
OECD TG 407 (EU B.7) Repeated dose 28-day oral toxicity study in rodents	Exposure for 28 days At least 3 dose levels plus control At least 5 males and females per group Preferred rodent species: rat	Clinical observations Functional observations (4th exposure week – sensory reactivity to stimuli of different types, grip strength, motor activity) Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, platelet count, blood clotting time/potential) Clinical biochemistry Urinalysis (optional) Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain, heart) Histopathology (full, at least control and high-dose groups - all gross lesions, brain, spinal cord, stomach, small and large intestines, liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs, gonads, accessory sex organs, urinary bladder, lymph nodes, peripheral nerve, a section of bone marrow)
OECD TG 410 (EU B.9) Repeated dose dermal toxicity: 21/28-day study	Exposure for 21/28 days At least 3 dose levels plus control At least 5 males and females per group Rat, rabbit or guinea pig	Clinical observations Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, clotting potential) Clinical biochemistry Urinalysis (optional) Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes) Histopathology (full, at least control and high-dose groups - all gross lesions, normal and treated skin, liver, kidney)

Test	Design	Endpoints
OECD TG 412 (EU B.8) Repeated dose inhalation toxicity: 28-day or 14-day study	Exposure for 28 or 14 days At least 3 concentrations plus control At least 5 males and females per group Rodents: preferred species - rat	Clinical observations Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, clotting potential) Clinical biochemistry Urinalysis (optional) Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes) Histopathology (full, at least control and high-dose groups - all gross lesions, lungs, liver, kidney, spleen, adrenals, heart)
OECD TG 408 (EU B.26) Repeated dose 90-day oral toxicity study in rodents	Exposure for 90 days At least 3 dose levels plus control At least 10 males and females per group Preferred rodent species: rat	Clinical observations Ophthalmological examination Functional observations (towards end of exposure period – sensory reactivity to stimuli of different types, grip strength, motor activity) Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, platelet count, blood clotting time/potential) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart) Histopathology (full, at least control and high-dose groups - all gross lesions, brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea and lungs, aorta, gonads, uterus, accessory sex organs, female mammary gland, prostate, urinary bladder, gall bladder (mouse), lymph nodes, peripheral nerve, a section of bone marrow, and skin/eyes on indication)

Test	Design	Endpoints
OECD TG 409 (EU B.27) Repeated dose 90-day oral toxicity study in non-rodents	Exposure for 90 days At least 3 dose levels plus control At least 4 males and females per group Preferred species: dog	Clinical observations Ophthalmological examination Body weight and food/water consumption Haematology (as in OECD TG 408) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (as in TG 408 - additional: gall bladder, thyroid, parathyroid) Histopathology (as in OECD TG 408 – additional: gall bladder, eyes)
OECD TG 411 (EU B.28) Subchronic dermal toxicity: 90-day study	Exposure for 90 days At least 3 dose levels plus control At least 10 males and females per group Rat, rabbit or guinea pig	Clinical observations Ophthalmological examination Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, clotting potential) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes) Histopathology (full, at least control and high-dose groups - all gross lesions, normal and treated skin, and essentially the same organs and tissues as in OECD TG 408)
OECD TG 413 (EU B.29) Subchronic inhalation toxicity: 90-day study	Exposure for 90 days At least 3 concentrations plus control At least 10 males and females per group Rodents: preferred species - rat	Clinical observations Ophthalmological examination Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total and differential leucocyte count, clotting potential) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (all animals - liver, kidneys, adrenals, testes) Histopathology (full, at least control and high-dose groups - all gross lesions, respiratory tract, and essentially the same organs and tissues as in OECD TG 408)

Test	Design	Endpoints
OECD TG 452 (EU B.30) Chronic toxicity studies	Exposure for at least 12 months At least 3 dose levels plus control Rodents : At least 20 males and females per group Non-rodents: At least 4 males and females per group Preferred rodent species: rat Preferred non-rodent species: dog	Clinical observations, including neurological changes Ophthalmological examination Body weight and food/water consumption Haematology (haematocrit, haemoglobin, erythrocyte count, total leucocyte count, platelet count, clotting potential) Clinical biochemistry Urinalysis Gross necropsy (full, detailed, all animals) Organ weights (all animals - brain, liver, kidneys, adrenals, gonads, thyroid/parathyroid (non-rodents only)) Histopathology (full, at least control and high-dose groups - all grossly visible tumours and other lesions, as well as essentially the same organs and tissues as in the 90-day studies (OECD TG 408/409))
OECD TG 453 (EU B.33) *Combined chronic toxicity*/carcinogenicity studies	Exposure for at least 12 months (satellite groups) or majority of normal life span (carcinogenicity part) At least 3 dose levels plus control At least 50 males and females per group Satellite group: At least 20 males and females per group Preferred species: rat	Essentially as in OECD TG 452

Test	Design	Endpoints
OECD TG 422[13] 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 groups, i.e. as in OECD TG 407)
OECD TG 424 (EU B.43) Neurotoxicity study in rodents	Exposure for at least 28 days Dose levels: not specified At least 10 males and females per group Preferred rodent species: rat Generally oral route of administration	Detailed clinical observations Functional observations (sensory reactivity to stimuli of different types, grip strength, motor activity, more 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) 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

[13] To date there is no corresponding EU testing method available

Test	Design	Endpoints
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)

Table 9: Overview of other *in vivo* test guideline studies giving information on repeated dose toxicity

Test	Design	Endpoints (general toxicity)
OECD TG 416 (EU B.35) Two-generation reproduction toxicity study	Exposure before mating for at least one spermatogenic cycle until weaning of 2nd generation At least 3 dose levels plus control At least 20 parental males and females per group	Clinical observations Body weight and food/water consumption Gross necropsy (all parental animals) Organ weights (reproductive organs, brain, liver, kidneys, spleen, pituitary, thyroid, adrenal glands, and known target organs) Histopathology (reproductive organs, previously identified target organ(s) - at least control and high-dose groups
OECD TG 415 (EU B.34) One-generation reproduction toxicity Study	Exposure before mating for at least one spermatogenic cycle until weaning of 1st generation At least 3 dose levels plus control At least 20 parental males and females per group	As in OECD TG 416
OECD TG 443 Extended one generation reproductive toxicity study	As described in OECD TG 443	As described in OECD TG 443

Test	Design	Endpoints (general toxicity)
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[14] 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 426[14] 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 are prohibited for the purposes of the BPR due to practical and ethical considerations involved in deliberate exposure of individuals to chemicals.

The following types of human data may already be available, however:

- Analytical epidemiology studies on exposed populations. These data may be useful for identifying a relationship between human exposure and effects such as

[14] To date there is no corresponding EU testing method available.

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. They examine differences in disease rates among human populations in relation to age, gender, race, and differences in temporal or environmental conditions. These studies may be useful for identifying priority areas for further research but not for 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 certain substances.
- Controlled studies in human volunteers. These studies, including low exposure toxicokinetic studies, might also be of use in risk assessment.
- Meta-analysis. In this type of study data from multiple studies are combined and analysed in one overall assessment of the relative risk or dose-response curve.

Human data in the form of epidemiological studies or case reports can contribute to the hazard identification process as well as to the risk assessment process itself. Criteria for assessing the adequacy of epidemiology studies include an adequate research design, the proper selection and characterisation of the exposed and control groups, adequate characterisation of exposure, sufficient length of follow-up for the disease as an effect of the exposure 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. These types of criteria have been described in more detail (Swaen, 2006 and can be derived from Epidemiology Textbooks (Checkoway *et al*., 1989; Hernberg, 1991; Rothman and Greenland, 1998).

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 relation to hazard identification, the relative lack of sensitivity of human data may cause particular difficulty. Therefore, negative human data cannot be used to override the positive findings in animals, unless it has been demonstrated that the mode of action of a certain toxic response observed in animals is not relevant for humans. In such a case a full justification is required.

1.7.3. Specific system/organ toxicity

1.7.3.1 General aspects

For some specific system/organ effects the testing methods of EU Annex V or the OECD may not provide for 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, hence, 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 EU Annex V repeated dose toxicity tests. Reproductive toxicity is specifically examined using special methods (EU Annex V). Specific investigation (or further investigation) of any organ/system toxicity (e.g. kidney, cardiac, adrenal, thyroid) may sometimes be considered 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. Also addressed herein, as a discrete issue, is lung overload and fibrosis.

1.7.3.2 Neurotoxicity

1.7.3.2.1 Definition of neurotoxicity

Neurotoxicity is the induction by a chemical 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

It is recommended that a hierarchical approach is taken in the investigation of the potential neurotoxicity of substances. The starting point for the testing strategy should be exposure considerations, *in vitro* data, SAR and should proceed via data already available from base set tests to more specific testing. Thus, any indications of specific or non-specific neurotoxicity in the acute and repeated dose toxicity tests should be carefully noted. In addition, if there are already alerts from SAR or available information on the substance or similar substances, and repeated dose toxicity is planned it would be of benefit to investigate neurotoxicity within the repeated dose test. The same would apply for consideration of developmental neurotoxicity investigations within the reproductive toxicity generation tests.

The present EU and OECD oral 28-day and 90-day tests (EU Annex V B7, Annex V B26, OECD TG 407, 1995; OECD TG 408, 1998) examine a number of simple nervous system endpoints (e.g. clinical observations of motor and autonomous nervous system activity, histopathology of nerve tissue), which should be regarded as the starting point for evaluation of a substance potential to cause neurotoxicity. It should be recognised that the standard 28-/90-day tests measure only some aspects of nervous system structure and function, while other aspects, e.g. learning and memory and sensory function is not or only superficially tested. SAR considerations may prompt the introduction of additional parameters to be tested in standard toxicity tests or the immediate request of studies such as delayed neurotoxicity (EU Annex V B37 or B38, OECD TG 418 or 419; see below). Any indication of potential neurotoxicity of substances can also be a trigger for testing for developmental neurotoxicity (see also ECHA Biocides Guidance, Vol. III, Part A (Information Requirements)).

If there are no indications of neurotoxicity in humans, and no indications in adequately performed acute and repeated dose toxicity tests, and none from SAR, it will not be necessary to conduct any special tests for 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), and carbamates (for cholinergic effects). Several estimation techniques are available, one of which is the rule-based DEREK system. The rule base comprises the following hazards and structural alerts: Organophosphate (for direct and indirect anticholinesterase activity); N-methyl or N,N-dimethyl carbamate (for direct anticholinesterase activity); gamma-diketones (for neurotoxicity).

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 seen at dose levels near or above those causing lethality should not be considered, in isolation, to be 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 or a few unrelated effects.

It is important to ascertain whether the nervous system is the primary target organ. The reversibility of neurotoxic effects should also be considered. The potential for such effects to occur in exposed humans (i.e. the exposure pattern and estimated level of exposure are "acute") should be considered in the risk characterisation. Reversible effects may be of high concern depending on the severity and nature of effect. In this context it should be kept in mind that 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 itself may not be harmful, but in relation to operation of machinery it is an effect of high concern). Furthermore the possibility that a permanent lesion has occurred cannot be excluded, even if the overt effect is transient. The nervous system possesses reserve capacity, which may compensate for the damage, but the resulting reduction in the reserve capacity should be regarded as an adverse effect. Compensation may be suspected if a neurotoxic effect slowly resolves during the lifespan. This could be the case for developmental neurotoxicants (see Section 5.3). Irreversible neurotoxic effects are of high concern and usually involve structural changes, though, at least in humans, lasting functional effects (e.g. depression, involuntary motor tremor) are suspected to occur as a result of neurotoxicant exposure, apparently without morphological abnormalities.

For the evaluation of organophosphate pesticides, Competent Authority experts agreed to use the WHO/FAO JMPR recommendations on "Interpretation of Cholinesterase Inhibition" (FAO, 1998; FAO, 1999). The applicability of these recommendations, outlined below, could also be extended to biocides and new/existing substances.

1.7.3.2.5 Recommendations from the WHO/FAO JMPR

The inhibition of brain acetylcholinesterase activity and clinical signs are considered to be the primary end-points 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 studies of repeated doses 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 considered not relevant. Regarding brain and erythrocyte acetylcholinesterase inhibition, the experts defined that statistically significant inhibition by 20% or more represents a clear toxicological effect and any decision to dismiss such findings should be justified. JMPR also agreed on the convention that statistically significant inhibition of less than 20% or statistically insignificant inhibition above 20% indicate that a more detailed analysis of the data should be undertaken. The toxicological significance of these findings should be determined on a case-by-case basis. One of the aspects to consider is the dose-response characteristic.

Certain substances and/or certain effects are best investigated in particular species. Pyridine derivatives are neurotoxic to humans and primates but not to rats. Among other neurotoxic compounds, organophosphorus compounds are a group with known delayed neurotoxic properties, which need to be assessed in a specified test for delayed neurotoxicity, to be performed preferentially in the adult laying hen according to the EU Annex V B.37 or OECD TG 418 (Delayed neurotoxicity of organophosphorus substances following acute exposure) and the EU Annex V B.38 or 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 anticholineesterase 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 Biocides Guidance, Vol. III, Part A (Information Requirements).

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. Alternatively, toxicity may 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) (IPCS, 1996). 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 dosages. However, an immunotoxic effect should not be disregarded until a thorough investigation has been performed.

The Guidance for Immunotoxicity risk assessment for chemicals by WHO/IPCS (WHO/IPCS, 2012) shall be consulted together with this Guidance when performing the assessment of this endpoint.

1.7.3.3.2 Introduction

The toxicological significance of immune responses is currently under discussion by several scientific groups (e.g. ECETOC, IPCS). Immunotoxicity is of particular concern for test substances that induce toxicity on the immune system at dose levels below those which induce toxicity at other target sites. If the immunotoxicity is the critical effect, it is recommended to assess immune effects in the risk assessment process as for any other toxic effect (IPCS, 1996). As the revised test methods (EU Annex V B.7 and B.26, OECD TG 407 and 408) become applied routinely, it is expected that the database on immunotoxic potential of substances will increase and experience on the evaluation of immune effects will improve. Primarily the test guidelines are intended as a screening for immunotoxicity, and depending on the results immediate 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 3 on Sensitisation.

1.7.3.3.4 Immunosuppression

The basis of the recommended approach to assessment of the potential immunotoxicity of a new substance is that many immunotoxic substances can be identified via the standard tests for systemic toxicity, particularly if the relevant additional measures of the updated EU and the OECD 28-day and 90-day test guidelines (see below) are used. As these additional measures do not comprise functional tests, it should be noted that discussions are currently taking place in the OECD as to whether these revised guidelines should be further enhanced by the inclusion of a function test (i.e. antibody response to sheep erythrocytes). 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. A tiered approach to the identification of immunotoxic hazard in routine toxicology is described in IPCS (1996).

The revised protocols of both the EU and the OECD 28-day and 90-day studies EU Annex V B.7 and B.26, equivalent to OECD TG 407 and OECD TG 408, respectively) now include the measurement of thymus and spleen weights and histopathological examination of certain lymphoid tissues (i.e. 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 required in the previous Annex V method. These tissues all have immunological function and changes to them can be indicative of adverse effects on the immune system.

The additional histopathological examinations listed above should be conducted on all control and high-dose animals. The stipulated tissues from all animals in all dose groups should be preserved. If tissues from high-dose animals show treatment-related changes, those from lower dose groups should also be examined to try to establish the NOAEL. The documentation of histopathology findings on immune organs can be improved by using a diagnostic system as developed by international collaborative studies (ICICIS, 1998; Kuper *et al*., 2000; Richter-Reichhelm and Schulte, 1996). In this system the lymphoid tissue is divided into compartments and the effects are assessed by application of a semiquantitative grading system. If there are changes in the bone marrow section, a bone marrow smear may be useful to quantify the changes: for a substance suspected to be immunotoxic (e.g. from SAR) it would be useful to prepare bone marrow smears in anticipation of this need. For these substances the study design could be further enhanced by adding parameters such as identification of lymphocyte subpopulations (flowcytometric analysis) and/or determination of serum immunoglobulin concentrations. Satellite groups could be included to conduct functional tests, e.g. antibody response to sheep erythrocytes.

If there are no indications of immunotoxicity in the 28-day (or 90-day) toxicity test, and also none from SAR, no further specific investigation for immunotoxicity will normally be required. However, when further studies of systemic toxicity are conducted on such substances, investigations for potential immunotoxicity, as described above should also be undertaken.

The need for further testing to examine in more depth the immunotoxicity of a substance giving rise to concern for immunotoxicity in the base-set 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 population(s) is exposed (e.g. workers and/or consumers) will be influencing factors.

Indications of immunotoxicity from standard repeated-dose studies include one or more of the following signs:

- 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 of changes of in haematology and of clinical chemistry parameters, together with an 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, it should be noted that the observation of the immunological changes discussed above may not necessarily reflect a primary immunotoxic effect but may be secondary to other effects.

Currently there are few methods for specific investigation of immunotoxic effects which are regarded as sufficiently validated for routine use (IPCS, 1996; Richter-Reichhelm *et al*., 2001). The plaque forming assay or the equivalent using the ELISA method are recommended to identify altered T-cell dependent humoral responses (Van Loveren *et al*., 1991; Temple *et al*., 1993). Of particular value for risk assessment are so called host resistance models, in which the clinical relevance of immunotoxicity can be evaluated (Van Loveren, 1995; IPCS, 1996). Other methods may also be of value to provide information on the mode of immunotoxic action (e.g. mitogen stimulation tests, leucocyte phenotyping). However, further work is needed on standardisation and validation of these test methods. For immunotoxicity testing a list of reviews of principles and methods from WHO/IPCS is provided in the Guidance for Information Requirements (WHO/IPCS, 2012) as well as a list of available test methods including assays for the assessment of autoimmunity.

1.7.3.4 Effects on the endocrine system

The endocrine system consists of a set of glands such as the thyroid, gonads and the adrenal glands, and the hormones they produce such as thyroxine, oestrogen, testosterone and adrenaline, which help guide the development, growth, reproduction and behaviour of animals, including human beings (EC Commission Communication, 1999).

Endocrine disruptors are believed to interfere with the endocrine system by one or more modes of action, depending on the individual substance. Individual modes of action can be assigned to one of at least three general possible ways as listed below:

- by mimicking the action of a naturally-produced hormone such as oestrogen or testosterone and thereby setting off similar chemical reactions in the body;
- by blocking the receptors in cells receiving the hormones (hormone receptors), thereby preventing the action of normal hormones;
- by affecting the synthesis, transport, metabolism and excretion of hormones, thus altering the concentration of normal hormones.

In relation to hazard identification, elements described with the JRC/IHCP Scientific report entitled "Key Scientific issues relevant to the identification and characterisation of endocrine disrupting substances" (available at: http://ihcp.jrc.ec.europa.eu/our_activities/food-cons-prod/endocrine_disrupters/jrc-report-scientific-issues-identification-endocrine-disrupting-substances) should be considered until further guidance is developed also in relation to the criteria for identification of endocrine disrupting chemicals. In addition, the following two OECD documents can be further considered for the evaluation of endocrine disruption potential of biocidal active substances:

- Detailed Review Paper on the State of the science on novel in vitro and *in vivo* screening and testing methods and endpoints for evaluating endocrine disruptors (OECD Series on Testing and Assessment, No 178, 2012) available at: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2012)23&doclanguage=en
- Guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption (OECD Series on Testing and Assessment, No 150, 2012) available at: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282012%2922&doclanguage=en

1.7.3.5 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 $mg \cdot m^{-3}$ (i.e. the atmospheric concentration should be $<1.6\ mg \cdot m^{-3}$ for a substance with a specific gravity of 1.6).

The principle outlined in the paragraph above does not, however, 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 (see Henderson, 1989)). 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 considered to be 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 the NOAEL for a new substance, 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 without further delay (e.g. immediately post-base-set).

The need for such repeated dose inhalation testing of an existing substance 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 and Section 4).

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 (i.e. LOAEL or LOAEC) and the highest level with no biologically or statically significant adverse effects (i.e. NOAEL or 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 the assessment of the uncertainty in the threshold of the critical effect(s).

The NOAEL is typically used as the starting point for the derivation of the threshold level (e.g. AEL, ADI). In case a NOAEL has not been achieved, a LOAEL may be used, provided the available information is sufficient for a robust hazard assessment and for Classification and Labelling. BMD may also be used as the starting point.

The selection of NOAEL or 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. Alternatively, mathematical curve fitting techniques or statistical approaches exist to determine the threshold for an adverse effect. The use of such approaches (e.g. BMD) to estimate the threshold should be considered on a case-by-case basis and are usually used for higher tier hazard characterisation refinement. For further guidance see Section 3 and Section 4 of this guidance.

1.7.4.2 Other considerations

Another situation may arise when testing is not technically possible, a waiving option indicated in Annex IV of the BPR (General Rules for the adaptation of the information requirements. In such cases 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, e.g. TTC might be considered for the starting point of a risk characterisation as risk management tools to estimate negligible exposure potential (see Appendix 1-4 of this guidance).

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. WoE is not to be interpreted as simply tallying the number of positive and negative studies, nor does it imply an averaging of the doses or exposures identified in individual studies that may be suitable as starting points for risk assessment. 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 the health hazards upon repeated exposure. In this process an assessment of all toxicological effect(s), 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 effect(s) 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. Further guidance to this issue can be found in publications of IPCS (IPCS, 1994; IPCS, 1999) and ECETOC (2002c).
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. Furthermore, 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 effect(s).
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 will be necessary unless there are indications for further risk.
Another consideration to be taken into account 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 at this stage.
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, the biological relevance, the reversibility, and the normal biological variation of an effect that may be shown by representative historical control values (IPCS, 1990).

1.7.6. 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, and consideration should be given also to the Guidance for the implementation of the CLP Regulation.

1.7.7. Concluding on suitability for risk assessment

Identification of the so-called dose descriptor: i.e. an appropriate threshold dose for the critical effect as the starting point for AEL, ADI derivation, i.e. a NOAEL or BMD. If a NOAEL can not be identified, the LOAEL may be used instead provided the data are adequate for a robust hazard assessment.
It is to be noted that the dose descriptor should be route-specific. Thus, in case only animal data with oral exposure are available and humans are exposed mainly via skin and/or inhalation, a threshold level for dermal route and/or for inhalation route are needed: i.e. route-to-route extrapolation is needed, if allowed. Guidance for this route-to-route extrapolation is provided in Section 2.

If this route-to-route extrapolation is not allowed, route-specific information is needed, possibly including testing, as a last resort (see ECHA Biocides Guidance, Vol. III, Part A (Information Requirements)).

1.8. Mutagenicity

The section on Mutagenicity of the ECHA Biocides Guidance, Vol. III, Part A (Information Requirements) should be considered together with the elements described in this section for the assessment of mutagenicity.

1.8.1. Definition

In the risk assessment of substances it is necessary to address the potential effect of "mutagenicity". It can be expected that some of the available data will have been derived from tests conducted to investigate harmful effects on genetic material ("genotoxicity"). Hence, both the terms "mutagenicity" and "genotoxicity" are used in this document.

The chemical and structural complexity of the chromosomal DNA and associated proteins of mammalian cells, and the multiplicity of ways in which changes to the genetic material can be effected make it difficult to give precise, discrete definitions.

A mutation means a permanent change in the amount or structure of the genetic material in a cell. The term 'mutation' applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term 'mutagenic' and 'mutagen' will be used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms (*Guidance on the Application of CLP Criteria*).

The term clastogenicity is used for agents giving rise to structural chromosome aberrations. A clastogen causes breaks in chromosomes that can result in the loss or rearrangements of chromosome segments. Aneugenicity (aneuploidy induction) refers to the effects of agents 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 multiple of the haploid number. For example, three number 21 chromosomes or trisomy 21 (characteristic of Down syndrome) is a form of aneuploidy.

The more general terms 'genotoxic' and 'genotoxicity' apply to agents or processes which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication. Genotoxic events might result but are not necessarily associated with mutagenicity. Thus, the tests for genotoxicity include tests which assess DNA damage (e.g. DNA strand breaks, DNA adducts), tests which provide an indirect indication of induced damage to DNA via effects such as DNA repair (unscheduled DNA synthesis) or mitotic recombination (sister chromatid exchange), as well as tests for mutagenicity (e.g. AMES test)."

The aims of testing for genotoxicity are to assess the potential of substances to induce genotoxic effects which may lead to cancer or cause heritable damage in humans. Genotoxicity data are used in risk characterisation and classification of substances.

Alterations to the genetic material of cells may occur spontaneously or be induced as a result of exposure to ionising or ultraviolet radiation, or genotoxic substances. In principle, human exposure to substances that are mutagens may result in increased frequencies of mutations above background.

Mutations in somatic cells may be lethal or may be transferred to daughter cells with deleterious consequences for the affected organism (e.g. cancer may result when they occur in proto-oncogenes, tumour suppressor genes and/or DNA repair genes) ranging from trivial to detrimental or lethal.

There is considerable evidence of a positive correlation between the mutagenicity of substances *in vivo* and their carcinogenicity in long-term studies with animals. Genotoxic

carcinogens are chemicals for which the most plausible mechanism of carcinogenic action involves genotoxicity.

Heritable damage to the offspring, and possibly to subsequent generations, of parents exposed to substances that are mutagens may follow if mutations are induced in parental germ cells. To date, all known germ cell mutagens are also mutagenic in somatic cells *in vivo.* Substances that are mutagenic in somatic cells may produce heritable effects if they, or their active metabolites, reach the genetic material of germ cells. Conversely, substances that do not induce mutations in somatic cells *in vivo* would not be expected to be germ cell mutagens.

1.8.2. Data to be used in the effects assessment

Genotoxicity is a complex endpoint and requires evaluation by expert judgement. For both steps of the effects assessment, *i.e.* hazard identification and dose (concentration)-response (effect) assessment, it is very important to evaluate the data with regard to their adequacy and completeness. The evaluation of adequacy shall address the reliability and relevance of the data in a way as outlined in the introductory section. The completeness of the data refers to the conclusion on the comparison between the available adequate information and the information that is required under the BPR. Such a conclusion relies on WoE approaches, mentioned in the EU Annex IV of the BPR, which categorise available information based on the methods used: guideline tests, non-guideline tests, and other types of information which may justify adaptation of the standard testing regime. Such a WoE approach also includes an evaluation of the available data as a whole, i.e. both *over and across* toxicological endpoints.

This approach provides a basis to decide whether further information is needed on endpoints for which specific data appear inadequate or not available, or whether the requirements are fulfilled.

1.8.2.1 Non-human data for mutagenicity

1.8.2.1.1 Non testing data for mutagenicity

In a more formal approach, documentation can include reference to a related chemical or group of chemicals that leads to the conclusion of concern or lack of concern. This can either be presented according to scientific logic (read-across) or sometimes as a mathematical relationship of chemical similarity.

If well-documented and applicable (Q)SAR data are available, they should be used to help reach the decision points described in the section below. In many cases the accuracy of such methods will be sufficient to help, or allow either a testing or a specific regulatory decision to be made. In other cases the uncertainty may be unacceptable due to the severe consequences of a possible error. This may be driven by many factors including high exposure potential or toxicological concerns.

WoE approaches that use expert judgement to include test results for close chemical analogues are ways of strengthening regulatory positions on the mutagenicity of a substance. Methods that identify general structural alerts for genotoxicity such as the Ashby-Tennant super-mutagen molecule (Ashby and Tennant, 1988) may also be useful.

Generally, (Q)SAR models that contain putative mechanistic descriptors are preferred; however many models use purely structural descriptors. While such models may be highly predictive, they rely on statistical methods and the toxicological significance of the descriptors may be obscure.

Another type of (Q)SAR model for mutagenicity attempts to predict (within their domain) diverse (non-congeneric) groups of substances. These are termed global (Q)SAR and are far more ambitious than the more simple local models. Global (Q)SAR are all computer programs which in essence first divide chemicals into local (Q)SAR and then make a conventional prediction.

Most global models for mutagenicity are commercial and some of the suppliers of these global models consider the data in their modelling sets to be proprietary. Proprietary means that the training set data used to develop the (Q)SAR model is hidden from the user. In other cases it means that it may not be distributed beyond use by regulatory authorities.

Further information on mutagenicity models (and other endpoints) can be found in the OECD Database on Chemical Risk Assessment Models, where they have been assembled as part of an effort to identify tools for use in research and development of chemical substances (www.oecd.fr).

Chemicals for which no test-data exist present a special case in which reliance on non-testing data may be absolute. Many factors will dictate the acceptability of non-testing methods in reaching a conclusion based on no tests at all. It is yet to be established whether weight-of evidence decisions based on multiple genotoxicity and carcinogenicity estimates can equal or exceed those obtained by one or two *in vitro* tests. This must be considered on a case-by-case basis.

1.8.2.1.2 Testing data for mutagenicity

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

(a) *In vitro* data

Table 10: *In vitro* test methods

Test method	Genotoxic endpoints measured/ Principle of the test method	EU/OECD guideline
Bacterial reverse mutation test	Gene mutations/The test uses amino-acid requiring strains of bacteria to detect (reverse) gene mutations (point mutations and frameshifts).	EU: B.12/13 OECD TG: 471
In vitro mammalian cell gene mutation test – hprt test	Gene mutations/The test identifies chemicals that induce gene mutations in the hprt gene of established cell lines.	EU: B.17 OECD TG: 476
In vitro mammalian cell gene mutation test – Mouse lymphoma assay	Gene mutations and structural chromosome aberrations/The test identifies chemicals that induce gene mutations in the TK gene of the L5178Y mouse lymphoma cell line. If colonies in a TK mutation test are scored using the criteria of normal growth (large) and slow growth (small) colonies, gross structural chromosome aberrations may be measured, since mutant cells that have suffered the most extensive genetic damage have prolonged doubling times and are more likely to form small colonies.	EU: B.17 OECD TG: 476
In vitro mammalian chromosome aberration test	Structural and numerical chromosome aberrations/The test identifies chemicals that induce chromosome aberrations in cultured mammalian established cell lines, cell strains or primary cell cultures. An increase in polyploidy may indicate that a chemical has the potential to induce numerical chromosome aberrations	EU: B.10 OECD TG: 473
In vitro micronucleus test	Structural and numerical chromosome aberrations/The test identifies chemicals that induce micronuclei in the cytoplasm of interphase cells. These micronuclei may originate from acentric fragments or whole chromosomes, and the test thus has the potential to detect both clastogenic and aneugenic chemicals.	EU: none OECD TG: 487

As noted earlier, accepted modifications to the standard test protocols have been developed to enhance test sensitivity to specific classes of substances. Expert judgement should be applied to judge whether any of these are appropriate for a given substance.

(b) Animal data

Table 11: Somatic cells - *in vivo* test methods

Test method	Genotoxic endpoints measured/ Principle of the test method	EU/OECD guideline
In vivo mammalian bone marrow chromosome aberration test	Structural and numerical chromosome aberrations/The test identifies chemicals that induce structural chromosome aberrations in the bone-marrow cells of animals, usually rodents. An increase in polyploidy may indicate that a chemical has the potential to induce numerical chromosome aberrations.	EU: B.11 OECD TG: 475
In vivo mammalian erythrocyte micronucleus test	Structural and numerical chromosome aberrations/The test identifies chemicals that cause micronuclei in erythroblasts sampled from bone marrow and/or peripheral blood cells of animals, usually rodents. These micronuclei may originate from acentric fragments or whole chromosomes, and the test thus has the potential to detect both clastogenic and aneugenic chemicals.	EU: B.12 OECD TG: 474
UDS test with mammalian liver cells in vivo	DNA repair/The test identifies chemicals that induce 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.	EU: B.39 OECD TG: 486
Transgenic animal models	Gene mutations/The tests can measure gene mutations in any tissue of an animal and may, therefore, also be used in specific site of contact tissues.	EU: none OECD TG: 488
In vivo alkaline single-cell gel electrophoresis assay for DNA strand breaks (Comet assay)	DNA strand breaks/The test can measure DNA strand breaks in any tissue of an animal and may, therefore, also be used in specific site of contact tissues.	EU: none OECD TG: none

A detailed review of transgenic animal model assays including recommendations on the conduct of such assays in somatic cells has been produced for the OECD (Lambert *et al.*, 2005).

Protocols for conducting the *in vivo* alkaline single-cell gel electrophoresis assay for DNA strand breaks (Comet assay) developed by an expert panel that met at the 2nd International Workshop on Genotoxicity Testing (IWGT, under the umbrella of the International Association of Environmental Mutagen Societies) are available (Tice *et al.*, 2000), as are recommendations for conducting this test developed by an expert panel who met in conjunction with the 4th International Comet Assay Workshop (Hartmann *et al.*, 2003).

Table 12: 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 and numerical chromosome aberrations/The test measures 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 chemical has the potential to induce numerical chromosome aberrations.	EU: B.23 OECD TG: 483

Test method	Genotoxic endpoints measured/ Principle of the test method	EU/OECD guideline
Rodent dominant lethal test	Structural and numerical chromosome aberrations/The test measures 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 lethals are due to structural and numerical chromosome aberrations. Rats or mice are recommended as the test species.	EU: B.22 OECD TG: 478
Transgenic animal models	Gene mutations/The tests measure gene mutations in spermatocytes of an animal and may, therefore, be used to obtain information about the mutagenic activity of a chemical in germ cells.	EU: none OECD TG: 488
In vivo alkaline single-cell gel electrophoresis assay for DNA strand breaks (Comet assay)	DNA strand breaks/The test measures DNA strand breaks in spermatocytes of an animal and may, therefore, be used to obtain information about the DNA-damaging activity of a chemical in germ cells.	EU: none OECD TG: none

A detailed review of transgenic animal model assays including recommendations on the conduct of such assays in germ cells has been produced for the OECD (Lambert *et al.,* 2005).

Evaluation of genotoxicity test data should be made with care. Regarding positive findings, responses generated only at highly toxic/cytotoxic concentrations should be interpreted with caution, and the presence or absence of a dose-response relationship should be considered.

Particular points to take into account when evaluating negative test results include:

- the doses or concentrations of test substance used (were they high enough?).
- was the test system used 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 active in the system including those in extra-hepatic organs.
- was the test substance taken up by the test system used for in vitro studies?
- for studies in vivo, is the substance reaching the target organ? (taking also toxicokinetic data into consideration, e.g. rate of hydrolysis and electrophilicity may be factors that need to be considered).

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

- conflicting results obtained in non-mammalian systems and in mammalian cell tests may be addressed by considering possible differences in substance uptake, metabolism or in the organisation of genetic material. Although the results of mammalian tests may be considered of higher significance, additional data may be needed to resolve contradictions.
- if the results of indicator tests (e.g. DNA binding; SCE) are not supported by results obtained in tests for mutagenicity, the results of mutagenicity tests are generally of higher significance.
- if contradictory findings are obtained in vitro and in vivo, in general, the results of in vivo tests indicate a higher degree of relevance. However, for evaluation of

negative results in vivo, it should be considered whether there is adequate evidence of target tissue exposure.

- the sensitivity and specificity of different test systems varies for different classes of substances. If available testing data for other related substances permits assessment of the performance of difference assays for the class of substance under evaluation, the result from the test system known to produce more accurate responses would be given higher priority.

Conflicting results may be also available from the same test, performed by different laboratories or on different occasions. In this case, expert judgement should be used to reach an overall evaluation of the data. In particular, 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-effect relationships, and biological relevance of the findings. The purity of the test substance may also be a factor to take into account. In the case where an EU/OECD guideline is available for a test method, the quality of a study using the method is regarded as being higher if it was conducted in compliance with the requirements stated in the guideline. Furthermore, studies compliant with GLP may be regarded as being of a higher quality.

When making an assessment of 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 with internationally established test protocols. For each test type and each genotoxic endpoint, there should be a separate WoE analysis. It is not unusual for positive evidence of mutagenicity to be found 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.

1.8.2.2 Human data on mutagenicity

Human data have to be assessed carefully on a case-by-case basis. The interpretation of such data requires considerable expertise. Attention should be paid especially to the adequacy of the exposure information, confounding factors, co-exposures and to sources of bias in the study design or incident. The statistical power of the test may also be considered.

1.8.3. Remaining uncertainty on mutagenicity

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, a certain level of uncertainty remains when extrapolating these testing data to the effect in humans.

1.8.4. Concluding on suitability for Classification and Labelling

In order to conclude on an appropriate classification and labelling position with regard to mutagenicity, the available data should be considered using the criteria according to EU Annex I of CLP Regulation.

1.8.5. Concluding on suitability for risk assessment

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

Considerations of the dose-response relationship and of possible mechanisms of action are important components of a risk assessment. The default assumption for genotoxic chemicals, in the absence of mechanistic evidence to the contrary, is that they have a

linear dose-response relationship. However, both direct and indirect mechanisms of genotoxicity can be non-linear or threshold and, consequently, sometimes this default assumption may be inappropriate.

Examples of mechanisms of genotoxicity that may be demonstrated to lead to non-linear or threshold dose-response relationships include extremes of pH, ionic strength and osmolarity, 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, high cytotoxicity, metabolic overload and physiological perturbations (e.g. induction of erythropoeisis). Assessment of the significance to be assigned to genotoxic responses mediated by such mechanisms would include an assessment of whether the underlying mechanism can be induced at substance concentrations that can be expected to occur under relevant *in vivo* conditions.

In general, several doses are tested in genotoxicity assays. Determination of experimental dose-effect relationships may be used to assess the genotoxic potential of a substance, as indicated below. It should be recognised that not all of these considerations may be applicable to *in vivo* data:

- A dose-related increase in genotoxicity is one of the relevant criteria for identification of positive findings. In practice, this 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-response related effect in some experimental systems.
- Genotoxicity tests are not designed in order to derive no effect levels. However, the magnitude of the lowest dose with an observed effect (i.e. the Lowest Observed Effect Dose or LOED) may, on certain occasions, be a helpful tool in risk assessment. This is true specifically for genotoxic effects caused by threshold mechanisms, like, e.g. aneugenicity. Further, it can give an indication of the mutagenic potency of the substance in the test at issue. Modified studies, with additional dose points and improved statistical power may be useful in this regard.
- Unusual shapes of dose-response curves may contribute to the identification of specific mechanisms of genotoxicity. For example, extremely steep increases suggest an indirect mode of action or 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. In fact, most – if not all tests used today – are developed and applied to identify mutagenic properties of the substance, i.e. identification of the mutagenic hazard *per se*. In today's regulatory practice, the assessment of human health risks from exposure to mutagenic substances is considered to be covered by assessing and regulating the carcinogenic risks of these agents. The reason for this is that mutagenic events underlie these carcinogenic effects. Therefore, mutagenicity data is not used for deriving dose descriptors for risk assessment purposes and the reader is referred to this aspect in Section on Carcinogenicity for guidance on how to assess the chemical safety for mutagenic substances.

1.9. Carcinogenicity

The section on Carcinogenicity of the ECHA Biocides Guidance, Vol. III, Part A (Information Requirements) should be considered together with the elements described in this section for the assessment of carcinogenicity.

1.9.1. Definition

Chemicals are defined as 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 (*Guidance on the Application of 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 (e.g., when inhaled, ingested, applied to the skin or injected), but carcinogenic potential and potency may depend on the conditions of exposure (e.g., 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). Each strategy for the identification of potential carcinogens is discussed in detail later in this report. The determination of the carcinogenic potential of a chemical is based on a WoE approach. Classification criteria are given in the CLP Regulation.

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

Carcinogenic chemicals have conventionally been divided into two categories according to the presumed mode of action: genotoxic or non-genotoxic. Genotoxic modes of action involve genetic alterations caused by the chemical interacting directly with DNA to result in a change in the primary sequence of DNA. A chemical can also cause 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 that may influence gene expression, altered cell-cell communication, or other factors involved in the carcinogenic process. For example, chronic cytotoxicity with subsequent regenerative cell proliferation is considered a mode of action by which tumour development can be enhanced: the induction of urinary bladder tumours in rats may, in certain cases, be due to persistent irritation/inflammation, tissue erosion and regenerative hyperplasia of the urothelium following the formation of bladder stones. Other modes of non-genotoxic action can involve specific receptors (e.g. PPARα, which is associated with liver tumours in rodents; or tumours induced by various hormonal mechanisms). As with other non-genotoxic modes of action, these can all be presumed to have a threshold.

The objective of investigating the carcinogenicity of chemicals is to identify potential human carcinogens, their mode(s) of action, and their potency.

With respect to carcinogenic potential and potency the most appropriate source of information is directly from human epidemiology studies (e.g. cohort, case control studies). In the absence of human data, animal carcinogenicity tests may be used to differentiate carcinogens from non-carcinogens. However, the results of these studies subsequently have to be extrapolated to humans, both in qualitative as well as quantitative terms. This introduces uncertainty, both with regard to potency for as well

as relevance to humans, due to species specific factors such as differences in chemical metabolism and TK and difficulties inherent 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 (see above).

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 discernible (e.g. 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 above mentioned modes of action; and a conclusion on this, in fact, depends on the outcome of mutagenicity/genotoxicity testing and other mechanistic studies. In addition to this, 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 be highly dependent on exposure conditions such as the route of exposure. A pulmonary carcinogen, for example, can cause lung tumours in rats following chronic inhalation exposure, but there may be no cancer hazard associated 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, a broad spectrum of non-testing techniques exist for elucidating mechanistic, toxicokinetic or toxicodynamic factors important in understanding the carcinogenic process. These range from expert judgement, to the evaluation of structural similarities and analogues (i.e. read-across and grouping), to the use of (Q)SAR models for carcinogenicity. Such information may assist with priority setting, hazard identification, elucidation of the mode of action, potency estimation and/or with making decisions about testing strategies based on a WoE evaluation.

Genotoxicity remains an important mechanism for chemical carcinogenesis and its definitive demonstration for a chemical is often decisive for the choice of risk assessment methodology.

It has long been known that certain chemical structures or fragments can be associated with carcinogenicity, often through DNA-reactive mechanisms. Useful guidance for structures and fragments that are associated with carcinogenicity via DNA reactive mechanisms has been provided by the FDA's "Guideline for Threshold Assessment, Appendix I, Carcinogen Structure Guide" (U.S. FDA, 1986); the Ashby-Tennant "super-mutagen model" (e.g. Ashby and Tennant, 1988); and subsequent builds on this model (e.g. Ashby and Paton, 1993; Munro *et al*., 1996a). Additional information on structural

categories can be found in the "IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man" (IARC, 2006a).

Models predicting test results for genotoxic endpoints for closely related structures are known as local or congeneric (Q)SARs. These congeneric models are less common for carcinogenicity than for mutagenicity. Franke *et al*. (2001) provide an example of such a model for a set of genotoxic carcinogens.

The situation is far more complex for non-genotoxic carcinogenicity due to the large number of different mechanisms that may be involved. However, progress is being made in predicting activity for classes of compounds that exert effect via binding to oestrogen receptors, induction of peroxisomal proliferation, and binding to tubulin proteins. Although many potentially useful models exist, their applicability will be highly dependant on the proposed mechanism and chemical class.

Several global (non-congeneric) models exist which attempt to predict (within their domain) the carcinogenic hazard of diverse (non-congeneric) groups of substances (e.g. Matthews and Contrera, 1998). These models may also assist in screening, priority-setting, deciding on testing strategies and/or the assessment of hazard or risk based on WoE. Most are commercial and include expert systems such as Onco-Logic® (currently made available by U.S. EPA) and DEREK, artificial intelligence systems from MULTICASE, and the TOPKAT program. Historically, the performance of such models has been mixed and is highly dependent on the precise definition of carcinogenicity among those substances used to develop and test the model. These have been reviewed by ECETOC (2003a) and Cronin *et al*. (2003).

Free sources of carcinogenicity predictions include the Danish EPA (Q)SAR database (accessible through http://ihcp.jrc.ec.europa.eu/our_labs/predictive_toxicology/qsar_tools). Predictions in this database for 166,000 compounds include eight MULTICASE U.S. FDA cancer models, a number of genotoxicity predictions, rodent carcinogenic potency, hepatospecificity, oestrogenicity and aryl hydrocarbon (AH) receptor binding. Another source of carcinogenicity predictions is the Enhanced NCI database "Browser", which is sponsored by the U.S. National Cancer Institute (NCI). This has 250,000 chemical predictions within it (http://cactus.nci.nih.gov), including general carcinogenicity, mutagenicity and additional endpoints, which may be of potential mechanistic interest in specific cases.

Further information on carcinogenicity models is available in the OECD Database on Chemical Risk Assessment Models where they are listed in an effort to identify tools for research and development of chemical substances. (http://www.olis.oecd.org/comnet/env/models.nsf/MainMenu?OpenForm).

It is noted that all the above mentioned sources may potentially inform on possible carcinogenic hazard and on the underlying mode of action, as well as on carcinogenic potency.

1.9.2.1.2 Testing data on carcinogenicity

(a) *In vitro* data

The following *in vitro* data, which provide direct or indirect information useful in assessing the carcinogenic potential of a substance and (potentially) on the underlying mode(s) of action, may be available. No single endpoint or effect in and of itself possesses unusual significance for assessing carcinogenic potential but must be evaluated within the context of the overall toxicological effects of a substance under evaluation. Except, standardised protocols do not exist for most of the *in vitro* endpoints noted. Rather, 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:

(i) Genotoxicity studies: the ability of substances to induce mutations or genotoxicity can be indicative of carcinogenic potential. However, correlations between mutagenicity/genotoxicity and carcinogenesis are stronger when effects are observed in appropriately designed *in vivo* as opposed to *in vitro* studies.

(ii) *In vitro* cell transformation assay results: such 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* (OECD, 2006). As *in vitro* assays, cell transformation 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.

(iii) Mechanistic studies, e.g.:

- cell proliferation: sustained cell proliferation can facilitate the growth of neoplastic/pre-neoplastic cells and/or create conditions conducive to 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, as assessed by a diverse array of assays for fluorescent dye transfer or the exchange of small molecules between cells, 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. Interactions of this nature are diverse and generally very compound specific.
- Other targeted mechanisms of action.
    - 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 this immune surveillance function and permit the growth of neoplastic cells induced by exogenous factors or spontaneous changes.
    - ability to inhibit or induce apoptosis: apoptosis, or programmed cell death, 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 mode(s) of action. For example, *in vitro* genotoxicity studies may provide information about whether or not the substance is likely to be genotoxic *in vivo*, and thus a potential genotoxic carcinogen, and herewith on the potential mode of action underlying carcinogenicity: with or without a threshold.

Besides genotoxicity data other *in vitro* data such as *in vitro* cell transformation can help to decide, in a WoE evaluation, whether a chemical possesses a carcinogenic potential. Cell transformation results in and of themselves do not inform as to the actual underlying mode(s) of action, since they are restricted to the detection of effects exerted at the level of the single cell and may be produced by mechanistically distinct processes.

Studies can also be conducted to evaluate the ability of substances to influence processes thought to facilitate carcinogenesis. Many of these endpoints are assessed by experimental systems that have yet to be formally validated and/or are the products of continually evolving basic research. Formalised and validated protocols are thus lacking for the conduct of these tests and their interpretation. Although it is difficult to give general guidance on each test due to the variety and evolving nature of tests available, it is important to consider them on a case-by-case basis and to carefully consider the context on how the test was conducted.

A number of the test endpoints evaluate mechanisms that may contribute to neoplastic development, but the relative importance of each endpoint will vary as a function of the overall toxicological profile of the substance being evaluated. It should further be noted that there are significant uncertainties associated with extrapolating *in vitro* data to an *in vivo* situation. Such *in vitro* data will, in many instances, provide insights into the nature of the *in vivo* studies that might be conducted to define carcinogenic potential and/or mechanisms.

(b) Animal data

A wide variety of study categories may be available, which may provide direct or indirect information useful in assessing the carcinogenic potential of a substance to humans. They include:

(i) Carcinogenicity studies (conventional long-term or life-time studies in experimental animals): Carcinogenicity testing is typically conducted using rats and mice, and less commonly in animals such as the Guinea pig, Syrian hamster and occasionally mini-pigs, dogs and primates. The standard rodent carcinogenicity bioassay would be conducted using rats or mice randomly assigned to treatment groups. Exposures to test substances may be via oral, inhalation or dermal exposure routes. The selection of exposure route is often dictated by *a priori* assumptions regarding the routes of exposure relevant to humans and/or other data sources (e.g. epidemiology studies or repeated dose toxicity studies in animals) that may indicate relevance of a given exposure route. Standardised protocols for such studies have been developed and are well validated (e.g. OECD TGs 451, 453 or U.S. EPA 870.4200).

(ii) Short and medium term bioassay data (e.g., mouse skin tumour, rat liver foci model, neonatal mouse model): multiple assays have been developed that 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 are generally regarded as adjuncts to conventional cancer bioassays, and while less validated and standardised, are applicable on a case-by-case basis for obtaining supplemental mechanistic and dose response information that may be useful for risk assessment (Enzmann *et al*., 1998).

(iii) Genetically engineered (transgenic) rodent models (*e.g.*, *Xpa*$^{-/-}$, *p53*$^{+/-}$, *ras*H2 or Tg.AC): animals can be genetically engineered such that one or more of the molecular changes required for the multi-step process of carcinogenesis has been accomplished (Tennant *et al*., 1999). This can increase the sensitivity of the animals to carcinogens 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

(Jacobson-Kram, 2004; Pritchard *et al*., 2003; ILSI/HESI, 2001). Data from these models may be used in a WoE analysis of a chemical's carcinogenicity.

(iv) Genotoxicity studies *in vivo*: the ability of substances to induce mutations or genotoxicity can be indicative of carcinogenic potential. There is, in general, a good correlation between positive genotoxicity findings *in vivo* and animal carcinogenicity bioassay results.

(v) Repeated dose toxicity tests: can identify tissues that may be specific targets for toxicity and subsequent carcinogenic effects. Particular significance can be attached to the observation of pre-neoplastic changes (e.g. hyperplasia or metaplasia) suspected to be conducive to tumour development and may assist in the development of dose-effect relationships (Elcombe *et al*., 2002).

(vi) 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 (Cohen and Ellwein, 1991; Cohen *et al*., 1991).

(vii) Studies on immunosuppressive activity: as noted earlier, suppression of normal immune surveillance functions can interfere with normal immune system functions that serve to identify and eliminate neoplastic cells.

(viii) 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 carcinogenic impacts may or may not exist, but such data can serve to focus carcinogenesis studies upon specific tissue types or animal species.

(ix) Other studies on mechanisms/modes of action, e.g. OMICs studies (toxicogenomics, proteomics, metabonomics and metabolomics): carcinogenesis is associated with multiple changes in gene expression, transcriptional regulation, protein synthesis and other metabolic changes. Specific changes diagnostic of carcinogenic potential have yet to be validated, but these rapidly advancing fields of study may one day permit assessment of a broad array of molecular changes that might be useful in the identification of potential carcinogens.

It is noted that the above tests inform differently on hazard identification, mode of action or carcinogenic potency. For example, conventional bioassays are used for hazard identification and potency estimation (i.e. derivation of a dose descriptor), whereas studies using genetically engineered animals are informative on potential hazard and possibly mode of action, but less on carcinogenic potency as they are considered to be highly sensitive to tumour induction.

In vivo data can give direct information about the carcinogenic potential of a substance, possible underlying mode(s) of action, and its potency.

The carcinogenicity testing should be addressed by an *in vivo* test according to OECD TG 451 or 453, unless the substance is classified as mutagen category 1A or 1B, and a conclusion is based on a comparison of the incidence, nature and time of occurrence of neoplasms in treated animals and controls. Other tests may contribute to a WoE evaluation, e. g. by providing supporting information or mechanistic data.

Knowledge of the historic tumour incidence for the strain of animal used is important (laboratory specific data are preferable). Also attention to the study design used 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. A qualitative assessment of the acceptability of study reports is therefore an important part of the process of independent evaluation. Sources of guidance in this respect can be found in IEH (2002), CCCF (2004) and OECD (2002). If the available study report does not include all the information required by the standard test guideline, judgement is required to decide if the experimental procedure is or is not acceptable and if essential information is lacking.

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. Such modifications to standard protocols can be considered as a function of the specific properties of the material under evaluation.

Carcinogenicity data may sometimes be available in species other than those specified in standard test guidelines (e.g. Guinea pig, Syrian hamster and occasionally mini-pigs, dogs and primates). Such studies may be in addition to, or instead of, studies in rats and mice and they should be considered in any evaluation.

Data from non-conventional carcinogenicity studies, such as short- and medium-term carcinogenicity assays with neonatal or genetically engineered (transgenic) animals, may also be available (CCCF, 2004; OECD, 2002). Genetically engineered animals possess mutations in genes that are believed to be altered in the multi-step process of carcinogenesis, thereby enhancing animal sensitivity to chemically induced tumours. A variety of transgenic animal models exist and new models are continually being developed. The genetic alteration(s) in a specific animal model can be those suspected to facilitate neoplastic development in a wide range of tissue types or the alterations can be in genes suspected to be involved in tissue specific aspects of carcinogenesis. The latter must be applied with recognition of both their experimental nature and the specific mechanistic pathways they are designed to evaluate. For example, a transgenic animal model sensitive to mesothelioma induction would be of limited value in the study of a suspected liver carcinogen. While such animal model systems hold promise for the detection of carcinogens in a shorter period of time and using fewer animals, their sensitivity and specificity remains to be determined. Due to a relative lack of validation, such assays have not yet been accepted as alternatives to the conventional lifetime carcinogenicity studies, but may be useful for screening purposes or to determine the need for a rodent 2-year bioassay. Several evaluations of these types of study have been published (e.g. Jacobson-Kram, 2004; Pritchard *et al*., 2003; ILSI/HESI, 2001).

When data are available from more than one study of acceptable quality, consistency of the findings should be established. When consistent, it is usually straightforward to arrive at a conclusion, particularly if the studies were in more than one species or if there is a clear treatment-related incidence of malignant tumours in a single study. If a single study only is available and the test substance is not carcinogenic, scientific judgement is needed to decide on whether (a) this study is relevant or (b) additional information is required to provide confidence that it should not be considered to be carcinogenic.

Study findings also may not clearly demonstrate a carcinogenic potential, even when approved study 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. Although less convincing than an increase in malignant and rare tumours, and recognising the potential over-sensitivity of this model (Haseman, 1983; Ames and Gold, 1990), a detailed and substantiated rationale should be given before such positive findings can be dismissed as not relevant.

Repeated dose toxicity studies may provide helpful additional information to the WoE gathered to determine whether a substance has the potential to induce cancer, and for potential underlying modes of action (Elcombe *et al*., 2002). For example, the induction of hyperplasia (either 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 for carcinogenic potential. Toxicity studies may also provide evidence for immunosuppressive activity, a condition favouring tumour development under conditions of chronic exposure.

Finally, toxicokinetic data may reveal the generation of metabolites with relevant 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 upon differences in absorption, distribution, metabolism and or excretion of the substance. Species specific differences mediated by such factors may be demonstrated through experimental studies or by the application of toxicokinetic modelling.

Positive carcinogenic findings on animals require careful evaluation and this should be done with reference to other toxicological data (e.g. *in vitro* and/or *in vivo* genotoxicity studies, toxicokinetic data, mechanistic studies, (Q)SAR evaluations) and the exposure conditions (e.g. route). Such comparisons may provide evidence for (a) specific mechanism(s) of action, a significant factor to take into account whenever possible, that may then be evaluated with respect to relevance for humans.

A conceptual framework that provides a structured and transparent approach to the WoE assessment of the mode of action of carcinogens has been developed (see Sonich-Mullin *et al*., 2001; Boobis *et al*., 2006). This framework should be followed when the mechanism of action is key to the risk assessment being developed for a carcinogenic substance and can be particularly critical in a determination of whether a substance induces cancer via genotoxic or non-genotoxic mechanisms.

For example, 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 mechanism(s) 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 (IARC, 2006b). The burden of proof in drawing such mechanistic inferences can be high but can have a significant impact upon underlying assumptions made in risk assessment.

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 (see OECD, 2002). 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, i.e. by mechanisms/modes of action not operating in humans or operative in humans under extreme and unrealistic conditions. Reviews are available for some of these tumour types providing a detailed characterisation that includes the key biochemical and histopathological events that are needed to establish these rodent-specific mechanisms that are not relevant for human health (IARC, 1999a). The IPCS has developed a framework and provided some examples on how to evaluate the relevance to humans of a postulated mode of action in animals (ILSI RSI, 2003; Boobis *et al*., 2006; Meek *et al*., 2013).

The information available for substances identified as carcinogenic based on testing and/or non-testing data should be further evaluated in order to identify underlying mode(s) of action and potency and to subsequently allow for a proper quantitative risk assessment (see Section 1.9.5). As already pointed out, the use of non-standard animal models (e.g. transgenic or neonatal animals) needs careful evaluation by expert judgement as to how to apply the results obtained for hazard and risk assessment purposes; it is not possible to provide guidance for such evaluations.

1.9.2.1. Human data for carcinogenicity

A number of basic epidemiological study designs exist and include cohort, case-control and registry based correlational (e.g. ecological) studies. 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 the intensity of exposure experienced by 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 are frequently nested within the conduct of cohort studies and can help increase the precision with which excess cancer can be associated with a given substance. Correlational or ecological studies evaluate cancer incidence/mortality in groups of individuals presumed to have exposure to a given substance but are generally less precise since measures of the exposure experienced by individuals are not available. Observations of cancer clusters and case reports of rare tumours may also provide useful supporting information in some instances but are more often the impetus for the conduct of more formal and rigorous cohort studies.

Besides the identification of carcinogens, epidemiological studies may also provide information on actual exposures in representative (or historical) workplaces and/or the environment and the associated dose-response for cancer induction. Such information can be of much value for risk characterisation.

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/or quality of information that is available regarding exposure history (e.g. measurements of individual exposure) or other determinants of health status within a cohort are often limited. Given the long latency between exposure to a carcinogen and the onset of clinical disease, robust estimates of carcinogenic potency can be difficult to generate. Similarly, 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 long established industry sectors (e.g. base metal production) now 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 and drinking habits, dietary patterns and ethnicity) influence the incidence of cancer but are often inadequately documented for purposes of adequate confounder control. Thus, modest increases in cancer at tissue sites known to be impacted by confounders (e.g. lung and stomach) can be difficult to interpret.

Techniques for biomonitoring and molecular epidemiology are developing rapidly. These newly developed tools promise to provide information on biomarkers of individual susceptibility, critical target organ exposures and whether effects occur at low exposure

levels. Such ancillary information may begin to assist in the interpretation of epidemiology study outcomes and the definition of dose response relationships. For example, monitoring the formation of chemical adducts in haemoglobin molecules (Albertini *et al*., 2006), the urinary excretion of damaged DNA bases (Chen and Chiu, 2005), and the induction of genotoxicity biomarkers (micronuclei or chromosome aberrations; Boffetta *et al*., 2007) are presently being evaluated and/or validated for use in conjunction with classical epidemiological study designs. Such data are usually restricted in their application to specific chemical substances but such techniques may ultimately become more widely used, particularly when combined with animal data that defines potential mechanisms of action and associated biomarkers that may be indicative of carcinogenic risk. Monitoring of the molecular events that underlie the carcinogenic process may also facilitate the refinement of dose response relationships and may ultimately serve as early indicators of potential cancer risk. However, as a generalisation, such biomonitoring tools have yet to demonstrate the sensitivity requisite for routine use.

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 using accepted causality criteria, such as that of Hill (1965). Particular attention should be given to exposure data in a study and to the choice of the control population. Often a significant level of uncertainty exists around identifying a substance unequivocally as being carcinogenic because of inadequate reporting of exposure data. Chance, bias and confounding factors can frequently not be ruled out. A clear identification of the substance, 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 an appropriate joint evaluation (meta-analysis) may be used in order to increase the sensitivity, provided the studies are sufficiently similar for such an evaluation. When the results of different studies are inconsistent, possible explanations should be sought and the various studies judged on the basis of the methods employed.

Interpretation of epidemiology studies must be undertaken with care and include an assessment of the adequacy of exposure classification, 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). A number of such factors can limit the sensitivity of a given 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. Efforts are ongoing to improve the sensitivity and specificity of traditional epidemiological methods by combining cancer endpoints with data on established pre-neoplastic lesions or molecular indicators (biomarkers) of cancer risk.

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, and/or may be useful in demonstrating an upper bound on the human cancer risk. Identification of the underlying mode(s) of action – needed for the subsequent risk assessment (see Section 1.9.3 below) – quite often depends critically on available testing and/or non-testing information.

1.9.3. Remaining uncertainty

As indicated in the previous sections, adequate human data for evaluating the carcinogenic properties of a chemical are most often not available, and alternative approaches have to be used.

In addition test systems for identifying genotoxic carcinogens are reasonably well developed and adequately cover this property. There is also agreement that animal carcinogens which act 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. Unclear, however, and herewith introducing some uncertainty, is the relationship between carcinogenic potency in animals and in humans.

There is, on the other hand, a shortage of sensitive and selective test systems to identify non-genotoxic carcinogens, apart from the carcinogenicity bioassay. In the absence of non-testing information on the carcinogenicity of structurally related chemicals, indications for possible carcinogenic properties may come from existing repeated dose toxicity data, or from *in vitro* cell transformation assays. However, whereas the former source of data will have a low sensitivity (e.g. in case of a 28-day study), there is a possibility that the latter may lead to an over-prediction of carcinogenic potential. Insufficient data are available to provide further general guidance in this regard.

Non-genotoxic carcinogens may be difficult to identify in the absence of animal carcinogenicity test data. However, it could be argued that current conservative (cautious) risk assessment methodology should cover the risk for carcinogenic effects via this mode of action as well: i.e. current risk assessments for many non-genotoxic carcinogens are based on NOAELs for precursor effects or target organ toxicity with the application of conservative assessment factors to address uncertainty. Once identified as a non-genotoxic carcinogen (from testing or non-testing data) there may be uncertainty as to the human relevance of this observation, i.e. to the human relevance of the underlying mode of action. In the absence of specific data on this, observations in the animal are taken as relevant to humans. However, additional uncertainty will exist for the relationship between carcinogenic potency in animals and in humans; this uncertainty, though, will be addressed in the procedure for deriving human standards (ILSI RSI, 2003).

Finally, conventional assays of carcinogenicity 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 that there will always be a possibility that a chemical could pose a carcinogenic hazard in humans but be missed in conventional animal studies. This is also true for other toxicological endpoints and should be taken into account by risk managers, especially when making decisions about the acceptability of scenarios showing particularly high exposures to workers and/or consumers.

1.9.4. Concluding on suitability for Classification and Labelling

In order to conclude on an appropriate classification and labelling position with regard to carcinogenicity, the available data should be considered using the criteria and guidance associated with the CLP Regulation.

1.9.5. Concluding on suitability for risk assessment

Besides the identification of a chemical as a carcinogenic agent from either animal data or epidemiological data, or both, dose response assessment is an essential further step in order 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, whether this induction of tumours is thought to be via a genotoxic mechanism or not.

In regulatory work, 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, i.e. it is not possible to define a no-effect level for carcinogenicity induced by such agents. However, in certain cases even for these compounds a threshold for carcinogenicity may be identified in the low-dose region: e.g. it has in certain cases been clearly demonstrated that an increase in tumours did not occur at exposures below those associated with local chronic cytotoxicity and regenerative hyperplasia. It is also recognised that 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 (agents that induce aneuploidy – the gain or loss of entire chromosomes to result in changes in chromosome number), or for chemicals that cause indirect effects on DNA that are secondary to another effect (e.g. through 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 many different modes of action thought to be involved in non-genotoxic carcinogenicity. Some appear to involve direct interaction with specific receptors (e.g. oestrogen receptors), whereas appear to be non-receptor mediated. Chronic cytotoxicity with subsequent regenerative cell proliferation is considered a mode of action by which tumour development can be induced: the induction of urinary bladder tumours in rats, for example, may, in certain cases, 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, have also been proposed to 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 (both *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. Epidemiological data seldom contribute to this.

Once the mode of action of tumour-formation is identified as having a threshold or not, a dose descriptor has to be derived for concluding the risk assessment.

If the mode of action of tumour formation is identified as non-threshold, dose descriptors such as T25, BMD10 or BMDL10 (see Section 2)within the section on dose response descriptors) can be established to allow, if needed, the derivation of a so-called DMEL (for guidance see the *Guidance on information requirements and chemical safety assessment Chapter R.8, Section R.8.5*), that could subsequently be used in the safety assessment to establish exposure levels of minimal concern as a risk management option.

Though mainly derived from animal data, epidemiological data may also occasionally provide dose descriptors that allow derivation of a reference value, e.g. Relative Risk (RR) or Odds Ratio (OR).

1.10. Reproductive toxicity

The Section on Reproductive Toxicity within the *Guidance on the BPR: Volume III Human Health, Part A Information Requirements* should be considered together with the elements described in this section for the assessment of reproductive toxicity.

1.10.1. Definition

At the population level the property of reproductive toxicity is of obvious high concern because the continuance of the human species is dependent on the integrity of the

reproductive cycle. Similarly, to the individual an impairment of the ability to reproduce and the occurrence of developmental disorders are self-evidently serious health conditions. Therefore, it is important that the potential hazardous properties with respect to reproduction are established for chemicals with relevant human exposure that may be present in the environment, at the workplace and in consumer products.

The term reproductive toxicity is used to describe the adverse effects induced (by a substance) on sexual function and fertility in adult males and females, developmental toxicity in the offspring and effects on or mediated via lactation, as defined in Part 3 of the Globally Harmonised System (GHS) of Classification and Labelling of Chemicals System (UN, 2005). In practical terms, reproductive toxicity is characterised by multiple diverse endpoints, which relate to impairment of male and female reproductive functions or capacity (fertility) and the induction of non-heritable harmful effects on the progeny (developmental toxicity). Effects on male or female fertility include adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or hormonal or physiological response, which would interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation. Developmental toxicity includes any effect interfering with normal development, both before and after birth. It includes effects induced or manifested either pre- or postnatally. This includes embryotoxic/foetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional effects, peri- and postnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.

The general objectives of the testing are to establish:

- whether exposure of humans to the substance of has been associated with adverse effects on reproductive function or capacity; and/or
- whether, in studies in animals, administration of the substance to males and/or females prior to conception and during pregnancy and lactation, causes adverse effects on reproductive function or capacity; and/or
- whether, in studies in animals, administration of the substance during the period of pre- or post-natal development induces non-heritable adverse effects in the progeny;
- whether the pregnant female is potentially more susceptible to general toxicity;
- the dose-response relationship for any adverse effects on reproduction.

Substance-related adverse effects on reproduction are always of potential concern, but it is important, where possible, to distinguish between a specific effect on reproduction as a consequence of an intrinsic property of the substance and an adverse reproductive effect which is a non-specific consequence to general toxicity (e.g. reduced food or water intake, maternal stress). Hence, reproductive toxicity should be assessed alongside parental toxicity in the same study. Further guidance on the assessment of developmental toxicity in relation to maternal toxicity is presented further below.

With respect to germ cell mutagens that meet the criteria for classification as Category 1 or 2 mutagens (according to Directive 93/21) (or Cat 1A or 1B according to CLP Regulation) and genotoxic carcinogens that meet the criteria for classification as both Category 3 mutagens and Category 1 or 2 carcinogens (or as Cat 2 and Cat 1A or 1B respectively according to CLP Regulation), the results of reproductive toxicity testing are unlikely to influence the outcome of the risk assessment. This is because the risk characterisation for such substances will be based on the assumption that a threshold exposure level for adverse health effects cannot be identified, which will normally lead to a recommendation for the most stringent risk management measures. Therefore, reproductive testing will not normally be required for germ cell mutagens and genotoxic carcinogens, unless there are case-specific reasons to indicate that the information

gained from testing will be needed for the risk characterisation. Germ cell mutagens and genotoxic carcinogens not tested for reproductive toxicity should be regarded as potentially toxic to reproduction.

1.10.2. Data to be used in the effects assessment

1.10.2.1 Non-human data

1.10.2.1.1 Non-testing data

(a) 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 cross 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.

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

The concept of structure-activity relationships (SAR) offers approaches for estimating the reproductive toxicity potential of a substance. By grouping substances with similar structures there is an opportunity for the toxicity potential of well-investigated substances to be extended to substances for which there are no or incomplete data. This is particularly the case where the toxicity profile (or lack thereof) can be associated with structural characteristics and reproductive toxicity potential may be extrapolated or interpolated across a homologous series or category.

QSAR systems approaches are currently not well validated for reproductive toxicity and consequently no firm recommendations can be made concerning their routine use in a testing strategy in this area. Therefore, a negative result from current QSAR models cannot be interpreted as demonstrating the absence of a reproductive hazard unless there is other supporting evidence. Another limitation of QSAR modelling is that dose-response information, for example the N(L)OAEL, required for risk assessment is not provided.

However, a positive result in a validated QSAR model could provide a trigger (alert) for further testing but because of limited confidence in this approach such a result would not normally be adequate as a primary support for a hazard classification decision.

Additionally, QSAR models could be used as part of a WoE approach, when considered alongside other data, provided the applicability domain is appropriate. Also, QSAR 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.

1.10.2.1.2 Testing data on reproductive toxicity

(a) *In vitro* data

Currently there is no officially adopted EU or OECD test guideline for *in vitro* tests of relevance to reproductive toxicity.

Most of the assays that are relevant to reproductive toxicity are designed to assess the ability of a chemical to bind and activate or block the androgen receptor (AR) or the oestrogen receptor (ER). These include cell-free or whole cell binding assays, cell proliferation assays and transcription assays. Also, tests for detecting the ability to interfere with steroidogenesis are currently being developed.

The latest information on the status of alternative methods that are under development can be obtained from the ECVAM website and other international centres for validation of alternative methods.

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.

At the present time *in vitro* approaches have many limitations, for example the lack of capacity for biotransformation of the test substance (Coecke *et al*., 2006). Consequently, no firm recommendations can be made for the exclusive use of *in vitro* methods in a testing strategy for reproductive toxicity. The combination of assays in a tiered and/or battery approach may improve predictivity, but the *in vivo* situation remains more than the sum of the areas modelled by a series of *in vitro* assays (see Piersma (2006) for review). Therefore, a negative result for a substance with no supporting information cannot be interpreted with confidence as demonstrating the absence of a reproductive hazard. Another limitation of *in vitro* tests is that a N(L)OAEL and other dose-response information required for a risk assessment is not provided.

However, a positive result in a validated *in vitro* test could provide a justification for further testing, dependent on the effective concentration and taking account of what is known about the toxicokinetic profile of the substance. However, because of limited confidence in this approach at this time, such a result in isolation would not be adequate to support hazard classification.

Additionally, validated and non-validated *in vitro* tests, provided the applicability domain is appropriate, could be used with other data in a WoE assessment approach to gathering 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 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.

Notably, the recent validation study of the three most promising tests for detection of developmental effects, the embryonic stem cell test, the limb bud micromass culture and the whole embryo culture, showed that these had high predictivity for the limited number of strongly embryotoxic chemicals included in the study (Genschow *et al*., 2002; Piersma, 2006; Spielmann *et al*., 2006). However, a number of weaknesses in the design of both the validation study and of the *in vitro* tests have been identified, such as the limited number and range of substances tested and absence of a biotransformation system, which have lead to the conclusion that the tests currently have limited value in a regulatory context. Nevertheless, as discussed above, the results of these tests can have a role, when considered alongside other data, in a WoE assessment and in support of read-across approaches, and can serve as a trigger for further testing.

(b) Animal data

Data may be available from a wide variety of animal studies, which give different amounts of direct or indirect information on the potential reproductive toxicity of a substance; e.g.:

- screening studies (such as OECD TGs 421 or 422)
- other short-term in vivo screening tests (e.g. Chernoff/Kavlock tests see Hardin *et al*. (1987), uterotrophic and Hershberger assays)
- one- or two- (or multi-) generation studies (such as B.35, OECD TGs 415 or 416,or EU B.34 or a 'F1-extended one-generation study OECD TG 443)
- prenatal developmental toxicity tests (such as EU B.31, OECD TG 414)
- developmental neurotoxicity studies (such as OECD TG 426)
- peri-postnatal studies
- male or female fertility studies of non-standard design

- repeated-dose toxicity studies, if relevant parameters are included, for example semen analysis, oestrous cyclicity and/or reproductive organ histopathology
- dominant lethal assay (EU B.22, OECD TG 478)
- mechanistic and toxicokinetic studies
- studies in non-mammalian species

Repeated-dose toxicity studies:

Although not aimed directly at investigating reproductive toxicity, repeated-dose toxicity studies (e.g. EU B.7, OECD TG 407) may reveal clear effects on reproductive organs in adult animals. However, if these findings occur in the presence of marked systemic toxicity (up to the highest dose level tested in a repeated-dose study) may lower concerns for effects on fertility and can contribute to decisions on further testing requirements. However, this does not rule out the possibility that the substance may have the capacity to affect fertility.

The observation of effects on reproductive organs in repeated-dose toxicity studies may also be sufficient for identifying a N(L)OAEL for use in the risk assessment. It should, however, be noted that the sensitivity of repeated-dose toxicity studies for detecting effects on reproductive organs may be less than reproductive toxicity studies because of the lower number of animals per group. In addition, a number of cases have demonstrated that effects on the reproductive system may occur at lower doses during the development of foetuses and young animals than in adults. Consequently, in cases where there are substantiated indications for adverse effects on the reproductive organs of adult animals the use of an increased assessment factor in the risk assessment process may be considered. Alternatively, further studies, for example a screening test (OECD TG 421) or a two-generation study (EU B.35, OECD TG 416) may be triggered based on a WoE assessment. Some effects seen in repeated-dose toxicity studies may be difficult to interpret, for example changes in sex hormone level, and should be investigated further as part of other studies.

Repeated-dose toxicity studies may also provide indications to evaluate the need to investigate developmental neurotoxicity and/or developmental immunotoxicity endpoints.

***In vivo* reproductive toxicity tests:**

The available OECD TGs (or drafts) specifically designed to investigate reproductive toxicity are shown in Table 13.

The purpose of Reproduction/Developmental Toxicity Screening Test (OECD TGs 421 and 422) is to provide information of the effects on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of conceptus and parturition. The observation of clear evidence of adverse effects on reproduction or on reproductive organs in these tests may be sufficient to meet the information needs for a classification and risk assessment (using an appropriate assessment factor), and providing a N(L)OAEL from which a AEL can be identified. However, the results should be interpreted with caution because OECD TGs 421/422 are screening assays that were not designed as an alternative or a replacement of the definitive reproductive toxicity studies (OECD TGs 414 and 416, EU B.31 and B.35). These screening tests are not meant to provide complete information on all aspects of reproduction and development. In particular, the post-natal effects associated with prenatal exposure (such as undetected malformations affecting viability or functional effects) or effects resulting from post-natal or lactational exposure are not covered in these studies. Furthermore, the exposure duration in these studies may not be sufficient to detect all effects on the spermatogenic cycle, although it is likely that in practice the 2-week exposure period will be sufficient to detect the majority of testicular toxicants (Ulbrich and Palmer, 1995). However, the number of animals per dose group is limited

which may affect the statistical power of the study to detect an effect. These screening tests may in some cases give indications for reproductive effects (e.g. fertility and post natal effects) that cannot be investigated in a prenatal developmental toxicity study (OECD TG 414, EU B.31). A negative result in a screening study may lower concerns for reproductive toxicity, but this will not provide reassurance of the absence of this hazardous property. However, a negative result can provide the basis for AEL derivation in relation to reproductive toxicity derived from the highest dose level used in the study and using an assessment factor that takes account of the limitations of this study. An evaluation of the OECD TG 421 or TG 422 has confirmed that these tests are useful for initial hazard assessment and can contribute to decisions on further test requirements (Reuter *et al*., 2003; Gelbke *et al*., 2004).

The two-generation study (OECD TG 416, EU B.35) is a general test which allows evaluation of the effects of the test substance on the complete reproductive cycle including libido, fertility, development of the conceptus, parturition, post-natal effects in both dams (lactation) and offspring and the reproductive capacity of the offspring.

The extended one generation reproductive toxicity study (OECD TG 443) addresses the main limitation of OECD TG 415 (EU B.34) by incorporating additional post-natal evaluations, which include clinical pathology, a functional observation battery, immunotoxicity endpoints, oestrous cyclicity and semen analysis, and using an extended F1 generation dosing period (to PND day 70) endpoints addressing developmental neurotoxicity. The study has a shortened F0 male premating dosing period, justified by the observation of no differences in the detection rates for adverse effects on fertility between 4- and 9-week pre-mating dosing periods in a number of studies (reviewed by Ulbrich and Palmer; 1995).

The prenatal developmental toxicity study (OECD TG 414, EU B.31) provides a focussed evaluation of potential effects on prenatal development, although only effects that are manifested before birth can be detected.
Positive results in these studies will be relevant to hazard classification and the human health risk assessment, unless there is information to show that effects seen in these studies could not occur in humans. N(L)OAELs can be identified from OECD TGs 414 (EU B.31), 415 (EU B.34), 416 (EU B.35), 426 and 443.

Developmental neurotoxicity studies (OECD TG 426 or OECD TG 443) are designed to provide information on the potential functional and morphological hazards to the nervous system arising in the offspring from exposure of the mother during pregnancy and lactation. These studies investigate changes in behaviour due to effects on the CNS and the peripheral nervous system. As behaviour also may be affected by the function of other organs such as liver, kidneys and the endocrine system, toxic effects on these organs in offspring may also be reflected in general changes in behaviour. No single test is able to reflect the entire complex and intricate function of behaviour. For testing behaviour, therefore, a range of parameters, a *test battery*, is used to identify changes in individual functions.

The severity and nature of the effect should be considered. Generally, a pattern of effects (e.g. impaired learning during several consecutive trials) is more persuasive evidence of developmental neurotoxicity than one or a few unrelated changes. The reversibility of effects should be considered, too. Irreversible effects are clearly serious, while reversible effects may be of less concern. However, it is often not possible to determine whether an effect is truly reversible. The nervous system possesses reserve capacity, which may compensate for damage, but the resulting reduction in reserve capacity should be regarded as an adverse effect. If developmental neurotoxicity is observed only during some time of the lifespan then compensation should be suspected. Also, effects observed for example during the beginning of a learning task but not at the end should not be interpreted as reversible effects. Rather the results may indicate that the speed of learning is decreased.

The experience of offspring especially during infancy may affect their later behaviour. For example, frequent handling of rats during infancy may alter the physiological response to stress and the behaviour in tests for emotionality and learning. In order to control for environmental experiences, the conditions under which the offspring are reared should be standardised within experiments with respect to variables such as noise level, handling and cage cleaning. The performance of the animals during the behavioural testing may be influenced by e.g. the time of day, and the stress level of the animals. Therefore, the most reliable data are obtained in studies where control and treated animals are tested alternatively and environmental conditions are standardised.

Equivocal results may need to be followed up by further investigation. The most appropriate methods for further investigations should be determined on a case-by-case basis. Additional Guidance is provided within the ECHA Biocides Guidance, Vol. III, Part A (Information Requirements).

Positive results in a developmental neurotoxicity study will be relevant to hazard classification and the human health risk assessment, providing a N(L)OAEL, unless there is information to show that effects seen in these studies could not occur in humans.

For more detailed reviews of how to interpret the test guidelines mentioned in this section, including a discussion of their strengths and limitations see the reports from Nordic Chemicals Group (2005), ECETOC (2002b) and WHO (2001).

Table 13: Overview of *in vivo* OECD test guidelines for reproductive toxicity

Test	Design	Endpoints
OECD TG 443 Extended one generation study	Exposure of 10 weeks prior to mating[2] (P) until post-natal day 90-120 (Cohorts 1A and 1B). If the extension of Cohort 1B is triggered, then until post-natal day 4 or 21 (F2)[3]. 3 dose levels plus control; highest dose level must be chosen with the aim to induce some toxicity. Preferred species rat Preferred route oral[1] N = sufficient mating pairs to produce 20 pregnant animals per dose group (P generation) N = 20 mating pairs (extension of Cohort 1B, if triggered) N = 10 males and 10 females per dose group (Cohorts 2A, 2B and 3, if triggered)	*Parental (P) generation:* Growth, survival, fertility Oestrus cyclicity and sperm quality Pregnancy length and litter size Histopathology and weight of reproductive and non-reproductive organs Haematology and clinical chemistry *Offspring (F1):* Growth, survival and sexual maturation Histopathology and weight of reproductive and non-reproductive organs (Cohort 1A) Weight of reproductive organs and optional histopathology (Cohort 1B) Haematology and clinical chemistry Fertility of F1 animals to produce F2 generation (extension of Cohort 1B) under certain conditions Developmental neurotoxicity (Cohorts 2A and 2B or a separate study) in case of a particular concern Developmental immunotoxicity (Cohort 3 or a separate study) in case of a particular concern
OECD TG 416 Two-Generation study	Exposure before mating for at least one spermatogenic cycle until weaning of 2nd generation 3 dose levels plus control N =20 parental males and females	Fertility Oestrus cyclicity and sperm quality Pregnancy outcome, e.g. dystocia Growth, development and viability Anogenital distance if triggered

Test	Design	Endpoints
		Sexual maturation Histopathology and weight of reproductive organs, brain and target organs Recommended: motor activity, sensory function, reflex ontology in F_1 generation
OECD TG 415 One-Generation Study	Exposure before mating for at least one spermatogenic cycle until weaning of 1st generation 3 dose levels plus control N =20 parental males and females	Fertility Growth, development and viability Histopathology and weight of reproductive organs, brain and target organs
OECD TG 414 Prenatal Developmental Toxicity Study (Teratology study)	At least from implantation to one or two days before expected birth 3 dose levels plus control N = 20 pregnant females	Implantation, resorptions Foetal growth Morphological variations and malformations
OECD TG 426 Developmental Neurotoxicity Study	At least from implantation throughout lactation (PND 20) 3 dose levels plus control N =20 pregnant females	Birth and pregnancy length Growth, development and viability Physical and functional maturation Behavioural changes due to CNS and PNS effects Brain weights and neuropathology
OECD TGs 421 and 422 Reproduction/ Developmental toxicity screening test	From 2 weeks prior to mating until at least day 4 postnatally 3 dose levels plus control N =8-10 parental males and females	Fertility Pregnancy length and birth Foetal and pup growth and survival until day 4 OECD TG 422 combines reproduction/developmental screen with repeated-dose toxicity investigations that are in concordance with the requirements of OECD TG 407

Developmental effects should be considered in relation to adverse effects occurring in the parents. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, etc., it is important that the effects observed should be interpreted in conjunction with possible concomitant maternal toxicity (ECB, 2004; Fleeman *et al*., 2005, Cappon *et al*; 2005). The nature, severity and dose-response of all effects observed in progeny and parental animals should be considered and compared together to achieve a balanced integrated assessment of available data on all endpoints relevant for reproductive toxicity.

1.10.2.1.3 Relation between maternal toxicity and developmental toxicity

Developmental toxicity may be mediated by maternal toxicity, as experience has shown. However, in most studies evidence for a causal relationship is lacking. Developmental toxicity occurring in the presence of maternal effects does not itself imply a causal relationship between the two and therefore it is not appropriate to discount developmental toxicity that occurs only in the presence of maternal toxicity. If a causal relationship can be established, it can be concluded that developmental toxicity does not occur at lower doses than the threshold for maternal toxicity, although the substance

can still be considered as a developmental toxicant. In the absence of proven causality, the nature and severity of the developmental versus the maternal effects may well warrant the conclusion that a substance should be considered as a specific developmental toxicant when the effects are only observed in the presence of maternal toxicity. The assessment of the interrelationship between developmental toxicity and maternal toxicity and its influence on decisions regarding hazard classification must be conducted on a case-by-case by basis, using a WoE approach, and with reference to the *Guidance on the Application of CLP*.

Because of possible differences in sensitivity between pregnant and non-pregnant animals, toxicity data from repeat dose studies have little use in the interpretation of maternal toxicity in reproductive studies. On the other hand, in reproductive toxicity studies, endpoints that were shown to be affected in repeated toxicity studies may be incorporated as maternal parameters. This may help to identify any differences in sensitivity to treatment between pregnant and non-pregnant animals due to pregnancy-induced changes in physiology.

1.10.2.1.4 Reproductive toxicity via lactation

Reproductive toxicity may occur through lactation in several ways. Substances may reach the milk and result in exposure of the newborn. On the other hand, the quality and quantity of the milk may be affected by maternal exposure to the substance, resulting in nutritional effects on the newborn. Three aspects are crucial in the risk assessment of lactational effects, as indicated below:

- the concentration of the substance transferred via the milk. Toxicokinetic aspects should be considered including the chemical-physical properties of the compound, the timing and duration of exposure, the bioavailability and the persistence of the substance. Fat-soluble chemicals that may be mobilized during lactation are of special concern;
- the sensitivity of the newborn as compared to the adult. A wide spectrum of toxic effects may occur in the newborn, ranging from general toxic effects which may present as reduced weight gain or delayed general development, to specific effects on the maturation of organs or physiological systems. The newborn may be more sensitive as compared to the adult, not only because of specific developmental endpoints, but also in view of a possibly higher intake of the substance per kg body weight and the immaturity of detoxification pathways and physiological barriers. Moreover, some effects may become apparent only later in life;
- effects on milk quality and/or quantity. These effects will usually be detected only through effects on the growth and development of the newborn. In addition, the underlying effect may be found in alterations in the anatomy and histology of the mammary gland which can be studied through histological analysis.

In general, the two-generation study (EU Annex V B.35 or OECD TG 416) or the extended one generation study (OECD TG 443) is the best guideline-based study available to identify effects on or via lactation. In case of specific questions regarding lactation the protocol may have to be amended in view of any existing information on the substance under study, including physico-chemical, toxicokinetic and general toxic properties. Cross-fostering may establish whether toxicity to the offspring is the result of lactational effects or via uterine exposure.

1.10.2.2 Human data on reproductive toxicity

Epidemiological studies, conducted 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) may also be available.

Epidemiological data require a detailed critical appraisal that includes an assessment of the adequacy of controls, the quality of the health effects and exposure assessments, and of the influence of bias and confounding factors. Epidemiological studies, case reports and clinical data may provide sufficient hazard and dose-response evidence for classification of chemicals as reproductive toxicants in Category 1 and for risk assessment, including the identification of a N(L)OAEL. In such cases, there will normally not be a need to test the chemical. However, convincing human evidence of reproductive toxicity for a specific chemical is rarely available because it is often impossible to identify a population suitable for study that is exposed only to the chemical of interest. Human data may provide limited evidence of reproductive toxicity that indicates a need for further studies of the chemical; the test method selected should be based on the potential effect suspected.

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 would have power to identify only those developmental toxins that caused at least a 2-fold increase (EMEA, 2006). Extensive, high quality and preferable prospective, data are necessary to support a conclusion that there is no risk from exposure to the chemical.

1.10.3. Remaining uncertainty on reproductive toxicity

Unless the effect is a very specific one of low "normal" incidence, there may be a high level of uncertainty in human studies of effects on reproduction (see above for a discussion of the evaluation criteria for human data).

It is obvious that there are limitations in many of the types of non-human studies relating to reproductive toxicity. Well-conducted tests according to EC methods B.35/B.31 or OECD TG 416/414 standard can be used with confidence to identify substances as, or not as, being toxic to reproduction in relation to the endpoints addressed in the test. However, other studies, including tests conducted according to the OECD TG 421 and 422, may provide clear (in the case of the OECD methods) or indicative evidence of reproductive toxicity, but will not provide sufficient evidence for confidence about the absence of reproductive toxicity. The WoE from other studies (including human data), toxicokinetic and/or mechanistic data, when available, can help in reducing this uncertainty.

1.10.4. Conclusions on reproductive toxicity

Reproductive toxicity endpoints should be considered collectively, using a WoE approach to establish the most relevant endpoint and its NOAEL or Critical Effect Dose to be used in risk assessment.

A WoE assessment involves the consideration of all data that is available and may be relevant to reproductive toxicity. There can be no firm rules to the conduct of a WoE assessment as this process involves expert judgment and because the mix and reliability of information available for a particular substance will probably be unique. Also, the WoE assessment should consider all toxicity endpoints together, and not look at reproductive toxicity in isolation.

One example of a WoE assessment is the pooling of information from several *in vivo* reproductive toxicity studies. Individually, these studies may have deficiencies, such as brief reporting, small group size, limited range of endpoints evaluated, the dose levels or the dosing schedule was not appropriate for a comprehensive evaluation of potential

effects on the reproductive cycle, the study was not in compliance with GLP. However, taking account of their reliability and relevance and consistency of findings, collectively these studies could provide a level of information similar to that of the EU or OECD test guideline studies, and therefore meet the information requirements needed for the classification decision and risk assessment.

1.10.5. Concluding on Classification and Labelling

In order to conclude on a proper C&L, all the available information needs to be taken into account, and considerations should be given to both EU Annex I of CLP Regulation and the various remarks (as they relate to classification and labelling) made throughout this guidance document.

1.10.6. Concluding on suitability for risk assessment

In order to be suitable for risk assessment appropriate threshold levels have to be established.

Appendix 1-1: Toxicokinetics – Physiological Factors

This inventory has been compiled to provide a source of information on physiological parameters for various species that may be useful for interpreting toxicokinetic data. The list is not exhaustive and data from other peer-reviewed sources may be used. If study-specific data are available then this should be used in preference to default data.

De Zwart *et al*. (1999) have reviewed anatomical and physiological differences between various species used in studies on pharmacokinetics and toxicology of xenobiotics. These authors presented selection of data that may be relevant in the context of the EU risk assessment (quoted below). The tables are adapted from De Zwart *et al*. (1999).

The authors, however, focus on the oral route of administration and data relevant for other routes may have to be added. Some of those are already quoted in the section on repeated dose toxicity and are therefore not repeated here.

Data on stomach pH-values

Qualitative Aspects to be considered in the stomach

Rodents have a non-glandular forestomach that has no equivalent in humans. It is thin-walled and transparent. In the non-glandular stomach the pH is typically higher than in the glandular part and it contains more microorganisms. The glandular stomach has gastric glands similar to the human stomach but is a relatively small part of the total rodent stomach. Data on stomach pH for different species are rare and most stem from relatively old sources.

Appendix table A1-1: Data on stomach pH for different species

	Human	Rhesus monkey	Rat	Mouse	Rabbit	Dog	Pig
Median	-	-	-	-	-	-	2.7 (3.75-4)
Median anterior portion	2.7 (1.8-4.5)	4.8	5.0	4.5	1.9	5.5	4.3
Median posterior portion	1.9 (1.6-2.6)	2.8	3.0	3.1	1.9	3.4	2.2
Fasted	1.7 (1.4-2.1)	-	-	-	-	1.5	1.6-1.8 (0.8-3.0)
Fed	5.0 (4.3-5.4)	-	-	-	-	2.1 ± 0.1 [1)]	<2 [2)]

[1)] Standard deviation
[2)] Data from one animal only

Data on intestine pH and transit times

Appendix table A1-2: Data on intestine pH

pH (fasted)	Human	Rat (Wistar)	Rabbit	Dog	Pig	Monkey
Intestine	-	6.5-7.1	6.5-7.1	6.2-7.5	6.0-7.5	5.6-9
Duodenum	5-7	6.91		4.5-7.5	7.2	-
Jejunum	6-7	-	-	-	-	-
Ileum	7-8	-	-	-	-	-
Jejunum/ileum	-	7.81	-	-	-	-

pH (fasted)	Human	Rat (Wistar)	Rabbit	Dog	Pig	Monkey
Caecum	5.9	6.8	6.6	6.4	6.3	5.0
Colon	5.5-7	6.6, 7.1 [1)]	7.2	6.5	6.8	5.1
Rectum	7	-	-	-	-	-

[1)] Fed state

Appendix table A1-3: Calculated transit times in the intestine

Transit time (hours)	Human	Rat	Rabbit	Dog
Small intestine	2.7 to 5 [1)] Children (8 to 14 years): 5.1-9.2	1.5	-	0.5-2
Colon	Children (8 to 14 years): 6.2-54.7	6.0-7.2	3.8	-

[1)] From various authors, after fasting or a light meal

Physiological parameters for inhalation

Appendix table A1-4: Comparison of physiological parameters relating to the upper airways of rat, humans and monkeys

Species	Body weight (kg)	Body surface area (m2)	Nasal cavity volume (cm3)	Nasal cavity surface area (cm2)	Relative nasal surface area	Pharynx surface area (cm2)	Larynx surface area (cm2)	Trachea surface area (cm2)	Tidal volume (cm3)	Breaths per min	Minute volume (l/min)
Human	70	1.85	25	160	6.4	46.6	29.5	82.5	750-800	12-15	9-12
Rhesus monkey	7	0.35	8	62	7.75	-	-	-	70	34	2.4
Rat	0.25	0.045	0.26	13.44	51.7	1.2	0.17	3	2	120	0.24

(from De Sesso, 1993)

The U.S. EPA in the Exposure factors handbook (U.S. EPA, 1997 (a)) has reviewed a number of studies on inhalation rates for different age groups and activities. The activity levels were categorized as resting, sedentary, light, moderate and heavy. Based on the studies that are critically reviewed in detail in the U.S. EPA document, a number of recommended inhalation rates can be derived. One bias in the data is mentioned explicitly, namely that most of the studies reviewed were limited to the Los Angeles area and thus, may not represent the general U.S. population. This should also be born in mind when using those data in the European context. The recommended values were calculated by averaging the inhalation rates (arithmetic mean) for each population and activity level from the various studies. Due to limitations in the datasets an upper percentile is not recommended. The recommended values are given below:

Appendix table A1-5: Summary of recommended values from U.S. EPA (1997 (a))

Population Long-term exposures	Mean ventilation rates [m^3/24 h]
Infants <1 year [1)]	4.5
Children 1-2 years [1)]	6.8

Population Long-term exposures	Mean ventilation rates [m^3/24 h]
3-5 years [1)]	8.3
6-8 years [1)]	10
9-11 years	-
males	14
females	13
12-14 years	-
males	15
females	12
15-18 years	-
males	17
females	12
Adults 19 – 65+ years	-
males	15.2
females	11.3
Short-term exposures	[m^3/h]
Children:	-
Rest	0.3
Sedentary activities	0.4
Light activities	1.0
Moderate activities	1.2
Heavy activities	1.9
Adults:	-
Rest	0.4
Sedentary activities	0.5
Light activities	1.0
Moderate activities	1.6
Heavy activities	3.2
Outdoor workers:	-
Hourly average	1.3 (3.3 m^3/h) [2)]
Slow activities	1.1
Moderate activities	1.5
Heavy activities	2.5

[1)] No sex difference found
[2)] Upper percentile

The document also mentions that for a calculation of an endogenous dose using the alveolar ventilation rate, needs to be considered only the amount of air available for exchange via the alveoli per unit of time, accounting for approximately 70% of the total ventilation. This should also be reflected in the risk assessment.

Using a respiratory tract dosimetry model (ICRP, 2002; Snipes *et al*., 1997) calculated respiration rates for male adults. Based on these breathing rates estimated daily volumes of respiration were derived for different populations:

- General population: 8h sleep, 8h sitting, 8h light activity: 19.9 m^3
- Light work: 8h sleep, 6.5h sitting, 8.5h light activity, 1h heavy activity: 22.85 m^3
- Heavy work: 8h sleep, 4h sitting, 10h light activity, 2h heavy activity: 26.76 m^3

The same authors also mention that human breathing pattern changes from nose breathing to nose/mouth breathing at a ventilation rate of about 2.1 m^3/h (60% through nose, 40% through mouth). At 5 m^3/h ventilation rate, about 60% of air is inhaled through the mouth and 40% through the nose. However, these model calculations seem to overestimate the ventilation rates compared to the experimental data reviewed by U.S. EPA (1992).

Physiological parameters used in physiologically-based kinetic modeling

Literature on physiologically-based kinetic modelling also contains a number of physiological parameters that are used to calculate tissue doses and distributions. Brown

et al. (1997) published a review of relevant physiological parameters used in physiologically-based kinetic models. This paper provides representative and biologically plausible values for a number of physiological parameters of common laboratory species and humans. It constitutes an update of a document prepared by Arms and Travis (1988) or U.S. EPA and also critically analyses a compilation of representative physiological parameter values by Davies and Morris (1993). Those references are therefore not reviewed here but given in the reference list for consultation. In contrast to the other authors Brown *et al*. (1997) also try to evaluate the variability of the parameters wherever possible, by giving mean values plus standard deviation and/or the range of values identified for the different parameters in different studies. The standard deviations provided are calculated from the reported means in different studies. In other words, they are a measure of the variation among different studies, not the inter-individual variation of the parameters themselves. This variation may therefore include sampling error, interlaboratory variation and differences in techniques to obtain data. The authors also provide some data on tissues within certain organs, which isn't quoted here.

Appendix table A1-6: Organ weights as percent of body weight

Adapted from Brown *et al*. (1997)- the values typically reflect weights of organs drained of blood

Organ	Mouse mean ± SD[6)]	Mouse range	Rat mean ± SD[6)]	Rat range	Dog mean ± SD[6)]	Dog range	Human reference value mean ± SD[6)]	Human range
Adipose tissue[1)]	-	5-14 [1a)]	-	5.5-7 [1b)]	-	-	13.6±5.3[1c)] 21.3[1d)] 32.7 [1e)]	5.2-21.6 [1c)]
Adrenals	0.048 [2)]	-	0.019 ± 0.007	0.01-0.031	0.009 ± 0.004	0.004-0.014	0.02 [3)]	-
Bone	10.73 ± 0.53	10.16-11.2	-	5-7 [4)]	8.10 [2)5)]	-	14.3 [3)]	-
Brain	1.65 ± 0.26	1.35-2.03	0.57 ±0.14	0.38-0.83	0.78 ±0.16	0.43-0.86	2.00 [3)]	-
Stomach	0.60 [2)]	-	0.46 ±0.06	0.40-0.60	0.79 ±0.15	0.65-0.94	0.21 [3)]	-
Small intestine	2.53 [2)]	-	1.40 ±0.39	0.99-1.93	2.22±0.68	1.61-2.84	0.91 [3)]	-
Large intestine	1.09 [2)]	-	0.84 ±0.04	0.80-0.89	0.67±0.03	0.65-0.69	0.53 [3)]	-
Heart	0.50 ±0.07	0.40-0.60	0.33 ±0.04	0.27-0.40	0.78±0.06	0.68-0.85	0.47 [3)]	-
Kidneys	1.67 ±0.17	1.35-1.88	0.73 ±0.11	0.49-0.91	0.55±0.07	0.47-0.70	0.44 [3)]	-
Liver	5.49 ±1.32	4.19-7.98	3.66 ±0.65	2.14-5.16	3.29±0.24	2.94-3.66	2.57 [3)]	-
Lungs	0.73 ±0.08	0.66-0.86	0.50 ±0.09	0.37-0.61	0.82±0.13	0.62-1.07	0.76 [3)]	-
Muscle	38.4 ±1.81	35.77-39.90	40.43 ±7.17	35.36-45.50	45.65 ±5.54	35.20-53.50	40.00 [3)]	-
Pancreas	No reliable	-	0.32 ±0.07	0.24-0.39	0.23±0.06	0.19-0.30	0.14 [3)]	-

Organ	Mouse mean ± SD[6)]	Mouse range	Rat mean ± SD[6)]	Rat range	Dog mean ± SD[6)]	Dog range	Human reference value mean ± SD[6)]	Human range
	data							
Skin	16.53 ±3.39	12.86-20.80	19.03 ±2.62	15.80-23.60	no representative value	-	3.71[3)] (3.1-female, 3.7-male)[3)]	-
Spleen	0.35 ±0.16	0.16-0.70	0.20 ±0.05	0.13-0.34	0.27±0.06	0.21-0.39	0.26 [3)]	-
Thyroid	no data	-	0.005 ±0.002	0.002-0.009	0.008 ±0.0005	0.0074-0.0081	0.03 [3)]	-

[1)] Defined mostly as dissectible fat tissue
[1a)] Strongly dependent on strain and age of mice
[1b)] Male Sprague Dawley rats equation: Fat content = 0.0199.body weight + 1.664
Male F344 rats: Fat content = 0.035.body weight + 0.205
[1c)] Males, 30-60 years of age
[1d)] ICRP, 1975 reference value for 70 kg man
[1e)] ICRP, 1975 reference value for 58 kg women
[2)] One study only
[3)] ICRP, 1975 reference value
[4)] In most of the studies reviewed by the authors
[5)] Mongrel dogs
[6)] Standard deviation (SD)
To derive the organ volume from the mass for most organs a density of 1 can reasonably be assumed. The density of marrow free bone is 1.92 g/cm^3 (Brown *et al*., 1997).

Brown *et al*. (1997) also give values for cardiac output and regional blood flow (as a percentage of cardiac output or blood flow/100 g tissue weight) for the most common laboratory species and humans. The data used are derived from non-anaesthetised animals using radiolabelled microsphere technique. The data for humans were compiled using various techniques to measure perfusion.

Appendix table A1-7: Cardiac output (ml/min) for different species
Adopted from Brown *et al*. (1997)

Mouse mean ± standard deviation	Mouse range	Rat mean ± standard deviation	Rat range	Dog mean ± standard deviation	Dog range	Human reference value
13.98± 2.85	12-16	110.4±15.60	84-134	2,936 [1)]	1,300-3,000[1)]	5,200 [1)]

[1)] One study only

According to the authors giving blood flow in units normalised for tissue weight can result in significant errors if default reference weights are used instead of measured tissue weights in the same study.

Appendix table A1-8: Regional blood flow distribution in different species
Adopted from Brown *et al*. (1997)
(ml/min/100 g of tissue)

Organ	Mouse mean ± standard deviation	Mouse range	Rat mean ± standard deviation	Rat range	Dog mean ± standard deviation	Dog range

Organ	Mouse mean ± standard deviation	Mouse range	Rat mean ± standard deviation	Rat range	Dog mean ± standard deviation	Dog range
Adipose tissue[1)]	-	-	33±5	18-48	14±1	13-14
Adrenals	-	-	429±90	246-772	311±143	171-543
Bone	-	-	24±3	20-28	13±1	12-13
Brain	85±1	84-85	110±13	45-134	65±4	59-76
Heart	781±18	768-793	530±46	405-717	79±6	57-105
Kidneys	439±23	422-495	632±44	422-826	406±37	307-509
Liver	131	-	-	-	-	-
Hepatic artery	20	-	23±44	9-48	21±3	12-30
Portal vein	111±9	104-117	108±17	67-162	52±4	42-58
Lungs	351	-	127±46 [1)]	38-147 [1)]	79±43 [1)]	36-122
Muscle	24±6	20-28	29±4	15-47	11±2	6-18
Skin	18±12	9-26	13±4	6-22	9±1	8-13

[1)] Bronchial flow
[2)] Based on animal studies

Appendix table A1-9: Regional blood flow distribution in different species
Adopted from Brown *et al*. (1997)
(% cardiac output)

Organ	Mouse mean ±standard deviation	Mouse range	Rat mean ±standard deviation	Rat range	Dog mean ±standard deviation	Human reference value mean, male	Human reference value mean, female	Human range
Adipose tissue [1)]	-	-	7.0 [2)]	-	-	5.0	8.5	3.7-11.8
Adrenals	-	-	0.3±0.1	0.2-0.3	0.22	0.3	0.32	-
Bone	-	-	12.2 [2)]	-	-	5.0	5.0	2.5-4.7
Brain	3.3±0.3	3.1-3.5	2.0±0.3	1.5-2.6	2.0 [2)]	12.0	12.0	8.6-20.4
Heart	6.6±.0.9	5.9-7.2	4.9±0.1	4.5-5.1	4.6 [2)]	4.0	5.0	3.0-8.0
Kidneys	9.1±2.9	7.0-11.1	14.1±1.9	9.5-19.0	17.3 [2)]	19.0	17.0	12.2-22.9
Liver	16.2	-	17.4	13.1-22.1	29.7 [2)]	25.0	27.0	11-34.2
Hepatic artery	2.0	-	2.4	0.8-5.8	4.6 [2)]	-	-	-
Portal vein	14.1	13.9-14.2	15.1	11.1-17.8	25.1 [2)]	19.0	21.0	12.4-28.0
Lungs	0.51	-	2.1±0.4 [1)]	1.1-3.01)	8.8 [1)2)]	2.51		
Muscle	15.9±5.2	12.2-19.6	27.8 [2)]	-	21.7 [2)]	17.0	12.0	5.7-42.2

Organ	Mouse mean ±standard deviation	Mouse range	Rat mean ±standard deviation	Rat range	Dog mean ±standard deviation	Human reference value mean, male	Human reference value mean, female	Human range
Skin	5.8±3.5	3.3-8-3	5.8 [2)]	-	6.0 [2)]	5.0	5.0	3.3-8.6

[1)] Bronchial flow
[2)] One study only

The blood flow to some organs, such as liver, is highly variable and can be influenced by many factors, including anaesthesia, posture, food intake and exercise.

Gerlowski and Jain (1983) have published a compilation of different organ volumes and plasma flows for a number of species at a certain body weight from other literature sources.

Appendix table A1-10: Organ volumes, plasma flow used in physiologically-based kinetic – models

Parameter	Mouse	Hamster	Rat	Rabbit	Monkey	Dog	Human
Body weight (g)	22	150	500	2 330	5 000	12 000	70 000
Volume (ml)	-	-	-	-	-	-	-
Plasma	1	6.48	19.6	70	220	500	3 000
Muscle	10	-	245	1 350	2 500	5 530	35 000
Kidney	0.34	1.36	3.65	15	30	60	280
Liver	1.3	6.89	19.55	100	135	480	1 350
Gut	1.5	12.23	11.25	120	230	480	2 100
Gut lumen	1.5	-	8.8	-	230	-	2 100
Heart	0.095	0.63	1.15	6	17	120	300
Lungs	0.12	0.74	2.1	17	-	120	-
Spleen	0.1	0.54	1.3	1	-	36	160
Fat	-	-	34.9	-	-	-	10 000
Marrow	0.6	-	-	47	135	120	1 400
Bladder	-	-	1.05	-	-	-	-
Brain	-	-	-	-	-	-	1 500
Pancreas	-	-	2.15	-	-	24	-
Prostate	-	-	6.4	-	-	-	-
Thyroid	-	-	0.85	-	-	-	20
Plasma flow (ml/min)	-	-	-	-	-	-	-
Plasma	4.38	40.34	84.6	520	379	512	3 670
Muscle	0.5	-	22.4	155	50	138	420
Kidney	0.8	5.27	12.8	80	74	90	700
Liver	1.1	6.5	4.7	177	92	60	800

Parameter	Mouse	Hamster	Rat	Rabbit	Monkey	Dog	Human
Gut	0.9	5.3	14.6	111	75	81.5	700
Heart	0.28	0.14	1.6	16	65	60	150
Lungs	4.38	28.4	2.25	520	-	512	-
Spleen	0.05	0.25	0.95	9	-	13.5	240
Fat	-	-	3.6	-	-	-	200
Marrow	0.17	-	-	11	23	20	120
Plasma flow (ml/min)	-	-	-	-	-	-	-
Bladder	-	-	1.0	-	-	-	-
Brain	-	-	0.95	-	-	-	380
Pancreas	-	-	1.1	-	-	21.3	-
Prostate	-	-	0.5	-	-	-	-
Thyroid	-	-	0.8	-	-	-	20

Appendix table A1-11: A number of physiological parameters for different species

Compiled by Nau and Scott (1987)

Parameter	Mouse	Rat	Guinea pig	Rabbit	Dog	Monkey	Human
Bile flow (ml/kg per day)	100	90	230	120	12	25	5
Urine flow (ml/kg per day)	50	200	-	60	30	75	20
Cardiac output (ml/min/per kg)	300	200	-	150	100	80-300	60-100
Hepatic blood flow (l/min)	0.003	0.017	0.021	0.12	0.68	0.25	1.8
Hepatic blood flow (ml/min per kg)	120	100	-	50	25	25	25-30
Liver weight (% of body weight)	5.1	4.0	4.6	4.8	2.9	3.3	2.4
Renal blood flow (ml/min per kg)	30	-	-	-	22	25	17
Glomerular filtration (ml/min per kg)	5	-	-	-	3.2	3	1.3

Gad and Chengelis (1992) have summarised a number of physiological parameters for different species. The most important data of the most common laboratory test species are summarised below:

Appendix table A1-12: A number of physiological parameters for different species (Blaauboer *et al*., 1996)

	Rat	Mouse	Guinea Pig	Rabbit	Dog (Beagle)
Blood volume whole blood (ml/kg)	57.5-69.9	78	75	45-70	-
Blood volume Plasma (ml/kg)	36.3-45.3	45	30.6-38.2	-	-
Respiratory frequency (min^{-1})	66-114	84-230	69-160	35-65	10-301
Tidal volume (ml)	0.6-1.25	0.09-0.38	1.8	4-6	18-351
Urine volume (ml/kg/24 h)	55	-	-	20-350	-
Urine pH	7.3-8.5	-	-	8.2	-

1) In Beagles of 6.8 to 11.5 kg bw

Appendix 1-2: Prediction of toxicokinetic integrating information generated in silico and in vitro

The methods are presented in this attachment in order to demonstrate the future use of *in silico* and/or *in vitro* methods in TK. Although promoting in the area of pharmaceutical research, most of the given examples have not been fully validated for the purpose of use outside this area. Further development and validation of these approaches are ongoing.

Techniques for prediction of pharmacokinetics on animals or on human have been used for many years in the pharmaceutical industry at various stages of research and development. A considerable amount of work has been dedicated to developing tools for prediction of absorption, distribution, metabolism, and excretion of drug candidates. The objective in drug development is to eliminate as early as possible the candidate drugs that are predicted to have undesirable characteristics, such as being poorly absorbed by the intended route of administration, being metabolised via undesirable pathways, being eliminated too rapidly or too slowly. These predictions are done at various stages of drug development, using all available evidence and generating additional meaningful information from simple experiments. Although these techniques were developed in the particular context of drug development, there is no *a priori* reason not to use them for safety assessment of chemicals. The generated TK information saves experimental efforts in terms of cost, time and animal use, in particular, by selecting substances which will be further developed, directing further testing and assisting to the experimental design,.

In practice, the prediction of the TK behaviour of a chemical rests upon the use of appropriate models, which are essentially physiologically-based compartmental pharmacokinetic models, coupled to the generation of estimates for the relevant model parameters. To estimate parameter values which are used to predict absorption, metabolic clearance, distribution and excretion, *in silico* models or *in vitro* techniques have been developed. Blaauboer reviewed the techniques involved in toxicokinetic prediction using physiologically-based kinetic models (Blaauboer *et al*., 1996; Blaauboer, 2002). Also, a general discussion on the *in silico* methods used to predict ADME is provided by Boobis *et al*. (2002).

All the models using predictions must be considered together with the accompanying uncertainty of the predictions made. The uncertainty has to be balanced against the objective of the prediction. Experimental validation of the predictions *in vivo* and refinement of the models is usually necessary (Parrott *et al*., 2005a; U.S. EPA, 2007), and has to be carefully planned on a case by case basis. Theil *et al*. (2003), Parrott *et al*. (2005b) and Jones *et al*. (2006) describe a strategy for integrating predicted and experimental kinetic information generated routinely during drug development. The principles presented by these authors are relevant to kinetics simulation and prediction in the field of chemical safety, since they allow for integration of the available kinetic or kinetically-relevant information from the very beginning of the risk assessment process. In the very first stages of development, simulations can be generated using only physico-chemical characteristics, which themselves can be derived from *in silico* models (QSARs/ QSPRs).

Jones *et al*. (2006) proposed a strategy for investigation of compound sets, which led to reasonably accurate prediction of pharmacokinetics for human in case of approximately 70% of the compounds. According to the authors, *these successful predictions were achieved mainly for compounds that were cleared by hepatic metabolism or renal excretion, and whose absorption and distribution were governed by passive processes. Significant mis-predictions were achieved when other elimination processes (e.g. biliary*

elimination) or active processes were involved or when the assumptions of flow limited distribution and well mixed compartments were not valid.

In addition to the parent compound, in a number of cases metabolites contribute significantly or even predominantly, to the overall exposure-response relationship. In such cases, the quantitative *ex vivo* prediction of metabolite kinetics after exposure to the parent compound remains difficult. A separate study program of the relevant metabolites may then become necessary.

Models used to predict absorption/bioavailability

Gastro intestinal absorption models

In order to be absorbed from the GI tract, substances have to be present in solution in the GI fluids, and from there they have to cross the GI wall to reach the lymph or the venous portal blood. Key determinants of GI absorption are therefore:

- release into solution from solid forms or particles (dissolution);
- solubility in the GI fluids;
- permeability across the GI wall into the circulatory system.

Dokoumetzidis *et al*. (2005) distinguish two major approaches in the modelling of the drug absorption processes involved in the complex milieu of the GI tract.

The first approach is the simplified description of the observed profiles, using simple differential or algebraic equations. On this basis, Amidon *et al*. (1995) developed the Biopharmaceutics Classification System (BCS), a simple classification for pharmaceutical substances, which rests on solubility and intestinal permeability considerations. BCS divides pharmaceutical substances into 4 classes according to their high or low solubility, and high or low intestinal permeability. BCS has been incorporated into FDA guidance (2000).

The second approach tries to build models incorporating in more detail the complexity of the processes taking place in the intestinal lumen, using either compartmental analysis, i.e. systems of several differential equations (Agoram *et al*., 2001;. Yu *et al*., 1996; Yu and Amidon, 1999), dispersion systems with partial differential equations (Ni wt al., 1980; Willmann *et al*., 2003; Willmann *et al*., 2004), or Monte Carlo simulations (Kalampokis *et al*., 1999a; Kalampokis *et al*., 1999b). Some of these approaches have been incorporated into commercial computer software (Coecke *et al*., 2006; Parrott and Lave, 2002), or are used by contract research organisations to generate predictions for their customers. An attractive feature of these models is the ability to generate a prediction of extent and often also the rate of absorption in data-poor situations (i.e. at the initial stage of data generation) using a simple set of parameters describing ionisation, solubility and permeability.

Factors potentially complicating the prediction of absorption are:

- intra luminal phenomena, such as degradation or metabolism, matrix effects, or chemical speciation. All of which may reduce the amount available for absorption, or generate metabolites which have to be considered in terms of toxicological and toxicokinetic properties;
- intestinal wall metabolism, which may have similar consequences;
- intestinal transporters (efflux pumps), which may decrease the permeability of the GI wall to the substance.

These factors have to be considered and incorporated into absorption/bioavailability models on a case-by-case basis.

Parameter estimation for GI absorption models

A discussion on the *in vitro* approaches used to generate absorption parameters can be found in Pelkonen *et al*. (2001).

Where relevant (i.e. when dissolution from solid particles may be the limiting factor for GI absorption) estimates for the dissolution rate parameters can be obtained experimentally *in vitro* or using a QSAR/QSPR approach (e.g. Zhao *et al*., 2002). Potentially rate-limiting steps preceding dissolution (e.g. disaggregation of larger solid forms) are usually studied in to a greater extent in the pharmaceutical field than in chemical safety assessment, because they can be manipulated via formulation techniques. However, pre-dissolution events may also have a determining role in the absorption of chemicals, by influencing either its rate or its extent.

Solubility parameters can be estimated experimentally or using QSAR/QSPR models. A discussion of *in silico* models can be found in Stenberg *et al*. (2002).

Permeability estimates can be obtained via:

- *in silico* models (QSAR/QSPRs);
- *in vitro* permeation studies across lipid membranes (e.g. PAMPA) or across a monolayer of cultured epithelial cells (e.g. CaCO-2 cells, MDCK cells);
- *in vitro* permeation studies using excised human or animal intestinal tissues;
- *in vivo* intestinal perfusion experiments on animals or humans.

Discussion of the various *in silico* and *in vitro* methods to estimate intestinal permeability can be found in Stenberg *et al*. (2002), Artursson *et al*. (2001), Tavelin *et al*. (2002), Matsson *et al*. (2005).

Dermal route

Percutaneous absorption through intact skin is highly dependent on physico-chemical properties of chemicals and in particular on molecular weight and lipophilicity. Molecules above a certain molecular weight are unlikely to cross intact skin. Substances which are either too lipophilic or too hydrophilic have a low skin penetration. Cut off points at a molecular weight of 500 and log P values < -1 or > 4 have been used to set a conservative default absorption factor at 10 % cutaneous absorption (EC, 2007). However, it should be emphasised that this is a default factor, and by no means a quantitative estimate of cutaneous absorption[15].

Predictive models have been developed to try and estimate the extent of dermal absorption from physico-chemical properties (Cleek and Bunge, 1993). An *in vitro* method has been developed and validated and is described in EU B.45 or OECD TG 428.

The EU founded project on the Evaluation and Prediction of Dermal Absorption of Toxic Chemicals (EDETOX) established a large critically evaluated database with *in vivo* and *in vitro* data on dermal absorption/penetration of chemicals. The data were used to evaluate existing QSARs and to develop new models including a mechanistically-based mathematical model, a simple membrane model, and a diffusion model of percutaneous absorption kinetics. A guidance document was developed for conduct of *in vitro* studies of dermal absorption/penetration. More information on the database, model and guidance documents can be found at http://www.ncl.ac.uk/edetox/.

[15] For Biocidal active substances and products the default factors for dermal absorption following the EFSA Guidance on Dermal Absorption (EFSA, 2012) and the OECD Guidance on Dermal Absorption (OECD, 2004 and OECD, 2011) should be followed.

Inhalation route

Together with physiological values (ventilation flow, blood flow), the key parameter needed to predict the passage into blood of inhaled volatile compounds is the blood/air partition coefficient (Blaauboer *et al*., 1996; Reddy *et al*., 2005). References to methods for estimating or measuring blood/air partition coefficients are indicated below together with the discussion of other partition coefficients. The parameters are included in physiologically-based models predicting the concentrations in the venous pulmonary blood, assimilated to the systemic arterial blood, and in the exhaled air.

Other factors may influence absorption by the inhalation route. For example, water solubility determines solubility in the mucus layer, which may be a limiting factor. Also dimensions of the particles are a key factor for the absorption of particulate matter.

Other routes

Other routes (e.g. via the oral, nasal or ocular mucosa) may have to be considered in specific cases.

Systemic bioavailability and first-pass considerations

After an oral exposure, systemic bioavailability is the result of the cumulative effects of the absorption process and the possible extraction of part of the absorbed dose from the portal blood by the liver (the latter is so called first-pass effect). The first-pass effect can be incorporated into a suitably defined physiologically-based TK model. The systemic bioavailability of the substance can be predicted using estimates of both the absorption rate and the intrinsic hepatic clearance. Metabolism at the port of entry can also occur within the gut wall, and this can be included in the kinetic models. However, it is often difficult to differentiate the gut wall metabolism from the liver metabolism *in vivo* at the model validation stage.

Similarly, metabolism may occur in the epidermis or dermis. The current skin absorption test (EU B.45, OECD TG 428) does not take cutaneous metabolism into account. Specific studies may be necessary to quantify skin metabolism and bioavailability by dermal route.

Pulmonary metabolism of some substances exist (Borlak *et al*., 2005) but few substances are reported to undergo a quantitatively important pulmonary first-pass effect.

Models to predict distribution

Blood binding

Blood cell partitioning

Partitioning of compounds into blood cells, and in particular into the red blood cells (RBC), is an important parameter to consider in kinetic modelling (Hinderling, 1997).

Partitioning into leukocytes or even platelets (i.e. thrombocytes) may have to be considered in rare cases. A significant influence of such partitioning has been described for some drugs, e.g. chloroquine (Hinderling, 1997).

Partitioning into blood cells can be measured experimentally *in vitro* (Hinderling, 1997), or estimated using a QSAR/QSPR approach based on physico-chemical properties.

Plasma protein binding

Plasma protein binding is an important parameter to be included in physiologically-based kinetic models because plasma protein binding can influence dramatically distribution, metabolism and elimination. Plasma binding with high affinity will often restrict distribution, metabolism and elimination. However, this is by no means systematic because the overall kinetics is a function of the interplay of all processes involved.

Distribution depends on the balance between affinity for plasma components and for tissues. The high plasma protein binding accelerates the elimination of compounds which have a very high intrinsic clearance (i.e. very effective elimination mechanisms), which causes that more compound is available for clearance in the blood compartment.

Plasma protein binding is measured using *in vitro* techniques, using either plasma or solutions of specific proteins of known concentrations. The most standard techniques are equilibrium dialysis and ultrafiltration but numerous other techniques have been described. Zini (1991) and Roberts (2001) give more detailed information and references. QSAR/QSPR methods have also been used to predict of protein binding affinity (e.g. Colmenarejo, 2003).

Tissue distribution

Blood flow-limited distribution

In physiologically-based kinetic models, the blood flow-limited distribution is the most common model to describe distribution between blood and tissue, i.e. the equilibrium between tissue and blood is reached within the transit time of blood through the tissue. In this model, the key parameters are the partition coefficients. Partition coefficients express the relative affinity of the compound for the various tissues, relative to a reference fluid which may be blood, plasma or plasma water. Tissue/blood, tissue/plasma, and tissue/plasma water partition coefficients are inter-related via plasma protein binding and blood cell partitioning. Partition coefficients are integrated in the differential equations predicting blood and tissue concentrations, or in equations of models predicting globally the compound's steady-state volume of distribution (Poulin and Theil, 2002a).

Permeability-limited distribution

However, in some cases the equilibrium between blood and tissue cannot be reached within the transit time of blood through the tissue due to a low permeability of the exchange surface between blood and a particular tissue (e.g. blood-brain barrier, placental barrier), and, therefore, a correction factor must be introduced in the differential equation describing distribution to that tissue. One common simple way of doing this is to use the permeability area cross product. Thus, the arterial concentration and the three factors; blood flow (physiological parameter), permeability per unit of surface (compound-specific parameter), and surface of exchange (physiological parameter) (see Reddy *et al.*, 2005), determine the distribution. Permeability-limited distribution makes prediction more difficult due to the lack of well-recognised, easy to use and robust models to quantify the necessary parameters.

Determination of partition coefficients

Krishnan and Andersen (2001) discuss the available experimental methods to obtain blood/air, tissue/air and blood/ tissue partition coefficients. *In vitro* methods include vial equilibration (for volatile compounds), equilibrium dialysis and ultrafiltration. However, these methods require *ex-vivo* biological material, are time-consuming and often involve use of a radiolabelled compound (Blaauboer, 2002).

Models to calculate predicted tissue/blood, tissue/plasma or tissue/plasma water partition coefficients from simple physico-chemical properties have been developed (Poulin and Theil, 2002b; Rodgers *et al*., 2005a; Rodgers *et al*., 2005b; Rodgers and Rowland, 2006). The necessary compound-specific input is limited to knowledge of the chemical structure and functionalities (e.g. neutral, acid, base, zwitterionic), the pKa or several pKa values, where applicable, and the K_{ow} at pH 7.4. Additional necessary parameters describe the tissue volumes and tissue lipid composition. Tissue volumes are usually available or can be estimated from the literature. There are less available direct data on tissue composition in terms of critical binding constituents, particularly for human, although some reasonable estimates can be made from the existing information.

QSAR/QSPR models developed for the estimation of blood/air and tissue/blood partition coefficients have also been reported (Blaauboer, 2002).

Prediction of metabolism

Numerous aspects of metabolism can and often should be explored using in vitro methods (Pelkonen *et al*., 2005)

Major objectives of the study of metabolism using *in vitro* methods are:

- determining the susceptibility of a chemical to metabolism (its metabolic stability);
- identifying its kinetically and toxicologically relevant metabolites in the species of interest (including human);
- obtaining a quantitative global estimate of its metabolic clearance, to be included in TK models.

Additional possible objectives are:

- characterising enzyme kinetics of the principal metabolic reactions, which can be used also for scaling up and predicting in vivo kinetics of a new chemical;
- estimating the ability of the chemical to act as a substrate for the different enzymes involved in biotransformation;
- exploring inter-species differences in metabolism;
- evaluating potential variability in metabolism in a given species, human in particular;
- identifying whether the chemical and/or its metabolite can act as an enzyme inducer;
- identifying whether the chemical and/or its metabolite can act as an enzyme inhibitor, and the type of inhibition involved.

Most methods have been developed in the pharmaceutical field and focused on the CYP because these are the major enzymes involved in drug metabolism. The extension of existing methods to a wider chemical space, and to other enzymatic systems, such as other oxidation pathways, acetylation, and hydrolysis, needs to be undertaken with caution, and methods are bound to evolve in this context. In any case, the study of metabolism *in vitro* is often an important step in the integrated risk assessment of chemicals. In many cases *in vitro* methods are the only option to study metabolism, due to the impracticality or sheer impossibility of *in vivo* studies.

Relative role of different organs in metabolism

Quantitatively, the most important organ for metabolism is by far the liver, although metabolism by other organs can be important quantitatively or qualitatively. The nature of the chemical and the route of administration must be taken into account when assessing which organs are the most relevant in terms of metabolism (Coecke *et al*., 2006).

In vitro methods to study metabolism

In vitro methods to explore the metabolism and particularly the hepatic metabolism of a substance are thoroughly discussed by Pelkonen *et al*. (2005) and Coecke *et al*. (2006). Depending on the objective, the different metabolising materials used are microsomes and microsomal fractions, recombinant DNA-expressed individual CYP enzymes, immortalised cell lines, primary hepatocytes in culture or in suspension, liver slices.

Quantitative estimation of the intrinsic clearance of a substance

The intrinsic metabolic clearance *in vivo* has to be incorporated into the kinetic models, as it is one of the most important pieces of information in order to simulate the TK of a substance. Intrinsic clearance can be estimated using quantitative *in vitro* systems

(purified enzymes, microsomes, hepatocytes) and extrapolating the results to the *in vivo* situation.

If only a single or few concentrations are tested, the intrinsic clearance can be expressed only as a single first-order elimination parameter, ignoring possible saturation phenomena. The latter can only be detected by testing a large enough concentration range in an appropriately chosen system. For instance, if a Michaelis and Menten model is applicable, both the Vmax and the Km of the system may be thus determined.

Of particular importance are:

- the quality and characterisation of the metabolising system itself;
- the quality and characterisation of the experimental conditions, in particular as regards the system's capacity for binding the substances under study (Blanchard *et al*., 2005) but obviously also as regards other parameters such as temperature, pH, etc.;
- the use of appropriate scaling factors to extrapolate to predicted clearance values in vivo.

Scaling factors must be chosen taking into account the *in vitro* system utilised. They incorporate in particular information on the *in vitro* concentration of chemical available to the metabolising system (unbound), the nature and amount of the enzymes present in the *in vitro* system, the corresponding amount of enzymes in hepatocytes *in vivo*, and the overall mass of active enzyme in the complete liver *in vivo*. Discussions on the appropriate scaling procedures and factors to be taken into account have been developed by Houston and Carlile (1997), Inoue *et al*. (2006), Shiran *et al*. (2006), Howgate *et al*. (2006), Johnson *et al*. (2005), Proctor *et al*. (2004).

In vitro screening for metabolic interactions

In vitro screening procedures for the prediction of metabolic interactions have been developed for pharmaceuticals. They involve testing an *in vitro* metabolising system for a number of well characterised compounds, with and without the new substance (Blanchard *et al*., 2004; Turpeinen *et al*., 2005).

Prediction of excretion

The most common major routes of excretion are renal excretion, biliary excretion and, for volatile compounds, excretion via expired air.

There is at present no *in vitro* model to reliably predict biliary or renal excretion parameters. Determining factors include molecular weight, lipophilicity, ionisation, binding to blood components, and the role of active transporters. In the absence of specific a priori information, many kinetic models include non-metabolic clearance as a single first order rate excretion parameter.

Expired air (exhalation clearance)

Excretion into expired air is modelled using the blood/air partition coefficient (Reddy *et al*., 2005).

Biliary clearance

Current work on biliary excretion focuses largely on the role of transporters (e.g. Klaassen, 2002; Klaassen and Slitt, 2005). However, experimentally determined numerical values for parameters to include into modelling of active transport are largely missing, so that these mechanisms cannot yet be meaningfully included in kinetic models. Levine (1978), Rollins and Klaassen (1979) and Klaassen (1988) have reviewed classical information on the biliary excretion of xenobiotics. Information in human is still relatively scarce, given the anatomical and ethical difficulties of exploring biliary excretion directly in human. Compounds may be highly concentrated into the bile, up to

a factor of 1,000, and bile flow in human is relatively high, between 0.5 and 0.8 ml/min, so that considerable biliary clearance values of several hundred ml/min, can be achieved (Rowland and Tozer, 1989; Rowland *et al*., 2004). It should be considered on a case-by-case basis whether biliary excretion and possible entero-hepatic recirculation should be included in the kinetic models used for prediction.

Renal clearance

In healthy individuals and in most pathological states, the renal clearance of xenobiotics is proportional to the global renal function, reflected in the glomerular filtration rate, which can be estimated *in vivo* by measuring or estimating the clearance of endogenous creatinine. Simple models for renal clearance consider only glomerular filtration of the unbound plasma fraction. However, this can lead to significant misprediction when active transport processes are involved. More sophisticated models have been described which include reabsorption and / or active secretion of xenobiotics (Brightman *et al*., 2006a; Brightman *et al*., 2006b; Katayama *et al*., 1990; Komiya, 1986; Komiya, 1987), but there are insufficient input or reference data to both implement such models and evaluate satisfactorily their predictivity.

Kinetic modelling programs

A number of programs for TK simulation or prediction are either available, or used by contract research companies to test their customer's compounds. A non-comprehensive list of such programs is given by Coecke *et al*. (2006). Available physiologically-based modelling programs purpose-built for toxicokinetic prediction include (non-comprehensive list):

- SimCYP® (SimCYP Ltd, www.simcyp.com);
- PK-Sim® (Bayer Technology Services GmbH, www.bayertechnology.com);
- GastroPlus™ (Simulations Plus Inc, www.simulations-plus.com);
- Cloe PK® (Cyprotex Plc, www.cyprotex.com);
- Noraymet ADME™ (Noray Bioinformatics, SL, www.noraybio.com).

Numerous other simulation programs, either general-purpose or more specifically designed for biomathematical modelling, can be used to implement physiologically-based kinetic models. A discussion on this subject and a non-comprehensive list can be found in Rowland *et al*., (2004).

Appendix 1-3: Physiologically-based kinetic modelling and development of assessment factors

A simple but fictional example of the development of an assessment factor for interspecies differences using physiologically-based kinetic modelling is presented. A fictional chemical, compound A, is a low molecular weight, volatile solvent, with potential CNS depressant properties. Evidence for the latter comes from a number of controlled human volunteer studies where a battery of neurobehavioural tests were conducted during, and after, exposure by inhalation to compound A.

Compound A is metabolised *in vitro* by the phase I, mixed-function oxidase enzyme, cytochrome P450 2E1 (CYP2E1) by both rat and human hepatic microsomes. There are also some *in vivo* data in rats exposed by inhalation to compound A, with and without pre-treatment with diallyl sulphide, an inhibitor of CYP2E1, that are consistent with metabolism of compound A by this enzyme.

Physiologically-based kinetic models for the rat and standard human male or female for exposure by inhalation to compound A are built. The rat model was validated by simulating experimentally determined decreases in chamber concentrations of compound A following exposure of rats to a range of initial concentrations in a closed-recirculated atmosphere exposure chamber. The removal of chamber concentration of compound A over time is due to uptake by the rat and elimination, primarily by metabolism. The human physiologically-based kinetic model was validated by simulating experimentally determined venous blood concentrations of compound A in male and female volunteers exposed by inhalation to a constant concentration of compound A in a controlled-atmosphere exposure chamber.

It is assumed that the following have been identified for the chemical:

1) the active moiety part of the chemical, and

2) the relevant dose-metric (i.e. the appropriate form of the active moiety part e.g. Cmax, area-under-the-curve of parent chemical in venous blood (AUCB), average amount metabolised in target tissue per 24 hours (AMmet), AMPeakMet, etc.). In this case, it is hypothesised that Cmax of compound A is the most likely surrogate dose metric for CNS concentrations of compound A thought to cause a reversible CNS depressant effect. However, Cmax, is dependent upon AMPeakMet. Therefore, the validated rat and human physiologically-based kinetic models were run to simulate the exposure time and concentrations of the human study where the neurobehavioural tests did not detect any CNS depressant effects. The dose metric, AMPeakMet for the rat would be divided by the AMPeakMet for the human. This ratio would represent the magnitude of the difference between a specified rat strain and average human male or female. This value may then replace the default interspecies kinetic value since it is based on chemical-specific data. Therefore, the derivation of an appropriate assessment factor in setting a threshold level (e.g. AEL) can be justified more readily using quantitative and mechanistic data.

Appendix 1-4: Threshold of toxicological concern TTC – a concept in toxicological risk assessment

Human Health Aspects

Risk assessment for human health effects is based on the threshold of a critical toxicological effect of a chemical, usually derived from animal experiments. Alternatively, a toxicological threshold may also be based on the statistical analysis of the toxicological data of a broad range of structurally-related or even structurally-different chemicals and extrapolation of the no effect doses obtained from the underlying animal experiments for these chemicals to levels considered to be of negligible risk to human health. This latter approach refers to the principle called TTC. Regarded in this way the TTC concept could be seen as an extension of such approaches read-across and chemical category. As such, the TTC concept has been incorporated in the risk assessment processes by some regulatory bodies, such as FDA and the UN JMPR and EFSA in the assessment of flavourings and food contacts articles (SCF, 2001), as an approach to identify exposure levels of low regulatory concern, and as a tool to justify waiving of generation of animal data.

This section will briefly discuss different TTC approaches, their limitations, criteria for use.

TTC approaches

The TTC was implemented by the FDA as the *Threshold of Regulation* from food contact materials since 1995; a TTC value of 1.5 µg per person per day was derived for a chemical database that covered carcinogenicity (i.e. their calculated one per million risk levels; Gold *et al*., 1995). This value is considered to be applicable for all endpoints except genotoxic carcinogens.

Munro *et al*. (1996a and 1996b) subsequently developed a structure-based TTC approach on principals originally established by Cramer *et al*. (1978). The structural classes of organic chemicals analysed showed significantly different distributions of NOEL's for subchronic, chronic and reproductive effects. Carcinogenic or mutagenic endpoints were not considered. Based on the chemical structure in combination with information on toxicity three different levels (90, 540 and 1800 µg per person per day, respectively) were derived. WHO/FAO-JECFA and EFSA have implemented these values in the regulations for flavourings as direct food additives whereas FDA uses these values for indirect food additives (food contact materials).

Another structure-based, tiered TTC concept developed by Cheeseman *et al*. (1999), extended the Munro *et al*. (1996a) 3 classes approach by incorporated acute and short-term toxicity, mutagenic and carcinogenic potency (but exempting those of high potency).

Kroes *et al*. (2004) evaluated the applicability for different toxicological endpoints, including neurotoxicity and immunotoxicity, and proposed a decision tree with 6 classes of organic chemicals. Allergens or substances causing hypersensitivity could not be accommodated due to the lack of an appropriate database (enabling statistical analysis for this category of substances).

Apart from the two indicated cases, the other approaches have not been adopted by any regulatory body.

ECETOC has proposed a Targeted Risk Assessment approach for REACH including a series of threshold values for a wide variety of organic and non-organic substances (both volatile and non-volatile), i.e. so-called GEV and GLEV for acute and repeated dose toxicity (ECETOC, 2004). Category 1 and 2 carcinogens, mutagens and reprotoxins were excluded. The GEV is a generic threshold values for occupational exposure (and derived dermal values), derived from some most stringent OEL. The GLEV is based on

classification criteria for repeated dose toxicity and extrapolation factors. It is noted that the derivation of GEV values was based upon an analysis of current published occupational exposure levels, and therefore also incorporated socio-economic and technical arguments in addition to the assessment factors applied to toxicological endpoints and other data on which the OELs were based. This approach has not been peer reviewed nor accepted by regulatory bodies.

EFSA has developed an opinion on exploring options for providing advice about possible human health risks based on the concept of TTC (http://www.efsa.europa.eu/en/efsajournal/pub/2750.htm) that defines also a number of exclusion categories of substances for which the TTC approach would not be used.

Basic requirements

The TTC concept discussed above requires a minimum set of information in order to be applied successfully. However, it should be noted that the application of TTC excludes substances with certain structural elements and properties including:

- Non-essential, heavy metals and polyhalogenated dibenzodioxins, -dibenzofurans, or-biphenyls and similar substances:
  This class of substances cannot be addressed by the TTC concepts due to the bio-accumulating properties. Although the TTC approach is able to accommodate other categories of substances with bio-accumulating potential, within the regulatory context, substances with potential for bioaccumulation are 'of concern' and need to be assessed on a case-by-case basis. Potentially bioaccumulating or persistent substances are also excluded from default environmental risk assessments.
- Genotoxic carcinogens:
  A case-by-case risk assessment is required for genotoxic carcinogens, even though some carcinogens can be accommodated within the TTC concept if the estimated intake is sufficiently low (<0.15 µg/day).
- Organophosphates and carbamate substances:
  According to the EFSA opinion (http://www.efsa.europa.eu/en/efsajournal/pub/2750.htm) 18 µg/person per day for organophosphate and carbamate substances with anti-cholinesterase activity.
- Proteins:
  This class of substances is a surrogate to address specifically potential (oral) sensitisation, hypersensitivity and intolerances. There are no appropriate databases available which allow the derivation of a generic threshold for this type of endpoint.

Additionally, another very critical criterion concerns the knowledge on the handling and use of the substance. TTC is only applicable in case there is detailed information available on all anticipated uses and use scenarios for which the risk assessment is provided.

Limitations

The TTC has several limitations. First of all, they are derived on data bases covering primarily systemic effects from oral exposure. This is especially important concerning occupational situations where inhalation or dermal exposure is the main route of contact. Only some cover mutagenic, carcinogenic and acute effects, and in fact none (except for the proposed ECETOC approach) addresses local effects such as irritation and sensitisation.

As all TTC approaches (except for the proposed ECETOC approach) have oral exposure as the principle route, further substantial efforts are needed to explore its potential use for the exposures routes inhalation and skin contact, before any application may become realistic.

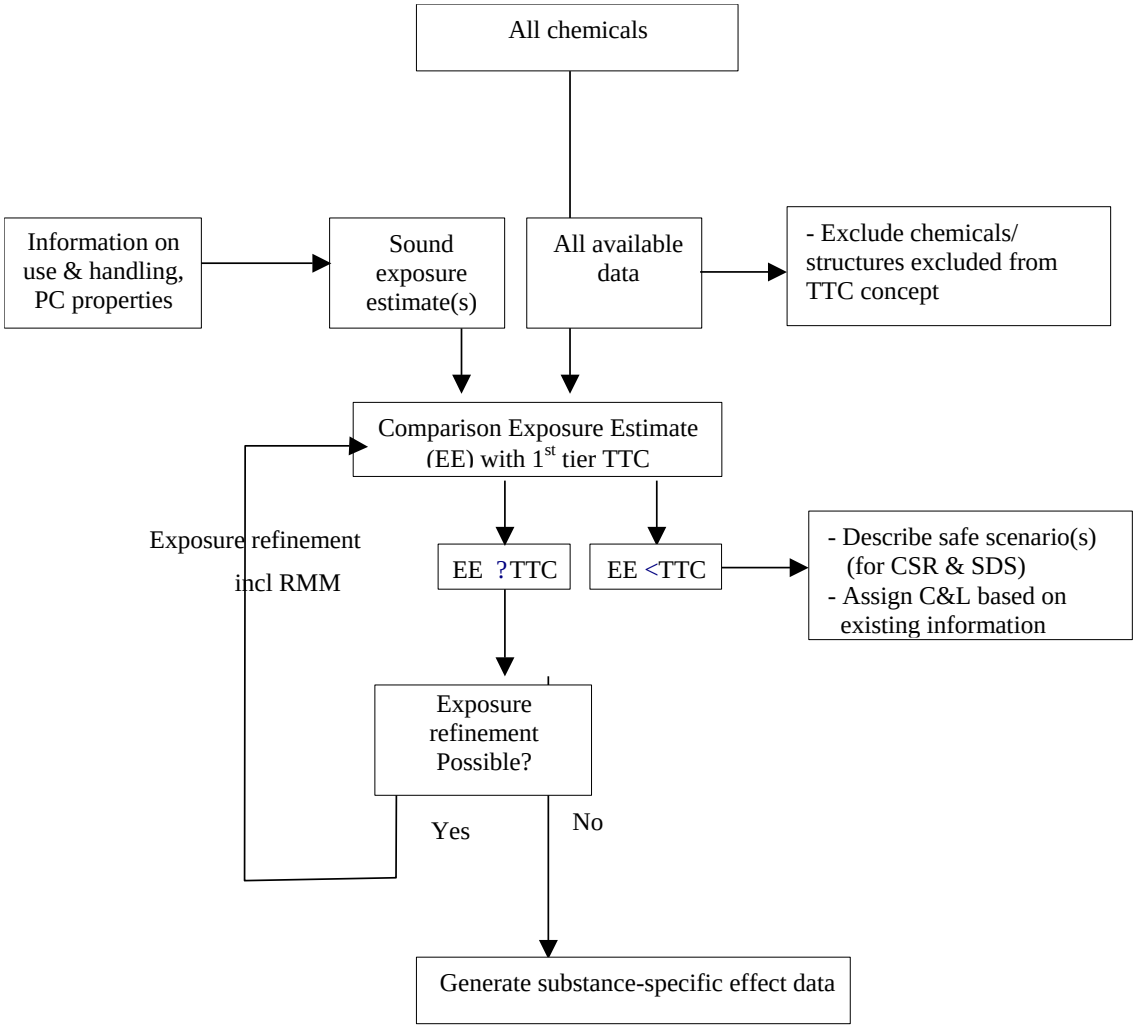
Several of the structurally-based approaches such as TTC have limitations in applicability domain and cannot accommodate every chemical class. For instance, proteins, heavy metals, polyhalogenated-dibenzodioxins, aflatoxin-like substances, N-nitroso-compounds, alpha-nitro furyl compounds and hydrazins-, triazenes-, azides-, and azoxy-compounds have been excluded by the approach of Kroes *et al*. (2004). As indicated, the TTC approach is only applicable in case there is detailed information available on all anticipated uses and use scenarios for which the risk assessment is provided. Based on the experience of the EU Risk Assessment Programme for Existing Substances, robust exposure estimates will require a significant effort, even in cases where the uses were well characterised. In case of a multitude of (dispersive) uses and applications, it may not be feasible to generate overall exposure estimate with detail and precision necessary for use in a risk assessment relying on the thresholds based on the TTC concept. Therefore, a TTC will in practice only be applicable in those cases where there are only a few number of exposure scenario's that allow well characterisation.

Furthermore, the use of the TTC approach does not provide information on classification and labelling of a chemical, or on its potency for a specific effect.

Use of the TTC concept

The TTC concept has been developed primarily for use within a risk assessment framework. As already indicated, the TTC concept is applied for regulatory purposes by the FDA and the EFSA and UN JECFA in the assessment of food contact articles and flavourings, respectively. These specific TTC approaches underwent a critical review before being accepted on these regulatory platforms. Clearly, in the same way, any other TTC approach should be agreed upon by the relevant regulatory body before use, and it should be clearly indicated for which endpoints, routes and population they apply.

Figure 6: Generic TTC scheme/concept (as described in ECHA REACH Guidance R7.C for the purpose of use under REACH)



The figure above illustrates the way a TTC can be used: it precedes any chemical-specific testing. One tier is shown, but one could apply additional tiering rounds (as clearly illustrated by the approach presented by Kroes *et al*., 2004) dependent upon the chemical of interest.

Potential use within Biocides

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 potential use of TTC concept would require good quality exposure data.

In the striving for alternatives to animal testing one suggested approach is the use of generic threshold values. However, application of TTC would imply that limited data may be generated and thus, that the level of protection might be influenced. From information on flavouring substances in the diet the TTC concept seems to be reasonable well based with respect to general toxicity and the particular endpoints examined. There may be some important differences between and substances used for food contact articles or flavourings, such as differences in use pattern and composition (for a further discussion see Tema Nord, 2005; COC, 2004).

2 Effects Assessment - Hazard Characterisation (Dose-Response/Concentration Relationship)

2.1 Introduction

For the derivation of Acceptable Exposure Levels (AELs) and other reference levels (e.g. external reference doses such as ADI and ARfD), all available hazard information regarding systemic toxicity and local effects needs to be evaluated (see Section 1) and, where possible, dose descriptors (N(L)OAEL, BMD, etc.) need to be established.

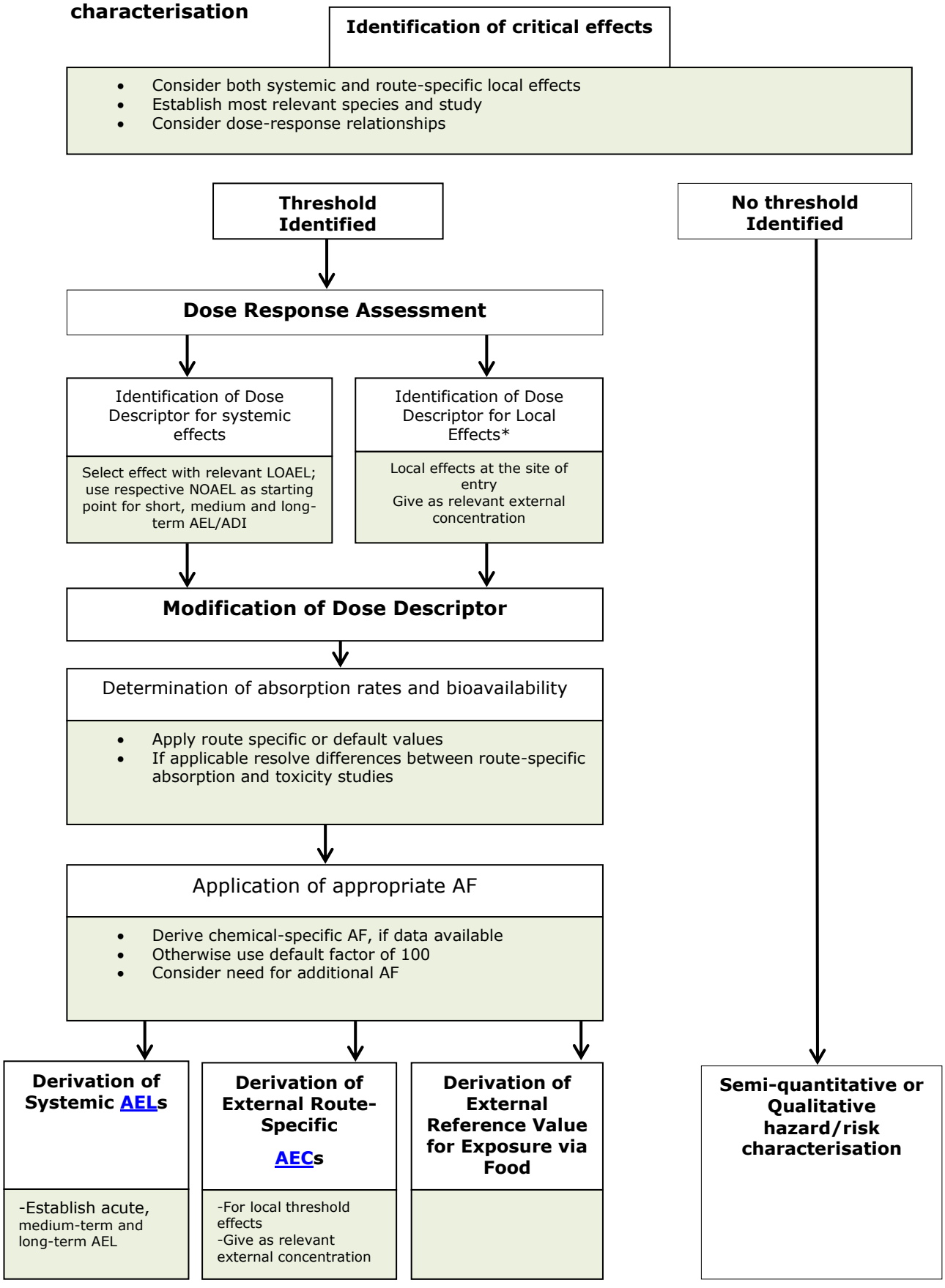
- A systemic effect is defined as an effect that is normally observed distant from the site of first contact, i.e. after having passed through a physiological barrier (mucous membrane of the gastrointestinal tract or of the respiratory tract, or the skin) and becomes systemically available.
- A local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

It should be noted, however, 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). Figure 7 provides a schematic representation of the steps for performing hazard characterisation.

Hazard characterisation involves the following steps:

1. Identification of critical effects (outcome of the hazard identification)
2. For effects where threshold identified:
    - Dose response Assessment – Derivation of N(L)OAEL/BMD: Identification of most relevant dose descriptor (e.g. NOAEL) for systemic threshold effects.
    - Identification of most relevant dose descriptor (e.g. NOAEC) for local effects and non-threshold effects if available or qualitative/semi-quantitative approach.
    - Modification of relevant dose descriptor: Determination of absorption rate/bioavailability - Application of appropriate assessment factors to the dose descriptor to address uncertainty - Derivation of systemic AELs, external route specific AECs (if available), external reference doses for exposure via food (ADI, ARfD).
3. For effects where threshold is not identified:
    - Qualitative or semi-quantitative assessment

Figure 7: Schematic representation of the steps for performing hazard characterisation



*For Local Effects and the decision logic on whether to perform a threshold and/or non-threshold based approach the guidance provided in Chapter 4 (section 4.3.2) should be followed

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 as well as the mode of action (if known) and its relevance for humans. For the latter WHO/IPCS has developed a framework for analysing the relevance of a non-cancer (Boobis *et al*., 2008) or cancer mode of action for humans (Boobis *et al*., 2006). The WHO/IPCS Framework on Mode of Action/Species Concordance analysis has been updated to take into account new developments in the field of risk assessment paradigm (Meek *et al*., 2013). This framework gives the opportunity to present in a transparent manner the evidence for the key events leading to an adverse effect and to identify a causal linkage (through dose-response and time concordance). Likewise, 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.

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 for which the derivation of a local threshold needs to be considered. Also indications for route-specific sensitivity and dose-response relationship shall be taken into account when considering the relevant critical NOAELs. If the data package allows, external reference values could be derived.

Before deriving reference levels (e.g. AELs, ADI, ARfD) on the basis of the dose descriptors, 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 a threshold is possible to derive (e.g. acute toxicity, local effects for irritation/corrosion and sensitisation).

If the substance exerts its effects by a threshold mode of action, reference values must be 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 a threshold is not possible to derive (e.g. local effects) a reference value cannot be derived and for these effects semi-quantitative approach has to be followed (e.g. DMELs) where relevant or a qualitative approach for hazard and risk characterisation.

It is to be noted that the decision on a threshold mode and a non-threshold mode of action may not always be easy to make, especially, when, although a biological threshold may be postulated (e.g. sensitisation), the data do not allow identification of it. If not clear, the assumption of a non-threshold mode of action would be the prudent choice.

For mutagens/carcinogens it should be stressed that Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work ("Carcinogens and Mutagens Directive") requires that occupational exposures are avoided/minimised as far as technically feasible; the approach to controlling workplace exposure should therefore 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 TK will provide information on the possible 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 it is needed or to address species-specific mechanisms if relevant.

Studies on, for example dermal absorption contribute significantly to the risk characterisation of biocides as dermal exposure is a major route of exposure. Guidance on TK is provided in Section 1.3 as well as within the *Guidance on the BPR: Volume III Human Health, Part A Information Requirements*.

2.2.1.2 Acute toxicity

Most exposure will probably be via the dermal route and also by inhalation. Risk characterisation could be quantitative since acute effects usually have a threshold, and thus can be based on a $LD(C)_0$ or $LD(C)_{50}$ value. While acute toxicity is usually not characterised by a NOAEL, NOAEC (or LOAEL, LOAEC), these can be used if available from sub-acute toxicity studies. $LD(C)_{50}$ values are the most frequently available data but are not suitable for risk characterisation since they are based on the endpoint of lethality. For the derivation of acute AELs information on acute effects from relevant studies (see section 2.3.1) can be used. Occasionally information from human case reports of poisoning may be available. The use of this for risk characterisation will depend upon expert judgement on the reliability of the reported information. Problems include the availability of an effect level but no information on a no-effect level or a dose-response relationship. Oral toxicity will have an impact upon risk assessment where ingestion may occur for example through poor occupational hygiene. A substance would normally have to be very toxic or toxic for this to be an issue of concern.

Non-professional users may use large quantities of some active substances on an occasional basis and with less control over exposure than professional users (for example, in wood preservative products and antifouling products). Non-professionals will not usually use protective equipment and, in fact, cannot depend on it to reduce risk to an acceptable level, so, in practice, dermal and inhalation exposure may be considerably greater than for professional users for the same pattern of use.

For the general public, in addition to acute exposure due to normal use of a biocidal product, another relevant acute exposure occurs via the oral route of exposure following accidental/intentional ingestion of an active substance. Risks based on oral toxicity of active substances shall be considered for all PTs due to the risk of accidental ingestion by young children. Dermal exposure to for example treated fabrics or soft furnishings would usually be low level and would be compared to data from a repeated dose study. Inhalation exposure is especially relevant where volatile active substances have been applied recently indoors.

2.2.1.3 Irritation and corrosivity

These toxicity endpoints are more significant for products for non-professional use since one must assume that no PPE is worn during application of products. Dermal contact could be significant depending on the formulation type and method of application for the product.

Formal quantitative risk characterisation is not usually possible but a semi-quantitative or a quantitative risk characterisation may be carried out in scenarios under specific conditions (see Section 4.3.2 for further guidance).

2.2.1.4 Sensitisation

The options on how to perform risk characterisation for skin and respiratory sensitisation is provided in Section 4.3.2. The qualitative risk characterisation approach is preferred; however the semi-quantitative and quantitative approaches are also described for potential use depending on the availability of data and taking into account specific limitations.

2.2.1.5 Repeated dose effects

Repeated dose effects (i.e. those detected in the 28-day study, 90-day study and long term toxicity study; for genotoxicity or carcinogenicity findings see following sections) will be of concern whenever exposure occurs on a regular and/or frequent basis and especially if the effects have been observed to be irreversible or only partially reversible.

Most effects can be assessed using quantitative risk characterisation and therefore depend upon the difference in dose levels at which adverse effects are seen in animals (or humans) and the estimated exposure for the active substance in the product. The key factors are the most sensitive, relevant NOAEL, the effects it is based upon and the dose response that occurs at higher doses.

Effects noted in repeated dose studies are critical endpoints for secondary exposure (man via the environment and via occupational settings) because exposure can be repeated for various reasons. It might be that the same individuals enter treated areas immediately following regular treatments (including staff in hospitals, offices, shipyards) or that they frequently handle treated goods (such as carpenters). Long-term, low level inhalation exposure is also possible from indoor use of treated material. Exposure in the diet via residues should normally be compared to chronic reference values (e.g. AELs, ADI).

2.2.1.6 Genotoxitity

Data from genotoxicity studies do not allow the derivation of a reference dose since a non-threshold mode of action is usually assumed for genotoxic substances. Therefore a qualitative and/or semi-quantitative (for genotoxic carcinogens) risk characterisation needs to be performed (see Section 4.3.1 for further guidance).

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 a non-threshold mode of action underlies the carcinogenicity observed (e.g. genotoxic carcinogens) the semi-quantitative or qualitative approach (see Section 4.3.1) for further guidance should be then followed.

2.2.1.8 Toxicity to reproduction and development

Effects on the reproductive system are often threshold-based allowing a quantitative risk characterisation to be carried out. However, effects on the development of offspring can be due to a genotoxic mechanism and the potential for this need to be considered since a qualitative risk characterisation would then be appropriate.

If AELs are based on severe reproductive effects, the need for an additional assessment factor should be considered. The assessment factor will depend upon the severity of effects, their relationship to toxicity observed in the mothers and the exposure level at which they occurred compared with effects seen in other animals. It should also be remembered that the general public is unprotected from exposure and that the people concerned may not be aware of their exposure, which implies the use of a very stringent assessment factor.

Fertility and developmental effects are relevant endpoints for exposure scenarios involving repeated exposure. However, developmental effects can occur following short-term exposure if this happens to coincide with the critical formative stages of embryonic and foetal development. Furthermore, effects on fertility have been reported already following short-term exposure so this risk should also be characterised where indicated.

2.2.1.9 Other Toxicity End-points

In addition to the above-mentioned effects, other effects such as endocrine disruption, immunotoxicity and neurotoxicity must be considered.

The toxicity endpoints neurotoxicity, immunotoxicity, behavioural toxicity and endocrine effects may be as significant for professional as for non-professional users. They may also be significant for secondary exposed persons, among them children, especially if the use of the biocidal product leaves residues that cannot, or are not intended to be, removed.

Judgment on the approval of an active substance for use in biocidal products is made on a case-by-case basis, taking into account the use pattern and consequent potential primary and secondary exposures.

The effects may be of concern after any type of exposure (ranging from acute to chronic); they may be reversible or irreversible. In any case, the acceptability of the effects will be reflected by the relevant AEL and the assessed exposure.

2.3 Threshold Effects

Usually the study in the most sensitive and relevant species resulting in the most relevant lowest dose descriptor (e.g. NOAELs, NOAECs, LOAELs, LOAECs, BMDs) will be selected for establishing the relevant critical dose descriptor for AEL derivation. Often, several studies addressing a certain endpoint are available for one species. Different dose spacing in these studies results in different dose descriptors (e.g. NOAELs, LOAELs, BMD). If study design and endpoints addressed are comparable, it might be appropriate to consider these studies together. When the studies are comparable, regarding study design (endpoints investigated, duration of exposure, route of exposure) and species/strain of animal, the 'overall NOAEL' should be the highest value identified in the available studies that provides a reasonable margin (≥2) over the lowest LOAEL, provided that due consideration is given to the shape of the dose–response curve (FAO/WHO, 2004).

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. However, the lowest dose descriptor may not always provide the lowest AEL value as it depends on the assessment factors that will be used for its derivation. Therefore the choice of the critical dose descriptor should be made having in mind that the resulting AEL should also be the critical one for use in risk characterisation. Alternatively, the assessor should derive all possible AELs from the available dose descriptors for each endpoint and then choose the most critical AEL for a relevant time frame. Multiple AEL values could be derived before selecting the critical ones for performing risk characterisation for each exposure scenario.

2.3.1 Relevant Time Frames in AEL derivation

A comparison of the relevant critical dose descriptor (NOAELs, NOAECs, LOAEL, LOAECs, BMD) for AEL derivation for different time-frames provides useful information on the influence of exposure duration on the severity and spectrum of toxicity. Therefore, an assessment of the entire data package is of high scientific value, as it helps in elucidating time-dependency of toxicity. This information is helpful to adjust human health risk assessment to varying time-frames for professional as well as consumer exposure.

The ILSI Health and Environmental Sciences Institute Task Force for Systemic Toxicity Assessment has also proposed the use of different time-frames for human exposure for which risk assessment might be required for PPPs (Table 14 (Doe *et al*., 2006)).

The proposed time-frames are considered useful for the quantitative risk assessment of active substances for approval for use in biocidal products especially with respect to non-professional users and the general public.

Three AELs are usually derived namely acute AEL, medium-term AEL and long-term AEL for the purpose of biocidal active substances risk characterisation. For professional users, evaluation often focuses on medium-term and long-term exposure. If intermittent/acute exposure needs to be evaluated, relevant dose descriptors for AEL derivation obtained from studies with daily subacute, subchronic or chronic administration of the test compound might in some cases be considered a conservative approach erring on the safe side. In this context, all available information on the time-dependency of toxicity should be taken into consideration.

Preferably, relevant critical dose descriptors for acute AEL derivation should be derived based on acute studies with single exposure, which are designed to establish a dose-response relationship including NOAELs. The appropriateness of using doses and end-points from sub-acute, sub-chronic and chronic studies to establish acute relevant dose descriptors needs to be carefully considered. Particular weight should be given to observations and investigations at the beginning of repeated-dose studies. However, in the absence of such initial information, all toxic effects seen in repeated-dose studies should be evaluated for their relevance in establishing the relevant critical NOAEL for acute AEL derivation.

Table 14: **Relationship between duration of human exposure and the studies required for hazard identification and establishment of relevant dose descriptors (e.g. NOAEL(C)s, LOAEL(C)s, BMD) for AEL derivation**

Estimated duration of human exposure	Basic toxicity studies	Relevant dose descriptors for AEL derivation
≤ 24 h	Single dose studies designed to determine dose descriptors* or repeated dose studies demonstrating relevant acute effects, e.g. • acute neurotoxicity • 28-d/90-d repeated-dose studies, acute effects • developmental toxicity, acute effects	Toxic effects relevant for acute exposure
>24 h – 3 months (max. 6 months)	Repeated-dose studies designed to determine dose descriptors, e.g. • 28-d/90-d repeated-dose studies • 90-d neurotoxicity • 12-m dog, depending on nature of effects • developmental toxicity • 2-generation study	Toxic effects relevant for medium-term exposure
> 6 months (min. 3 months)	Chronic studies or repeated dose studies designed to determine dose descriptors and demonstrating relevant chronic effects, e.g. • 18-m/24 m chronic/carcinogenicity • 2-generation study (or extended one	Toxic effects relevant for long-term exposure

Estimated duration of human exposure	Basic toxicity studies	Relevant dose descriptors for AEL derivation
	generation study), chronic effects • developmental toxicity • 12-m dog, depending on nature of effects	

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

In principle, the following four situations could arise when deriving an acute AEL:

(1) A relevant acute dose descriptors for acute/short-term AEL derivation is not allocated, since no acute toxic effects have been identified.

(2) A relevant acute dose descriptor for acute/short-term AEL derivation is based on an appropriately designed single-dose study.

(3) A relevant acute dose descriptor for acute/short-term AEL derivation is based on a repeated-dose study (including developmental/embryotoxicity studies), since the critical effect is also considered relevant for a single exposure.

(4) A conservative relevant acute dose descriptor for acute/short-term AEL derivation is based on a repeated-dose study if the critical effect was not adequately evaluated in a single dose study.

Most often, the relevant critical dose descriptor for medium-term AEL derivation will be based on a repeated dose toxicity study (28-day or 90-day) or studies investigating specific end-points, e.g. reproductive toxicity, developmental toxicity or sub-acute neurotoxicity. If there are indications that effects only become evident in chronic toxicity studies but might be initiated by sub-acute or sub-chronic exposures, the dose descriptor for these effects in the long-term studies should be considered in selecting medium-term relevant dose descriptors for AEL derivation. For the medium-term time frame the estimated duration of human exposure can be from >24 h to 3 (max. 6) months. The decision on whether the estimated duration of human exposure for this time frame should be 3, 4, 5 or 6 months, will be a case by case decision. The toxicokinetic properties of the active substance, such as slow elimination, potentially leading to prolonged internal exposure even after cessation of external contact with the biocidal product or the reversibility of the repeated-dose and chronic effects have to be considered.

In most cases, the relevant critical dose descriptor for long-term AEL derivation will be based on a long-term toxicity study, generally a lifetime study in rats or mice, or studies investigating specific end-points such as reproductive toxicity or hormonal effects. Depending on the nature of effects the dose descriptor from studies of shorter duration (e.g. one-year dog study or developmental toxicity study) can be used for the derivation of the long-term AEL if the dose descriptor is lower than the one based on a chronic toxicity study. In principle 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 other observations from other studies. If the dose descriptor (e.g. NOAEL) derived from relevant effects in a valid developmental toxicity study is lower than those from short-term repeated dose toxicity studies and this cannot be explained by dose spacing, the dose descriptor (e.g. NOAEL) from the developmental toxicity study should be used for the derivation of the AEL value. This will apply to the

global population (thus protecting both pregnant and non-pregnant women). Developmental studies are often the only studies to use gavage dosing with the aim of determining a dose descriptor (e.g. NOAEL). This can give rise to Cmax related effects, such as certain clinical signs, that might not be relevant to dermal exposures where a spike of absorption is not normally seen. Maternal effects can be regarded as critical effects for deriving both medium - and long-term AELs if they are deemed relevant in comparison to other critical effects observed in other valid repeated dos toxicity studies.

2.3.2 Dose Response Assessment

The quantitative extrapolation of hazard from the animal experiment to exposed humans is based on the most relevant endpoints. In most cases, these endpoints should correspond to relevant dose descriptors (e.g. NOAEL, NOAEC, LOAEL, LOAEC, BMD). Generally, a whole set of relevant dose descriptors are established with respect to different exposure time-frames and exposure routes. Relevant dose descriptors for AEL derivation should be identified for use in risk characterisation with regard to all relevant exposure scenarios characterised by duration, frequency as well as route of exposure, and by the exposure profile for the target (sub-) population exposed. It should not be concluded from the absence of a particular exposure scenario for a given product that a relevant NOAEL is not needed, because different exposure scenarios might become relevant with subsequent product authorisations on Member State level. As specified in Article 47 of the BPR the holder of an authorisation for a biocidal product shall notify the Competent Authority, that granted the national authorisation and the Agency or, in the case of a Union Authorisation, the Commission and the Agency, of information concerning an active substance or a biocidal product containing it, which may affect continuing authorisation.

2.3.2.1 Identification of Dose Descriptors for systemic effects

It is generally agreed that many of the adverse health effects caused by substances are not expressed until the substance, or an active metabolite, reaches a threshold concentration in the relevant organ. Whether or not this threshold concentration is reached is related to the level of exposure of the organism (human or test animal) to the substance: for a given route of exposure, there will be a threshold exposure level which must be attained before effects are induced. The threshold exposure dose or concentration may vary considerably for different routes of exposure, and for different species because of differences in TK and possibly also in mechanisms of action. The observed threshold dose or effect level in a toxicity test will be influenced by the sensitivity of the test system and is a surrogate for the true so-called NAEL.

The NOAEL identified in a particular test will be simply the highest dose level or concentration of the substance used in that test at which no statistically significant adverse effects were observed, i.e. it is an operational value derived from a limited test. For example if the dose levels of 5, 10, 50, 200 mg.kg-1.day-1 of a substance have been used in a test and adverse effects were observed at 200 and 50 mg.kg-1.day-1 but not at 10 or 5 mg.kg-1.day-1, the derived NOAEL will be 10 mg.kg-1.day-1. Thus, the NOAEL and LOAEL (lowest observed adverse effect level) values for a given study will depend on the experimental study design, e.g. the selection of dose levels and the spacing between doses.

If there are several studies addressing the same effects from which different NOAELs could be derived, normally the lowest relevant value should be used in reference value (e.g. AEL) derivation. When it is not possible to identify the NOAEL in a repeated dose study, the "lowest observed adverse effect level" (LOAEL) should be used in the risk characterisation. If a NOAEL becomes available subsequently, from another test, the risk characterisation should be re-addressed and revised, if necessary, in the light of the new information.

The sensitivity of a study (which is related to the toxicological endpoint, the potency of the toxic substance, the exposure period and frequency, the variability within the species, the number of dose groups and the number of animals per dose group), may limit the extent to which it could be possible to derive a reliable NOAEL from a particular test. In these cases where it is impossible to derive a NOAEL, at least a LOAEL should be identified.

It is recognised that the NOAEL is not very accurate with respect to the degree to which it corresponds with the (unknown) true NAEL. Also, the data obtained at one dose (NOAEL) are used rather than the complete dose response data set. In case sufficient data are available, the shape of the dose response curve should be taken into account. In the case of a steep curve the derived NOAEL can be considered as more reliable (the greater the slope, the greater the reduction in response to reduced doses); in the case of a shallow curve, the uncertainty in the derived NOAEL may be higher and this has to be taken into account in the reference value (e.g. AEL) derivation. If a LOAEL has to be used, then this value can only be considered reliable in the case of a very steep curve. In response to the general call for consideration of the dose response curve as a whole rather than to use only the data obtained at one dose (NOAEL) for risk characterisation, alternatives for dose-response assessment have been proposed such as the BMD concept (Crump, 1984; Gaylor, 1988; U.S. EPA, 1995; Slob and Pieters, 1998) and categorical regression (Hertzberg, 1989).

The BMD methodology involves fitting a mathematical curve (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) of the BMD is often taken as the starting point ("point of departure") for determining reference values. The BMD_L and BMD_u and their ratio provides a measure of the uncertainty around the BMD and in the experimental data.

Advantages of this approach over the NOAEL are:

- The BMD is derived using all experimental data and reflects the dose-response pattern to a greater degree. It makes extended quantitative use of the dose-response data from studies in experimental animals or from observational epidemiological studies (EFSA Scientific Opinion, 2009), rather than utilising a single dose defined as the NOAEL;
- The BMD is independent of predefined dose levels and spacing of dose levels, resulting in a more consistent point of departure which reflects more accurately the true potency of the substance (as a consequence of the specified benchmark response) (EFSA Scientific Opinion, 2009);
- The BMD approach provides a quantification of the uncertainties in the dose-response data (EFSA Scientific Opinion, 2009);
- The BMD approach makes more reasonable use of sample size, with better designs resulting in higher BMDs. It takes into account the spread of the data at each dose level rather than relying on a mean. Outlying values can be identified and excluded from the analysis;
- The BMD approach unveils the uncertainties in the response level hidden in the NOAEL, which is not a dose level with no effects (Slob, 1999);
- Use of the BMD approach leads to a more precise and more transparent risk estimate (Slob, 1999), which, in turn, may lead to improved risk communication between risk assessors, risk managers, policy-makers and the public.

A perceived weakness of the BMD approach is the uncertainty with respect to the reliability of the approach when results are obtained from toxicity studies performed

according to the requirements defined in current testing guidelines. For the derivation of reliable dose-response relationships, the classical study design of three dose groups and a vehicle control group is not ideal, especially if one considers the unfavourable possibility that in a particular experiment, adverse effects may be identified only at the highest dose level.

An improved Benchmark model fit would be possible by increasing the number of dose groups without changing the total number of animals in the test. However, such a change in study design would generally no longer allow a proper derivation of a NOAEL. It should be noted that the current standard testing designs are not a major limitation for the application of the BMD approach as, although it is true that the BMD approach obtained from three dose groups rather than six is more uncertain, the uncertainty will be reflected in the confidence limits (ratios of BMD_L and BMD_u). In addition, the same uncertainty applies to the NOAEL identified from any such study but the degree of uncertainty is hidden. Yet, there is a large number of practical obstacles to the increased use of the BMD approach in a regulatory context, including lack of statistical and modelling expertise, huge range of models and no agreed critical effect size or benchmark response. Given these barriers, perhaps it is time to recognise that in the near future the BMD approach will not entirely replace the NOAEL approach in general use for the routine evaluation of existing studies. One practical way forwards could be for NOAELs and expert judgment to guide an evaluator to the most critical study and critical endpoint for a given chemical and at this point for the BMD approach to be invoked as a higher tier or supplementary approach.

However, despite potential practical problems, the scientific supremacy of the BMD approach compared to the NOAEL approach should be an incentive to apply it at least as a higher tier or supplementary method when the critical study for the derivation of a reference value has been identified.

The BMD can be used in parallel to derivation of a NOAEL or as an alternative when there is no reliable NOAEL. In addition, the BMD approach is, when possible, preferred over the LOAEL-NAEL extrapolation (See also U.S. EPA, 1995; Barnes *et al*., 1995; Slob, 1999; Vermeire *et al*., 1999, for further details on the BMD approach).

Unless a threshold mechanism of action is clearly demonstrated, it is generally considered prudent to assume that thresholds cannot be identified in relation to mutagenicity, genotoxicity, and genotoxic carcinogenicity, although a dose-response relationship may be shown under experimental conditions. Details on the practical derivation of different dose descriptors (T25, BMD(L)10) based on animal studies for non-threshold carcinogens are given in APPENDIX R.8-6 of *Guidance on information requirements and chemical safety assessment Chapter R8.*

It is possible that for a particular endpoint data from more than one study are available (e.g. in different species, with different durations), and that these studies are all relevant and appropriate (with respect to conduct, tested species relevant for humans, etc.). The dose descriptor can also be set based on human data (ECHA, 2012a). Since it is not possible to know beforehand which of these dose descriptors will turn out to be critical for the endpoint-specific reference level (i.e. AEL), it might sometimes be relevant to derive AELs for more than one study per endpoint. The choice of key studies and derivation of AELs will depend on expert judgement, including the use of a WoE approach. In any case the choice of one or more dose descriptors should be justified.

2.3.2.2 Identification of Dose Descriptor for local effects

As compared to the straight-forward derivation of AELs for systemic toxicity, it may be more difficult for the endpoints acute toxicity, irritation/corrosion, and skin sensitisation. For instance, whereas the ideal starting point for the derivation of the acute toxicity AEL should be the NOAEL or LOAEL for sub-lethal effects, such as local respiratory irritation caused by cytotoxicity or CNS depression, oftentimes only data from 'LD_{50}-studies' are

available. Likewise, there is usually no strict NOAEL or NOAEC identified in studies on irritation, corrosion, or sensitisation. Therefore, in many, or even most cases, the lack of NOAEL(C), dose-response or indication of potency will require that a more qualitative approach is followed. Additional guidance for risk characterisation for local effects is provided in Section 4.3.2.1.

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

In a few situations, the effects assessment is not directly comparable to the exposure assessment in terms of exposure route, units and/or dimensions. In these situations, it is necessary to convert the dose descriptor for the threshold effect (e.g. N(L)OAEL, BMD) into a correct starting point (i.e. correct the unit of exposure, e.g. corrected N(L)OAEL). This applies to the following situations:

1. If for a given human exposure route there is a dose descriptor for the same route in experimental animals but for that particular exposure route there is a difference in bioavailability between experimental animals and humans at the relevant level of exposure.
2. If for a given human exposure route there is not a dose descriptor for the same route (in experimental animals or humans).
3. Differences in human and experimental exposure conditions.
4. Differences in respiratory volumes between experimental animals (at rest) and humans (light activity).

It should be noted that modification is not appropriate in cases where human exposure is evaluated based on biological monitoring data. In such cases (availability of valid biomonitoring data), the calculation of AEL/AEC values can be straightforward if studies in animals or humans are available which relate the effect directly or indirectly to the biomonitoring metric.

Further Guidance on modification of dose descriptor is provided in the *Guidance on information requirements and chemical safety assessment Chapter R.8*, Section R. 8.4.2 with worked examples.

2.3.4 Application of appropriate Assessment Factors

Derivation of reference values such as AELs, requires the choice of AFs, which account for extrapolation from animal toxicity data to the exposed human population.

At present, with the exception of genotoxic carcinogens and non-threshold mutagens, hazard assessment for different toxicological end-points is based on the assumption of a threshold.

The setting of the overall AF is a critical step, which considers, but is not limited to, inter-species variation and intra-species variation.

In the absence of sufficient chemical-specific data a default 100-fold AF is applied to the relevant NOAEL for AEL derivation in the first tier of risk characterisation (see Figure 6. The basis for this approach is a 10-fold factor for inter-species variation and a 10-fold factor for intra-species variation. Variability is governed by toxicokinetic as well as toxicodynamics factors.[16]

[16] The default value of 100 was included in the TNsG on Annex I inclusion (April 2002) and thus applied in previous evaluations of biocidal active substances. It is also included in the AOEL guidance document in the context of risk assessment of plant protection products as well as in FAO/WHO (JEFCA, JMPR) and U.S. EPA evaluations.

Chemical-specific AFs as proposed by IPCS (WHO/IPCS, 2005) can be introduced to replace a default AF if specific information is available on:

1. Inter-species differences in TK
2. Inter-species differences in toxicodynamics
3. Human variability in TK
4. Human variability in toxicodynamics

Additionally allometric scaling and PBPK modelling (as described further below in this section) can be considered for replacement of default AFs on a case by case basis.

The use of scientifically valid human data reduces the level of uncertainty in comparison to extrapolation from animal models and is seen as a valuable contribution to science-based decision making. Biomonitoring studies, epidemiological data and medical poisoning records can be some of the sources of human data. Human volunteer studies should not be performed for the purposes of the BPR. However, human monitoring data can be requested for products already authorised for use under the BPR. As a prerequisite for the consideration of the use of human volunteer studies that have been performed for the purpose of regulatory frameworks other than the BPR, studies in humans should include clear statements that they were performed in accordance with internationally accepted ethical standards (Charnley and Patterson, 2004), e.g. the Declaration of Helsinki (World Medical Association, 1997). In some cases, the use of human data in regulatory safety assessment might lead to more stringent exposure limits for some biocides than those that would have been derived on the basis of animal data only. If human data are used for AEL derivation, the 10 fold inter-species AF is omitted and the 10-fold AF for intra-species variation is regarded adequate.

In addition to uncertainties in inter-species differences and intra-species variability, additional AFs for the following elements should be considered:

1. the nature and severity of the effect
2. the human (sub-)population exposed
3. deviations between the exposure in the study providing the NOAEL and the estimated human exposure as regards frequency or pattern (e.g. 6 hours in animals and 8 hours or 24 hours in humans)
4. duration extrapolation: AFs for duration extrapolation should be handled on a case by case basis, to use the best available data in derivation of reference values. It is specifically noted that the possibility for duration extrapolation duration extrapolation cannot be used in justifying study waiving.
    - subchronic to chronic: AF of 2
    - subacute to subchronic: AF of 3
    - subacute to chronic: such an extrapolation should normally not be necessary. In exceptional cases, e.g. if the chronic data is considered to be of insufficient quality for derivation of reference values, but it can nevertheless be concluded that chronic exposure does not result in more severe effects, an AF of 6 can be used.
5. Dose-response relationship
    - extrapolation from LOAEL to NOAEL
    - the slope of the dose-response curve
6. the overall quality of the toxicity data package

If the severity of the critical effect at the LOAEL (even if a NOAEL has been identified) was judged to be of particular significance an additional AF might be considered necessary. So far, this AF has been from 2 to 10. Quantification should be determined on a case-by-case basis taking into account the dose-response data.

If the derivation of the AEL was based on a LOAEL and not a NOAEL, an additional AF has to be considered. This factor will vary depending on the slope of the dose-response curve and the magnitude of the effect at the LOAEL. This extrapolation step should be based on expert judgement. BMD concept can also be used when data allows and it is deemed appropriate. Guidance for using the BMD approach can be found Section 2.4.1 of this document. The use of LOAEL s to set AELs should be a last resort; however, where the effects at the LOAEL are of moderate magnitude and not severe, the use of a LOAEL and an appropriate assessment factor reduces the need for additional animal studies.

For local effect at the port of entry (skin, eye, and GI tract) it is sometimes justified to assume that either toxicokinetics or –dynamics (or both) do not contribute significantly to interspecies differences (as for example in the case of direct/pH-driven chemical action on tissue/cell membranes). In such cases, based on sound scientific reasoning, the 10-fold interspecies default factor might be reduced dependent on the mode of action. With regard to local acute effects on the respiratory tract, guidance is available e.g. from the EU project ACUTEX (ACUTEX TGD, 2006), which proposes to apply reduced interspecies AFs when extrapolating data obtained in rats to humans. However, given that there could be significant quantitative differences in deposition, airflow patterns, clearance rates and protective mechanisms between humans and animals and when there is no data to inform on this uncertainty, it is prudent to assume that humans would be more sensitive than animals to effects on the respiratory tract. In such a situation, the default interspecies dynamic factor of 2.5 should be applied.

For other risk evaluation programmes in the EU (e.g. DNEL methodology in the context of REACH) slightly different default approaches concerning inter- and intra-species variability are applied. As a main difference, the DNEL methodology in the context of REACH extrapolate inter-species differences 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. For the rat, given the usual average body mass, the overall inter-species default factor is 10 and thus similar to the approach outlined above (4 × 2.5 = 10). For the dog, the default value is lower (1.4 × 2.5 = 3.5); for the mouse higher (7 × 2.5 = 17.5). Allometric scaling can be used for biocides, generally as a refinement step in derivation of reference values for use in risk characterisation. The ECHA REACH Guidance should be used when applying allometric scaling factors (*Guidance on IR+CSA Chapter R.8.4.3.1*). Allometric scaling can be used when the toxic effect is essentially determined by the area under the (plasma) concentration curve over time, as opposed, for example, to the peak plasma concentration or another pharmacokinetic variable. Allometric scaling should not be applied (or should be adjusted) if there are indications of significant inter-species differences in the bioavailability of the substance, if its clearance is known not to scale approximately with the body weight to the power of 0.75, if the kinetics cannot be assumed as dose-proportional over the dose-range considered, or if the animal species can be considered especially susceptible or unsusceptible to the effects in question. Whenever substance specific data is available, it should be used instead of the default values and approaches.

In addition, when available, data from the use of PBPK modelling shall be used for the purpose of refining the assessment factors. PBPK models will not remove all of the uncertainty from the risk assessment process. The rationale for using PBPK models in risk assessment is that they provide a documentable, scientifically defensible means of bridging the gap between animal bioassays and human risk estimates. Guidance on the

use of PBPK modelling is available from the WHO/IPCS project on the Harmonization of Approaches to the Assessment of Risk from Exposure to Chemicals, and should be followed (WHO/IPCS, 2010).

The rationale for the choice of the AFs should be explained in detail in the dossier or report.

2.3.5 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, e.g. 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 taking into account use patterns and exposure scenarios. In principle, these AELs should be derived independently of the route of exposure. Such AELs represent the internal (absorbed) dose available for systemic distribution from any route of exposure and are expressed as internal levels (mg/kg b.w/day).

AELs for biocidal active substances can be interpreted as daily or interrupted exposure levels of the general human population or a specific sub-population likely to be without an appreciable risk of adverse effects during a specified period of time. AELs should be established for all relevant time-frames of exposure (acute, medium-term, and long-term) based on the full toxicological data package available.

The derivation of AELs should follow the same common scientific principles as the derivation of the AOEL proposed by the European Commission Health and Consumer Protection Directorate-General (DG SANCO) (EC, 2006), which are applied also in other regulatory frameworks, e.g. for PPPs.

The majority of studies submitted for inclusion of active substances into the Union List are oral studies. However, risk assessment mainly focuses on the dermal and the inhalation exposure routes.

To avoid additional experimental testing by other relevant routes of human exposure, systemic AELs will usually be set on the basis of oral studies, i.e. the external NOAEL is converted to an internal NOAEL with help of the oral absorption value.

If systemic AELs are derived from dermal or inhalation studies, the external dermal and inhalative NOAELs must also be converted to systemic dose descriptors by use of dermal- and inhalation-specific absorption rates. On that background, any additional information from route-specific studies is of high value for derivation of reference values for use in risk characterisation because it reduces the uncertainties associated with route-to-route extrapolation.

In case local effects at the port of entry are observed, or there are indications of route-specific differences in toxicity, which are not reflected by absorption data, then additional considerations on appropriate route-specific reference values for use in risk characterisation are necessary (see Section 4.2).

For the purpose of human health risk assessment of active substances for approval for use in biocidal products, the AEL should generally be derived for acute, medium-term, and long-term exposure

Even in cases where the complete toxicological data package does not indicate any acute hazard, setting an acute AEL would be required for the risk characterisation of acute scenarios for certain PTs. In this case, the acute AEL may be the same as the medium-term AEL value. On the other hand, if setting a long-term AEL is not supported by the

data package, e.g. due to waiving of long term studies based on exposure considerations, this should also be clearly indicated in the report with any restrictions clearly explained in the Union List inclusion description.

Data waiving arguments are quite common in biocide dossiers. Therefore, it is clearly stated in the ECHA Biocides Guidance, Vol. III, Part A (Information Requirements) that the exposure pattern for a particular biocide may lead to the conclusion that a certain type of data are not needed and can be waived. Thus, there might be a lack of data for a certain type of study, route of exposure, or exposure duration. In these cases, caution should be taken, e.g. establishing a long-term reference value based on a NOAEL from a short-term study or a medium-term study (see Section 2.3.1. above).

2.3.6 Derivation of External Reference Values for Route-Specific Effects (AEC)

During handling and/or use of active substances and biocidal products there is a high probability of dermal and inhalation exposure. Active substances or biocidal products may produce local effects on the skin or the respiratory tract independently of systemic toxicity (e.g. irritation or corrosion). For this type of effects the derivation of a (systemic) AEL might be inappropriate as the actual (external) exposure to the active substance and not the systemic dose is the determinant of the response. Instead, an external reference value (AEC), derived as local concentration in mg/m^3 air or mg/cm^2 skin should be derived for the quantitative evaluation of actual exposure data where appropriate (see Section 4 for further details).

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 4.3).

A route-specific reference value is also needed if data are available showing that toxicity at a specific route (e.g. inhalation) is critically different from what is expected by absorption data in combination with oral studies. Most probably the best choice in this case would be to derive an external reference value for the route in question. For inhalation at the workplace this would typically reflect OEL (EC, 1999).

2.3.7 Derivation of External Reference Values for Exposure via Food

For certain PTs and use patterns, especially if the active substance can enter the food chain, ADI and, if necessary, ARfD should be derived. Intake estimations might be needed to calculate the TMDI and to recommend the need for setting specific MRL for the active substance and metabolites.

If residues in food and feeding stuffs are expected to arise from the use of biocidal products, toxicological reference values should be set according to the principles of ADI and ARfD derivation for PPPs. The ADI is usually based on NOAEL s from long-term or sub-chronic studies divided by an appropriate AF whereas the ARfD is appropriate for assessing risk posed by short-term exposure to acutely toxic residues. ADI and ARfD are usually based on the same NOAEL as the $AEL_{chronic}$ and AEL_{acute} respectively. They are external reference doses and expressed as mg/kg b.w.

For risk assessment of biocidal active substances, ADI and ARfD values for the inclusion of active substances in in plant protection products (Commission Implementation Regulation (EU) 540/2011) or in foodstuffs of animal origin (Regulation (EEC) No 470/2009) should be taken into consideration whenever possible.

2.3.8 Deriving reference levels (AELs) when a community/national OEL is available

When an EU IOEL exists, under conditions described in the *Guidance on information requirements and chemical safety assessment Chapter R.8 (Appendix R 8-13),* the basis for the IOEL can be considered during the derivation of a reference value for biocide active substances with the application of methodology as described in this document for biocidal active substances. Other Occupational exposure limits can be considered as additional information in the derivation of reference values for biocidal active substances but not for direct application as reference values (e.g. AELs).

2.4 No threshold Identified

2.4.1 Semi-Quantitative or Qualitative Hazard Characterisation

When no reliable dose descriptor can be set for a given endpoint, a more qualitative approach has to be chosen. This usually applies for irritation/corrosion, sensitisation and mutagenicity/carcinogenicity.

For local effects (irritation/corrosion and sensitisation) additional guidance for qualitative and/or semiquantitative risk characterisation is provided in Section 4.3 of this guidance.

In case of mutagens and carcinogens where no threshold is possible to be identified a semi-quantitative approach can be considered if it is feasible to derive a DMEL (see Section 2.4.1.1).

2.4.1.1 Semi-quantitative hazard characterisation for non threshold carcinogens

As required by the Directive 2004/37/EC 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 mitigation measures to be used. If the data on the substance is considered of sufficient quality, a semi-quantitative risk assessment can be performed. This will provide quantitative information on the residual exposure levels (that will occur despite the application of risk mitigation measures) and on whether these are tolerable/acceptable or should be further reduced.

The semi-quantitative risk assessment for a non-threshold carcinogenic biocidal substance should be performed when the data allow, as an additional risk management tool to judge the efficiency of risk mitigation measures already in place in achieving minimal exposure, according to the methodologies described in ECHA REACH Guidance (ECHA 2012a, ECHA 2012b). Two methodologies can be used, the 'linearised' approach referring to the lifetime cancer risk and the 'Large Assessment Factor' approach as originally proposed by EFSA (EFSA, 2005). The relevance of the mode of action for humans should always be considered (Boobis *et al*., 2006).

- The 'linearised' approach 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 and using further assessment factors if necessary. Extrapolation factors for specified cancer risk levels are given in the REACH guidance. 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 (ECHA, 2012a). Using the 'Linearised' approach, different DMEL values can be calculated, representing different risk levels, e.g. 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).

- The 'Large Assessment Factor' approach: as in the 'linearised approach', the dose-descriptor is selected and modified to adjust for the differences in human and animal exposure routes, conditions etc. Starting from this modified dose descriptor, a set of AF is 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. The intraspecies AF is always 10 instead of 5 that is used for workers in REACH. The resulting overall assessment factor is generally much higher than overall assessment factors for threshold effects.

Both approaches result in derivation of a DMEL which in most cases is similar regardless of the choice of methodology used to derive it. The risk-related reference values thereby obtained can be used in judging the significance of any exposure that would remain after introducing the strict risk management measures. It can thus provide information to be used in further targeting the risk management measures. Exposure levels below the DMEL are considered to represent a risk level where the likelihood of effects (cancer) is appropriately low and the risk may be considered to be of very low concern.

Narrative description of the overall quality of the data has to be provided. Special attention should be given to judging whether the exposure assessment is reliable and representative of the actual exposure situations.

The REACH guidance cited above should be applied only to the assessment of the non-threshold carcinogenic effect. It should be done on a case-by-case basis, considering all biocide-specific guidance as well. Conclusions on the cancer risk should be indicated in a clear, explicit and transparent manner, and special consideration has to be given to risk mitigation measures. Expert judgment will play a considerable role in the assessment.

In case it is not possible to derive a DMEL for a non-threshold carcinogen/mutagen due to the absence of cancer data the Guidance provided in section R.8.5.3 within the *Guidance on information requirements and chemical safety assessment Chapter R.8* should be considered. In this case the following possibilities may be explored to derive a DMEL:

- Read-across
- Use of subchronic studies
- The TTC concept (see Appendix 1-4 within Repeated Dose Toxicity in Section 1)

3 Exposure Assessment

3.1 Introduction

The BPR requires a risk assessment of biocidal products before these can be placed on the European 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.

Not all tasks that may be carried out with biocidal products are covered with suitable experimental exposure data or databases/approaches. In such cases suitable information on exposure is required (to be provided by industry to the evaluating CA) to build a risk assessment to indicate appropriate safety for humans during use.

This section on Exposure Assessment presents a tiered approach (see section 2.4) 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 (as described in Section 4 Risk Characterisation), needs to be considered either for hazard or exposure assessment or for both.

This section outlines the principles of exposure assessment and the procedure that needs to be followed for the assessment of exposure from biocidal products. It is applicable for both the review of active substances programme and for product authorisation applications.

For the actual estimation of exposure, additional technical guidance on types of generic models, calculations and default parameters is provided in the document Biocides Human Health Exposure Methodology available on the ECHA Ad hoc Working Group – Human Exposure webpage [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/human-exposure]

NOTE to the reader:

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

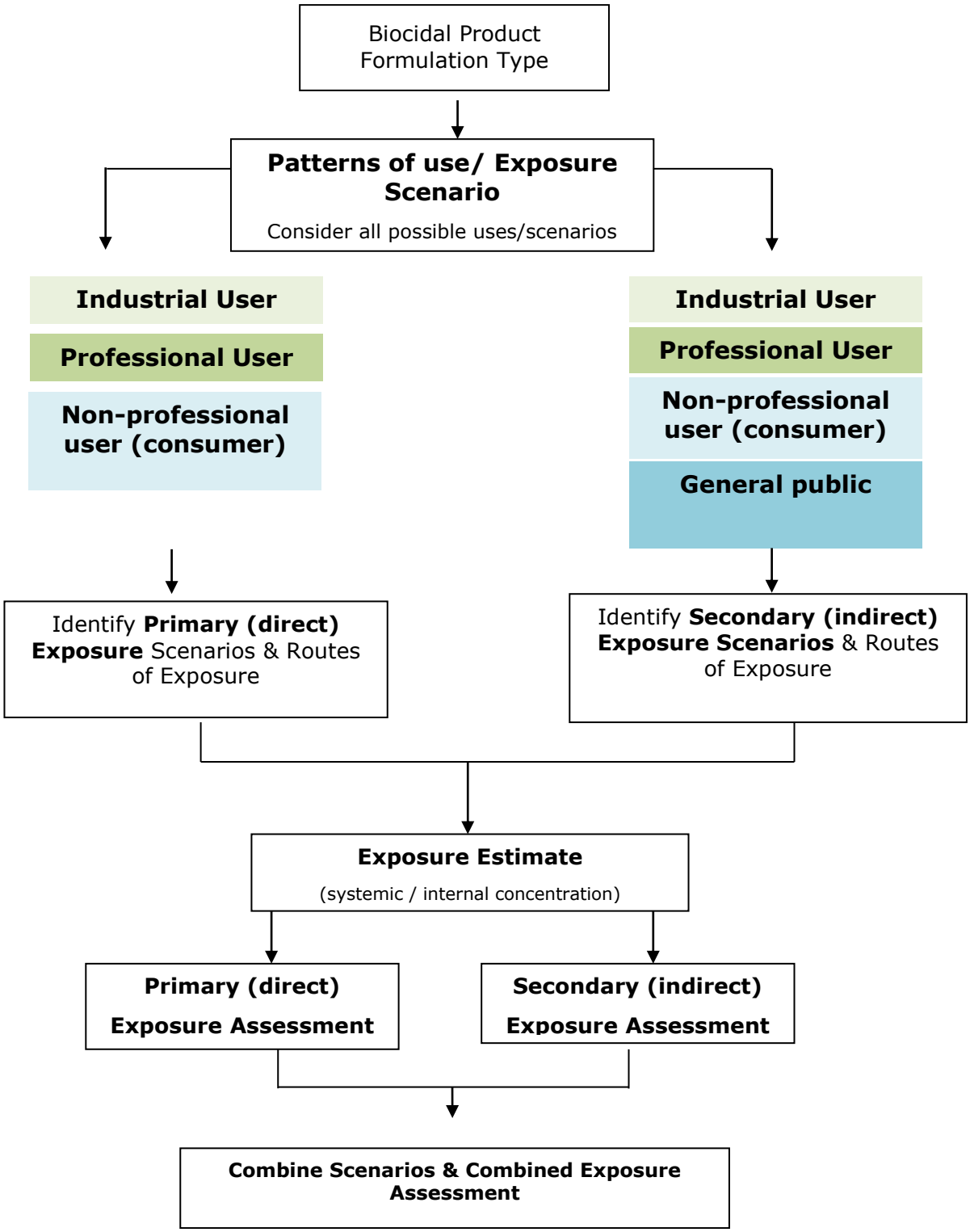
3.2 General Principles of Exposure Assessment

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 determination 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.

Figure 8 provides the general workflow for the exposure assessment.



Product type/formulation type

Understanding of the source of exposure is the first step in preparing the exposure assessment.

Identification of the product type(s) where the active substance is contained, is needed to enable mapping of the patterns of use with specific product type(s) and/or formulations and the corresponding exposure via different routes of each exposed population.

3.2.2 Patterns Of Use / Exposure Scenario (Identification Of The Uses and Users and Exposed Population

For the purpose of exposure assessment, the different types of potential users (intentional use of a biocidal product) as well as the exposure of individuals via secondary (indirect, unintentional exposure) pathways of exposure need to be considered. As a first step, depending on the product type 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).

The industrial users are in essence a subcategory of the professional users (i.e. professional users performing tasks at industrial settings). For the structure of the guidance, in order to align with the Competent Authority Report (CAR) template, the terms "industrial users" and "professional users" are used to indicate the area where a task is performed (within or outside industrial settings respectively).

3.2.2.1 Industrial and Professional users

The industrial users (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.) or professional users (those using end-products outside industry) are users that 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 Personal Protective Equipment (PPE). However, 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 (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. 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 (e.g. pest control operators).

3.2.2.2 Non-professional users (consumers)

The non-professional user is the consumer, i.e. a member of the general public who may primarily be exposed to biocides by using a consumer product. The consumer is unlikely to take informed measures to control exposure and may not follow exactly the instructions for using the biocidal product. In addition, the non-professional pattern of use is expected to show 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 of products or articles, a separate assessment of other reasonably foreseeable uses should also be made. For example, consumers will experience relatively high exposures when they use biocidal products in poorly ventilated indoor areas. When use under these circumstances is foreseeable, an exposure assessment for this situation should be carried out.

Another important aspect of consumer practice is the very limited use of PPE to control exposure. Consumers will not normally use PPE unless it is very strongly recommended by the manufacturer and/or provided with the product. As a result only typical clothing should be assumed when carrying out consumer exposure assessments.

3.2.2.3 General public (adults, infants, children)

The general public are the individuals that are likely to be inadvertently exposed to the biocidal active substance directly or indirectly via the environment and via different routes of exposure without actually using the biocidal product themselves.

The general public would cover both residents (those living in areas treated with biocides when longer exposure is expected) and bystanders (those adjacent to an area treated with a biocide that would be exposed for short periods, thus acute exposure).

The general public covers all adults, infants and children.

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, it needs to be defined what type of exposure is expected. The type of exposure expected for each of the identified exposed populations should be characterised as primary (direct) or secondary (indirect). **Primary exposure** to biocidal products occurs to the individual who actively uses the biocidal products, i.e. the user. 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 (see Section 3 for further information on primary exposure assessment).

Secondary exposure is exposure that 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* secondary exposure scenarios 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 (see Section 4 for further information on secondary exposure assessment).

It is important to note that the user of a product may be subject to both primary and secondary exposure whereas the "non-user" (i.e. the general public) will only experience secondary exposure. Primary exposures are invariably higher than secondary exposures, however, 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 (occular route).

The second step in the exposure assessment process is therefore to determine the likelihood of the biocides entering the body by the three major routes: being inhaled (inhalation), being absorbed through the skin (dermal), or being swallowed (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 in this second step it is indicated that exposure via one or more of the pathways does not occur, no further assessment is needed for that route of exposure and the conclusion can be mentioned in the risk assessment phase. Where one or more routes of exposure have been identified then an appropriate exposure assessment is required for each route.

Once all the exposure assessments from all possible 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

Inhalation exposure is often a small component of total exposure to biocides but can in some cases become the predominant route of exposure (e.g. use of a 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 a stipulated period of time. By its nature this concentration represents an assessment of potential exposure. The potential inhalation exposure can be reduced by technical measures such as local exhaust ventilation or by using respiratory protective equipment. The resulting actual exposure takes the effectiveness of these risk mitigation measures into account. Inhalation exposure stops at the end of the work shift when exposure ends.

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 over some defined period of time. The most common metric measurement for biocides is the amount of biocidal product that deposits per unit time (mg/min)[17] or task (mg/cycle);

[17] 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

- Actual dermal exposure is an estimate of the amount of contamination that actually reaches the skin. It is dependent on the effectiveness of clothing and is often expressed simply as a weight of biocidal product on skin (mg on skin).

  Actual dermal exposure arises through:

  - direct deposition on exposed skin such as the face;
  - 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 (including protective gloves).

For the assessment of dermal exposure (professional and non-professional) it is estimated that the calculated external dose (mg/min x duration of exposure resulting in mg per person) will stay on the skin for the whole shift or even longer, since it is generally not possible to rely on personal cleaning procedures/ washing habits as a reducing factor. This means 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. Unfortunately, at present there are no good or established ways to estimate oral exposure to humans, unless with biomonitoring (where oral, dermal and inhalation exposure are integrated).

3.2.3.2.4 Systemic exposure

The estimates of exposure, via the three major routes outlined above, relate to external exposure, i.e. the amount of the substance ingested, the amount in contact with the skin and the amount inhaled. For risk characterisation purposes, two approaches can be taken.

The first is to calculate the internal (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. systemic AEL (AcceptableExposure Level)) using relevant absorption data for each route of uptake.

Guidance and default values regarding dermal absorption and physiological factors are given in Section 1 on Hazard Identification within the toxicokinetics section of this Guidance, as well as in the *Guidance on the BPR, Volume III Human health Part A Information Requirements.* In addition the "Default Human Factor Values for Human Health Exposure Assessment" within the Biocides Human Health Exposure Estimation Methodology should also be consulted.

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weight/volume concentration of active substance, will avoid the need for making a correction for density.

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 an exposure assessment, it is not usual to consider an active substance, but instead to consider a biocidal product containing the active substance. This may be a liquid or a solid and the concentration may be given in percentage (for a solid) or as w/w or w/v for liquids. Care should be taken to interpret these values appropriately, as shown in the following example:

Example

Say the active substance concentration in the biocidal product is 0.56 % w/v. This means there is 0.56g of active substance in 100 ml of the biocidal product.

If the density of the biocidal product is 0.8g/ml then, 100ml of the biocidal product weighs 0.8 x 100 = 80g of biocidal product.

Consequently, for 0.56g of active substance in 100ml (i.e. in 80g of biocidal product) then in 1g of biocidal product there is 0.56 ÷ 80 = 0.007g of active substance.

Thus, there is 0.007 x 100 = 0.7g of active substance in 100g of biocidal product. This is equivalent to a concentration of 0.7% w/w active substance in the biocidal product.

An important further issue is to consider absorption for each relevant route of exposure. This again is not so much relevant for the active substance, but for the product type containing the active substance.

For inhalation, the absorption is usually taken as 100%, when no further details are known. The same may apply for dermal absorption, although the actual absorption may in practice be much lower and will also depend on the concentration in use; this may vary appreciably between concentrates and in-use dilutions. Further guidance on the use of dermal absorption values is provided within the *Guidance on the BPR, Volume III Human health Part A Information Requirements* and Section 2 on Hazard Assessment within the toxicokinetics section of this Guidance.

3.2.4 Tiered approach in human exposure assessment

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 risks (unacceptable effects), the assessment (for that human population) can be stopped and no further refinement of the exposure estimate is required. However, if the outcome is that the use of a biocidal product presents a risk (unacceptable effects), the assessment must, if possible, 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 process to risk assessment and uses the available information thus reducing unnecessary requirements for human exposure surveys or studies. The three Tiers described below provide an illustration of how this iterative risk assessment process might progress.

The tiering scheme should be read together with Section on 3.3 regarding refinement options for exposure assessment.

The tiering (from low to higher tiers) can include either options regarding exposure controls (including PPE for professional users) or higher tier methodology (e.g. use of more complex mathematical models and probabilistic approaches versus deterministic ones used in lower tiers) or both.

Tier 1

This is the screening Tier in the risk assessment process and should be kept simple. The assessor should select the top end value from a single exposure study or 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 time budget information (i.e. 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 this exposure assessment produces an unacceptable outcome in risk assessment, a refined exposure estimate will be required.

Tier 2

The second Tier in the exposure estimation process 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 time budgets, transfer factors and the effects of exposure reduction measures (e.g. technical measures such as LEV or mechanical ventilation, or PPE) may be used to modify the exposure assessment. However, the use of PPE by non-professional users (consumers) should only be considered in very limited situations for example, where gloves are to be supplied with the product, such as antifouling products. 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, after this remodelling the predicted exposure is still unacceptable, then a third iteration of the exposure assessment will be required.

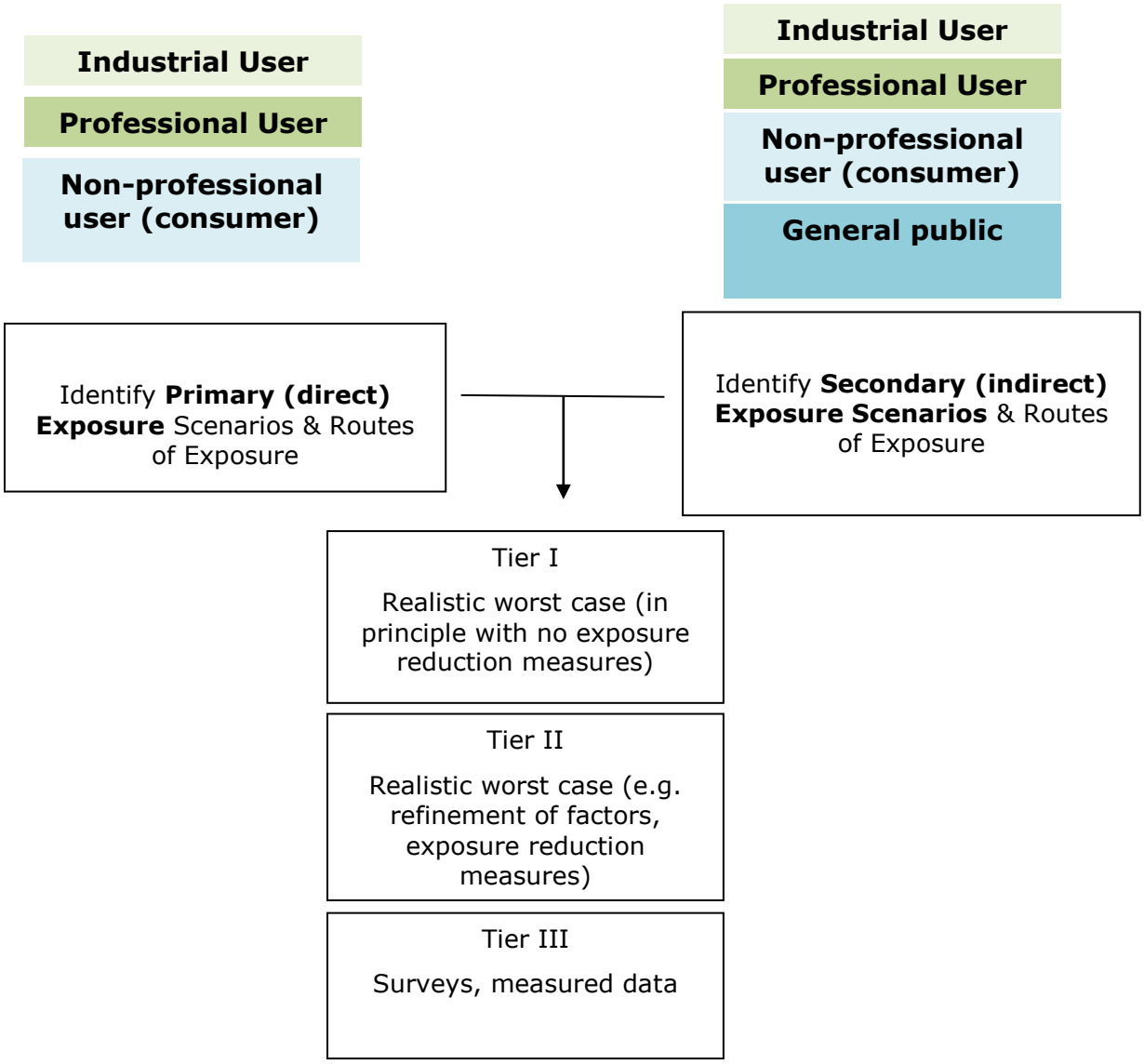
Tier 3

The most detailed level of risk assessment requires 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.

It should be noted that where biological monitoring is not included in the study, unless the specific scenario of the study is more representative than the generic model, simply generating further potential inhalation and dermal exposure data may not allow refinement of the exposure assessment. Obviously where no generic data, and hence a model are available, then a field study is required. Where field studies are done the OECD guidance on exposure studies[18] should be followed and biomonitoring studies should be carried out in accordance with the Helsinki Declaration (Describing the Ethical Principles for Medical Research Involving Human Subjects).

[18] OCDE/GD(97) 148 (OECD, Paris, France, 1997

Figure 9: Schematic representation of Tiering for exposure assessment



3.2.5 Exposure Estimation (Types of Exposure Data / Approaches)

Although substance specific measured data (where available) are preferred over modelled data, it could contain considerable uncertainty due to temporal and spatial variations as well as deficiencies in the quality and/or quantity of the available measured data. In such circumstances it may be very useful 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/or modelled data for the situation/use scenario/conditions under consideration. This will require substantial expert judgement and should always be based on reasoned arguments.

3.2.5.1 Deterministic and Probabilistic Approaches

When performing estimation of exposure there are two approaches that can be followed. The first one is the deterministic approach which provides an estimate that is 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.

Another approach is the probabilistic analysis in which distributions are assigned to represent variability or uncertainty in quantities. The form of the output of a probabilistic analysis is likewise a distribution.

3.2.5.2 Product specific exposure data

Measured exposure data for the specific product and associated information describing these 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 before use 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.

3.2.5.3 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 regulatory tool in this scheme, 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 generic data and a suitable model exist, modelling is the initial, and often the only, basis for the exposure assessment. Generic exposure models may also be used instead of, or as well as, exposure data for the specific product if there is significant uncertainty associated with the quality and/or quantity of these data.

Generic exposure data can also be used to develop more complex computer based data models.

3.2.5.4 Mathematical models

In the absence of product specific and/or generic exposure data for a particular biocidal use/scenario Competent Authorities and Approval Holders should make use of the available mathematical exposure models for assessing human exposure to biocidal products. As in the case of generic exposure models, mathematical exposure models may also be used instead of, or as well as, 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.

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 of the real world as the full range of real variables cannot be accounted for and are therefore assigned very conservative defaults. However, although mathematical models are usually meant to be conservative, this does not hold true for all models or assessed scenarios. For some models and some scenarios, model outcomes

may also underestimate exposure substantially. In general, 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 of these types of 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

Commonly, mathematical models are based on mass balance equations. Mathematical mechanistic models are often used for assessing inhalative 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;
- their 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;
- 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 are probably best 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, which can lead to skepticism over the answer.

Statistical Mathematical Models

Such models use empirical relationships to predict exposures from statistical indicative distributions together with historical data. In principle, 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 the uncertainty of a variable. From this point of view it enables 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.

Many exposure data are needed to establish a distribution and allow 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 are adequate, a probabilistic analysis is advocated, at least to underpin a deterministic presentation of the results.

3.2.5.5 Reverse reference scenarios

In the absence of suitable product specific data or generic exposure data or suitable mathematical model the reverse reference scenario can be used to determine the upper acceptable exposure level.

The 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 No Observed Adverse Effect Level (NOAEL), it is possible to compute the amount of product that would lead to that dose by a specific route. That amount can be related to the amount of exposure that is realistically likely, as determined from experimental or other data. An example on how to use the reverse reference scenario is provided in Appendix 3-3 of this section.

3.2.5.6 Suitability of exposure data sources

Any data source that describes relevant exposures can be used in the exposure assessment, when the detailed descriptions of the circumstances (contextual information) of the data source is available. The main criterion is the similarity in the tasks being considered. Good data are thus representative and robust, i.e. covering a reasonable large sample for the full range of circumstances. One might have a suitable exposure model or database with measurements at hand that cover similar scenarios. One might even have a series of measurements for the scenario to be assessed. The combination of all this information should really be done at expert level, covering all relevant parameters and circumstances, i.e. contextual information.

Another important issue is the combination of tasks, since human exposures are distributions, not single values. But single values must be drawn from the distributions in order 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 procedure can be reasonably estimated by a percentile from the data distribution. However, if the procedure is done several times, simple addition of percentile values can show gross deviations in the final estimate, especially with high or low percentiles.

This argument applies to:

- summing the data for several daily treatment cycles;
- summing the data for the inhalation and dermal exposure routes;
- adding the phase of use estimates;
- combining primary and secondary exposure;
- aggregate exposure from all sources of the particular chemical.

For practical reasons, 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 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. The probabilistic estimation technique is currently not fully integrated in the risk assessment process (for more details see Ann. Occup. Hyg. 45 Suppl. 1, 2001).

3.3 Primary (Direct) Exposure Assessment for Industrial & Professional Users and Non Professional Users

In this section, a summary of the main components from the pattern of use that are needed in the different types of exposure scenarios is presented.

The essentials of exposure assessment for primary (direct) exposure for industrial/professional and non-professional users are:

- Product composition & physicochemical properties (physical state, concentration, vapour pressure of the active substance);
- Type of user: By whom the product will be used (for primary exposure);
- Duration and frequency of use (for each stage of use) (see Section 3.1;
- Method of application / 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 not acceptable (see Section 3.3).

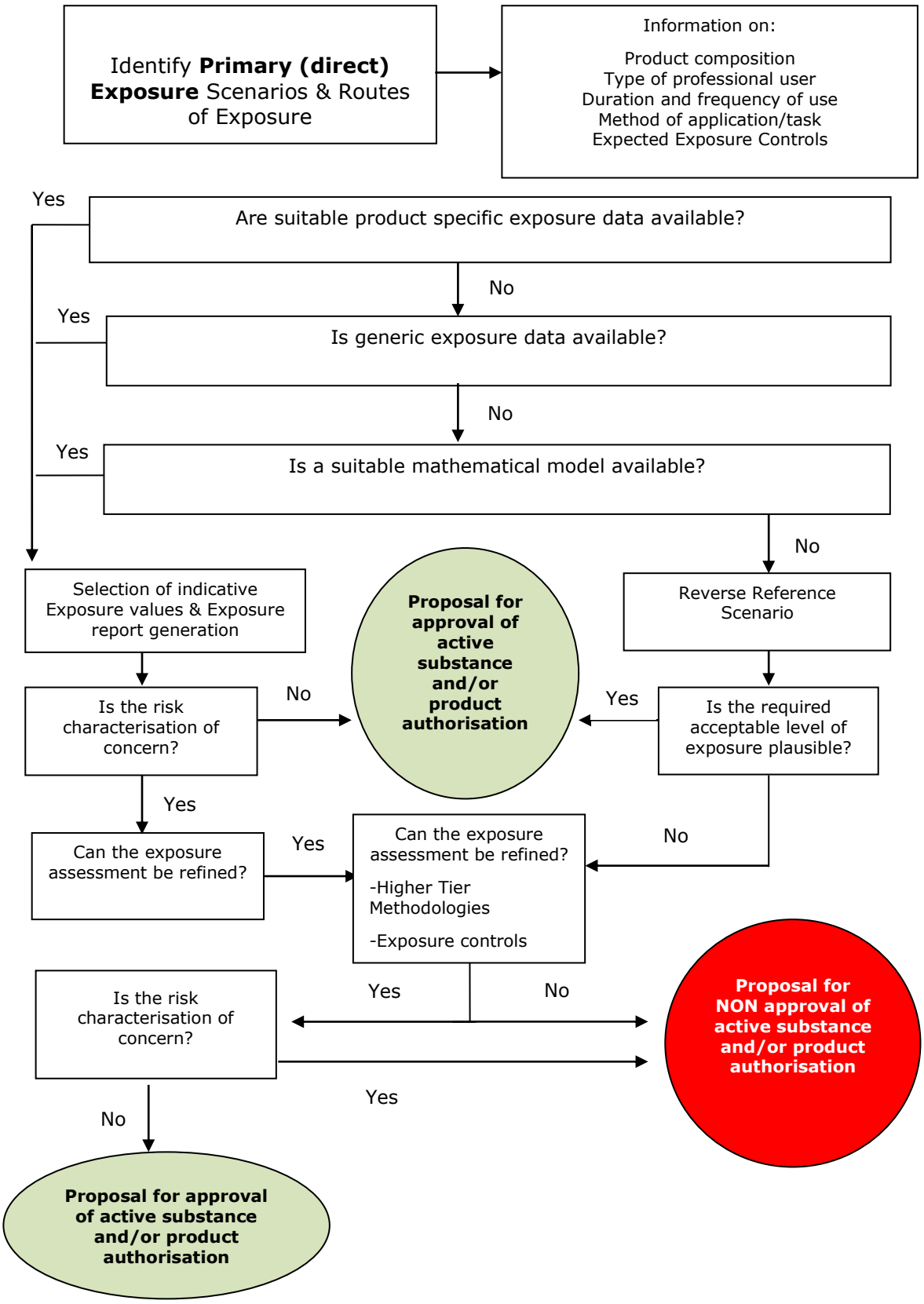
In Figure 10 a flow chart on how to perform in a stepwise approach primary (direct) exposure assessment for industrial/professional and non-professional users respectively is shown. Additional information on the methodology that applies in Figure 10 is available within the Biocides Human Health Exposure Methodology Document.

Depending on the data/information available at the time of the assessment, it maybe that suitable product specific exposure data are available.

In the absence of product specific data, the next choice would be the use of default parameters (generic exposure data) or specific models available for the exposure scenario under consideration.

When the exposure assessment estimate is compared to the corresponding hazard threshold, if no risk is identified no further refinement is needed. However if risk is identified, refinement of exposure should be performed. This can be done taken 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 also include PPE but this cannot be the case for non-professional users), generation of product specific data (e.g. measured data), or uncertainty assessment of the various steps of the exposure assessment performed.

Figure 10: Flow chart for primary (direct) exposure scenario/assessment for industrial, professional users, and non-professional users



Information on the pattern of use can be gathered through surveys or generic data from similar products. Specific information on patterns of use for many biocidal product types is limited and those placing biocidal products on the market will need to conduct research into patterns of use directly with the users if actual or surrogate data are not available.

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
- 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 per 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

Data Requirements	Priority	Comment
- frequency per task	Essential	events per day
- duration of task	Essential	event duration
- quantity used	Essential	not always relevant
- area / volume treated	Essential	not always relevant
- timing	Desirable	seasonality etc.
Post-application phase		
- task	Essential	description, continuous / intermittent / event
- frequency per 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		
User sector	Essential	
- mode of exposure	Essential	inhaled / via skin / ingested, by task
- proximity to exposure source	Desirable	hand / arm's length / more distant
- operators per task	Desirable	
Data may be better expressed as ranges and likely values, rather than as single values.		

3.3.1 Duration and frequency of use (for each stage of use)

The frequency and duration of a task are major determinants influencing the level of exposure. The frequency of a task is variable and is critical in deciding whether the exposure is chronic or acute for risk characterisation purposes. Frequency of exposure should be expressed as events per day (with precision as to how many days per year the user of biocides is exposed).

Duration of exposure (duration intervals) should be expressed as minutes or hours per day.

When determining the pattern of use, by default a harmonised approach is followed. There are however cases where variability in pattern of use (e.g. different user groups; professional user versus non-professional user/consumer), across the EU may be based such as:

- regional differences;
- climatic differences.

Competent Authorities will need to ensure the relevance of a stated pattern of use, especially in product authorization and appropriate justification should be provided if it is

not in line with the harmonized approach (see the Biocides Human Health Exposure Methodology Document for further information).

3.3.2 Method of application / task: where and how the product will be used

Primary exposure is experienced by industrial users, professionals and non-professionals (consumers) who use and apply a biocidal product. It is related to the task and 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/or the introduction of product to the application apparatus/system.
2. Application — Involves all uses of biocidal products, including application by hand, by hand-held tools, by dipping, by spraying, handling treated articles, and in machining. This phase of use can lead to the exposure of people who are present during the product application (secondary exposure).
3. Post-application — Includes exposure through separately cleaning and maintaining process equipment and tools. Secondary exposure is also included in the post-application phase.

The contribution to each route of exposure may vary considerably between these phases with any given biocidal product and method of application, given that mixing and loading can reflect exposure to a concentrate, application to a dilute product, post-application to vapour or dried residue and removal to waste material (e.g. removing and disposing of a preserved coating). In practice, exposure data often relate 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

This Section introduces concepts of how to control exposure to biocides.

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;
- Modification of a process or substance to reduce emission or release.

For biocides, with the myriad of application methods available, preventing exposure is not, in many cases, reasonably practicable. Exposure must therefore be controlled.

3.3.3.1.1 Control options

There are control options that evaluators can invoke, to abate exposure. In order of priority according to Dir. 98/24/EC, art.6, para.2, the options to consider are:

- structure related;
- engineering;
- technical (especially for consumers);
- administrative;
- personal.

Structure related control of exposure (applies to both residential environments and workplaces)

Structure related control means the reduction of exposure by inhalation afforded by general ventilation, for example, opening windows. Structure related control of exposure can also be achieved by spatial separation of the exposure source and the worker, for example, by installing control elements for a vacuum impregnation chamber in a separate room. This can reduce inhalative as well as dermal exposure.

Engineering control of exposure (applies to workplaces only)

Engineering control in the professional setting means the abatement of exposure by local exhaust ventilation (LEV) at the point of emission, or by containment in pipework or other systems from which minor emissions only are anticipated.

Technical measures for control (for consumers)

Bait boxes and child-resistant fastenings are good examples of technical measures to reduce possible exposure.

Administrative control of exposure (applies to both residential environments and workplaces, but in different ways)

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 has several levels to consider:

- proper supervision and training of workers;
- 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 minimum risk. For example, the risk is likely to be high in operations such as maintenance and 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.

Personal control of exposure (applies to both residential environments and workplaces, but in different ways)

The personal approach refers to the use of PPE, which can be defined as 'all equipment which is intended to be worn or held by a person and which protects them against one or more risks to their health or safety'. The user, taking specific steps to limit inhalation and skin exposure, uses PPE as a means of reducing primary exposure. PPE is relevant to primary exposure only. The impact of the use of PPE as part of the exposure assessment is complicated and needs to address:

- proper functioning, i.e. designed and tested to result in reproducible, quantifiable reduction of exposure;
- proper use, i.e. wearers use PPE according to guidelines to ensure adequate protection under conditions of use.

Industrial workers, Professional workers and workplaces

Workers are covered by additional regulatory control mechanisms and as a consequence are more likely to use PPE if it is required. In many cases PPE has to be supplied and used at work wherever there are risks to health and safety that cannot be adequately controlled in other ways. However, the selection of PPE should not only be triggered by the assessed risk and the required level of protection. The assessor should also ensure that the PPE is appropriate for the assessed workplace and the knowledge and training of

the worker. For instance, from the perspective of risk characterization wearing of RPE may be necessary for a specific disinfection task in a hospital, but in such a workplace this would be unusual or even unacceptable, in particular when patients are present.

Moreover, PPE providing a high level of protection does not always meet the ergonomic requirements of the workplace (e.g., heavy duty chemical protective suits may not be acceptable for longer tasks performed in a warm environment).

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. For example, non-professional users applying antifoulants to leisure craft in warm weather, would most likely be wearing sandals and shorts rather than long trousers and boots or the recommended protective clothing. For inhalation exposure, no exposure reduction should be assumed

3.3.3.1.2 Use and Selection of Appropriate PPE

There are three points to acknowledge when considering the implications of using PPE in the field of biocides. These are:

- what default values for the protection offered by PPE, should be used when undertaking an exposure assessment (this requires proper functioning)?
- what impact does the recommendation to use PPE have on the operator (this requires proper use)?

It is also important to remember that we are primarily concerned with the user of the biocide, however for the use of PPE to be successful both employer and employee need to take an active part in the selection and use of PPE.

Default values for the use of PPE are available in the Biocides Human Health Exposure Estimation Methodology Document.

Specific requirements to consider when recommending use of PPE

There are eight key issues to consider when considering recommendation for the use of as described in this section. This Section should also be read in conjunction with the section on the principles of good control practice in Appendix 3-1.

1. Provision of suitable PPE.

It must be remembered that PPE should always be regarded as the `last option' to protect against exposure to biocides. The provision of appropriate engineering controls and safe systems of work should always be considered first and this should be the basis of the users risk assessment. However, where there are no reasonably practicable other means of adequately controlling the risks, as will often be the case for the application of a biocide, then PPE will still be needed. The PPE which is provided should be appropriate for the risks involved and take into account ergonomic requirements (i.e. the nature of the job and the demands it places on the user), and the state of health of the person who may wear it. It must fit the wearer correctly and be effective to prevent or adequately control the risk.

2. Ensuring that where more than one item of PPE has to be worn to control risks, then the PPE is compatible and is effective against the risks.

Where the presence of more than one health and safety risk makes it necessary for a user to wear or simultaneously use more than one item of PPE, then the PPE must be compatible and continue to be effective against the risks, for example, certain types of respirators may not fit properly and give adequate protection if a safety helmet is worn.

3. Assessment of PPE to determine whether it is suitable.

Where PPE has to be provided to adequately control the risks, then an assessment has to be made to determine what PPE is suitable before it is chosen. This will ensure that the PPE is correct for the particular risks involved and for the circumstances of its use. The assessment should assess the risks to health which have not been avoided or sufficiently reduced by other means and should also define the characteristics the PPE must have in order to be effective against the assessed risks. It should then compare the characteristics of the PPE available against the defined effective characteristics needed. The person making the assessment of PPE should always seek the help from the manufacturer of the PPE and/or the manufacturer of the biocidal product when selecting PPE.

4. The maintenance and replacement of PPE.

Any PPE provided to users must be maintained in an effective and efficient condition and be in working order and in good repair. To ensure the equipment continues to provide the degree of protection for which it is designed, an effective maintenance system is essential and should include, cleaning, disinfection, examination, replacement, repair and testing as appropriate. The details of the maintenance procedures to be followed and their frequency should normally follow manufacturers' maintenance schedules and should be documented together with details of the person who has the responsibilities for carrying out the maintenance. Where appropriate, records of tests and examinations should also be kept; this may depend on the type of PPE, for example, gloves may only require periodic inspection by the user. Generally speaking, PPE should be examined to ensure it is in good working order before it is issued to the wearer and also be examined before it is put on and should not be worn if it is found to be defective or has not been cleaned. A sufficient stock of proper spare parts, where appropriate, should be available to wearers.

5. Provision of appropriate accommodation for PPE when it is not being used.

Where PPE is required, then appropriate accommodation when it is not being used has to be provided. Storage of PPE should be adequate to protect it from contamination, loss or damage by harmful substances, damp or sunlight. If it is likely that the PPE will become contaminated during use, then the accommodation should be separate from any provided for ordinary clothing. The accommodation required will obviously depend on the equipment and, in some cases, need not be complex or fixed, for example, pegs would be suitable for weatherproof clothing and safety spectacles could be kept by the user in a suitable carrying case.

6. Provision of adequate and appropriate information, instruction and training.

Employees have to be provided with adequate and comprehensible information, instruction and training in order that they know the risks which the PPE will avoid or limit, the purpose and manner in which the PPE is to be used and any action the employee has to take to ensure it remains in an efficient state, in efficient working order and in good repair. Everyone who is involved in the use or maintenance of PPE should be appropriately trained. A systematic approach to training, including the elements of theory as well as practice, in accordance with the recommendations and instructions supplied by the manufacturer, is required in order that: users are trained in its correct use; users know how to correctly fit and wear it and know its limitations; managers and supervisors are aware of why PPE is being used and how it is used properly, and training is given to those people who are involved in its maintenance, repair, testing and selection for use.

The instruction and training provided will obviously depend on the complexity and performance of the PPE but should typically include:

- An explanation of the risks present and why PPE is needed;

- The operation, performance and limitations of the equipment;
- List instructions on the selection, use and storage of PPE related to the intended use. Written operating procedures such as Permits to Work involving PPE should be explained;
- Factors which can affect the protection provided by the PPE, e.g. other PPE, personal factors, working conditions, inadequate fitting, defects, damage and wear;
- Recognition of PPE defects and arrangements for reporting loss or defects;
- Practice in putting on, wearing and removing the equipment;
- Practice and instruction in inspection and, where appropriate, testing of the PPE before use;
- Practice and instruction in the maintenance, which can be done by the user, such as cleaning and the replacement of certain components; and
- Instruction in the safe storage of equipment.

7. Ensuring that PPE provided to employees is properly used.

Employers have a duty to take all reasonable steps to ensure that any PPE equipment provided to users is correctly used and adequate levels of supervision should therefore be provided to ensure that the training and instructions are being followed. Users have a duty to ensure they use the PPE in accordance with any training and instructions they have received and to take all reasonable steps to ensure that the PPE is returned to the accommodation provided for it after use.

8. Duties on employees provided with PPE to report any loss or obvious defects to his employer.

All employees who have been provided with PPE have a duty to report immediately any loss or obvious defect to their employer. Arrangements should therefore be made to ensure that employees can report the loss of, or defects in, PPE and these arrangements should also ensure that defective PPE is replaced or repaired before the employee concerned re-starts work.

Protective gloves

Protective gloves are available in a wide range of materials; however, there is no single glove material (or combination of glove materials) able to provide unlimited resistance to any user or against any chemical substance or combination of chemical substances. There are three ways in which any protective glove will, at some stage, fail to protect the wearer from exposure to any chemical substance and these are:

- 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.

Selecting suitable protective gloves

The selection of suitable protective gloves is a complicated procedure and the degree of protection they give is not always easy to establish. When choosing gloves, always seek expert help from the manufacturer/distributor of the chemical substance and protective glove. They can provide glove performance test data, which can be used to assist in

predicting the permeation, penetration and degradation of specific glove materials by specific chemical substances.

There are four requirements which must be met for any protective glove to be considered suitable. The glove must:

- be appropriate for the risk(s) and the conditions where it is used;
- take into account the ergonomic requirements and state of health of the person wearing it;
- fit the wearer correctly, if necessary, after adjustments;
- either prevent or control the risk involved without increasing the overall risk.

Chemical protective gloves are Cat. III PPE in accordance with the PPE Directive (Directive 89/686/EEC the approximation of the laws of the Member States relating to personal protective equipment) and should be labeled with the Erlenmeyer flask symbol.

Selection should therefore take into consideration the wearer, the workplace conditions and the protective glove itself. Employees need to be trained in the correct way to put on, wear and then take off protective gloves to ensure maximum protection. If protective gloves are selected or worn incorrectly there is every possibility that this may increase the wearer's overall risk to health because:

- contaminant may get inside the glove to reside permanently against the skin, which could cause greater exposure than if a glove had not been worn at all;
- wearing a glove for extended periods can lead to the development of excessive moisture (e.g. sweat) on the skin, which in itself will act as a skin irritant;
- wearing gloves manufactured in natural rubber (latex) can cause an allergic reaction in susceptible individuals, causing the skin disease contact urticaria to occur.

Selecting protective gloves must be part of an overall health and safety risk assessment for the relevant tasks. The risk assessment must clearly demonstrate that exposure to the health risk is unavoidable and that other methods of control are not reasonably practicable. Gloves should be used as a control measure as a last option where other methods of control are not reasonably practicable. This is because:

- gloves only protect the wearer – they do not remove the biocide from the workplace environment;
- some types of glove are inconvenient and interfere with the way people work;
- wearing gloves interferes with the wearer's sense of touch;
- the extent of protection depends upon good fit and attention to detail;
- if protective gloves are used incorrectly, or badly maintained, the wearer may receive no protection;
- for glove design to be effective, the glove needs to be used correctly in the workplace.

Glove selection is a complex issue and the importance of using a material which provides suitable and sufficient protection, depends on the nature of the chemical and extent of exposure. Where there is a choice of glove material, the extent of exposure to the chemical substance will be a significant factor in choosing between, for example, a neoprene glove or a less costly glove: if workers' gloves are significantly contaminated for extended periods, the neoprene glove may be required; if however, there is only occasional splashing of the chemical substance onto the glove, then the less costly glove may be adequate. Other factors to consider are the manual dexterity required for the job and the required physical length of the glove, for example are gauntlet gloves

required? If workers cannot do their job because the glove material is too thick or too stiff then they may decide not to wear them.

Always remember that if the inner surface of a glove becomes contaminated, it will not matter how much care, attention and expertise has gone into the selection process of the protective gloves, exposure will occur. If, for example, contaminated gloves are removed temporarily, then the operators' hands may become contaminated from handling the gloves; if the same pair of gloves is then put back on, there could be transfer of the chemical substance to the inside surface of the glove. To prevent this, the gloves should be thoroughly washed before being taking off.

Detailed information on the selection of chemical protective gloves can be found in the BG Information BGI/GUV-I 868 E "Chemical protective gloves" (DGUV, 2009). This document is available in English language on the homepage of the DGUV: [http://publikationen.dguv.de/dguv/pdf/10002/i-868-e.pdf]

Selecting suitable Respiratory Protective Equipment (RPE)

The decision to use Respiratory Protective Equipment (RPE) should only be made after a justification has been made via a risk assessment. Examples of when RPE can be used include:

- where an inhalation exposure risk remains after other realistic controls have been put in place (i.e. there is a residual risk);
- short term or infrequent exposures (e.g. cleaning of equipment) where it is decided that other controls at source are not reasonably practicable;
- when other control measures are being put in place (e.g. interim measures);
- where there is a need to provide RPE for safe exit from an area where hazardous substances may be released suddenly in the event of a control systems failure (e.g. use of sulphurylfluoride);
- emergency work or temporary failure of controls where other means of controls are not reasonably practicable.

Ideally, the approval of a biocidal product will not rely on the use of RPE. However, in some cases at the approval stage, for example, when there is residual risk, it may be necessary to recommend the use of RPE. This should not be because other control measures are inadequate on their own, but should be to provide additional protection. During the exposure assessment there is an assumption that the user of the product will have put into place all eight principles of good control practice (see Appendix 3-1). When RPE is necessary there must be a system to demonstrate that selection of RPE has been made via a transparent and consistent procedure. Detailed information relating to selection of RPE can be found in HSE Guidance '*Respiratory protective equipment at work – A practical guide'* (HSE, 2013, available via http://www.hse.gov.uk/pubns/books/hsg53.htm).

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:

1. "Guidance Document on Characterising and communicating uncertainty in exposure assessment" (available at:

http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf)

2. "Harmonisation Project Document No: 3, Principles of Characterising and Applying Human Exposure Models"

(available at: http://whqlibdoc.who.int/publications/2005/9241563117_eng.pdf)

3.4 Secondary Exposure Scenarios

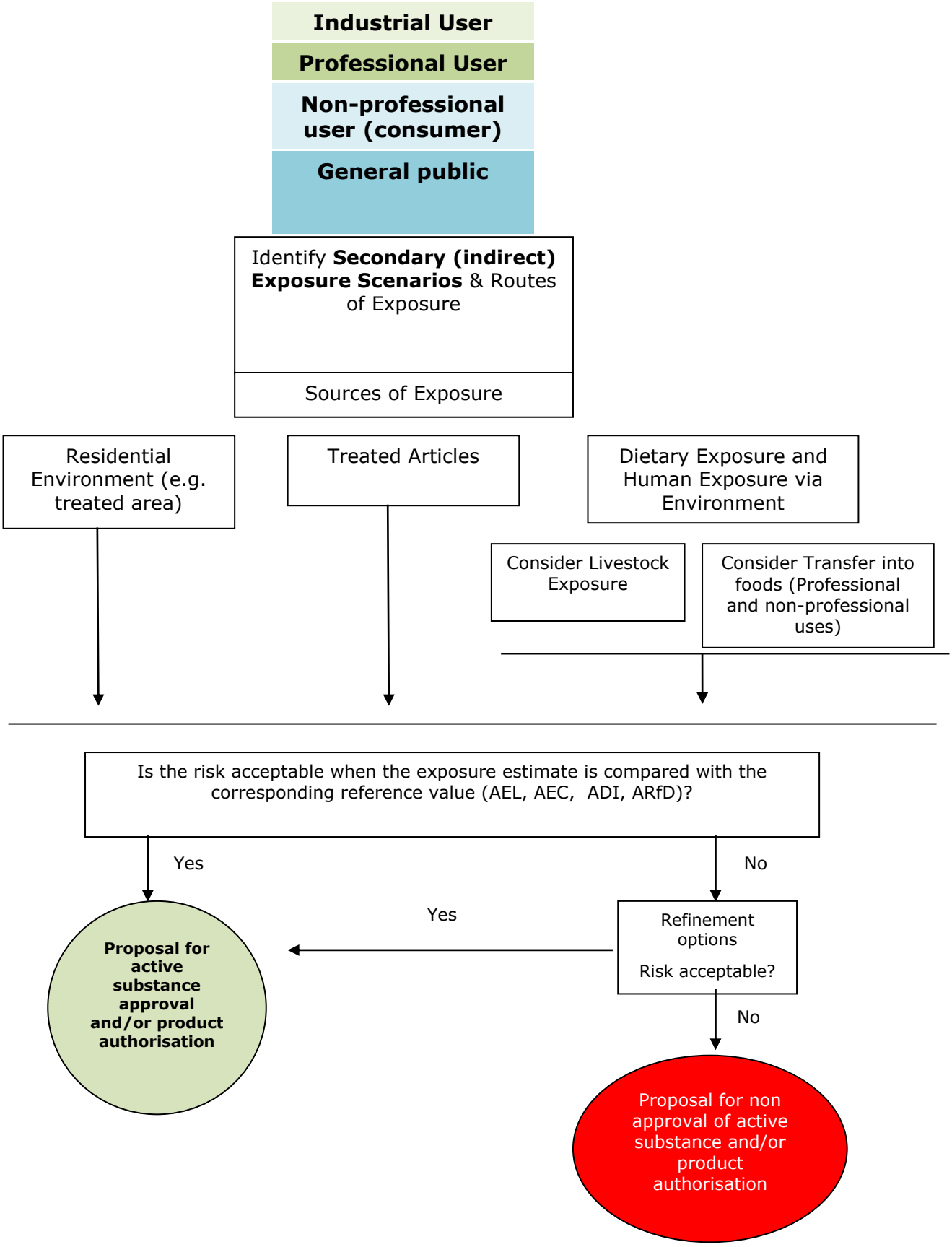
There can be three main categories that need to be considered as being potential source of secondary (indirect exposure).

These are environmental sources from the point of view of treated areas with biocidal products (e.g. a room fumigated with a biocidal product, swimming pool treated with disinfectants), treated articles and dietary exposure sources (covering potential of exposure via consumption of food where residues of biocidal products may be present).

Figure 12 provides an outline of the potential secondary exposure scenarios that need to be considered in the exposure assessment for each population.

When the exposure assessment estimate is compared to the corresponding hazard threshold, if no risk is identified no further refinement is needed. However if risk is identified, refinement of exposure should be performed. This can take into account refinement of parameters (defaults) used in the exposure assessment (with appropriate justification), generation of product specific data (e.g. measured data), or uncertainty assessment of the various steps of the exposure assessment performed.

Figure 11: Schematic flowchart of secondary (indirect) exposure assessment



3.4.1 Residential Environment

This includes exposure of people who are present during or following the use of a biocidal product (residents or bystanders). The post application phase is particularly important for non-professional exposure assessment because:

- some residues will remain in the treated area following application of the biocidal product;
- there can be prolonged contact in the residential environment because people live there;
- children, the elderly and other sensitive subgroups are present in the residential environment.

The task based approach does not apply to post application phase, because there are no well defined tasks in post application exposure. Instead, a scenario approach is proposed, containing the following two post-application scenarios for the residential environment:

1. Children playing on the floor where biocides have been applied. In this scenario, they transfer the biocide to their skin by 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, exposed to the residues in air and on surfaces.

The exposed population is anyone in the environment who may:

- inhale residual aerosols (sprays only, during or immediately after application);
- inhale vaporised biocide from deposits (any application);
- dermal contact deposits (both recently applied and dried);
- ingest dislodged deposits (inadvertently by adults, for example during smoking or eating/drinking; ingestion of dislodged deposits by infants).

Experience indicates that post application exposure of children may be the most important exposure to a biocidal substance. This is because 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 they may have a prolonged duration of contact, in the order of days to weeks. During application, concentrations are higher, but duration of contact is significantly shorter (minutes to tens of minutes typically).

In the above sense, post-application is subtly different from secondary exposure. The post application exposure is a consequence of the application of a biocide. It is secondary in the sense that the children are not aware of their exposure. However, the use of copper chrome arsenic (CCA)-treated wood, for instance, would constitute a secondary exposure but does not fit post-application exposure.

The mentioned defaults for frequency and duration of exposure should serve as a starting point for exposure assessment and should be used in the absence of accurate scenario data only. Whenever more detailed information for use scenarios is available, these data should be used instead, but always on the basis of a valid argument, for example, in case a survey has been carried out.

In addition to Table 15 (see Section 3) the following elements (see Table 16) should be considered / reported when performing secondary (indirect) exposure assessment:

Table 16: Data for Secondary (Indirect)Exposure Assessment

Data Requirements	Priority	Comment
Secondary exposure		
- population (acute phase)	Essential	include mode and likelihood of exposure
- population (chronic phase)	Essential	include mode and likelihood of exposure
- removal of product	Desirable	include mode of exposure

An overview of possible secondary exposure scenarios that might be considered when doing risk assessments for specific biocidal products in view of their uses within a certain Product Type, is available within the Biocides Human Health Exposure Methodology Document.

Additional information on secondary scenarios for consideration can be found within ECHA *Guidance on IR&CSA Chapter 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 (e.g. fish, crops, meat and milk) and drinking water, inhalation of air and ingestion of soil.

The indirect exposure 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 there are three more specific areas where estimation of risk via exposure needs to be addressed for specific product types and specific guidance is currently under development. It should however be noted that for use scenarios from additional product types (that are 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.

Relevant for the following product types:

- PT4 (Food and Feed area disinfectants);
- PT5 (Drinking water disinfectants);
- PT6 (Preservatives for product during storage);
- PT18 (Insecticides, acaricides & products to control arthropods).

2. Estimating Transfer of Biocidal Active Substances into Foods – Professional Uses.

Relevant for the following product types:

- PT3 (Veterinary hygiene products);
- PT4 (Food and Feed area disinfectants);
- PT8 (Wood preservatives);
- PT12 (Slimicides);
- PT14 (Rodenticides);
- PT18 (Insecticides, acaricides & products to control arthropods);

- PT19 (Repellents & attractants).

3. Estimating Livestock Exposure to Biocidal Active Substances

Relevant for the following product types:

- PT3 (Veterinary hygiene products);
- PT4 (Food and Feed area disinfectants);
- PT5 (Drinking water disinfectants);
- PT8 (Wood preservatives);
- PT12 (Slimicides);
- PT18 (Insecticides, acaricides & products to control arthropods);
- PT19 (Repellents & attractants);
- PT21 (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 chemical constituents of the active substances are released in any way. Exposure from treated articles during service life may be the most significant exposure to certain active substances (e.g. PT 7, 8, 9, 10). Specifically, articles consisting of different types of polymers can be used in a large range of consumer applications, which makes the exposure situation very complex. The diversity of applications has consequences for the exposure situation. Therefore, it can also be necessary to model the aggregated exposure of different articles used at the same time (please see further under section 5).

During direct contact with various materials that may have been treated with biocidal products, transfer may occur to the skin. This is due to the fact that the biocidal product may be dislodgeable, i.e. can be removed from the surface.

In addition to the dermal route of exposure, the possibility of transfer via the oral route should also be taken into account. This can be relevant for cases where an exposure scenario such as mouthing by infants or children or leaking from treated articles is identified.

In order to identify the potential that individuals may be exposed to an active substance via a secondary (indirect) route from treated articles, information from the patterns of use/exposure scenarios could also provide information on the potential of exposure from treated articles. In addition, the recommendations provided within the Biocides Human Health Exposure Estimation Methodology Document should be first consulted.

Furthermore, for specific product types and applications in relation to treated articles, guidance developed for the implementation of Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food ("Food Contact Materials Regulation") or WHO for the work of insecticides, can be also considered for the secondary (indirect) exposure assessment via treated articles from biocides.

3.4.4 Refinement Options

The principles described in Section 3.3 and the Tiering approach in Section 2.4 apply, with the exception of use of PPE which is not applicable for secondary exposure scenarios.

3.5 Combined Scenarios & Combined Exposure Assessment

The (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 job (for primary exposure related tasks) is added up from the exposure arising from the individual tasks through the different phases of use. In practice, the exposure estimates from the different routes of exposure (inhalation, dermal, oral) per scenario are added together to provide a total systemic (internal) dose. If relevant the total estimates from different scenarios are 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), for spraying a formulation and for 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 add primary and secondary exposure estimates before doing so.

Aggregate exposure to a specific substance includes both primary and secondary exposure and exposure to the same chemical in different products and matrices including treated articles.

Combined residential uses should also be considered if relevant (secondary exposure assessment), 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 in all cases to aggregate the personal daily exposure to a chemical substance through all such sources. Further guidance on aggregate exposure assessment is provided in Section 4.4of this Guidance.

For combined exposure assessment (cumulative and aggregate exposure assessment) principles please see Section 4.4 (risk characterization for combined exposures) of this Guidance.

The principles of exposure assessment for combined exposure assessment are the same as for the exposure assessment from a single biocidal product.

The tiering approach needs to be followed both in terms of exposure refinement and hazard refinement where relevant.

3.6 Assessment of Data Quality

3.6.1 Criteria for quality assessment of reports concerning exposure data

The criteria to judge the quality of exposure surveys and study reports are set out below. It is not acceptable to use inadequate data from inadequate reports in exposure estimation and so it is imperative that all data generated are adhering to thoughtfully designed protocols and carefully conducted studies.

Initially, to build a database from past studies, it may be necessary to use less stringent quality criteria. However, these "barely adequate" data must, in time, be superseded by more acceptable data so that they can serve as entries into a generic data base. Inappropriate data may trigger over-conservative default assumptions.

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 current test protocols and requirements.

Documentation is adequate when studies have been carried out in compliance with Good Laboratory Practice and Good Exposure Assessment (Hawkins et al., Am. Ind. Hyg. Ass. J. 53:34-41, 1992), and defined in terms of the following eight components. All components should be present:

1. A 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. A statement on the study model which bridges the actual observed data and the general application be it deterministic, empirical or statistical.
4. A fully described study design, containing all forms of data handling (sampling, chemical and statistical analysis). It is essential not only to describe what is done and how, but also to show that the procedures are adequate for reaching the study goal.
5. A quality assurance procedure, including external audits.
6. A 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.
8. The need for communication and confidentiality of results, when relevant or appropriate.

In practice it is recognised that a pragmatic approach to study acceptability would have to be developed to deal with the sparse data for exposure to biocides.

3.6.3 Criteria

Each study submitted should be evaluated by comparison with pragmatic data acceptability criteria as set out below.

This evaluation forms the basis for the decision whether or not to include a study in the database, which study information to include and which study exposure records (data points) to include in sub-sets for deriving surrogate values or distributions for use in predictive models. It would also form a basis for Competent Authorities to evaluate studies submitted in support of authorisation of specific biocidal products.

To provide transparency on the individual judgements, each study should be summarised in a standardised note format. The information in this summary should contain:

- study number (unique number);
- documentation (comment on adequacy or otherwise);
- contextual information about the scenario and tasks;
- database contribution (number of records);
- participants (number and definition);
- replicates (number per worker);
- time/surface/volume (relevant measure, as related to a work cycle or shift);
- equipment (and/or other relevant information);
- information, training;
- engineering measures in use;
- recommended (or in use) PPE;

- matrix-matched recovery data (field and laboratory);
- limits of detection and quantification;
- inhalation (technique and sampling media, collection efficiency, particle size, if applicable);
- dermal (body) (technique and sampling media);
- hands (technique and sampling media);
- bulk concentrate and in-use biocide concentrations;
- analytical aspects (technique and documentation);
- container size/type;
- formulation (type);
- activities involved;
- notes (other relevant information);
- judgement (proposed decision on inclusion of exposure records to be included);
- environmental conditions;
- calculations and data analysis;
- plausibility analysis;
- discussion of results.

The pragmatic acceptance criteria are set out in the following table (Table 17). These are set out as essential requirements, desirable attributes and rejection criteria. For example, it is considered essential that a study report should contain a description of the aims of the work and, ideally, there should be a written protocol for the study, including a justification/ reasoning for the chosen design.

Table 17: Recommended pragmatic acceptance criteria for human exposure studies

Essential Requirements	Desirable Requirements	Rejection criteria
Aims of survey or study strategy[19]	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	Analytical method, validation,	No recovery data (unless

[19] 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
recovery data	recovery, storage, detection limits	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
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 17
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 or otherwise of studies submitted. All studies that are reported in the present document have met the criteria of acceptability, unless noted otherwise.

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 18). 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 18: 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	Some indication of the dustiness of solids being handled or the

Data item	Desirable amount of detail to be recorded
of biocidal product	volatility of liquids; qualitative details of the viscosity of liquid biocidal products
Mass of product used	The total mass of product used during the task or tasks
Biocide concentration	Record of the concentration of the active biocide, both in use and before any dilution
Proportion of the task exposed to biocide	Percentage time the person is exposed (by inhalation or dermal contact) to the biocide
Time near to the source	Proportion of the task where the person is close (within 1m) to the source of the biocide
Description of the handling of the biocide	Details of the process or activity; for example, handling contaminated objects, spraying, brushing, wiping, immersion etc.; details of the process, e.g. spray technology, spray pressure, nozzle diameter, etc.
Process temperature	Temperature of the biocide in use
Description of local 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

It is realised that most studies of human exposure to biocides that have previously been undertaken will not report detailed data for many of the above. However, it is considered that in the future further efforts should be made to collect such data.

3.7 Selection of Indicative Exposure Values

The following general 'rules' are presented for selection of indicative exposure values from available 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. 90% confidence intervals for the 75th percentile are typically less than a factor of 2. For these datasets the 75th percentile is proposed as an indicative exposure value.

2. Considerable uncertainty. The dataset is of smaller size and/or the variability 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 is proposed as an indicative exposure value.
3. High uncertainty. The dataset is of small size and/or the variability is great. The lognormal approximation to the exposure dataset may not be verifiable and so confidence intervals based upon this assumption might be misleading. The exposure distribution is poorly characterised and so the maximum exposure value is proposed as an indicative value, or else none whatsoever.

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: Principles of Good Control Practice

The following text details the principles of good practice for the control of exposure to substances hazardous to health according to Directive 98/24/EC (especially Art.6/Paragraph 2) and the "Practical Guidelines of a non-binding nature on the protection of the health and safety of workers from the risks related to chemicals agents at work" (available at: http://bookshop.europa.eu/is-bin/INTERSHOP.enfinity/WFS/EU-Bookshop-Site/en_GB/-/EUR/ViewPublication-Start?PublicationKey=KE6805058). As such the principles should be followed when considering preventing / controlling exposure to biocides. The focus is on inhalation exposure.

The following table (Table 3-A1-1) provides a good summary of "Specific prevention methods and their prioritisation" (as available within the "Practical Guidelines of a non-binding nature on the protection of the health and safety of workers from the risks related to chemicals agents at work" Section 3.1):

Table 19: Specific prevention methods and their prioritisation

Priority	Objective	Area of Application			
		Chemical agent	**Process or installation**	**Workplace**	**Work method**
1	*Risk elimination*	Total substitution of the chemical substance	Modification of the process Use of intrinsically safe equipment (1)		Automation
2	*Risk reduction-control*	Partial substitution of the agent Change of form or physical state (2)	Closed process Local extraction	Safe storage Segregation of dirty departments Ventilation by dilution Fire prevention	Safe handing Safe internal transport
3	*Worker protection*			Eyebaths and showers Fire protection Explosion prevention and protection	Respiratory, skin and eye PPE

Notes:
(1) Applicable for eliminating the risk of fire or explosion
(2) For example, handling of a solid material in a wet state, in the form of a paste or gel or encapsulation may reduce inhalation risk

Adequate control

Considerable emphasis should be placed on using good control practice and that it would be considered adequate if:

- the principles of good control practice are applied;
- a workplace exposure limit is not exceeded.

The primary emphasis for achieving adequate control relies on the application of eight principles of good control practice.

Principles of good control practice

'To be effective in the long-term, control measures must be practical, workable and sustainable'.

There are eight principles (a - h) that have to be followed to develop effective control measures. The principles should be regarded as a 'package', which must all be properly applied in order to achieve effective, reliable and sustainable control of exposure. Applicants and evaluators cannot pick and choose which principles to apply – they are all important in achieving adequate control. Principle (a) is not more important than principle (h), although there is a logical progression in how they are presented and should be considered.

Principle a: Design and operate processes to minimise emission, release and spread of contaminants.

It is more effective to reduce the emission of a contaminant at source, rather than to develop ways of removing the contaminant from the workplace, once it has been released and dispersed. Clearly, with the way that many biocides are applied this approach is often not possible. However, it is possible to consider reducing in number the size, emission or release rate, as much as possible. Indeed it is often not possible to obtain adequate and reliable control unless this is done. Consequently, to identify how people are exposed during the application of biocides, it is essential to recognise the principal sources and how the contaminant is transferred within the workplace. It is easy to miss significant sources and causes of exposure. Application of biocides will lead to the emission and release of contaminants. The way this occurs and the scale of release needs to be understood because only then can alterations be developed to minimise emission, release and spread of the biocide. This is best done at the design stage. Other people, workers or bystanders, may be significantly exposed even though those applying are protected; for example, by wearing PPE. In such circumstances, the most practical option to protect those people not directly involved in application may be to segregate the process.

Once the number and size of sources has been minimised, consideration should be given to whether further reduction can be made by enclosing the process. If enclosure is possible (e.g. by sealing a building prior to fumigation), the enclosure should be big enough and robust enough to cope with the application process. For airborne contaminants, properly designed exhaust ventilation applied to the enclosure may be needed to minimise leakage into the workplace. Work methods should be designed and organised to minimise the number of people exposed, the duration, frequency and level of exposure. For example, when treating a large article with a wood preservative, containment may not be feasible; natural ventilation may, however, with the right precautions, be relied on to disperse vapour. Clearly this would be best done at the end of a shift, in controlled circumstances and when fewer people will be present.

In addition to identifying significant sources, it is essential to identify and consider all work groups and bystanders that may be exposed. It is easy to miss or underestimate the exposure of those engaged in non-routine activities such as work done by maintenance personnel and contractors. Control measures at the outset should be designed for ease of use and maintenance. If they include working methods that are difficult to follow or involve hardware that is difficult to repair, the control measures will probably not be maintained or sustained. Inevitably their effectiveness will fall and exposure will rise.

Principle b: Take into account all relevant routes of exposure – inhalation, skin absorption and ingestion – when developing control measures

The physical and chemical properties of a biocide, in the circumstances of use, have a great bearing on which route (inhalation, dermal or ingestion) of exposure, or

combination of routes, is most important. If there is no exposure, there is no risk to health, but for many biocides the usage pattern nearly always leads to some exposure. There is therefore a need to consider:

- the health effects that the biocide can cause;
- the way the biocide is used;
- the degree of exposure;
- how exposure occurs.

An adequate risk assessment considers all routes by which the biocide might enter the body and, in the case of direct contact, how a biocide might affect the skin and eyes. In some cases, it might be immediately obvious that not all routes apply. Therefore, for the exposure assessment there is a need to:

- identify all sources and routes of exposure;
- rank these routes in order of importance.

Where inhalation is the most relevant route, the main focus for control will be sources of emission to air. Where the main concern is ingestion or effects on, or as a result of penetration through the skin, the main focus for control will be sources of contamination of surfaces or clothing and direct contamination of the skin. The exposure assessment should identify and, if possible, grade or rank the contribution of all routes of exposure to total exposure. In this way control effort can be directed at the main sources and causes of exposure. Skin contact should be prevented, if possible, where contamination may lead to skin absorption, ingestion or direct health effects on the skin. Regular cleaning of surfaces that can become contaminated, for example, the outside of a knapsack sprayer, should be undertaken. The frequency of cleaning should be based on the rate at which the surfaces become contaminated and how often skin is likely to come into contact with them. Gloves are often used to provide protection against skin contact with biocides. However, transfer of contamination from the outside of protective gloves to the inside is common. The risk assessment should identify the fact that if gloves are to be worn then users have to be trained in the correct technique for putting on and taking off their gloves. If biocides are applied in a room, which may become contaminated, and this contamination may contribute significantly to exposure, people should not increase their exposure by activities such as:

- eating;
- drinking;
- smoking;
- using cosmetics in the workplace.

If the workroom is liable to be contaminated, people should have clean areas to rest, eat or drink. Where skin contact is relevant it will be necessary to provide:

- adequate and accessible welfare facilities for washing and changing;
- laundered or disposable workwear. The frequency of laundering will depend on the degree of contamination and the hazardous nature of the biocide;
- separate storage for day-wear and work-wear;
- clean facilities;
- segregation of clean and dirty areas if the risk of contamination is severe.

It is good practice to keep workplaces clean, however cleaning methods should not lead to spread of contamination. If dust exposure from contaminated work clothing could be significant, clothing should be used that is made from low dust-retention and low dust-release fabric.

Principle c: Control exposure by measures proportionate to the health risk

The more severe the potential health effect and the greater the likelihood of it occurring, the stricter the measures to control exposure will be required. Control measures that are adequate will take into account the nature and severity of the hazard and the magnitude, frequency and duration of exposure. They will therefore be proportionate to the risk. The consequences of failing to control exposure adequately should be considered. If the health effects arising from exposure are less serious, such as simple, reversible irritation, and are not likely to cause long-term harm, it may be sufficient to reduce exposure by simple low-cost measures, such as replacing lids on vessels. In such cases, it may be unnecessary to go to greater trouble and expense to reduce the risks even further. Where the health effects arising from exposure are more serious then exposure will need to be reduced to low levels. How low these levels need to be will depend on the nature of the hazard, the likelihood of harm occurring and the degree of confidence in the information on potential health effects. The control measures necessary in this case might be extensive, take time to develop and implement, and be relatively costly. The measures should control the risk of both long-term (chronic) and short-term (acute) health effects.

Sometimes, control measures may be selected that reduce exposure more than is strictly necessary. Usually, this occurs because some controls are more convenient and acceptable. For instance, people may prefer to wear air-fed respiratory protective equipment rather than filtering devices, although the protection offered by the latter would be adequate, if well fitted. Such cases do not undermine the general principle that, overall, control measures should reduce exposure to a level which minimises any risk to health. Control measures should be kept under review to ensure they remain effective enough in the light of new information. Knowledge and understanding of the potential health risks from the biocide may change. Advances in the application process and control technology and work organisation may enable changes to be made to reduce exposure.

Principle d: Choose most effective and reliable control options, which minimise escape and spread of contaminant from sources

Some control options are inherently more reliable and effective than others. For example, the protection afforded by personal protective equipment (PPE) is dependent upon good fit and attention to detail. In contrast a very reliable form of control is changing the process so that less of the biocide is emitted or released. For example, application by brush may be easier to control than by spraying. The most effective and reliable control option for particular circumstances should be chosen and these should be directed at the main source and cause of exposure. There is a broad hierarchy of control options available, based on inherent reliability and likely effectiveness. These include:

- elimination of the biocide;
- modification of the biocide, application process and/or workplace;
- applying controls to the process, such as enclosure;
- ways of working to minimise exposure;
- equipment or devices worn by individuals.

Clearly, for many biocidal products, some of the above control options are not feasible. However, raising the profile of the hierarchy of control means that the Applicant should have considered the possibility of elimination and asked the question; can the biocide be eliminated or replaced with something else? Elimination means exposure cannot occur and, as an option, should always be considered first. If it were not possible to eliminate then a reliable form of control would be to change the process so that less biocide is released. Controls applied to the process might be effective, but will require maintenance and are unlikely to be as reliable as elimination. The key message is that

there is a hierarchy of reliability of control options and this hierarchy is often linked to their effectiveness. Many of these decisions will be made by the user and not the Applicant.

Providing PPE, such as gloves or respirators, may appear to be a quick and easy option. In practice, it is likely to be the least reliable and effective option. Indeed, it may not actually be the cheapest if a PPE programme is compared like-for-like with the cost of providing other control options. What is required is the development of a set of integrated control measures that are effective and reliable enough to control exposure adequately. The 'hierarchy' of control should not be seen as a marker of reliability and effectiveness so rigidly that some control options are viewed automatically as 'good' while others are seen as 'bad'. This 'good-bad' view can hinder the development of what is needed, that is, effective, reliable, practicable and workable control measures. There is a large range of control options available. Each will have its own characteristics as to when it can be applied, how much it can reduce exposure, and how reliable it is likely to be. As a matter of principle, the aim should be to select from the most reliable control options. Again, it is important not to be too fixed in one's thinking as, in many cases, an effective set of control measures will turn out to be a mix of options – some more reliable than others.

Principle e: Where adequate control is not reasonably practicable by other means, provide suitable PPE in combination with other measures

Effective control measures usually consist of a mixture of process and/or workplace modifications; applied controls, such as LEV, and methods of working that minimise exposure and make the best use of controls. Sometimes the mix includes PPE, such as respirators, workwear or gloves. PPE tends to be less effective and reliable than other control options, because it:

- has to be selected for the individual;
- has to fit the individual and not interfere with their work or other PPE worn at the same time;
- has to be put on correctly every time it is worn;
- has to remain properly fitted all the time the individual is exposed;
- has to be properly stored, checked and maintained
- tends to be delicate and relatively easily damaged;
- fails to danger, sometimes without warning.

The possibility of failure at each of the steps needed for successful use of PPE makes it difficult to achieve sustained and effective exposure control across a population of people. Even if a reliable, defined sustained reduction in exposure is achieved using PPE, it offers no protection to others working nearby not wearing PPE. Control options, such as change of process or applied controls, are likely to be more effective and reliable than PPE. They will probably be cheaper long term, but it may take longer to plan and organise them. It is important not to rely solely on PPE as the only control option and believe exposure is adequately, effectively and reliably controlled. Unless, that is, PPE really is the only feasible control option. Normally, PPE should be used to secure adequate control in addition to the application process, operational or engineering measures, and where adequate control of exposure cannot be achieved straight away, or solely by application or use of these other measures.

With respect to biocides PPE may be the essential element for controlling exposure; in which case a programme to organise and manage this element will be required. PPE, including RPE, requires proper:

- selection;
- fitting;

- use;
- storage;
- checking and maintenance;
- training for use.

A PPE programme involves the careful, routine training of the behaviour of people, including wearers and supervisors. If used, it must be set up carefully, managed properly and checked regularly. Clearly, the type of PPE provided should be both adequate and suitable. Adequate, in this context, means technically capable of providing the required degree of protection; appropriate selection is therefore very important. Suitable, means correctly matched to the needs of the wearer, the job and the work environment. Choice, comfort, user trials and supervision will all be important. Sometimes the PPE chosen may offer protection that is more than adequate, but is chosen for its suitability. For instance, an airline hood may be more comfortable and, therefore, more acceptable than a full-face mask, even though the additional protection is not indicated from the risk assessment. As with gloves, shoes and clothing, one size of respirator will not fit everyone. People must be offered a choice of device. This is especially the case for half-mask devices, which need a good and complete fit against the face of the wearer to work effectively.

Principle f: Check and review regularly all elements of control measures for continuing effectiveness

Once an effective set of workable control measures have been devised, they need to be put in place and managed. This includes training all relevant people in the use and maintenance of the control measures. The requirement for maintenance covers all elements of the measures to achieve effective and sustained control of exposure. These include any defined methods of working, for example, supervisory actions and record keeping, (i.e. the 'software' of control) as well as the 'hardware' of control, such as PPE. Certainly, whatever hardware is involved must be checked and must continue to function as intended. In addition a similar approach needs to be taken to check the actions people must take and the methods of working they need to adopt. The effectiveness of control measures should be checked regularly. Which checks, and how often, will depend on the particular control measures. The consequences if the measures fail or degrade significantly should be considered. Process changes are likely to be more stable and reliable than, say, LEV. In turn, LEV is likely to be more stable and reliable than controls that rely on routine human behaviour. In practice, it is necessary to draw up a simple practical programme for checking essential elements in each set of control measures. For instance, it may be necessary to check every week that operators are still adopting the correct methods of working. Checking on the working of the LEV may only be needed every month. Checking the continuing effectiveness of the process changes may only be needed every six months.

It is however important not to miss the basic checks. It may be very obvious that an important element of a set of control measures has failed and the operator may well be in the best position to check this.

The frequency of checks should be adjusted to what is needed to keep the control measures effective. There is nothing more likely to cause people to ignore or not take checks seriously than routinely measuring and recording 'no change' over long periods of time. Checks have to have some purpose and meaning. Exactly what checks should be done will depend on:

- the control measures in use;
- how reliably they control exposure;
- how well characterised they are;

- the consequences of control degradation or failure.

When control measures are known to be reliable and effective, the focus of attention should be on checking the critical elements of the measures to ensure continued effectiveness. Where reliability and effectiveness are not known, it may, ultimately be necessary, to measure exposure to the biocide in question.

Principle g: Inform & train all employees on hazard and risks from substances and use of control measures

For control measures to be effective, operators need to know how to use them properly. Most importantly, operators need to know why they should be bothered to work in a certain way and use controls as specified; they need to be motivated. Motivation comes from understanding what the health risks are and, therefore, why the control measures are important. It also comes from the user having confidence in the control measures and believing that they will protect their health. If the health risk is serious and is chronic or latent in nature, a good appreciation of the risk is especially important. With latent or delayed risks, exposure can often be excessive, with no short-term warning, such as smell or irritation, to indicate that anything is amiss. People exposed during application of a biocide need to be told, clearly and honestly, why they should use the control measures, and the consequences, in terms of ill health, if they do not use them.

Operators need to know how control measures work to use them correctly, and to recognise when they are not working properly. This means training the operators that are directly involved, as well as supervisors and managers. This is so that everyone can identify when controls are being used in ways that reduce their effectiveness. It is important to know whether the individual is working in a way that reduces the effectiveness of control measures because:

- there is no other way of doing the job;
- because they do not know any better.

If the control measures are difficult to use or get in the way of doing the job, they will need redesigning. If the control measures are well designed and tested but are still misused, then the individual needs retraining and motivating. Most control measures involve methods of working, which means that, at the design stage, it is essential to ask workers and supervisors for their views on how best to do the work so exposure is minimised. They should be asked whether a proposed method of working is practical and how to get the best out of the proposed control measures. Easily followed, convenient and simple procedures, which minimise exposure, and are built-in to the working method, are more likely to be followed.

Principle h: Ensure introduction of control measures does not increase overall risk

Process changes, enclosures, ventilation, new methods of working, PPE and other changes to control exposure can introduce new risks. For instance, process changes may mean that equipment cannot be fully decontaminated before maintenance staff are given repairs to do. New methods of working may create risks of musculoskeletal injury. LEV has to be maintained, introducing possible risks of access and manual handling of heavy parts, while PPE can restrict movement, feel and vision. People designing control measures should look for these 'new' risks and minimise them. They must not only focus on the risk from biocides hazardous to health. A good control solution is one which minimises the health risk while reducing maintenance burdens, being relatively foolproof, and not introducing other risk.

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

The correct selection and use of exposure percentiles in a risk assessment is essential in order to avoid excessive conservatism whilst also providing reassurance that highly exposed workers are incorporated into the assessment. As uncertainty increases with small datasets it is generally the case that 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 considered when making 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:

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

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

Tables A2-1 and A2-2 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^{-1} from a dataset of 50 measurements with a GSD of 6 a 90% confidence interval for the percentile is 63 mg min^{-1} (100 /v1.59) to 159 mg min^{-1} (100v$\times$v1.59). Confidence

intervals become wider (less certain) with greater exposure variability and narrower with increasing sample size.

Table 20: 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 21: 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.

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 exposure to the active substance, which would allow for an acceptable Assessment Factor (AF) based on an appropriate NOAEL and then assess the likelihood that exposures will exceed this level.

The maximum amount of active substance allowable can be calculated by dividing the NOAEL by the appropriate AF. Assuming a NOAEL of 25mg kg^{-1} d^{-1} and an AF of 100, the maximum amount of active substance is given by:

$$NOAEL/AF = 25/100 = 0.25mg\ kg^{-1}\ d^{-1}$$

For a non-volatile paste it is assumed that inhalation exposure is negligible and so assuming dermal absorption of 10%[20], to exceed an AF of 100, active substance contamination to the skin would need to exceed:

$$0.25mg\ kg^{-1}\ d^{-1} \times 10 = 2.5mg\ kg^{-1}\ d^{-1}$$

[Although in many cases the AF is 100, the value of the AF should always be considered first and 100 is not to be taken as a default.]

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

$$2.5mg\ kg^{-1}\ d^{-1} \times 60kg = 150mg\ d^{-1}$$

As the maximum concentration of active substance in the ready-for-use paste formulation is 0.5% w/w, then the weight of paste product containing 150mg active substance will be

$$150/0.5 \times 100 = 30{,}000mg$$

Assuming that dermal exposure will be predominantly to the hands and that gloves are worn, then rate of actual dermal exposure to the hands inside gloves is required to exceed:

$$30{,}000\ mg\ /30\ min = 1{,}000\ mg\ min^{-1}$$

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 360mg min^{-1} with a 95^{th} percentile of 23mg min^{-1}. On this basis, for chronic exposure, it is concluded that a margin of safety of a least 100 will be achieved. This calculation is presented in the standard format in Table 3-A3-1.

[20] The correction for dermal absorption is only necessary if in the study the NOAEL is derived from absorption through the used route of uptake is 100% (e.g. an oral study). If the study were a dermal study, then there should not be a correction for dermal absorption.

Table 22: 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**

4 Risk Characterisation

4.1 Introduction

According to EU Annex VI of the BPR, risk characterisation is defined as: *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 the context of the BPR the risk characterisation is, thus, an assessment of the risk associated with the exposure to the active substance through the use of the biocidal products.

Risk characterisation for human health is based on a comparison of the critical toxicity endpoints of the active substance and resulting reference values (e.g. AELs) with the exposure levels to the active substance for the proposed pattern(s) of use.

The methodology for risk assessment of the active substance can be defined as the combined processes of (a) hazard identification, (b) hazard characterisation (identification of the dose-response relationship), (c) exposure assessment and (d) risk characterisation. The hazard characterisation and the dose-response relationship for the active substance will be elucidated once during the evaluation of the biocidal active substance. The agreed AELs/AECs will then used in the biocidal product evaluations.

Where a critical effect is **threshold-based** and exposure data are reliable, quantitative risk assessment should be carried out for each exposed population, product-type, and method of application relevant for the respective biocidal products as indicated by the exposure assessment. The risk characterisation method should follow the general principles of the AOEL approach in the risk assessment of PPP and the DNEL approach developed for industrial chemicals where relevant. The acute, medium-term and long-term AELs are used as general health-based reference values. The term AEL resembles the AOEL. The omission of the term operator underlines that the AEL is the reference value for the human population as a whole.

A tiered approach for human health risk characterisation of biocides has to be followed. These tiers follow the same principles as the ones used for exposure assessment and described in the Guidance for Human Exposure (Section 3).

In general, in the **first tier** systemic AELs should be derived for acute, medium-term, and long-term exposure, based on the systemic toxicity of the active substance using appropriate AFs. The derived AELs 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 for the inclusion in the Union List is necessary. If qualitative risk characterisation (e.g. for local effects) needs to be performed in parallel to the quantitative risk characterisation for systemic effects and this requires the use of risk management measures (including PPE where relevant), these risk management measures should be taken into account already within the quantitative risk characterisation for systemic effects when estimating the exposure estimates within each relevant exposure scenario.

In general, in the first tier a reasonable worst-case estimate of exposure is calculated not taking into account risk reduction measures such as PPE. However, it might be possible that certain assumptions on exposure reduction, e.g. as result of technical specifications and operational conditions, are already included in the assessment at this stage.

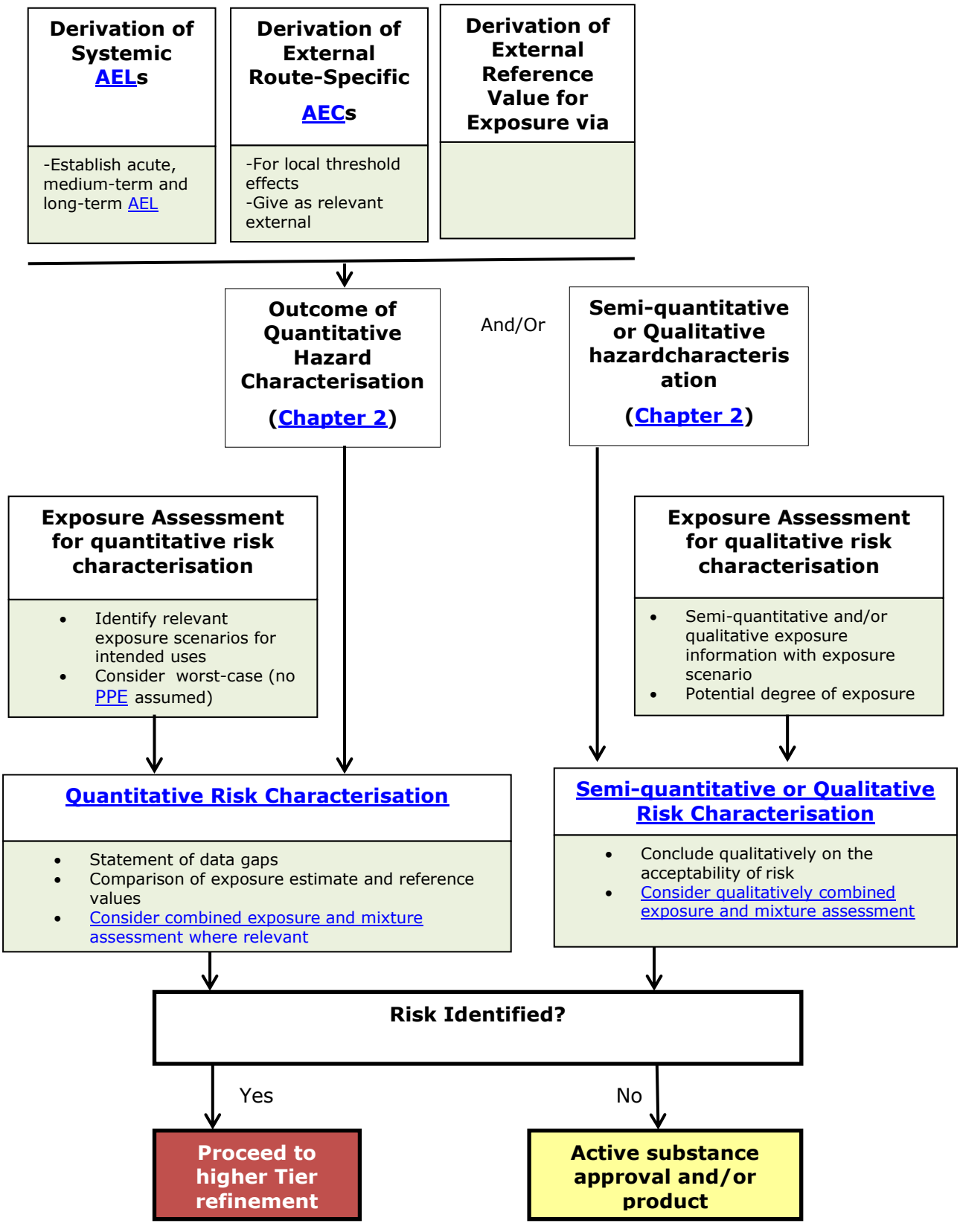
Local effects at the port of entry should be dealt with separately and in principle by a qualitative approach. If an unacceptable level of risk is identified for any of the scenarios in the first tier a refinement of the exposure assessment and/or the assessment factors (e.g. allometric scaling or CSAF) and more specific absorption rates might be performed in the **second tier** 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. If the active substance can enter the food chain, ADI and, if necessary, ARfD should be derived analogously to the procedures for PPPs.

During the approval of an active substance for use in biocidal products, the realistic combination of some uses or scenarios should also be addressed. In addition 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 4.4).

Risk assessment must also address exposure via treated articles via specific PTs where relevant.

The outcome of hazard characterisation (see Section 2), that can be reference values for quantitative risk characterisation (for threshold effects) or qualitative estimates (e.g. hazard classification) or semi-quantitative estimates (e.g. DMEL), and the outcome of exposure assessment are taken forward in risk characterisation as shown in Figure 12 **: Risk Characterisation on the basis of output from hazard characterisation (Dose-response/concentration relationship)**. Risk characterisation depending on the input from both hazard and exposure assessment can be either quantitative or qualitative or a combination of the two (mainly for mutagens/carcinogens, irritation/corrosion and sensitisation).

Figure 12 : Risk Characterisation on the basis of output from hazard characterisation (Dose-response/concentration relationship)



4.2 Quantitative Risk Characterisation

Where a critical effect is threshold-based and exposure data are reliable, quantitative risk characterisations for the active substance in the product should be carried out for each exposed population, PT and method of application relevant for the accompanying product(s) as indicated by the exposure assessment. The most appropriate AELs for use in risk characterisation must also be identified and then compared with the exposure estimates for the active substance in the product for the relevant use situations.

In quantitative risk characterisation the exposure estimates are compared to the corresponding AEL (exposure/AEL ratio) for each use and relevant time-frame. If the Exposure/AEL ratio is <1, the risk ratio is considered acceptable. In case of the Exposure/AEL ration >1, the risk is considered unacceptable and further refinement is needed with respect to exposure and/or hazard assessment including risk mitigation measures (see Section 4.6).

4.3 Semi-quantitative and Qualitative Risk Characterisation

The purpose of the qualitative risk characterisation is to assess the likelihood that effects are avoided when implementing the operational conditions and risk mitigation measures that define each scenario. The qualitative risk characterisation approach has to be followed when there is no basis for setting an AEL, AEC or DMEL for a certain human health endpoint, i.e. when the available data for this effect do not provide quantitative dose response information, but there exist toxicity data of a qualitative nature. The endpoints for which the available data may trigger a qualitative risk characterisation are: irritation/corrosion, sensitisation, carcinogenicity and mutagenicity.

When data are available that allow the derivation of a reference level (e.g. AEL) for an endpoint (including irritation/corrosion, sensitisation, carcinogenicity and mutagenicity), the quantitative or semi-quantitative approach should be followed; for local effects the uncertainties described in Section 4.3.2 , the first step is to define RMMs and OCs. In the semi-quantitative approach for non-threshold carcinogens, the DMEL methodology should be used to judge the remaining/residual likelihood of risks after these RMMs and OCs are implemented.

For a substance where reference levels are derived and quantitative assessment is possible but at the same time for some endpoints qualitative assessment is triggered (e.g. local effects), it may not be straightforward to identify the critical effect for the relevant exposure patterns. It cannot be excluded that the quantitative approach will be more protective for the exposure pattern than the qualitative one, except for non-threshold genotoxic substances and possibly respiratory sensitisation. In cases where both quantitative and qualitative approaches need to be followed (e.g. systemic and local effects), these should complement each other at risk management measures level, both demonstrating adequate control of risks.

Additional guidance on the conditions under which risk characterisation for local effects needs to be performed is provided in Section 4.3.2 whereas for non-threshold mutagens and carcinogens in Section 4.3.1.

4.3.1 Non threshold mutagens and carcinogens

Genotoxicity

Since it is usually assumed that a threshold does not exist for genotoxicity (with the possible exception of aneuploidy) genotoxicity studies cannot provide any quantitative input to the risk characterisation. However, a conclusion that potential for genotoxic activity exists 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.

The risk to the general public from secondary exposure to these substances would also usually be unacceptable.

When it comes to category 2 mutagens, these 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

The acceptability of the risk from active substances contained in biocidal products for which carcinogenic potential exists will depend upon the appropriate category of carcinogenic classification, the likely mechanism of carcinogenicity and the extent of exposure.

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 risk to the general public from secondary exposure to these substances would also usually be unacceptable. The inclusion of active substances meeting the criteria for category 1B classification will be strongly dependent upon the mechanism and levels of exposure. If the 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 (see section 2.11) which provides a means to assess the efficiency of risk management measure ensuring negligible exposure. In the latter case, the derived reference dose (e.g. DMEL) is compared to the exposure estimate to conclude whether the risk is as low as reasonably practicable.

4.3.2 Local effects (irritation/corrosion, sensitisation) – Qualitative and semi-quantitative risk characterisation

RC for local effects is triggered only when the biocidal product is classified for local effects. RC for local effects is not required when the active substance and/or co-formulants in a product are classified for local effects but are present at concentrations that do not trigger classification of the product according to the CLP criteria (*Guidance on the Application of CLP Criteria*).
RC for local effects should be performed for all relevant exposure scenarios. For situations where the biocidal product is classified but the in-use dilution isn't, a description of the exposure scenario involving the in-use dilution still needs to be provided.
It is critical that RC for local effects focuses on the product, rather than the active substance only. If all essential information on the product composition and respective

local hazards of the active substance aren't available at the evaluation stage, but local effects (when fulfilling one of the conditions outlined below) are observed or expected from the representative biocidal products, then the RC for local effects of the representative products shall nevertheless be performed at this stage (on the basis of the limited information). In this case, the RC may need to be refined at product authorisation stage.
A classification proposal or a self-classification or an adaption proposal for specific classification limits for the active substances, co-formulants or products is sufficient for triggering RC for local effects. The availability of a harmonised classification for the active substance or co-formulants should not be a pre-requisite for conducting risk characterisation for local effects.
Local effects that need to be considered for risk characterisation following the guidance within this section should fulfill at least one of the following conditions:

- Irritation or corrosive effects that lead to classification with H314 (Causes severe skin burns and eye damage) H315 (Causes skin irritation), H318 (Causes serious eye damage) or H319 (Causes serious eye irritation), are usually the result of acute studies with a single concentration.
- Irritation effects on the respiratory tract that lead to classification with STOT SE 3 – H335 (May cause respiratory irritation) or with EUH071 (Corrosive to the respiratory tract) or STOT RE (respiratory tract, eyes, skin, GIT) are usually based on observations in single and repeated exposure studies in animals or from human data.
- Effects that lead to classification with EUH066 (Repeated exposure may cause skin dryness or cracking) are usually based on specific relevant evidence.
- Sensitising effects that lead to classification with H334 (May cause allergy or asthma symptoms or breathing difficulties if inhaled) or H317 (May cause an allergic skin reaction) are usually the result of human data or animal studies, respectively. Some studies may provide certain dose response information that tends to be suitable for potency estimates only.
- Other local effects that do not lead to any classification are not considered as severe enough to require any type of risk assessment for local effects.

4.3.2.1 Definitions for risk characterisation for local effects

Quantitative local RC: The hazard as well as the exposure and risk part of the RC, are quantitative. This means that an AEC is compared with quantitative exposure estimates.

Qualitative local RC: The hazard as well as the exposure and risk part of the RC is qualitative. This means that for the hazard characterisation part, primarily classification is used to assign the substance to one of four categories of hazard severity (very high, high, medium, and low). 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, and duration of exposure, potential degree of exposure (amount and concentration of substance used) and relevant RMMs. Acceptability or non-acceptability of the risk is described as a list of qualitative arguments.

In practice the RC for local effects may also be an intermediate step/combination between these two approaches and this may be termed **semi-quantitative RC.**

- **Risk characterisation for local effects versus risk characterisation for systemic effects**

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 (see e.g. approach for formaldehyde releasers). Furthermore, the local toxicity of the active substance is particularly influenced by the potential of the other product ingredients and solvents to induce local effects as well as the pH of the product. This means that for different products and/or different intended uses or PTs, local or systemic effects may be more critical.

Also, in case of local effects present, it is often unclear whether the observed systemic effects are true primary effects or secondary to the local toxicity of the substance.

Therefore, where RC for local effects is triggered, a RC for systemic effects should always be performed in addition.

However, if it can be shown by a first tier systemic risk assessment that local effects are much more critical than systemic effects; higher tier assessments for systemic effects could be omitted, if full justification is provided.

4.3.2.2 Uncertainties to be considered for risk characterisation for local effects

i) Uncertainties for all exposure routes

Data that are potentially useful for a quantitative RC for local effects contain usually several types of additional uncertainties compared to those considered for systemic effects.

- Uncertainties due to LOAEC to NOAEC extrapolation, exposure-time extrapolation, intra-species and interspecies differences are usually addressed with assessment factors. However, data-based probabilistic information on the extrapolation uncertainties are only available for systemic effects; in contrast, assessment factors proposed for local effects (presented e.g. ECHA REACH Guidance
- IR+CSA Chapter R.19) are not informed by probabilistic data-bases and, thus, their application is substantially more uncertain (for some literature references addressing knowledge about uncertainties of local thresholds, see Appendix 4-1).
- Other uncertainties usually not considered for systemic AEL derivation are very important for local AEC estimation, especially substance - product differences. The pH of the product and the presence of co-formulants or other irritant substances may strongly influence the potential of the active substance to induce local toxicity, rendering any local AEC established for the active substance inappropriate for the hazard characterisation of the active substance in the product (see Appendix 4-3).

ii) Additional considerations for the dermal route

- Co-exposure to additional dermal stressors is also particularly important in relation to local effects. Fluhr *et al*. (2008) emphasise the necessity to consider synergistic effects with mechanical and physical stress on the skin, e.g. from water at wet work places. Endpoint uncertainty is another issue to be considered; skin irritation or sensitisation may be quantified by various methods and parameters (heat, redness, swelling and dysfunction) showing different sensitivity (see e.g. Fluhr *et al*., 2008; recommending a multiparametric approach). In addition, the relevance of semi-occlusive conditions and amount of substance per treated skin area in the animal test to the real human exposure situation represents another source of uncertainty.
- Exposure models or measurements usually provide highly uncertain dermal local exposure values which are not suitable for the risk assessment of local effects. These tend to be averaged values over time and skin surface. In contrast, peak

and localised skin area concentrations (e.g. in wrinkles) of the substance are known to drive local effects (Schaafsma *et al*., 2011).

iii) Additional considerations for the respiratory route

- Airway anatomy, respiratory rate, deposition patterns and consequently local and total clearance rates differ between the animal models and humans and have to be accounted for. Considering increased respiratory rate of small animals compared to humans and considering modelling data for the species-specific deposition patterns, ECETOC (2003b) concluded that effects in the rat nasal cavity are likely to overestimate effects in humans by a factor of 2 to 4. However, the *Guidance on information requirements and chemical safety assessment Chapter R.19* considered that data informing on animal-human respiratory differences are insufficient and it is prudent to assume that humans are more sensitive than animals; a default dynamic factor of 2.5 was proposed, as would be the case for systemic effects. The empirical data base informing this interspecies uncertainty factor for local respiratory effects is very weak (see Appendix 4-1)[21].
- Active substance and products may be different with regard to their physical form; gas or aerosol exposure may lead to different distributions in the respiratory tract and consequently different effects.
- With aerosol exposure, different active substance concentrations in the aerosol, different aerosol mass per air volume and different aerosol droplet size distribution may lead to different effects. Not all of the potentially relevant combinations of aerosol concentrations and mass per air volume can be tested.

iv) Additional considerations for the oral route

In principle, the relevance of the rat forestomach irritation is questionable for human risk assessment (Wester *et al*., 1988; IARC, 1999b; ECETOC, 2006; Proctor, 2007). 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. In contrast, 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 has a medium pH= 4.5 - 6, the human oesophagus has a pH= 7 and the human stomach a pH= 1 - 2 (fasting). But probably most important, the contact time between the oesophagus epithelium and ingested material is negligible in humans when compared to the rodents' forestomach, which 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. Therefore, it was suggested by Harrison (1992) that NOELs or concentrations for irritant effects should be determined in those parts of the GI tract having a counterpart in humans, such as oral cavity, pharynx and oesophagus, glandular stomach or intestine.

[21]However, it is acknowledged that also the assessment factor for systemic effects represents a policy decision: While the standard factor is 10 for rat-human extrapolation, data based probabilistic assessment factors range from 4 (geometric mean) to 40 (95th percentiles) (Schneider et al., 2006; Bokkers and Slob, 2007).

v) Additional considerations for sensitising effects

- For respiratory sensitisation no internationally accepted tests are available, though models that can indicate respiratory sensitisation potential are being developed.
- Skin sensitisation tests simulate the induction and/or challenge phase of allergic contact dermatitis in humans/type IV hypersensitivity reactions in animals (= sensitisation and elicitation). In the literature, the quantitative risk assessment method focuses on the induction phase but the available approaches need further scientific clarification. However, where induction was not effectively prevented and where people are already sensitised these approaches would not protect against the challenge of elicitation because of the lower doses required.
- Animal-based skin sensitisation tests, whilst indicative of potency, were not validated to predict thresholds for human sensitisation, but to qualitatively identify and classify skin sensitising substances. However, there are publications proposing that the EC3 value obtained from the LLNA could be used as a point of departure for AEC derivation (ICCVAM, 2011; Basketter *et al*., 2005b).
- The advantages and drawbacks of various study types for skin sensitisation are summarized Table 23 below.

Table 23: **Advantages and drawbacks of skin sensitisation tests**

	Advantages	Drawbacks
HRIPT*	- data directly obtained on the species of interest (human)	- groups of people are chosen, relatively low numbers; may not represent the diversity observed in the general population - new HRIPT should not be conducted due to ethical reasons - risk of iatrogenic sensitisation - usually poor transparency of the test method, unknown reproducibility and relevance of endpoint for human disease (Basketter, 2009; EAHC, 2009)
LLNA	- the results are objective - reduced number of animals used compared to M&K - a dose-response curve can be obtained - the critical doses correlate with human test results; there is support for the correlation between EC3 and human thresholds (Schneider & Akkan, 2004; ICCVAM, 2011; Basketter *et al*., 2005b) - data can be used to categorize a sensitisation potential (ICCVAM, 2011) - data can be used for risk assessment	- high number of false positive results for some chemicals (surfactants, fatty acid type substances, siloxanes) - false negatives for some metals (nickel) - vehicle impact on results
M&K	- can be used to define a dose-response relationship for challenge in some cases - high volume of data collected on a wide range of contact allergens for humans (historical database)	- animal suffering - one single concentration tested in general - results expressed in reaction

	Advantages	Drawbacks
	- can be considered as a reference procedure to detect contact allergens and tempt to extrapolate results to humans (but not the gold standard)	intensity - subjectivity of the results - tests on coloured substances non appropriated : results can be distorted
Buehler Method	- produces less false positive results than M&K -sensitive enough to detect moderate or strong sensitisers	- reduced sensitivity - bipolar test and only qualitative - results are not often extrapolated

HRIPT*: Some HRIPTs are only designed to detect cutaneous irritation and do not detect the sensitising potential.

4.3.2.3 Decision logic for performing (semi)-quantitative or qualitative risk

i. Respiratory and Skin Irritation/Corrosion

In view of the uncertainties described above, usually no local AEC should be derived and the RC for local effects should not be quantitative but qualitative.

However a semi-quantitative or a quantitative RC for local effects may be carried out in scenarios where the uncertainties described above are sufficiently controlled, e.g. if:

- Dose response information is available (usually for the active substance); and
- the active substance is the same as the biocidal product or the products are simple dilutions of the active substance without relevant changes of pH and solvent; and
- a NOAEC is available from relevant and reliable data covering the exposure period of concern.

- The first and last indents are more often true for respiratory irritation/corrosion than for dermal irritation/corrosion. Therefore, for respiratory irritation/corrosion, a local semi-quantitative or a local quantitative RC may be more appropriate than a qualitative RC on a more frequent basis.

ii. Respiratory and Skin Sensitisation

For respiratory sensitisation and dermal sensitisation only a qualitative RC should be carried out.

The qualitative approach may be complemented with a semi-quantitative or quantitative approach, depending on the available data and possibilities to control the described uncertainties. Quantitative methodologies for dermal sensitisation are available but need further scientific clarification, e.g. as described in Appendix 4-4. No established quantitative methodologies are available for respiratory sensitisation.

iii. Gastrointestinal Irritation

It is expected that a specific risk assessment for local GI(T) effects will be required only in very exceptional cases, since:

- Risk assessment for local effects is triggered by classification of the product. Theoretically, STOT RE classification may be possible for local GI(T) effects. However, this tends to be rare.
- GI(T) exposure to concentrated products classified for local effects is most likely to be accidental.

- It is often not easy to distinguish local GI(T) effects from secondary systemic effects. Therefore, risk assessment for local GI(T) effects will expectedly be often covered by the risk assessment for systemic effects and systemic AEL values derived from oral studies.

Since risk assessment for local GI(T) effects will be carried out very rarely, no specific guidance appears necessary so far. Assessment may follow case specific approaches respecting the specific available data and assessment needs as well as the most recent state of science. Principle concepts may be borrowed from this guidance. In any case, the assessments would be reviewed by the BPC.

4.3.2.4 (Semi-) Quantitative RC for local respiratory and skin effects

i. Respiratory and eye irritation 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 proposed by the *Guidance on information requirements and chemical safety assessment Chapter R.19*, shall be applied with the exception that the same intraspecies AF shall be applied **for professionals and non-professionals** (referred to as "workers" and "general population" in REACH, see box below).

Assessment factors for respiratory exposure:
Interspecies AF = 2.5 (default)
Intraspecies AF = 10

Deviation from the default AF values proposed in REACH should be considered on a case-by-case basis, and the scientific reasoning/justifications should always be given.

This AEC should then be compared with the external inhalation exposures, also expressed in mg/m^3.

ii. Skin irritation effects - Semi-quantitative approach

For dermal irritation effects a full quantitative RC (using assessment factors) is difficult, because dermal exposure models and measurements in terms of dermal dose per surface area are often not available and if available they are considerably uncertain: It is assumed that skin irritation is strongly influenced by peak exposure. However, exposure measurements are usually integrated or averaged over time and peak exposure in wrinkles remains unknown. In addition there is considerable uncertainty on the dose per surface area of the body coming into contact with the substance when personal protection (clothing, coveralls and gloves) is worn. Therefore tier II control measures cannot be taken into account in a quantitative manner.

Consequently, for local dermal effects, semi-quantitative hazard and exposure information could be used to support the qualitative risk assessment and resulting decision-making. The NOAEC (or the LOAEC if a NOAEC cannot be established) identified from the available animal or human data should be expressed as a percentage concentration (%). This NOAEC should then be compared directly with the in-use concentration (%) of the active substance in the representative product in each scenario without applying assessment factors. This comparison is meant to provide only an approximation of the magnitude of the risks rather than a precise, quantitative measure of the risks involved.

iii. Addressing uncertainties of quantitative or semi-quantitative risk assessment for local effects

In line with the *Guidance on information requirements and chemical safety assessment Chapter R.19 (uncertainty analysis)* an evaluation of the uncertainties in the hazard and exposure assessment may be carried out in addition to the quantitative or semi-quantitative risk assessment for local effects. This could start from a general checklist (see Appendix 4-2), that is tailored to case-specific needs and indicates which uncertainties were addressed by assessment factors and which of the remaining individual uncertainties tend to over- or underestimate the risk estimate or may influence the risk estimate in either direction.

In addition, the uncertainty analysis as described in Section 4.6 for higher tier refinement of risk assessment should be considered.

4.3.2.5 Qualitative RC for local effects

In case a qualitative RC for local effects is necessary, all available information on potential local effects and possible exposure shall be taken into account.

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* shall be applied. With regard to local dermal effects, the refined approach published by Schaafsma *et al*. (2011) should be taken into consideration.

The following steps should be followed:

- Describe the local hazards (that lead to classification under the DPD or CLP Regulation) of the (representative) biocidal product and its in use dilutions, using results from acute test data on the active substance and co-formulants or the product itself and additional relevant information (e.g. including special information on formulation, see Appendix 4-3).
- Assignment of hazard categories described by its classification to one of four categories (very high, high, medium, or low) as indicated in Section (i) below and in Table 24.
- Identification of the exposure scenarios: persons and animals exposed (industrial or professional workers, general public, children, infants, pets, farm animals, etc.); tasks/uses/processes involved; relevant exposure routes. Describe for each exposure scenario the frequency and duration of potential exposure and a potential degree of exposure (if available and relevant, use Tier I estimates from systemic exposure assessment) and illustrate the operational conditions, RMMs and PPE already in use.
- Acceptability or non-acceptability of the risks (supporting arguments) is determined on the basis of qualitative arguments, as suggested in Section (iii) below and Table 25.
- Concluding qualitatively on the acceptability of risk: Guidance to decide on the acceptability of exposure for each of the hazard categories is given in Section (iv) below and in Table 26 for the general public and Table 27 for professionals. The guidance takes into account: (1) frequency and duration of potential exposure, (2) potential degree of exposure, (3) necessary operational conditions and other RMMs, (4) necessary PPE. For each exposure scenario the minimum requirements for all 4 indicators of exposure should be met to support that the risk is acceptable. Expert judgment is necessary when evaluating (a) if the RMMs and PPE given in the tables can be met in the specific exposure scenario and (b) if deviations from the frequency and duration of potential exposure and degree of

exposure as well as deviations from the minimum RMMs and PPE required (including e.g. missing RMM/PPE, substitution by other means) may be acceptable. The conclusion on the acceptability of the risk should be accompanied by a narrative of the uncertainties in the data underpinning the conclusion.

The examples tabled in Appendix 4-5 may be used as templates to describe the hazard, exposure, risk and related uncertainties.

i. Assignment of Hazard categories

For local effects where no quantitative dose-response information of relevance to the product/in use solutions is available, a qualitative assessment needs to be performed. The general approach when no AEC can be derived aims at reducing/avoiding contact with the product/in use solutions. However, implementation of RMMs, engineering controls and other risk mitigation measures needs 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.

Consequently, the approach suggested in this section is based on the principle that the greater the potential hazard, the stricter the controls. Conversely, this implies that the lower hazard categories require less strict controls.

To provide practical guidance for the qualitative approach, a categorization of hazards (very high, high, medium, and low) is proposed (see Table 24, below).

It is suggested to allocate the local hazards identified for the product/in-use dilution to one of four categories, which are based on two key factors:

- The seriousness of the resultant health effect in terms of irreversibility, life-threatening and long-term consequences. For example, sensitisation is considered to be more serious than mild irritation because of its potential irreversibility and long-term consequences.
- The potency of the product/in use solution in relation to a particular toxicological endpoint. For example, more stringent control would be advocated for a strong skin sensitiser than for a moderate one. The same is also true for a strong corrosive in relation to an irritant.

To ensure consistency in the allocation of products/in-use solutions to the four hazard categories of very high, high, medium and low, a simple and transparent approach to hazard identification is required. It is proposed that the EU hazard classification system (both that under the DSD/DPD and that under the CLP Regulation) is used as a descriptor of the hazards since the classification system for these local effects tend to reflect the qualitative and semi-quantitative nature of the information that is usually available for these endpoints.

Table 24: Hazard categorisation of local effects

Hazard category	Relevant local effect	R-phrase under the DSD/DPD assigned to the biocidal product and/or its in-use solutions	Equivalent CLP hazard classification assigned to the biocidal product and/or its in-use solutions
Very high	Extreme skin sensitiser[1]	R43	Skin Sens 1A or Skin Sens 1 (H317) and potency evaluated as "extreme" according to CLP guidance, or Equivalent level of hazard

Hazard category	Relevant local effect	R-phrase under the DSD/DPD assigned to the biocidal product and/or its in-use solutions	Equivalent CLP hazard classification assigned to the biocidal product and/or its in-use solutions
	Strong respiratory sensitiser	R42	Resp Sens 1A (H334) or Resp Sens 1 and potency evaluated as strong according to the CLP
	Strong corrosive	R35	Skin Corr 1A (H314)
High	Strong skin sensitiser[2]	R43	Skin Sens 1A (H317) or Skin Sens 1 (H317) and potency evaluated as "strong" according to CLP guidance, or Equivalent level of hazard
	Moderate respiratory sensitiser	R42	Resp Sens 1B (H334) or Resp Sens 1 and potency evaluated as moderate according to the CLP
	Corrosive	R34	Skin Corr 1B/1C (H314)
	Corrosive to the respiratory tract	N.A.	EUH071
	Severe eye irritant	R41	Eye Dam 1 (H318)
Medium	Moderate skin sensitiser[3]	R43	Skin Sens 1B (H317) or Skin Sens 1 (H317) and potency evaluated as "moderate" according to CLP guidance, or Equivalent level of hazard
	Specific target organ toxicity – repeated dose	R48/23, R48/24 or R48/25	STOT RE1 (H 372, local effects skin, eye, RT, GIT)
Low	Irritant to skin	R38	Skin Irrit 2 (H315)
	Irritant to eye	R36	Eye Irrit 2 (H319)
	Irritant to respiratory tract	R37	STOT SE 3 (H335)
	Causes skin dryness	R66	EUH066
	Specific target organ toxicity – repeated dose	R48/20 or R48/21 or R48/22	STOT RE 2 (H373, local effects skin, eye, RT, GIT)

[1] Substances or mixtures can be placed in this category either when they can be sub-classified in the "Extreme skin sensitiser" category on the basis of a skin sensitisation test (using criteria from the CLP Guidance), or if no test is available, on the basis of an expert judgement which by taking into account all available information (concentration of the sensitising substance compared to its SCL, physical form, formulation of the product,...), establishes that the "Extreme sensitiser" classification is warranted.

[2] Substances or mixtures can be placed in this category either when they can be sub-classified in the "Strong skin sensitiser" category on the basis of a skin sensitisation test (using criteria from the CLP Guidance), or if no test is available, on the basis of an expert judgement which by taking into account all available information (concentration of the sensitising substance compared to its SCL, physical form, formulation of the product,...), establishes that the "Strong sensitiser" classification is warranted.

[3] Substances or mixtures can be placed in this category either when they can be sub-classified in the 1B/"Moderate skin sensitiser" category on the basis of a skin sensitisation test (using criteria from the CLP Guidance), or if no test is available, on the basis of an expert judgement which by taking into account all available information (concentration of the sensitising substance compared to its SCL, physical form, formulation of the product,...), establishes that the "Moderate sensitiser" classification is warranted.

It is proposed that in the "very high" hazard category, skin sensitisers and corrosives with very high potency (extreme skin sensitisers and strong corrosives) and strong respiratory sensitisers are included. Strong respiratory sensitisers are allocated to this category on the basis that exposure to such products/in-use dilutions should be strictly contained because current methodologies do not allow us to adequately assess the risks associated with their use. Extreme skin sensitisers and strong corrosives are assigned to this category because they cause serious, potentially irreversible effects at extremely low concentrations.

The "high" hazard category includes strong skin sensitisers, moderate respiratory sensitisers and corrosives (including corrosives to the respiratory tract and severe eye irritants) with significant potency. These products/in use solutions are allocated to this category because they cause serious, irreversible effects at relatively low concentrations.

The "medium" hazard category includes moderate skin sensitisers. These products/in-use dilutions are allocated to this category because they cause significant, possibly irreversible effects at not so low concentrations.

The "low" hazard category includes the moderate irritants and the products/in use-dilutions which cause skin dryness. They are allocated to this category because they cause moderate, reversible effects at relatively high concentrations.

The potency evaluation and hazard categorization as described above for biocidal substances, products and in-use solutions could potentially result in two products with very different concentrations of active substance being categorized in the same way. It should, therefore, be based on careful scientific considerations including not only results from formal classification rules but using all relevant available information including tests on substances and on products, concentration of the chemical, physical form, physico-chemical interactions and taking into consideration any possible formulation effects (see Appendix 4-3).

ii. Identification of exposure scenarios - Exposure indicators

The following qualitative information on the **exposure scenario** should be provided:

- **Who is exposed:** general public (adults, children, infants, pets), professionals or industrial workers
- **Tasks/uses/processes:** for example, spraying on floors outdoors, dilution by pouring into pond (see examples in Appendix 4-5)
- **Potential exposure route:** skin, eye, respiratory tract, gastrointestinal tract

The following semi-quantitative and qualitative **exposure information** should be provided separately **for each exposure scenario**:

(1) frequency and duration of potential exposure

A realistic worst case indication for the maximal likelihood of exposure should be provided. The likelihood of exposure increases with the frequency and duration of the task/use/process. The duration of potential exposure might be significantly lower than the duration of task/use/process and may also be different for different exposure routes.

(2) potential degree of exposure

If exposure estimates are available, e.g. from the exposure assessment for systemic effects, these may be described, e.g. in terms of ml/m^3 air or ml/cm^2 skin or mg/person. For the qualitative assessment, this semi-quantitative information is not decisive, but may be of value for the overall conclusion on the acceptability of exposure. If no reliable exposure estimates are available, they do not need to be generated.

(3) operational conditions and other RMMs already in use or additionally required

(4) PPE already in use or additionally required

Operational conditions in terms of technical and organisational provision and other RMM (including e.g. special formulations with microencapsulation, special packaging and others, see Appendix 4-3) as well as PPE should be considered. The RMMs and PPE should be indicated that are already in use and, if necessary, those that may be realistically described in the CAR. Potentially relevant RMMs and PPE are listed in Tables 26 and 27.

iii. Acceptability or non-acceptability of risk – Supporting Arguments

Table 25: Examples of qualitative arguments supporting acceptability or non-acceptability of risk

Support for acceptable risk	Support for non-acceptable risk
For products or in use dilutions that are not classified the risk for local effects should always be considered as acceptable.	
+ reversible effect + adverse effect expected only after repeated, prolonged exposure (e.g. STOT-RE and EUH066)	- irreversible and/or severe effect[22] (e.g. Cat. 1 effect) - 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 (liquid or solid) aerosol formation - high viscosity of product (aerosol formation and potential for splashes reduced) - high ventilation expected, e.g. due to outdoor use - no direct contact with skin, eye, GT expected	- high exposure (approximate information) : • high amount used per event • high vapour pressure • high (liquid or solid) aerosol formation • low viscosity of product • low ventilation expected (e.g. indoor use) • direct contact with skin, eye, GT expected • high exposure level compared to

[22] Severity of the effect can be assessed if available, e.g. for skin irritation/corrosion according to the Draize score, for eye irritation based on the degree of inflammation, for skin sensitisation based on e.g. chronic dermatitis, generalised (systemic/whole body) dermatitis and/or hospitalisation, and for respiratory sensitisation based on the duration and degree of the symptoms. Scores from specific *in vitro* tests may also be used as an information source.

- low exposure level compared to adverse effect concentration (LOAEC) or no adverse effect concentration (NOAEC) if available	adverse effect concentration (LOAEC) or NOAEC, if available
+ 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	- necessary operational RMMs not applicable, not feasible 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 for proper 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
+ proper 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 Appendix 4-3) reduce or eliminate exposure and/or expression of the hazard	- special formulation effects increase exposure and/or expression of the hazard

iv. Concluding qualitatively on the acceptability of risk

Tables 26 and 27 provide guidance for the acceptable maximum frequency and duration of potential exposure and potential degree of exposure for each effect in each hazard category.

This approach should be applied to each exposure scenario (who is exposed, task/use/process, exposure route).

Some of the descriptions for the different exposure indicators are rather vague. This is to allow a flexible application of the guidance. It should be noted that the duration of the actual exposure might be significantly lower than the duration of task/use/process and may also be different for different exposure routes.

The degree of potential exposure under best practice conditions is described qualitatively in terms of tasks and related expected exposures. In addition, RMMs and PPE recommended for products/in use dilutions assigned to each of the hazard categories are indicated.

The minimum requirements for all four indicators of exposure (four columns: frequency, potential degree of exposure, RMM, PPE) should be met to support that the risk is under control for the assessed hazard category and exposure scenario. Expert judgment is necessary when evaluating (a) if the RMMs and PPE given in the tables can be met in the specific exposure scenario and (b) if deviations from the maximum frequency and

duration of potential exposure and potential degree of exposure as well as deviations from minimum RMMs and PPE (including e.g. missing RMMs/PPE, substitution by other means) may be acceptable.

It should be noted that for the Biocides CA meeting (September 2013) has adopted an opinion/guidance for skin sensitising biocidal products requiring PPE for non-professional uses, outlining the conditions to be considered at product authorisation stage for such biocidal products and exposure patterns. This CA adopted opinion on this topic should be used for this particular scenario.

Table 26: Guidance for concluding qualitatively on the acceptability of the risk for general public

Hazard		Exposure information		
Hazard Category	**Effects**	**Frequency and duration of potential exposure[23]**	**Degree of potential exposure under best practice conditions**	**Relevant RMMs (PPE not relevant)**
Very high	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 the CLP Skin corr. 1A (H314)	n.r.[24]	n.r.	Products normally must not be sold to general public[25]
High	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 the CLP Skin corr. 1B,C (H314)	Equal to or less than once per week and equal to or less than few minutes per day	Practically no exposure, e.g. use of toilet cleaner	Labelling, instructions for use Child proof closure Packaging eliminating exposure Labelling, instructions for use Child proof closure Packaging eliminating exposure Labelling, instructions for use Child proof closure

[23] Duration of potential exposure might be significantly lower than the duration of task/use/process

[24] n.r. = not relevant

[25] Exceptional situations may arise e.g. where: (1) exposure is so low that the risk to public health is considered negligible; (2) a particular hazard is not relevant due to the route of exposure; (3) there is a clear benefit to public health such that withdrawal of the product may result in other more serious health concerns.

Hazard		Exposure information		
Hazard Category	**Effects**	**Frequency and duration of potential exposure[23]**	**Degree of potential exposure under best practice conditions**	**Relevant RMMs (PPE not relevant)**
	Eye dam. 1 (H318) Corrosive to the respiratory tract, EUH 071			Packaging eliminating exposure Labelling, instructions for use Child proof closure Packaging eliminating exposure Labelling, instructions for use Child proof closure Packaging eliminating exposure Products with high viscosity
Medium	Skin sens. 1B, H317, or Skin Sens 1 (H317) and potency evaluated as "moderate" according to CLP guidance STOT RE1 (local effects skin, GI(T)) STOT RE1 (local effects RT, eyes)	Equal to or less than once per week and equal to or less than few minutes per day	Practically no exposure, e.g. use of toilet cleaner	Labelling, instructions for use Child proof closure[26] Packaging minimising risk for exposure
Low	Skin irrit. 2, H315 EUH066 - Repeated exposure may cause skin dryness or cracking Eye irrit. 2, H319	Equal to or less than one hour per day	e.g. use of dish cleaning product or low volume outdoor spray application	Labelling, instructions for use that minimise exposure or possible health effects

[26]According to the CLP regulation EC 1272/2008, child resistant fastening is not formally required for substances and products classified for sensitisation or irritation. Therefore, this requirement has to be especially carefully discussed and agreed between applicant and competent authority.

Hazard		Exposure information		
Hazard Category	**Effects**	**Frequency and duration of potential exposure[23]**	**Degree of potential exposure under best practice conditions**	**Relevant RMMs (PPE not relevant)**
	STOT SE 3, H335 (may cause respiratory irritation) STOT RE2 (local effects skin, GI(T)) STOT RE2 (local effects RT, eyes)			

Table 27: Guidance for concluding qualitatively on the acceptability for professional exposure

VERY HIGH HAZARD				
Hazard	**Exposure**			
Effects	**Frequency and duration of potential exposure[27]**	**Degree of potential exposure under best practice conditions**	**Relevant RMM (copy from REACH guidance, part E)**	**PPE (copy from REACH guidance, part E)**
Skin Sens. 1A (H317) or Skin Sens. 1 (H317) and potency evaluated as "extreme" according to CLP guidance	few minutes per day or less	Very high level of containment, practically no exposure; e.g. exposure similar to that arising from connecting tubes with technical RMM and PPE	**all measures to eliminate exposure as much as possible, such as:** **Technics** - Very high level of containment required, except for short term exposures e.g. taking samples; - Design closed system to allow for easy maintenance; - If possible keep equipment under negative pressure; - Regular cleaning of equipment and work area; **Organisation** - Control staff entry to work area; - Ensure all equipment well maintained; - Permit to work for maintenance work; - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed;	- All skin and mucous membranes with potential exposure protected with appropriate PPE
Resp. Sens. 1A (H334) or Resp Sens. 1 and potency evaluated as strong according to CLP				-Appropriate respirator mandatory unless complete containment is verified for all phases of the operation
Skin corr. 1A, H314				- Face shield; - Substance/task appropriate gloves; - protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure); - Chemical goggles.

[27] Duration of potential exposure might be significantly lower than the duration of task/use/process

VERY HIGH HAZARD				
Hazard **Effects**	**Exposure** **Frequency and duration of potential exposure[27]**	**Degree of potential exposure under best practice conditions**	**Relevant RMM (copy from REACH guidance, part E)**	**PPE (copy from REACH guidance, part E)**
			- Training for staff on good practice; - Procedures and training for emergency decontamination and disposal; - Good standard of personal hygiene - Recording of any 'near miss' situations. Sensitisers - Pre-employment screening and appropriate health surveillance	
Skin sens. 1A (H317) or Skin Sens. 1 (H317) and potency evaluated as "strong" according to CLP guidance	few minutes per day or less	Very high level of containment, practically no exposure; e.g. exposure similar to that arising from connecting tubes with technical RMM and PPE	As in VERY HIGH HAZARD, see the table above	- All skin and mucous membranes with potential exposure protected with appropriate PPE
Resp. Sens. 1B (H334) or Resp Sens. 1 and potency evaluated as moderate according to CLP		Very high level of containment, practically no exposure; e.g. exposure similar to that arising from connecting tubes with technical RMM and PPE		-Appropriate respirator mandatory unless complete containment is verified for all phases of the operation

VERY HIGH HAZARD				
Hazard **Effects**	**Exposure** **Frequency and duration of potential exposure[27]**	**Degree of potential exposure under best practice conditions**	**Relevant RMM (copy from REACH guidance, part E)**	**PPE (copy from REACH guidance, part E)**
Skin corr. 1B,C, H314	few minutes per day or less	High level of containment, practically no exposure; no splashes, no hand to eye transfer, no (liquid or solid) aerosol formation e.g. exposure below or similar to brief contact with technical RMM and PPE, as touching of contaminated surfaces	**Measures to ensure well controlled exposure, such as:** **Technics** - Containment as appropriate; - Segregation of the emitting process; - Effective contaminant extraction; - Good standard of general ventilation; - Minimisation of manual phases; - Regular cleaning of equipment and work area; - Avoidance of contact with contaminated tools and objects; **Organisation** - Minimise number of staff exposed; - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice; - Good standard of personal hygiene.	- Substance/task appropriate gloves; - Skin coverage with appropriate barrier material based on potential for contact with the chemicals; - Substance/task appropriate respirator; - Optional face shield; - Eye protection
Eye dam. 1, H318		High level of containment, practically no exposure; no splashes, no hand to eye transfer, no (liquid or solid) aerosol formation e.g. exposure below or similar to brief contact with technical RMM and PPE as touching of contaminated surfaces		- Chemical goggles
Corrosive to the respiratory tract, EUH 071		High level of containment, practically no exposure; no splashes, no (liquid or solid) aerosol formation e.g. exposure below or similar to brief contact with technical RMM and PPE		- Substance/task appropriate respirator;

MEDIUM HAZARD				
Hazard	**Exposure**			
Effects	**Frequency and duration of potential exposure[28]**	**Degree of potential exposure under best practice conditions**	**Relevant RMM (copy from REACH guidance, part E)**	**PPE (copy from REACH guidance, part E)**
Skin sens. 1B, H317 or Skin Sens. 1 (H317) and potency evaluated as "moderate" according to CLP guidance	few minutes per day or less	high level of containment, practically no exposure; e.g. potential exposure below or similar to brief contact with technical RMM and PPE as touching of contaminated surfaces	**Measures to ensure well controlled exposure, such as:** **Technics** - Containment as appropriate; - Segregation of the emitting process; - Effective contaminant extraction; - Good standard of general ventilation; - Minimisation of manual phases; - Regular cleaning of equipment and work area; - Avoidance of contact with contaminated tools and objects; **Organisation** - Minimise number of staff exposed; - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice; - Good standard of personal hygiene	- Substance/task appropriate gloves; - Skin coverage with appropriate barrier material based on potential for contact with the chemicals; - Substance/task appropriate respirator; - Optional face shield; - Eye protection;
STOT RE1 (local effects, RT, eyes, skin)	few minutes per day or less	high level of containment, practically no exposure; e.g. potential exposure below or similar to brief contact with technical RMM and PPE as touching of contaminated surfaces		-Substance/task appropriate protection (select from box above)

[28] Duration of potential exposure might be significantly lower than the duration of task/use/process

LOW HAZARD				
Hazard	**Exposure**			
Effects	**Frequency and duration of potential exposure[29]**	**Degree of potential exposure under best practice conditions**	**Relevant RMM (copy from REACH guidance, part E)**	**PPE (copy from REACH guidance, part E)**
Skin irrit. Cat 2, H315	More than few minutes but equal to or less than few hours per day[30]	controlled exposure, e.g. respiratory exposure below or similar to spray application with high ventilation or technical RMM and PPE e.g. cleaning and maintenance work with high ventilation or technical RMM and PPE	**Measures to control exposure, such as:** **Technics** - Minimisation of manual phases/work tasks, - Minimisation of splashes and spills; - Avoidance of contact with contaminated tools and objects; - Regular cleaning of equipment and work area; **Organisation** - Management/supervision in place to check that the RMMs in place are being used correctly and OCs followed; - Training for staff on good practice. - Good standard of personal hygiene	- Face shield; - Substance/task appropriate gloves; - protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure)
Eye irrit. Cat 2, H319				- Chemical goggles
EUH066 - Repeated exposure may cause skin dryness or cracking				- Face shield; - Substance/task appropriate gloves; - protection coverall (EN 13034, 13962, 14605 or 943 according to pattern of exposure)
STOT SE 3, H335 (may cause respiratory irritation)				- Substance/task appropriate respirator
STOT RE2 (local effects, RT, eyes, skin)				-Substance/task appropriate protection (select from boxes above)

[29] Duration of potential exposure might be significantly lower than the duration of task/use/process

[30] If duration of potential exposure are less than few minutes per day – no RMM and PPE are necessary

4.3.2.6 Risk due to co-formulants

If the classification of the product (for local effects) is triggered by the active substance, then if unacceptable risks are identified, approval of the active substance would not be possible. However, if the classification of the product (for local effects) is triggered by a co-formulant, then if unacceptable risks are identified, approval of the active substance would still be possible with the provision that the local effect risks posed by the co-formulant are addressed further at Product Authorisation stage.

4.3.2.7 Concluding remarks

This guidance should be used with the necessary flexibility until more experience on the RC of local effects has been gained. Expert judgment should be used to avoid the risk of disproportionate results, taking always into consideration a weight-of-evidence approach and any realistic exposure scenarios.

4.4 Risk Characterisation for combined exposures

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 shall also be taken into account. Furthermore, BPR Article 8 (3) (Chapter II of BPR, Approval of Active Substances) refers also to the necessity for consideration of cumulative effects from the use of biocidal products containing the same or different active substances, whereas for the authorisation of biocidal products cumulative and synergistic effects shall be taken into account (BPR Article 19, 2(c)).

In the past, various terms have been used to describe assessment of effects from exposure to multiple chemicals.

With the aim of international harmonisation, WHO/IPCS within a framework developed for the risk assessment of combined exposure to multiple chemicals (Meek *et al*., 2011) recommends specific terms to be used in this work area.

In line with this, the following terms can describe the different scenarios that can occur from exposure to one or multiple chemicals from the use of biocidal products:

1. Combined Exposure to multiple substances by one source of release(s) and/or use(s)

Guidance on risk assessment from combined exposure to multiple biocidal substances within a single biocidal product is provided in Section 4.4.1. It follows the tiering principles of refinement as described also under Section 4.6 as well as within the WHO/IPCS Framework on Combined Exposures (Meek *et al*., 2011). This Guidance has been developed to assist in the evaluation of biocidal products.

2. Combined Exposure to multiple substances by different sources of release(s) and/or use(s) (also described as cumulative assessment for substances with common mode of action for the purposes of BPR)

For combined exposure to multiple substances by different sources of release and/or uses similar methodology as described in Section 4.4.1 can be considered with modifications taking into account the various exposure scenarios and cumulative effects. The WHO/IPCS framework should be used to build the risk assessment of such cases whereas additional methodology for assessment is provided within the European Commission Report on State of the art report on mixture toxicity (European Commission, 2010).

The WHO/IPCS Combined Exposures Framework (Meek *et al*., 2011) provides risk assessors means to do risk assessment for combined exposure to multiple chemicals using a tiered approach (with refinement in each tier using more sophisticated methods

of assessment) with exposure and hazard components being assessed simultaneously. Important aspects that need consideration for performing risk assessment of mixtures (combined exposure to multiple chemicals) include the decision process of the grouping of chemicals (which substances need to be addressed in the assessment and based on which criteria; common mode of action is the usual practice) and good exposure information (data on exposure) for the mixture components.

Experience from other regulatory frameworks on decision process for performing combined exposure to multiple substances by different uses (cumulative assessments) should be considered (e.g. EFSA, U.S. EPA).

3. Aggregated exposure: Exposure to a single substance from different sources of release(s) and/or use(s)

Risk assessment of biocidal products consists of calculation of risk level by comparison of internal exposure levels with the derived AEL. Therefore the combination of routes of exposure (oral, dermal, inhalation) is already performed by conversion of external exposure levels to internal systemic available concentrations and the ratio of Internal Exposure/AEL is taking into account already all routes of exposure. In case of multiple biocidal PTs containing the same active substance aggregated exposure can be assessed by combining the exposure estimates from uses/releases from the different PTs. However, in particular for articles treated with an active substance this is a complicated assessment since the consumer may be exposed to a vast range of treated articles for which the use frequency as well as the ratio of first-time use versus repeated use (and thus the leaching rate) needs to be considered.

Risk assessment of combined exposure to multiple substances can be based on individual component or whole mixture data.

The term mixture should be used as defined by the CLP Regulation, and consists of at least two substances that are intentionally or unintentionally mixed. Therefore, biocidal products can be considered as mixtures in most cases, with the principles for classification and labelling of mixtures being applicable as described in the *Guidance on the Application of CLP Criteria*.

The term mixture toxicity and mixture assessment refers to the hazard assessment of multiple chemicals/mixtures including hazard characterisation.

Current methodologies for the hazard assessment of mixtures include as a first step the identification of whether the chemicals present in the mixture interact and produce an increased or decreased overall response compared to the expected sum of the effects if each chemical acts 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.

In the case of **non-interaction** two cases are distinguished:

- Dose addition: Two ore more chemicals with same effect on the body and which differ only on potency and for which the combined effect can be estimated from the total dose of all chemicals together.
- Independent action: Chemicals with differing effects on the body and for which the combined effect of two agents equals to the separate effect of each agent alone.
- In the case of interaction two cases are distinguished:
- Synergism: The combined effect of two chemicals is greater than that when no interaction occurs.

- Antagonism: the combined effect of two chemicals is less than that when no interaction occurs.

Methods available for hazard assessment in mixture toxicity as presented reviews made at national and international level and include:

- Hazard index – Hazard Quotient
- Point of departure Index
- Margin of exposure for mixtures
- Toxic Equivalent Factor
- Relative Potency Factor
- Response addition
- Interaction-based Hazard Index

The methods mentioned above are listed in order of increased complexity for their application. In addition use of PBPK modelling to elucidate possible TK interactions of chemicals in mixtures is being proposed as additional methodology when performing hazard assessment of mixtures and combined exposures.

Further guidance needs to be developed regarding procedural aspects on when combined exposure to multiple chemicals (cumulative assessment) needs to be performed for active substances under the BPR.

4.4.1 Risk Characterisation from combined exposure to several active substances or substances of concern within a biocidal product

Hazard assessment of biocidal products would only rely on data on individual ingredients of the product regarding systemic toxicity effects (repeated dose toxicity, carcinogenicity, and reproductive toxicity).

This section on combined exposure to multiple active substances within a biocidal product is based on different recent documents of the Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Consumer Safety (SCCS) Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (SCENIHR, 2011) and some publications found through a literature review.

Two worked examples illustrating the application of the methodology described in this section are available in Appendix 4-7 and Appendix 4-8.

In addition, Annex A presents a practical scheme for the authorisation of biocidal products containing substances of concern and for such cases it should be read together with the principles elaborated in Section 4.4 and in the current section.

For the purpose of this document, mixtures are considered to be multi-substance biocidal products, i.e. containing at least two substances (active or of concern), and the definition is followed according to the CLP definition of mixtures. This means that for these substances of concern, sufficient data should be available to derive Toxicological Reference Values (e.g. AELs). The principles described for classification of mixtures within the CLP Regulation and the *Guidance on the Application of CLP Criteria* also apply in addition to the elements described in this section.

In this section a tiered approach is provided for practical risk assessment of biocidal product dossiers. In addition, a decision tree is presented on how to perform risk assessment of a biocidal product containing several active substances, is proposed. The tiering scheme follows the principles of tiering for refinement of hazard and/or exposure assessment as also presented for the risk assessment of biocidal active substances in Section 4.6.

The preliminary tier aims to identify potential synergistic effects induced by the mixture.

Tier 1 is an intermediary step to verify risk acceptability for each substance[31] used in the product, as currently performed.

It can then be followed by **Tier 2**, which involves assessing the combined exposure to the substances of the mixture/biocidal product. This step is easy to implement and relies on the worst-case scenarios, compared to **Tier 3**, which is more complex but considered to be more realistic regarding the risks to be assessed. Therefore, if a risk is considered acceptable in Tier 2, Tier 3 will not be necessary. Alternatively, Tier 2 can be omitted and mixture effects only be assessed by Tier 3. It is, therefore, recommended to begin by Tier 2 to save resources to assess mixture/biocidal product which are really of concern.

Tier 1: Risk Assessment of substance by substance of the mixture/biocidal product

This step must be undertaken in accordance with the methodology that is currently used for the assessment of products. Each product and each substance is assessed in terms of risks to primary and secondary exposure following all the scenarios which are relevant to the product use.

Exposure for each substance will be assessed taking into account the required level of PPE (determined in the assessment of each substance or imposed by classification). The most protective level of PPE should be taken into account for all substances.

The decision-making criterion for acceptability of risk remains as in the case of quantitative risk characterization (see Section 4.2) unchanged: the estimated level of exposure to each substance must be lower than its AEL[32] or – if an AEL is not available a European validated value (e.g. DNEL for the purpose of REACH implementation but only if a European Authority has assessed and validated this value e.g. Commission, ECHA or an EU Competent Authority for biocides) - in the considered scenario or the HQ. The Hazard Quotient is defined by the ratio of internal exposure and AEL.

HQ= Internal Exposure / AEL

If HQ <1: the risk from the individual components is considered acceptable and the effects of the biocidal product/mixture must be assessed (as outline in Tier 2 below).

If HQ >1: the risk from the individual components is not considered acceptable and before proceeding to Tier 2 refinement of hazard and/or exposure assessment needs to be performed first so that the HQ <1.

It is noteworthy that this methodology for biocidal product assessment can be applied only in order to assess systemic risks. It is not relevant risk assessment for local effects of mixtures (CLP rules would apply in such cases).

It will be necessary to briefly specify the modes of action and the target organs of the substances (SCENHIR, 2011), taking into account the data in the dossier for each substance and to check the studies available in the literature or in a dossier submitted (ecotoxicological studies or efficacy assays by example) on the mixture, to identify potential effects of the mixture (synergy, antagonism) (Boobis *et al*., 2010).

Further guidance on how to establish mode of action is provided by the WHO/IPCS Framework on mode of action (Meek *et al*., 2013; Boobis *et al*., 2008).

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[31] For the rest of the document, the term substance will be used, meaning active substance and substance of concern.

[32] Depending on the scenario, different AELs will be used: acute AEL, mid-term AEL or long term AEL.

If no synergistic effects have been reported, the toxicological effects of the combined substances are considered to be concentration or dose-additive (see definition in Appendix 4-9) by default in Tier 2. This assumption can be considered a worse-case scenario compared to independent action (see definition in Appendix 4-9).

If synergistic effects have been identified between the substances contained in the products, the risk will only be considered acceptable if the decision-making criterion specified in Tier 2 is met.

Tier 2: Assessment of combined exposure to mixture by concentration (dose) addition

The effects used to establish the AELs for each of the substances in the mixture/biocidal product are considered concentration or dose-additive. This approach is known to be conservative but corresponds to a pragmatically approach avoiding wasted time in a regulated context with many dossiers to assess.

The assessment will be performed with the same parameters as in the first tier. HQ for each substance will be used to calculate a HI for the mixture/biocidal product according to the following method:

HI = Σ HQa.s.

The HI being the sum of the HQs for each substance.

The Hazard Quotient is defined as: estimation of internal exposure/AEL.

If HI ≤1 the risk related to use of the mixture will be considered acceptable;

If HI >1 the risk related to use of the mixture will be considered unacceptable and refinement is needed.

When HI >1, both risk refinement, considering RMM and Tier 3 could be performed in parallel[33].

Refinement with RMM: When RMM are considered, the required level of RMM (determined in the assessment of each substance or imposed by classification) can be increased, taking into account that the conditions related to the different uses should remain realistic. HI is then re-calculated using the new estimate of internal exposure of each substance.

Specific case of synergistic effects

If synergistic effects have been identified or are suspected between the substances in the product, the risk related to use of the mixture will be considered acceptable if the value of HI is less or equal to a reference HI (HI_{ref}). The reference HI should be derived on a case by case basis on the available data. If data is too limited the worst case pragmatic factor of 10 could be used. Consequently, the value of this reference HI would be below to 1 (reference HI to which will be added a safety factor of 10 at a maximum; it is noted that this value of 10 is conservative based on the publication of Boobis *et al.*, 2010 showing that the magnitude of synergy at low doses did not exceed the levels predicted by additive models by more than a factor of 4).

As a result, the decision-making criterion is in this case:

If HI ≤HI_{ref} the risk related to use of the mixture will be considered acceptable;

[33] If a substance has already been assessed to determine specific target organ, AEL and PPE should be considered at the latest level and not in parallel anymore.

If HI >HI_{ref} the risk related to use of the mixture will be considered unacceptable.

Tier 3: Confirmation of concentration (dose) addition in the mixture/biocidal product

Initially, the effects used to establish the AEL for each of the substances are considered concentration or dose-additive by default. This step will either confirm or refute this assumption.

Tier 3 is divided in 3 steps of refinement:

- **Tier 3A**: Combined exposure assessment by grouping the substances with common target organ/mode of action (with the non refined AEL of each substance);
- **Tier 3B**: Combined exposure assessment with specific AEL by target organ/mode of action;
- **Tier 3C**: Combined exposure assessment by considering mechanism of action (if available) (see definition in Appendix 4-9).

Tier 3A:

As a first step, target organ(s)/mode of action(s) for each substance are listed.

Substances are then grouped related to their common target organ(s)/mode of action(s). For each group of target organ, HQ_{to} are summarized for each substance and **approx.** HI_{to} calculated.

Approx. $HI_{to} = \Sigma HQ_{to}$

The decision-making criterion will also be the same; all adjusted HI_{to} values must be ≤1 to consider the risk as acceptable.

If one or more HI_{to} >1, risk is considered unacceptable and Tier 3B could be envisaged.

When a target organ or mode of action is observed for only one substance, there is no need to perform a Tier 3A.

Tier 3B:

In each group for which risk is not acceptable, if AEL are not established on the same organs/modes of action, it will secondly be necessary to determine specific AELs for each identified target organ/mode of action and each substance ($AEL_{a.s.\text{-}to)}$, on the basis of the data used for the European assessment of each active substance or data available for SoC.

AEL by target organ/mode of action will be determined using the same principles as for the AEL defined for the substance (with the same safety factors, according to Section 2 on Hazard Characterisation).

Based on the exposure estimates calculated in Tier 1, $HQ_{a.s.}$-to by target organ will be calculated for each substance and for each common target organ/mode of action: adjusted $HQ_{a.s.}$-to.

An HI for each common target organ/mode of action (HI_{to}) will be calculated using the same principle as in Tier 2:

adjusted $HI_{to} = \Sigma$ HQa.s.-to

The decision-making criterion will also be the same; all adjusted HI_{to} values must be less than 1 in order for the risk to be considered acceptable (or less than the reference HI defined in the second tier if synergistic effects were identified).

If one or more adjusted HI_{to} > 1, risk is considered not acceptable.

In this case, it might be possible to refine the risk assessment, by considering either hazard assessment if data are available and allow to perform refinement (for example skin absorption data for the mixture if default values were used in Tier 1, or exposure assessment (e.g. data under actual conditions of use or any other data or study that may be used to refine the risk (justification of non-additivity of effects, etc.). The principles of higher tier refinement as described under Section 4.6 should be investigated for applicability in refinement of risk assessment also for mixture assessment.

Tier 3C:

When the mechanism of action is known, data could be used to refine the HI_{to}. It will be very rare in practice to have this information in the dossier.

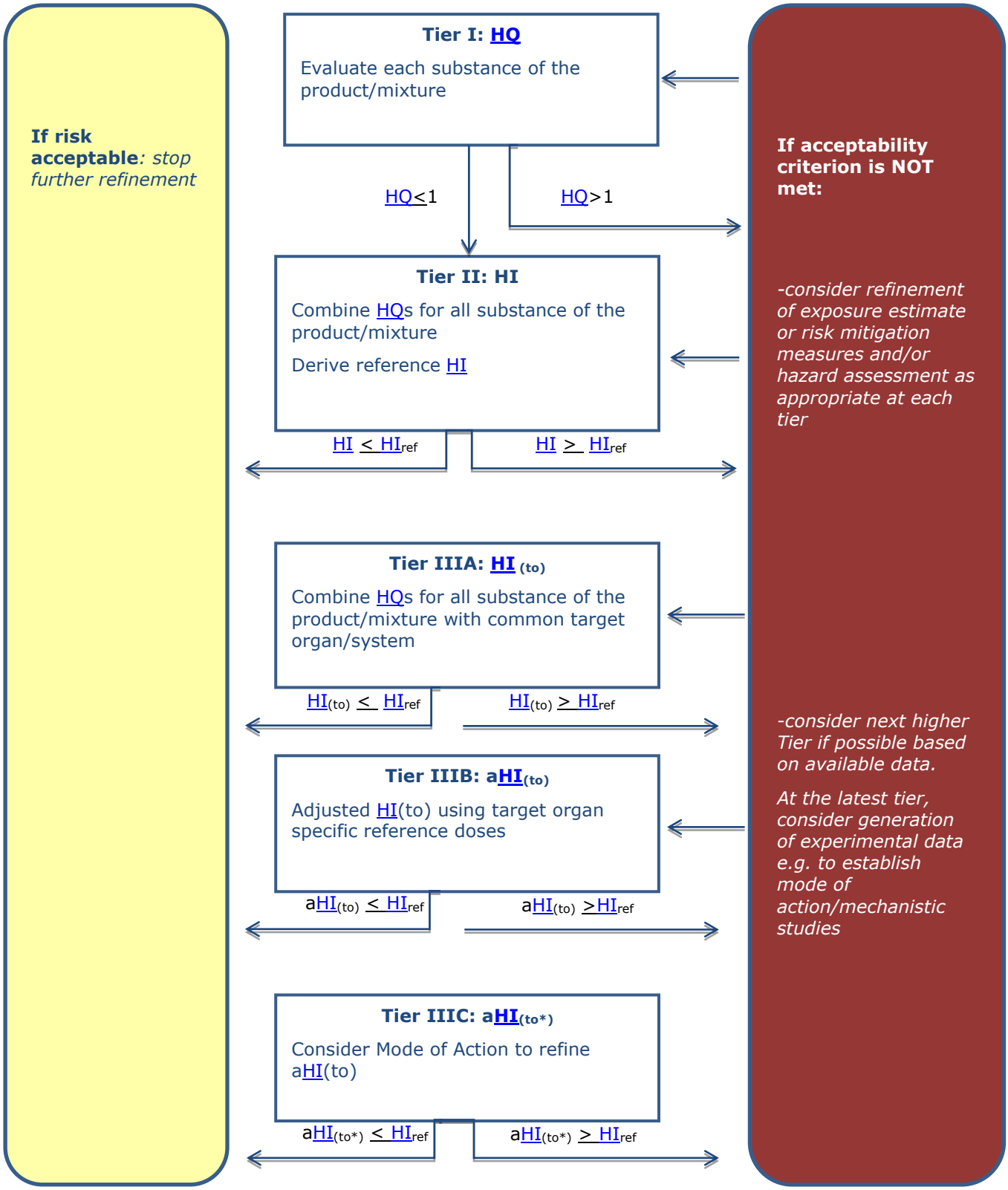
If there is no target organ or mode of action in common, the concentration (dose) addition is not confirmed thus, the effects are considered dissimilar. Consequently, independent action is the rule and the risks are, in this case, covered by Tier 1 of this approach: assessment made substance by substance.

Examples illustrating the approach are described in Appendix 4-7 and Appendix 4-8 from France (Anses) and Germany (BfR).

In conclusion:

- For active substance, to make a list of all the target organs in the CAR of each active substance
- For AEL by target organ/mode of action (see definition in Appendix 4-9), to refer to a common table referencing all AEL specific for target organs by substances avoiding rework nationally and to have harmonized AEL for each substance (see Appendix 4-6). These values should be validated at the Biocides working groups or at another European working group or another proposal could be to discuss only the values for which another Member States disagrees (to define).

Figure 13: Simplified overview of the assessment method: The diagram below shows the risk assessed for each population type: primary and secondary exposure.



A reference HI (HI_{ref}) is used as acceptability criterion. Usually, HI_{ref} is 1 if evaluation of all available data leads to the conclusion that no synergism is expected. If synergism is possible, the HI_{ref} is set on a case by case basis to an appropriate value.
HQ: Hazard Quotient; HI: Hazard Index; $HI_{(to)}$: target organ specific HI (RfDs used as derived); $aHI_{(to)}$: adjusted $HI_{(to)}$ using organ specific RfDs; *: Substance sharing common MoA

4.5 Risk characterisation of exposure via food

So far, quantitative risk characterisation for biocides does not take into consideration additional residues in food and feeding stuffs, e.g. from the use of PPP and VMP. To conduct an overall risk assessment, it would be necessary to cover the total amount of residues from all sources.

4.6 Refinement of risk characterisation in tier approach

If there is a borderline situation or already clear concern, refinement of the risk characterisation should be performed in a second tier. If both quantitative and qualitative risk characterisation approaches were followed in the first tier refinement, it can address both of the approaches, or one of them.

In principle when refinement is needed both aspects of the risk ratio, hazard and exposure need to be considered. Uncertainty analysis can be used as a tool to provide more accurate estimates for hazard or exposure side. For the purpose of using higher tier analysis either in exposure or in the hazard component of the risk assessment guidance is provided within *Guidance on information requirements and chemical safety assessment Chapter R.19* and by WHO/IPCS (WHO/IPCS, 2008; WHO/IPCS, 2013).

In this second tier a **refined exposure estimate** is established by introducing risk management tools. This would concentrate, primarily for professional users, on the input from risk mitigation measures actually used and not yet included in the first tier. Also additional options for exposure reduction, if e.g. addressed by the Applicant, could be taken into account. A refined exposure assessment is obtained then which presumably gives lower values. This estimate is again compared to the relevant reference values (e.g. AELs) to conclude on concern. The modified scenario will lead to a new risk characterisation for approval of the active substance.

Exposure data based on surveys or studies with the actual product or with a surrogate may allow further refinement of the exposure assessment as described in the tier 3 of exposure assessment in the Section for Exposure Assessment (Section 3). When such data is available it should be considered as a further way of refinement if needed at tier 2 of the risk characterisation.

In the second tier **refined hazard assessment** should also be considered together with refinement of exposure estimates where relevant. In this respect considerations on the sensitivity of the subpopulation in question will be integrated in this decision. Thus, adjustment of AFs might be applicable (see also Section 1, Section on TK), if only specific sub-population will be exposed based, on restrictions included in the Union List. If refinement of assessment factors is required the allometric scaling principle (see also Section 2) or data available from the use of PBPK modelling (WHO/IPCS, 2010) can be used[34] when deriving the reference values (e.g. AELs). Reassessment of mode of action and uncertainty analysis (WHO/IPCS, 2013; Meek *et al*., 2013; Boobis *et al*., 2008) or incorporation of refined information on mode action should be taken into account by consideration of kinetic and dynamic parameters suing probabilistic estimates.

There is a need to harmonise the outcome of the hazard assessments for industrial chemicals, plant protection products and biocides. It is proposed that in borderline cases the results from other regulatory frameworks are taken into consideration to give support for the decision. This is subject to the second tier of risk characterisation (see schema below).

[34] The TM has agreed that the intraspecies factors of 10 for professional users cannot be lowered to 5 and no adjustment is possible.

- **Risk Reduction Measures**

If also in this second tier, concern cannot generally be excluded, one possible result of the evaluation could be to request certain risk mitigation measures as essential for approval of the active substance. It might also be concluded that certain data would be necessary for product authorisation, e.g. a dermal absorption study with a real product. Finally certain exposure scenarios could be excluded from Union List inclusion.

The decision to what extent data from the active substance are applicable for the evaluation of risks from use of products, should be made under careful consideration of: (1) route–to–route extrapolation; (2) high dose–low dose extrapolation, as the absorbed percentage generally decreases with increasing concentration; (3) additional substances in the product, e.g. dermal absorption might change if a biocidal product contains solvents acting as skin penetration enhancers; and (4) differences in physical state between active substance and product, e.g. using granular vs. dissolved a.s. in the biocidal product.

Additionally, in depth characterisation of specific situations might be necessary, e.g. concerning a specific inhalation exposure scenario, including considerations, which do not usually belong to the standard repertoire and include a proposal for exposure mitigation.

A flexible risk characterisation methodology is needed to respond to modifications in input parameters, especially if new exposure scenarios are submitted after the Union List inclusion in the national authorisation process or to facilitate the evaluation of route-specific protection measures for occupational risk assessment.

For non-professionals, assumptions on the protective effect of risk mitigation measures, which require a minimum level of knowledge, skill and concerted action, e.g. the use of personal protection equipment, cannot be made. Even the use of gloves cannot usually be expected. Risk mitigation measures for non-professionals have to be conceived in a mode, that the biocidal product is provided to the non-professional/consumer in a state, in which the exposure is reduced or excluded without the need of any concerted action by the user (e.g. effective technical measures like bait boxes for rodenticides and insecticides, safety locks on bait stations).

Thus, exposure reduction by risk mitigation measures for non-professional users is limited to specific cases and cannot generally be included in the risk characterisation procedure.

For professional users the situation is different. Professional users come into contact with active substances in the biocidal products as a consequence of 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 principle should be employed (this is the so-called STOP-principle which stands for Substitution, Technical measures, Organisational measures, Personal protection and which ranks these exposure-mitigating measures in order of priority. Priority is given to technical and organisational measures over personal protective equipment). There are also specialised professional users, who will have expert knowledge and skills in handling hazardous biocidal products. It can well be assumed that for these users the variability in exposure for a certain task is comparably low thereby reducing the uncertainty in risk characterisation.

However, some workers will have limited knowledge and skills to handle hazardous biocidal products – particularly if the use of the biocidal product is not routinely required in their workplace. The exposure conditions of these users might be similar to those of non-professional users. In addition, it has to be taken into account that the extent of

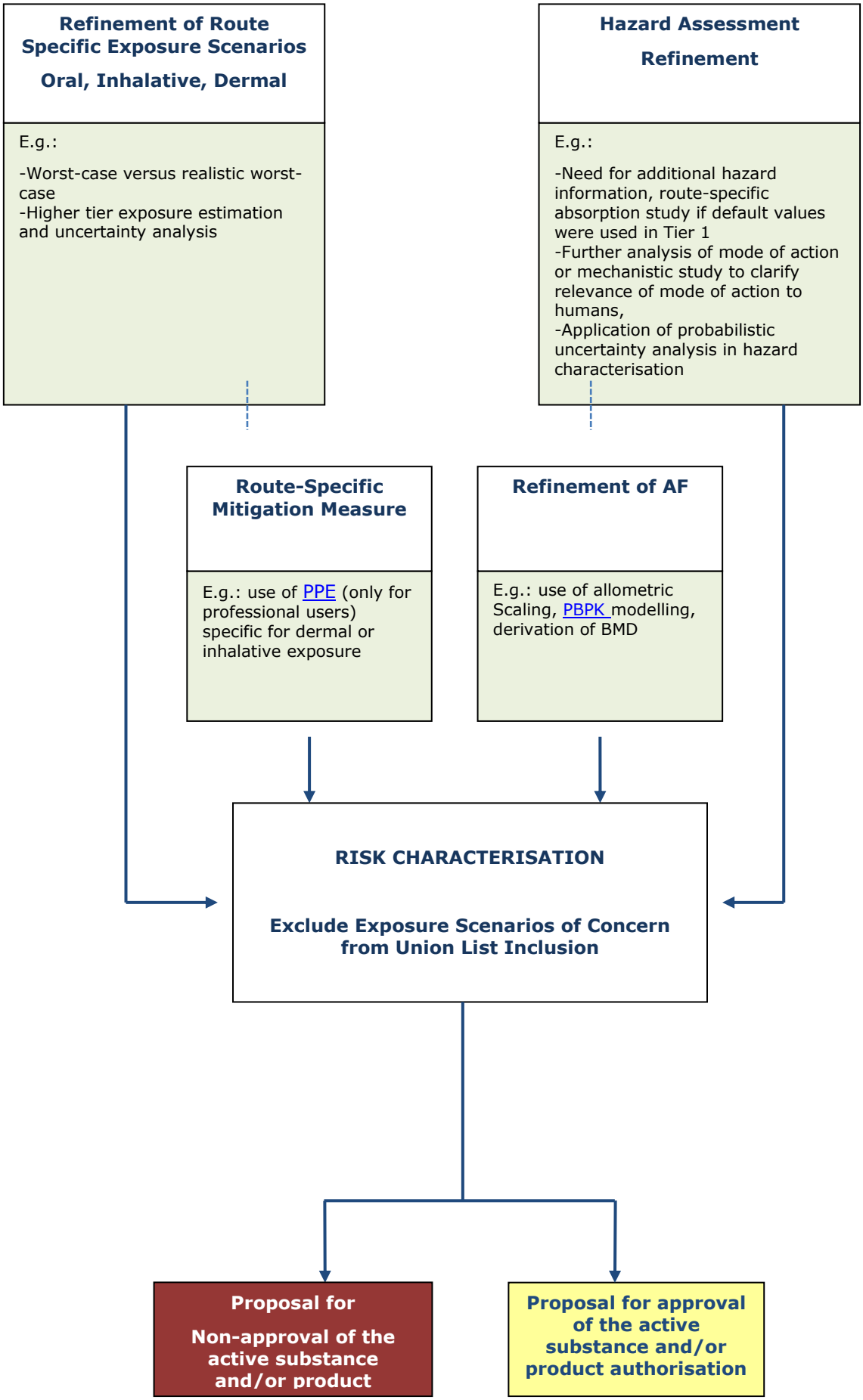
exposure reduction by a certain measure might critically depend on the exposure route and might be different for different parts of the body.

With respect to the time-frame, risk reduction measures for professionals, as a general rule, are oriented either to mitigate single exposure peaks or to reduce shift average values. Therefore, AELs for acute toxicity and chronic toxicity are mostly fully sufficient for the selection of suitable protection measures. In case a certain intermittent exposure scenario is to be evaluated the time-dependency of toxicity should be considered as additional information for the choice of an appropriate risk management strategy. The medium-term AEL will be helpful when evaluating occupational risks, but further support by toxicity data from different time frames might be needed to allow sound extrapolations to the exposure situation in question.

In summary for non-professional users risk reduction by personal protection measures usually cannot be assumed. For professional users the extent of exposure reduction seems to depend on their knowledge, training and skills to handle hazardous substances. Whereas exposure for users with limited knowledge might be similar to those of non-professionals, it can be assumed that for specialised professional users worker protection is effective. It seems essential to consider the degree and reliability of exposure reduction by protection measures case by case before further demanding risk mitigation measures are proposed. The refinement of the exposure assessment therefore resembles an essential element of the second tier in risk characterisation (see schema below)

The following schema summarises the proposed refinement tier approach for human health risk characterisation of biocides.

Figure 14: TIER II - Refinement



4.7 Definitions

AEL: General health-based reference value for the human population as a whole, including sensitive sub-populations. The term AEL resembles the AOEL. According to Directive 97/57/EC establishing Annex VI to Directive 91/414/EEC), the AOEL is defined as "... the maximum amount of active substance to which the operator may be exposed without any adverse health effects. The omission of the term Operator, however, underlines that the AEL is an overall reference value for the human population as a whole. As stated in the draft guidance document on the setting of AOELs: "*The term "AOEL " under Directive 91/414/EEC implies particular reference to "operators" which are represented by mixers/loaders, applicators and re-entry workers. However, according to Directive 97/57/EC, the AOELs established shall also be used to evaluate the possible exposure of non-occupationally exposed groups (bystanders). Therefore, based on the current Community legislation, the AOELs set for operators and workers should be established in such a way that they are also applicable for bystanders.* "Regarding the use of biocides the terms operator (occupational) and bystander (non-occupational) can be misleading in the way that biocides are often used in non-occupational settings and therefore, the user is not bystander but operator. Thus, the omission of Operator for biocidal risk assessment refers to particularities in the use of biocides as compared to plant protection products

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, a particular severity of effect, or a poor data base. A non-exhaustive list of expressions which have been used in the past as synonyms or for specific types of AFs would include any of the following terms: uncertainty factor, extrapolation factor, modifying factor or safety factor.

DMEL: 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.

Non-professional user: Non-professional users belong to the general population, which primarily is exposed to the biocidal products they are applying, mainly consumer products intended for domestic use. Non-professional users include also employed persons at work places, 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 the rest room of a kindergarten or a restaurant by regular employees). To distinguish between professionals and non-professionals might be difficult. Therefore, a clear definition of use and user is required.

OEL: OEL values are set by competent national authorities or other national institutions as limits for concentrations of hazardous compounds in workplace air. Only health effects are taken into account, not other safety issues such as flammable concentrations.

Overall assessment factor: In order to obtain a health-based reference value for human risk characterisation (e.g. AEL or AEC), the overall assessment factor is applied to a dose descriptor (in general a NOAEL/LOAEL) observed in an experimental study for the most relevant critical effect. It is calculated by multiplication of all individual assessment factors. [See also definition of AF]

Professional user: The professional or industrial user comes into contact with the biocidal product as a consequence of their professional life. 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 although a last line of defence, may include the use of PPE. However, 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 (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. There are also specialised professional users, who will probably have expert knowledge and skills in handling hazardous biocidal products and their pattern of use will show greater frequency and/or duration of use (e.g. pest control operators).

Reference values: This term is used for dose levels which serve as reference for judgment whether a particular exposure scenario can be considered to be without appreciable risk to human health. In general, (toxicological) 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 of a solution applied to human skin) 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.

STOP: The STOP principle gives a hierarchy for the selection of risk mitigation measures at the workplace in the order of priority: **S**ubstitution, **T**echnical measures, **O**rganisational measures, **P**ersonal protection.

Appendix 4-1: Literature references informing on the uncertainty of local effects

This Appendix provides and briefly summarises some primary literature references that may be useful in judging the uncertainty of RC for local effects, in the selection and discussion of AF, and when considering qualitative *vs.* quantitative RC and data requirements.

- ECETOC (2003b)

This paper provides references on how to model inter-species differences from respiration rate, airway anatomy and deposition pattern and how this may be used to inform inter-species uncertainty estimates. It is concluded that effects in the rat nasal cavity are likely to overestimate effects in humans by a factor of 2 to 4. Model uncertainty and variability is not explicitly reported. Intra human variability of irritation of the eye and respiratory tract are reported for formaldehyde, ammonia and chlorine (GSDs in the range of 1.47 to 2.52).

- Kalberlah (2002)

The tabled review indicates the variation between rat and human NOAEL/LOAELs over 33 ATSDR reports (e.g. particle exposure: factor >100 for 32% and factor 5-100 for 21% of reports; gas exposure: factor >100 for 6% and factor 5-100 for 42% of reports). The author comments that the comparability of the human observations and the endpoints analysed within the animal studies is problematic and limits his interpretation of the data to supporting that humans on average are marginally more sensitive than rats.

- Jirova *et al* 2007. Comparison of human skin irritation and photo-irritation patch test data with cellular in vitro assays and animal in vivo data. AATEX 14, Special Issue, 359-365.
- Basketter *et al* 2004. Determination of skin irritation potential in the human 4-h patch test. Contact Dermatitis, 51:1-4.

With regard to skin irritation, Jirova and Basketter indicate that acute dermal irritation studies in rabbits show a sensitivity of about 100% but specificity of or below 50% for the prediction of 4h-human-patch-test data. The new *in vitro* human skin method EU-B46 (full replacement of *in vivo* method) seems to perform superior. However no discussion is available of the implications of these data for interspecies uncertainty estimates for local dermal repeated dose NOAECs.

- Basketter *et al* 1997. The classification of skin irritants by human patch test. Food Chem Toxicol. 35(8):845-52
- York *et al* 1996. Evaluation of a human patch test for the identification and classification of skin irritation potential. Contact Dermatitis 34(3): 204-12
- Robinson *et al* 2001. Validity and ethics of the human 4-h patch test as an alternative method to assess acute skin irritation potential. Contact Dermatitis 45(1): 1-12)

Basketter, York and Robinson inform on the protocol for the 4h-HPT: 30 human volunteers are exposed to the substance with 0.2g/25mm plain Hill chamber for up to 4 hours. As soon as weak but unequivocal erythema is observed exposure is stopped in the respective individual and counted as positive response. The substance is considered as skin irritant (R38), when the incidence of positive irritation reactions to the undiluted test substance is statistically significantly ≥ the level of reaction in the same panel of volunteers to 20% SLS.

- Basketter *et al* 1996. Individual, ethnic and seasonal variability in irritant susceptibility of skin: the implications for a predictive human patch test. Contact Dermatitis 35, 208-213

With regard to skin irritation Basketter reports substantial human intraspecies differences for acute local effects with SLS: while up to 76% of humans showed irritation with up to 20% of SLS still up to 9% of humans showed irritation with 0.25%. Also seasonal effects are reported.

- Fluhr (2008)

Fluhr *et al* reviews that dermal irritation is not an immunologic inert process but involves different cytokines and intercellular interactions, however, he provides just qualitative information on individual and environment related variables

- Falk-Filipsson *et al* 2007. Assessment factors-applications in health risk assessment of chemicals. Environ. Res. 104, 108-127

Appendix 4-2: Uncertainties check list for (semi-) quantitative RA for local effects

In line with *Guidance on information requirements and chemical safety assessment Chapter R19 (uncertainty analysis)* in addition to the quantitative or semi-quantitative risk assessment for local effects an assessment of the uncertainties in the hazard and exposure assessment may be carried out. This could start from a general checklist (see below) that is finally tailored to case-specific needs and indicates which uncertainties were addressed by assessment factors and which of the remaining uncertainties tend to over- or underestimate the risk estimate or may influence the risk estimate in both directions.

Table 28:

	Sources of hazard uncertainty		Compensated by	Influence on risk estimate[1]
HAZARD ASSESSMENT	**Model**	**inter-strain and inter-species differences**	-	-
		toxicokinetic (e.g. from anatomy, respiratory rate, deposition pattern, local clearance rates, ...)	-	-
		toxicodynamic(e.g. inflammatory/immune reactions, …)	-	-
		human intra-species differences from age, size, weight, genetic background, background burden, life style, psychological conditions, disease, ...	-	-
		toxicokinetic	-	-
		toxicodynamic	-	-
		reproducibility of the test results	-	-
		"biostatistical chance"	-	-
		effects of feeding, housing and care	-	-
		bio-statistical setup influencing sensitivity of the model: number of samples and animals or humans, number of doses, dose-spacing ...	-	-
		background incidences	-	-
		in historical controls	-	-
		strain/laboratory specific trends in historical controls	-	-
		endpoints analysed	-	-
		relevant endpoints covered by the model: e.g. heat, redness, swelling,	-	-

	Sources of hazard uncertainty		Compensated by	Influence on risk estimate[1]
		dysfunction?, histology? BAL?		
		effects so far not detectable with standard animal tests	-	-
		applicability domain	-	-
		specific chemical groups leading to false results	-	-
		evaluation of complex study results	-	-
		validity-criteria, weighting of study findings, biological significance	-	-
		use of different statistical models and definition of statistical significance	-	-
	Input Parameter	**exposure time extrapolations** frequency and duration, recovery periods?	-	-
		exposure route extrapolations	-	-
		exposure dose extrapolation	-	-
		LOAEL to NOAEL	-	-
		was dose / concentration analytically confirmed?	-	-
		RT: was product concentration in air [mg/L air] generated with product/in use solution to be evaluated or with higher or lower concentrated product [mg/L in solvent]? RT: was the product tested as gas or aerosol – is extrapolation to the expected exposure necessary?	-	-
		skin: was product concentration on skin [mg/cm^2] generated with product/in use solution to be evaluated or with higher or lower concentrated product [mg/L in solvent]? skin: occlusive or semi-occlusive conditions?	-	-
		mixture uncertainties	-	-
		substance to product differences: pH, solvents, other ingredients	-	-
		co-exposure to other dermal stressors like mechanical and physical stress, e.g. from wet work places	-	-
		additive, synergistic and antagonistic reactions with other substances (and metabolites	-	-

	Sources of hazard uncertainty		Compensated by	Influence on risk estimate[1]
		thereof) within products, environmental compartments or human body fluids;		
EXPOSURE ASSESSMENT	Overall effect on hazard estimate E.g.: Mainly affected by overestimation from occlusive conditions and underestimation from substance to product differences and knowledge from inter-species differences, which is uncertainty that may be reduced by…			
	Scenario	e.g. did the measurements cover all potential scenarios, like application, use and cleaning? frequency, duration, amount used?	-	-
	Model	skin: mg product/cm^2 – average measurement over large surface and time or also very local values available?	-	-
		RT: gas or aerosol?	-	-
	Input Parameter	e.g. is model based on sufficient sample sizes over a sufficient time period? Were there measurement errors?	-	-
	Overall effect on exposure estimate E.g.: Mainly affected by underestimation due to uncertainty from all potentially relevant scenarios and model uncertainties from average measurements over time and surface and unavailability of local maximum values. Scenario uncertainty may be reduced by limiting the acceptable intended uses or... Data on variability of averaged skin surface measurements indicate dominant model uncertainties that could be addressed by...			
Risk Characterisation	Overall effect on risk estimate E.g.: The risk estimate appears to be overestimated mainly based on assumptions in exposure assessment that may be revised on the basis of further investigation…			

[1] **+** …Aspect of uncertainty is likely to overestimate hazard; **-** Aspect of uncertainty is likely to underestimate hazard; **+/-** Aspect of uncertainty may over- or underestimate hazard. Eventually this may be extended to a semi-quantitative analysis, e.g. +++ or + or --...

Appendix 4-3: Considerations on formulations of biocidal products provided by CEFIC

Consideration on formulations

The formulation of biocidal products is usually driven by the need to obtain an optimal performance for the product for its end use or by the will to reduce toxicity of the product to the end-user or the environment (e.g. by use of soluble bags, encapsulation). Arguably, formulation represents the forefront of innovation in the preservation sector and an active substance will usually be formulated in a multitude of diverse matrices, tailor-made amongst other things to maximize biocidal capacity, improve durability, extend shelf life, reduce toxicity and reduce wastage.

The effect of formulation will be particular to the active substance due its inherent physico-chemical characteristics. Formulation may or may not therefore result in hazards and exposures not normally associated with the pure active substance itself. Essentially formulation effects may consist of:

1) Increased or reduced local exposure to the active substance, e.g. physical state effect (liquid, aerosol, powder, pellets), particle size, encapsulation, chemical and physical interaction inside the product (partitioning, adsorption), soluble bags, vapour, etc.
2) Increased systemic exposure to the active substance, e.g. increased absorption through dermal layers, disposition changes caused by increased droplet size or reduced surface tension, etc.
3) Enhancement or reduction of the toxicity of each individual substance by additive, synergistic or antagonistic effects

When considering the risk assessment of a formulated product, the work of the regulator is to assess the potential for hazard and exposure (and therefore risk) associated with this specific product on a case by case basis, and the uncertainties associated with the information provided on that product.

Availability of data, Uncertainty and Data Requirements

Acute toxicity tests on biocidal products including irritation and sensitisation tests can be used to evaluate potential hazards associated with a product. However, as the matrix effects are largely related to physico-chemical interactions, other types of information including in-vitro tests not (yet) formally accepted by OECD or EU shall be evaluated by the Member States and, if deemed sufficiently reliable, included in the risk evaluation in a WoE approach.

Pragmatic approach to quantitative RC for local effects

Arnold S.M., Collins M.A., Graham C., Jolly A. T., Parod R. J., Poole A., Schupp TT., Shiotsuka R. N., Woolhiser M. R. (2012). Risk assessment for consumer exposure to toluene diisocyanate (TDI) derived from polyurethane flexible foam. Regulatory Toxicology and Pharmacology 64 pp504-515

This paper reviews several regulatory approaches to decision making on assessment factors (see Table 1 of the reference above). There is also an additional approach, provided by the authors, applying adjusted assessment factors based on additional considerations such as variability.

Appendix 4-4: Quantitative RC for sensitisation

For respiratory sensitisation and dermal sensitisation only a qualitative RC should be carried out.

The qualitative approach may be complemented with a quantitative approach, depending on the available data and possibilities to control the described uncertainties. Quantitative methodologies for dermal sensitisation are available but need further scientific clarification. No established quantitative methodologies are available for respiratory sensitisation.

The ECHA approach under REACH and a refined approach proposed by France as well as a The Netherlands reference to an evaluation of the methodology are presented here.

(i)Approach under REACH

Please see the guidance provided in *Guidance on information requirements and chemical safety assessment Chapters R8-R10,* appendix.

(ii)Approach proposed by France

As mentioned in Section 4.3.2 , for dermal sensitisation a qualitative RC should be carried out. However, in some cases the qualitative RC may be refined by the following quantitative method. For example:

- it might confirm that the use of PPE is really necessary,
- it can confirm that the use of PPE is necessary or not for weak sensitisers
- it can confirm if the use of PPE is needed for diluted products, or at what concentration the use of PPE is needed.

The use of this quantitative risk assessment approach for biocides can be limited by the lack of available tests in the active substances or biocidal products dossiers permitting a quantitative assessment. Indeed, few LLNA studies are available in the dossiers while it is the most relevant method avoiding the most uncertainties (possible dose-response correlation). Moreover, RMMs based on results from this test may prevent the induction of sensitisation as mentioned before and consequently avoid the occurrence of sensitisation.

If data are only available that are not (yet) agreed to be used for sub-categorization according to the CLP Regulation), LLNA DA, LLNA BrdU Buehler test (3 and 9 applications) positive test results should by default lead to the requirement of appropriate RMMs including PPEs.

The quantitative risk assessment has originally been developed for cosmetics (Api. *et al* 2008). The aim of the approach is to obtain an AEC and to compare it to an exposure value.

An AEC is determined by dividing a critical dose by SAF as follows:

AEC = NESIL / SAF

The critical dose is called NESIL. This value is supposed to be small enough to prevent the induction of skin sensitisation. The NESIL is issued from skin sensitisation tests on products or active substances. For example, the effective concentration for a stimulation index of 3 in proliferation of lymph node cells (EC_3) obtained with a LLNA test[35] is used.

[35]LLNA on products are preferred to LLNA on a.s. for the derivation of a NESIL because of possible interaction between the a.s. and substance of concern in the product. If no LLNA is available on the product but on the a.s. derivation of NESIL should take into account the sensitising substances present in the product. If data on mixtures are used "care shall be exercised in evaluating data on mixtures, that the dose used does not render the results inconclusive" (CLP Regulation EC 1272/2008, Annex I, 3.4.3.1.1.)

The (EC_3) unit is the percentage which has to be converted into a µg/cm² unit. This conversion takes into account the volume of vehicle and test item mixture (25 µl) and the (EC_3) percentage (Safford, 2008; ICCVAM 2011 - Appendix B). A French institute also proposed to add a factor considering the hydrophilic or hydrophobic properties of the vehicle for this unit conversion (INERIS, 2009). The data can thus be compared to the exposure value, which is also in µg/cm² (see example below) This conversion of unit is also used for the M&K test data, the (EC_3) value replaced by the intradermal test item concentration value in percentage (INERIS, 2009).

SAFs have been taken from the literature (Griem *et al*., 2003; Api *et al*., 2008;; INERIS, 2009) as well as *Guidance on information requirements and chemical safety assessment Chapter R.19* and adapted to biocidal uses (Appendix table 2). They take into account:

- the type of test used (HRIPT, LLNA, M&K),
- the intra-species factor (the high variability between individuals have been considered),
- inter-species factor (determined by the local metabolism and penetration factor),
- if a dose-response curve can be determined (depending on the critical dose obtained: LOAEL-NOAEL, BMD)
- the matrix (taking into account the irritation potential of the substance/product, hydrophilic nature of the matrix, penetration parameters) (detailed in the table Appendix table 3 – this table is a pragmatic proposal from France, to cross the different parameters and to propose an associated SAF),
- use conditions: this safety assessment factor is built by comparing situations in real life to the situation of the test. It takes into account the area of skin exposed (for example the hand, which is a relatively small area), the duration of the exposure (for example short-time exposure during mixing and loading) and the skin thickness (for example, the skin is less permeable, maybe dry) or abraded skin. It is considered that no PPE are worn.
- NB: SAF used with HRIPT data can be elevated because of the lack of information (for example the vehicle used) on the test.

The SAF have been taken from the literature and are already used in different domains (cosmetic for example). However, the scientific origin of these values is unknown but the values used are the standard values for safety factors.

Table 29: SAF for skin sensitisation AEC construction

SAF	HRIPT	LLNA	M&K
Inter species	1	1 / 2.5	2.5
Intra species	10	10	10
Dose response	10	1 / 3	10
Matrix	1	a.s./Product	a.s./Product
Use conditions*	3	3	3

Use conditions*: A high number of scenarios for biocidal products uses exist. The SAF may thus vary between 1, 3 and 10 (values usually used in the literature for SAF) depending on the parameters described above.

Table 30: Pragmatic proposal from France concerning the specific parameters of "matrix" SAF when a LLNA /M&K is used in skin sensitisation quantitative risk assessment

	Irritation	Test vehicle	AS penetration in function of the Product	SAF
AS	NI	L	nd	1
	NI or I	L	Weak (<10%)	3
	I	L	nd	3
	NI or I	H	nd	10
	NI or I	H	Weak (<10%)	10
	NI or I	**L or H**	Strong (>10%)	10
	Irritation	**Matrix**	**Product penetration**	**SAF**
Product	**NI or I**	L	Weak (<10%)	1
	NI	L	Default value	1
	I	L	Default value	3
	NI or I	H	Weak (<10%)	3
	NI or I	H	Default value	10
	NI or I	**L or H**	Strong (>10%)	10

NI: Non irritant, I : Irritant (R38, H315) ; L : lipophilic, H : hydrophilic, nd : No data;

This AEC is compared to an exposure value. If the ratio is under 1 the risk is considered acceptable, if it is over 1 the risk is considered unacceptable.

Ratio = Exposure / AEC

Example of the quantitative assessment approach for dermal sensitisation

We consider a product tested with a LLNA and no available data regarding a critical dose in humans.

The vehicle used in this assay was hydrophobic but in real life, the product is diluted in water and workers are mostly exposed on their hands.

The resulting (EC_3) from this assay is 0.5%.

An AEC can be derived to carry out a quantitative risk assessment of the dermal sensitisation effect with the following calculation:

AEC= NESIL / SAF

1) The (EC_3) is used as a NESIL. In order to obtain the NESIL in µg/cm², a conversion has to be done as following:

NESIL (µg/cm²) = (EC_3) (%) *conversion factor [36]
NESIL (µg/cm²) = 0.5 x 200
NESIL (µg/cm²) = 100

The calculated NESIL corresponds to 100 µg/cm².

2) The safety assessment factors have then been determined. They vary between the values of 1, 2.5, 3 and 10.

SAF = IRS*IS*DR*M*UC
SAF = 2.5*10*3*3*3
SAF = 675

Table 31:The SAFs are explained in the following table:

SAF	LLNA	Justifications
Inter species (IRS)	2.5	The toxicodynamic effect may be different between animal and humans and considering the toxicokinetic effect as similar between species.
Intra species (IS)	10	Considering the variability between individuals' sensitivity,
Dose-response (DR)	3	As the LLNA provide an (EC_3) and that the (EC_3) can be considered as a LOAEL, a safety factor of 3 has been taken.
Matrix (M)	3	The vehicle used in the assay is hydrophobic and different from the in-use situation. Then a factor of 3 has been chosen.
Use conditions (UC)	3	The worker is exposed on his hands. The hands have a skin quite thick and workers are exposed during a short period, mixing and loading for example. The medium factor of 3 is chosen.

3) The AEC can now be derived as following:
AEC = NESIL/SAF

AEC = 100/675

AEC = 0.15 µg/cm²

In conclusion, the AEC used for quantitative assessment is 0.15 µg/cm².

(iii)Observations provided by the Netherlands

RIVM Report 320015003/2010: Observations on the methodology for quantitative risk assessment of dermal allergens; W. ter Burg, S.W.P. Wijnhoven, A.G. Schuur (freely available in internet)

[36] A conversion factor of 200 is proposed for hydrophobic vehicles. It is obtained considering the applied volume of 25 µL (protocol's volume) and the application area (mice ear) of 1cm² (estimation). Moreover, the liquid density is 0.8.

A conversion factor of 250 is proposed for hydrophilic vehicles. It is obtained considering the applied volume of 25 µL (protocol's volume) and the application area (mice ear) of 1cm² (estimation). Moreover, the liquid density is 1.

Appendix 4-5: Risk Characterisation for local effects including sensitisation - Examples

Example 1: Qualitative risk assessment matrix for local effects

Primary exposure: use of product

Hazard			Exposure							Risk	
Hazard Category	effects in terms of 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 (↑↓)
low	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 matrix for local effects

A) Primary exposure: use of product

Hazard			Exposure							Risk	
Hazard Category	effects in terms of 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 (↑↓)
low	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 for professionals packaging reducing risk	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	
					for eye exposure by splashes washing of hands after use			

B) Primary exposure: use of application solutions

Hazard			Exposure							Risk	
Hazard Category	effects in terms of 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 (↑↓)
low	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 (↑↓)

Hazard	Exposure					Risk	
As above	professionals	As above	not daily, but ≥ 1 / week ~ 60 min/ day	As above	Like for general public + instructions for use minimizing exposure <u>for professionals</u>	Acceptable: +Reversible effect +low intensity: outdoor use, low intensity compared to additional hazard information +professionals following instructions for use +experience expected	

[1]With eye irritation also RT irritation is expected but no RT threshold is available, therefore acute product test data are used as additional information for semi-quantitative RC.

Example 3: Qualitative risk assessment matrix for local effects

Primary exposure: use of product

Hazard			Exposure							Risk
Hazard Category	effects in terms of 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
medium	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 matrix for local effects

A) Primary exposure: use of product

Hazard			Exposure							Risk
Hazard Category	effects in terms of 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
Very high	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

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

B) Primary exposure: use of application solutions

Hazard			Exposure							Risk
Hazard Category	effects in terms of 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
low	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	Acceptable: +reversible effects +installed RMM at place +trained workers +use of appropriate

									gloves, eye protection, filter mask	PPE

Appendix 4-6: Example to determine AELa.s.-to from NOAEL for each target organ for a substance

Organs	NOAEL														NOAEL retained
	28d rat mg/kg/d	21d rabbit demal mg/kg/d	28d rat dermal mg/kg/d	90d rat mg/kg/d	90d mouse mg/kg/d	90d mouse mg/kg/d	90d rat inhalation	24 months rat (carcino) mg/kg/d	52 weeks mouse mg/kg/d	18 months mouse mg/kg/d	2 generations rat	develop mental rat	develop mental rat	developmental rabbit	
liver	LOAEL: 50	1000	100	76.1-77.6	2.7	2.8		500 ppm	10.04-10.79	11	8				8
white line	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
kidney	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
brain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hemato-logy	150	-	-	76.1-77.6	-	-	-	-	-	-	-	-	-	-	-
GI tract	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
thymus	-	-	-	-	-	-	-		-	-	-	-	-	-	-
adrenals	-	-	-	-	-	-	-	3.60-4.57	-	-	-	-	-	-	-
eyes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pancreas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
spleen	-	-	-	76.1-77.6	-	-	-	-	-	-	-	-	-	-	-
neurotox	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
lymph nodes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Organs	NOAEL														NOAEL retained
	28d rat mg/kg/d	21d rabbit demal mg/kg/d	28d rat dermal mg/kg/d	90d rat mg/kg/d	90d mouse mg/kg/d	90d mouse mg/kg/d	90d rat inhalation	24 months rat (carcino) mg/kg/d	52 weeks mouse mg/kg/d	18 months mouse mg/kg/d	2 generations rat	develop mental rat	develop mental rat	developmental rabbit	
ovaries	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
uterus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
toxicity on fœtus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
terato-genicity	-	-	-	-	-	-	-	-	-	-	-	30	LOAEL: 300	-	-
bones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
fertility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
general toxicity	150	200	-	15.9-16.8	194	199	male: 21 mg/m3 female: deviation	3.60-4.57	-	11	-	30	LOAEL: 300	100	-
thyroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
heart	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pituitary	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
salivary glands	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
lung	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Organs	NOAEL														NOAEL retained
	28d rat mg/kg/d	21d rabbit demal mg/kg/d	28d rat dermal mg/kg/d	90d rat mg/kg/d	90d mouse mg/kg/d	90d mouse mg/kg/d	90d rat inhalation	24 months rat (carcino) mg/kg/d	52 weeks mouse mg/kg/d	18 months mouse mg/kg/d	2 generations rat	developmental rat	developmental rat	developmental rabbit	
mammary glands	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NOAEL	**SF**	**AEL**												
AEL acute-term	30	100	0.3	developmental study	-	-	-	-	-	-	-	-	-	-	-
AEL medium-term	8	100	0.08	2 generation study	-	-	-	-	-	-	-	-	-	-	-
AEL long-term	4	100	0.04	carcinogenicity study	-	-	-	-	-	-	-	-	-	-	-

Appendix 4-7: Example of cumulative risk assessment (theoretical)

A theoretical example of the approach is described below.

Considering a product containing 1 active substance from PT8, a biocidal substance from PT18 considered as a substance of concern and one co-formulant considered also as a substance of concern; called S1, S2 and S3 respectively.

Table 32: AEL has been derived for each substance:

	PT8 S1	PT18 (SoC) S2	Formulant (Soc) S3
AEL acute	0.1 mg/kg based on a NOAEL of 10 mg/kg in an acute neurotoxicity study in rats with a SF of 100	0.2 mg/kg based on a NOAEL of 20 mg/kg in a developmental toxicity study in rabbits with a SF of 100	0.5 mg/kg based on a NOAEL of 50 mg/kg in an acute neurotoxicity study in rats with a SF of 100
AEL chronic	0.05 mg/kg/d based on a NOAEL of 5 mg/kg in a 2-year study in rats with a SF of 100	0.01 mg/kg/d based on a NOAEL of 1 mg/kg in a 2-year study in rats with a SF of 100	0.02 mg/kg/d based on a NOAEL of 2 mg/kg in a 2-year study in rats with a SF of 100

Table 33: Primary exposure – Professional users:

	S1	S2	S3
Value of exposure (without PPE)	0.0125 mg/kg	0.0075 mg/kg	0.01 mg/kg
Value of exposure (with PPE)	0.005 mg/kg	0.0025 mg/kg	0.003 mg/kg

Table 34: Secondary exposure – general public:

	S1	S2	S3
Acute value of exposure	0.005 mg/kg	0.02 mg/kg	0.035 mg/kg
Chronic value of exposure	0.01 mg/kg	0.0065 mg/kg	0.007 mg/kg

Preliminary step: no indication of synergy in literature search, in acute studies performed with the product (toxicology, ecotoxicology, efficacy).

TIER 1 and TIER 2:

Tier 1 is an intermediary step to verify risk acceptability for each active ingredient used in the product and is followed by Tier 2 to assess the mixture effect.

For the toxicological section, primary exposure has been considered and exposure estimations were compared to the chronic AEL for each substance. Secondary exposure was performed according to 2 scenarios: acute and chronic using acute and chronic AEL respectively.

Table A4-8: Results of the risk assessment are summarized below:

Scenario 1 Primary exposure	S1	S2	S3	Conclusions
Without PPE				
TIER 1	25% AEL	75% AEL	50% AEL	Acceptable
TIER 2	0.25	0.75	0.50	Not Acceptable
	HI = 1.5			
With gloves during application				
TIER 1	10% AEL	25% AEL	15% AEL	Acceptable
TIER 2	0.1	0.25	0.15	Acceptable
	HI = 0.5			
Scenario 2 **Secondary exposure**	**S1**	**S2**	**S3**	**Conclusions**
Acute				
TIER 1	5% AEL	10% AEL	7% AEL	Acceptable
TIER 2	0.05	0.1	0.07	
	HI = 0.12			Acceptable
Chronic				
TIER 1	20% AEL	65% AEL	35% AEL	Acceptable
TIER 2	0.2	0.65	0.35	
	HI = 1.2			Not Acceptable*

* for secondary exposure, use of PPE cannot be recommended.

Conclusion:

For primary exposure:

TIER I: Risk assessment is acceptable for each substance individually in the product without PPE.

TIER 2: For Mixture risk assessment: risk assessment is not acceptable in T2 without PPE but is acceptable with gloves during application. A Tier 3 will however be performed according to the decision of Biocides TMIII 2012.

For secondary exposure:

Risk assessment is acceptable for each substance individually in the product (acute or chronic).

TIER 2: For Mixture risk assessment:

Acute risk assessment is acceptable.

Chronic risk assessment is not acceptable. Use of PPE cannot be recommended for general public. A Tier 3 is necessary.

For Tier 3, it is necessary to find information in the CAR of each active substance and also on the formulant (according to the guidance document, sufficient data should be available on the formulant to allow the derivation of AELs, in this case, acute and chronic AEL).

A summary of the critical NOAELs for each substance is detailed in the following table.

The lowest relevant values have been reported and can be used to derive organ AELs, in bracket with a SF of 100 (same SF than the one taken for the generic AELs for each substance).

Table 35: NOAELs

Target organ / Mode of Action	NOAELs S1 (AEL) Chronic AEL: 0.05 mg/kg/d (kidney and liver effect) Acute AEL: 0.1 mg/kg (neurotoxicity)	NOAELs S2 (AEL) Chronic AEL: 0.01 mg/kg/d (thyroid effect) Acute AEL: 0.2 mg/kg (malformation)	NOAELs S3 (AEL) Chronic AEL: 0.02 mg/kg/d (liver effect) Acute AEL: 0.5 mg/kg (neurotoxicity)
Liver (chronic)	5 mg/kg/d (0.05 mg/kg/d)	2 mg/kg/d (0.02 mg/kg/d)	2 mg/kg/d (0.02 mg/kg/d)
Thyroid (chronic)	10 mg/kg/d (0.1 mg/kg/d)	1 mg/kg/d (0.01 mg/kg/d)	NA
Kidney (chronic)	5 mg/kg/d (0.05 mg/kg/d)	NA	10 mg/kg/d (0.1 mg/kg/d)
Eye (cataract) (chronic)	10 mg/kg/d (0.1 mg/kg/d)	NA	NA
Fertility (chronic)	NA	20 mg/kg/d (0.2 mg/kg/d)	NA
Malformation (acute)	NA	20 mg/kg/d (0.2 mg/kg)	NA
Neurotoxicity (acute)	10 mg/kg (0.1 mg/kg)	NA	50 mg/kg (0.5 mg/kg)

TIER 3 is performed according:

- Grouping substances by target organs (without refining AEL) as a first step: T3a
- If risk assessment is not acceptable for a group, by refining AEL for the target organ/mode of action specific of the group.

Table 36: Tier 3a:

Scenario 1	HQ S1	HQ S2	HQ S3	HI
Liver	0.25	0.75	0.5	1.5
Thyroid	0.25	0.75	-	1
Kidney	0.25	-	0.5	0.75
Eye	0.25	-	-	0.25
Fertility	-	0.75	-	0.75
Scenario 2 Chronic	**HQ S1**	**HQ S2**	**HQ S3**	**HI**
Liver	0.2	0.65	0.35	1.2
Thyroid	0.2	0.65	-	0.85
Kidney	0.2	-	0.35	0.55
Eye	0.2	-	-	0.2
Fertility	-	0.65	-	0.65

According to this table, T3a is not acceptable for scenario 1 and for scenario 2 (chronic) for liver toxicity.

Table 37: Tier 3b: AEL can be refined by target organ:

	S1	S2	S3
Liver (chronic)	5 mg/kg/d (0.05 mg/kg/d)	2 mg/kg/d (0.02 mg/kg/d)	2 mg/kg/d (0.02 mg/kg/d)

Scenario 1	Organ HQ S1	Organ HQ S2	Organ HQ S3	HI
Liver	0.25	0.375	0.5	1.125
Scenario 2 Chronic	**Organ HQ S1**	**Organ HQ S2**	**Organ HQ S3**	**HI**
Liver	0.2	0.325	0.35	0.875

After organ AEL refinement risk assessment is acceptable for scenario 2 (chronic).

As risk assessment is not acceptable in Tier 3b, PPE can be proposed for scenario 1. Then risk is acceptable (see T2).

Appendix 4-8: Example illustrating the division of Tier 3 as proposed by BfR at TMIII12

Remarks:

a) We propose to further elucidate Tier 2, 3A, 3B, 3C on the basis of **hypothetical examples** as follows. Substance characteristics resemble these of biocidal a.s. under evaluation, but identity is not disclosed.

b) All RfDs used in this example were values for **long-term exposure only**. This approach might be performed for all time frames.

c) Potential for **synergistic effects is not considered** and the reference HI (HI_{ref}) is set to 1.00. It is noted that some of the substances may behave synergistically, e.g. C and D acting through different MoA on the nervous system.

Tier 2:

Calculation of $HI_{long\text{-}term}$:

Mixture of 5 substances A + B + C + D + E

Table 38: Characteristics of components A, B, C, D, and E in the mixture

Substance	$RfD_{long\text{-}term}$ (mg/kg bw/d)	Exposure Level (mg/kg bw/day)	HQ*
A	0.20	0.050	0.25
B	0.25	0.100	0.40
C	0.02	0.008	0.40
D	0.02	0.009	0.45
E	0.22	0.020	0.09
HI = **Σ**HQ	-	-	1.59

*: HQ= Exposure level/$RfD_{long\text{-}term}$

Summation of HQs for each active substance on the basis of derived RfDs (see **Error! Reference source not found.**):

HI = ∑HQ = 1.59 > 1; risk not acceptable.

Tier 3A:

If the RfDs have not been established for all substances on the basis of the same target organs/systems, grouping of substances by target organs/systems is performed. The existing RfDs and individual HQs already used in Tier 2 are used for risk characterisation in Tier 3A. Hence, no new HQs will be calculated in this Tier.

Example:

Table 39: Target Organs of components A, B, C, D, and E

a.s.	Critical Target Organ	Other target organs	HQ	HQ_{liver}	$HQ_{adrenals}$	$HQ_{nervous\ system}$	HQ_{kidney}
A	Liver	Kidney, thyroid, testes, adrenals	0.25	0.25	0.25	-	0.25
B	Liver	Kidney, GI effects, reduced bw gain	0.40	0.40	-	-	0.40
C	Nervous System	Liver	0.40	0.40	-	0.40	-
D	Nervous System	None	0.45	-	-	0.45	-
E	Adrenals	Liver	0.09	0.09	0.09	-	-
HI_{TO}	-	-	-	1.14	0.34	0.85	0.65

Note: No new HQs are calculated in this Tier.

Calculation of target organ specific $HI_{long\text{-}term}$ ($HI_{long\text{-}term,\ to}$):

Note: Tier 3A must include accurate evaluation of toxic effects including those to organs which were not identified as the most critical ones.

1. Target organ Liver:

Substances A and B were all shown to be hepatotoxic with the liver being the most sensitive target organ. However, HI_{liver} must also include substances C and E.

HI_{liver} = 0.25 + 0.4 + 0.4 + 0.09 = 1.14. > 1; risk not acceptable.

2. Target organ: Adrenal glands

Adrenal glands were the most critical target organ for substance E. In addition, adrenal glands were also identified as target organ for substance A.

$HI_{adrenals}$ = 0.25 + 0.09 = 0.34. < 1; risk acceptable.

3. Target organ: Nervous system

Substances C and D were neurotoxic.

$HI_{nervous\ system}$ = 0.40 + 0.45 = 0.85 < 1; risk acceptable

4. Target organ: Kidney

Substances A and B were also nephrotoxic.

HI_{kidney} = 0.25 + 0.40 = 0.65 < 1; risk acceptable

Tier 3B:

Tier 3B considerations apply only for target organs for which a risk was identified in Tier 3A. Here, this is the liver.

Grouping of substances is adopted from Tier 3A.

In addition, derivation of adjusted reference values for each identified target organ/system for each substance (RfD_{to}) is required.

For substances A and B, RfDs were derived on the basis of hepatotoxicity, whereas for substance C and E, RfDs were calculated from the NOAELs for neurotoxic and adrenal effects, respectively. At Tier 3B level, HI can be adjusted for substances C and E to reference doses[37] regarding the organ specific effect (here: hepatotoxicity):

Consequently, a new reference dose for hepatotoxicity must be calculated for substance C:

For instance for example C, if hepatotoxicity was seen in the 1y dog study at 150 mg/kg bw/d (LOAEL) and a NOAEL of 50 mg/kg bw/d was derived, an organ specific RfD of 0.5 mg/kg bw/d would result (no adjustment for oral absorption required, safety factor of 100 applied).

Calculation of adjusted HI_{to}:

Table 40: Adjusted $HQ_{long\text{-}term,\ liver}$ for components A, B, C, and E

a.s.	Critical Target Organ*	Other target organs	HQ	aHQ_{liver}
A	Liver	Kidney, thyroid, testes, adrenals	0.25	0.25
B	Liver	Kidney, GI effects, reduced bw gain	0.40	0.40
C	Nervous System	Liver	0.40	0.02
D	Nervous System	None	Not relevant	-
E	Adrenals	Liver	0.09	0.03
HI_{TO}	-	-	-	0.70

RfD substance C: 0.5 mg/kg bw/day, exposure level 0.008 mg/kg bw/d (unchanged), HQ= 0.02

RfD substance E: 0.67 mg/kg bw/day, exposure level 0.02 mg/kg bw* day (unchanged), HQ = 0.03.

aHI_{liver} = 0.25 + 0.4 + 0.02 + 0.03 = 0.73. < 1; risk acceptable

[37] It is noted that these organ specific reference doses do not represent the most critical effect used for the single substance for Annex I inclusion.

Tier 3C:

In this tier, the grouping of substances is further refined: those substances are selected, which cause effects via common mode/mechanisms of action. The RfDs derived in Tier 3B are used for the risk characterisation: An HI for each common target organ/mechanism of action (HI_{to}) will be calculated using the same principle as in Tier 3B.

In this example concerning substances affecting the liver, Tier 3C is not feasible. Due to the uncertainties in the mode of action no further refinement would be possible.

Substance A + B share the same mode/mechanism of action, whereas the mechanisms of action of E and C are not enough clarified to exclude additivity in hepatotoxicity.

Note: Applicability of Tier 3C requires (assured) independent mode/mechanisms of action of two or more substances if these independent MoA were not expected to lead to dose additivity.

For example, substances C and D are both neurotoxic but they do not share a common mode/mechanism of action. Substance C mediates its neurotoxic effects via prolonged opening of sodium channels whereas substance D is an acetyl choline esterase inhibitor. However, Tier 3C would not be performed as it is not known if these two substances exhibit their neurotoxic effects completely independently and whether the effects might influence each other.

Note: Before refinement at Tier 3C level can be performed an appropriate justification is required which plausibly demonstrates that individual components of the mixture do not lead to dose additivity and that identified processes do not influence each other.

Appendix 4-9: Definitions for Section 4.4.1

Active substance: A substance or micro-organism including a virus or a fungus having general or specific action on or against harmful organisms.

Substance of concern: Any substance, other than the active substance, which has an inherent capacity to cause an adverse effect on humans, animals or the environment and is present or is produced in a biocidal product in sufficient concentration to create such an effect.

Concentration (dose) addition approach assumes that all substances (active or "of concern") from the biocidal product act as if they were dilutions or concentrations of each other differing only in relative toxicity.

It is considered that substances act similarly if they produce similar effect(s) on the same target organ/tissue. As in most cases similar action could not be ensured with certainty, the best approach would be to use the concentration (dose) addition concept as a first tier, instead of the independent action since the former is more conservative than the latter (Kortenkamp *et al*., 2009).

Independent action: When considering substances with dissimilar effects, it is necessary to independently characterise the risk for each of them on the basis of available data. If the risk for each substance of the mixture is considered acceptable, it is considered that the product will lead to an acceptable risk. On the contrary, if one of the substances induces an unacceptable risk, it will be assumed that the mixture will also lead to an unacceptable risk.

Synergism: A situation where expected effects are higher than those expected with concentration (dose) addition approach.

Mode of action: Key events by which a chemical exerts its biological effects.

Mechanism of action: Molecular sequence of events that produce a specific biological outcome.

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Annex A: Substances of Concern – Proposed Human Health (Toxicology) Assessment Scheme for Authorisation of Biocidal Products

NOTES to the reader

This Annex is an attempt to provide guidance in the interest of consistency, and has been drafted by the Commission services responsible for biocidal products with the aim of finding an agreement with all or a majority of the Member States' Competent Authorities for biocidal products. Please note, however, that Member States are not legally obliged to follow the approach set out in this document, since only the Court of Justice of the European Union can give authoritative interpretations on the contents of Union law.

This Annex addresses exclusively the identification and evaluation of substances of concern in relation to human health (toxicological) endpoints. Guidance concerning substances of concern in relation to physical-chemical and environmental endpoints is under development and will be published at a later time point.

The text has been edited by ECHA to add cross references to the relevant sections of this guidance and some minor editorial revisions.

The Commission document reference is CA-Nov14-Doc.5.11.

Text cited from Regulation (EU) No. 528/2012/EC is indicated in green boxes.

This Annex should be read together with the section on general guidance in Section 4 on combined exposure to several active substances or substances of concern (Section 4.4 and Section 4.4.1) and the examples of cumulative risk assessment in Appendix 4-7 and Appendix 4-8. This Annex provides, in addition to Section 4.4.1, a practical way for dealing with the assessment of SoC at product authorisation stage.

Identification of Substances of Concern

A substance of concern (SoC) is defined in Art 3(f) of Regulation (EU) No. 528/2012/EC or the Biocidal Product Regulation (BPR) as follows:

Article 3 (f) 'substance of concern' means any substance, other than the active substance, which has an inherent capacity to cause an adverse effect, immediately or in the more distant future, on humans, in particular vulnerable groups, animals or the environment and is present or is produced in a biocidal product in sufficient concentration to present risks of such an effect.

Such a substance would, unless there are other grounds for concern, normally be:

- *a substance classified as dangerous or that meets the criteria to be classified as dangerous according to Directive 67/548/EEC, and that is present in the biocidal product at a concentration leading the product to be regarded as dangerous within the meaning of Articles 5, 6 and 7 of Directive 1999/45/EC, or*
- *a substance classified as hazardous or that meets the criteria for classification as hazardous according to Regulation (EC) No 1272/2008, and that is present in the biocidal product at a concentration leading the product to be regarded as hazardous within the meaning of that Regulation*
- *a substance which meets the criteria for being a persistent organic pollutant (POP) under Regulation (EC) No 850/2004, or which meets the criteria for being persistent, bio-accumulative and toxic (PBT) or very persistent and very bio-accumulative (vPvB) in accordance with Annex XIII to Regulation (EC) No 1907/2006;*

Therefore, a SoC is a co-formulant in a biocidal product which meets at least one of the conditions specified in Art 3(f) of the BPR, i.e. a classified co-formulant present in the biocidal product above the respective specific or generic concentration limit of Directive 1999/45/EC and/or the CLP Regulation and thus, leading to its classification. However, as it can be seen from Art 3(f) of the BPR, the legal text is vague on what constitutes a SoC on the basis of "other grounds for concern". It has been proposed that in addition to the three cases (three indents in the green box above) of clearly defined SoCs specified in Art 3(f) of the BPR, the following co-formulants present in a biocidal product should be considered as SoCs:

1. Classified substances that are taken *into consideration* when determining the classification of the product according to Directive 1999/45/EC, Article 3(3) or according to Article 11(2) of the CLP Regulation. It should be noted that impurities might affect the classification of any such substances. This criterion partly overlaps with the requirements of Art 3(f) of the BPR. Ultimately, this criterion will additionally identify classified co-formulants that contribute, by additivity, to the classification of the biocidal product. It is noted that since the additivity principle of Directive 1999/45/EC or CLP Regulation applies only to acute toxicity and irritation/corrosivity, SoCs identified by this criterion would be co-formulants classified for these endpoints, which are present in the biocidal product at concentrations insufficient to trigger the classification of the product by themselves, but that together with other co-formulants/active substance(s) contribute to the classification of the product. Conversely, as the additivity principle of Directive 1999/45/EC or CLP Regulation does not apply to the other toxicological hazards under the scope of these legislations, co-formulants classified for these other hazards, which are present in the biocidal product at concentrations insufficient to trigger the classification of the product by themselves are not considered SoCs on the basis of this criterion. Concentrations for classified substances taken into consideration when determining the

classification of the product are specified in the relevant legislation (Directive 1999/45/EC and CLP Regulation).

2. Active substances, other than those included in Annex I of the BPR, for which a draft final Competent Authority Report (CAR) (with agreed reference values) is available (including draft final CARs for Product Types other than the one of the actual biocidal product under evaluation). This criterion identifies other active substances in the biocidal product that act as co-formulants (e.g. in-can preservatives). It is noted that active substances (acting as co-formulants in a product) should be regarded as SoCs because, due to their intrinsic biological activity, they are likely to possess toxicological activity. It is also noted that as many active substances do not hold harmonised classifications under the CLP Regulation, they may fail to be identified as SoCs by the first two indents of Art 3(f) of the BPR. These substances should be considered SoCs if they are present in the biocidal product at a concentration ≥ 0.1%.
3. Substances that enhance the effect of the active substance in the product, e.g. synergists. For such substances, critical information/data shall relate to the interaction between the active substance and the synergist, not only to the synergist itself. In such situations, an appropriate evaluation of the risks posed by the active substance in the presence of the synergist rather than an evaluation of the risks posed by the synergist itself should be undertaken. A generic concentration cut-off value (for their presence in a product) applicable to all synergists cannot be specified. On a case-by-case basis, a synergist should be considered a SoC, if it is present at a concentration that enhances the toxicity of the active substance, as indicated by the available data.
4. Substances that have been included in the list (the candidate list) established in accordance with the REACH Regulation, Article 59(1) or fulfil the criteria for inclusion in the candidate list, if not already covered by the criteria of Article 3(f) of the BPR. These substances should be considered SoCs if they are present in the biocidal product at a concentration ≥ 0.1%. It is noted this criterion will ultimately capture, over and above the clearly-defined SoCs specified in Art 3(f) of the BPR, endocrine disruptors (EDs) and substances with hazards of equivalent concern to CMR 1A or 1B (under the CLP Regulation).
5. Substances for which there are Community workplace exposure limits. A generic concentration cut-off value (for their presence in a product) applicable to all such substances cannot be specified. This should be determined on a case-by-case basis depending on the hazard profile, potency and exposure potential of the substance.

Evaluation of identified SoCs

Annex VI of the BPR lays down the common principles for the evaluation of dossiers for biocidal products. The following is stated at paragraphs 3, 4, 5, 6, 7, 14, 16 and 17 of this Annex:

Annex VI

3. In order to ensure a high and harmonised level of protection of human health, animal health and the environment, any risks arising from the use of a biocidal product shall be identified. To achieve this, a risk assessment shall be carried out to determine the acceptability or otherwise of any risks that are identified. This is done by carrying out an assessment of the risks associated with the relevant individual components of the biocidal product, taking into account any cumulative and synergistic effects.

4. A risk assessment on the active substance(s) present in the biocidal product is always required. This risk assessment shall entail hazard identification, and, as appropriate, dose (concentration) - response (effect) assessment, exposure assessment and risk characterisation. Where a quantitative risk assessment cannot be made a qualitative assessment shall be produced.

5. Additional risk assessments shall be carried out, in the same manner as described above, on any substance of concern present in the biocidal product. Information submitted in the framework of Regulation (EC) No 1907/2006 shall be taken into account where appropriate.

6. In order to carry out a risk assessment, data are required. These data are detailed in Annexes II and III and take account of the fact that there are a wide variety of applications as well as different product-types and that this has an impact on the associated risks. The data required shall be the minimum necessary to carry out an appropriate risk assessment. The evaluating body shall take due consideration of the requirements of Articles 6, 21 and 62 in order to avoid duplication of data submissions. Data may also be required on a substance of concern present in a biocidal product. For in-situ generated active substances, the risk assessment includes also the possible risks from the precursor(s).

7. The results of the risk assessments carried out on the active substance and on the substances of concern present in the biocidal product shall be integrated to produce an overall assessment for the biocidal product itself.

14. A risk assessment on the active substance present in the biocidal product shall always be carried out. If there are, in addition, any substances of concern present in the biocidal product then a risk assessment shall be carried out for each of these.

16. For each active substance and each substance of concern present in the biocidal product, the risk assessment shall entail hazard identification and the establishment of appropriate reference values for dose or effect concentrations.

17. The results arrived at from a comparison of the exposure to the appropriate reference values for each of the active substances and for any substances of concern shall be integrated to produce an overall risk assessment for the biocidal product. Where quantitative results are not available the results of the qualitative assessments shall be integrated in a similar manner.

Therefore, the BPR requires that a risk assessment is performed for all active substances and SoCs in a biocidal product. Clearly paragraph 5 of Annex VI of the BPR implies that a risk assessment should be conducted for SoCs in the same manner as it is performed for the active substance. However, the text in the Regulation contains several caveats. Paragraph 4 of Annex VI indicates that qualitative rather than quantitative risk

assessments may be performed where a quantitative one cannot be produced. The 'where' part of paragraph 4 is important as, in certain circumstances, it allows applicants to demonstrate that the risk is likely to be acceptable with qualitative arguments or more simplistic calculations (e.g. Tier I exposure assessment).

Performing full risk assessments for every SoC in every formulation is not only impractical, unworkable and unsustainable but also not justified from a scientific point of view. A tiered approach is therefore required to assess the risks posed by SoCs in a proportionate manner.

It should be noted that the onus is on applicants to identify SoCs, provide appropriate information/data and perform risk assessments, if necessary. To identify SoCs, applicants should take into account all available information, including data in the open literature and information from predictive approaches such as (Q)SAR ((quantitative) structural activity relationship), read-across from structural analogues and category approaches. It should also be noted that although SDSs (Safety Data Sheets) for individual co-formulants represent the primary source of hazard information on potential SoCs, useful information could also be obtained from a number of specialised databases and portals such as the eChemPortal, the C&L Inventory, ECHA dissemination website (database of registered substances under REACH), R4BP 3 (Register for Biocidal Products), Annex VI of the CLP Regulation and cosmetic databases. It should be noted that, wherever relevant data for the assessment is covered by proprietary rights, it is the responsibility of the applicant to obtain the right to use these data.

The following toxicological assessment scheme for SoCs is proposed to be used together with ECHA Guidance for Human Health Risk Assessment (Volume III, Part B). The scheme takes into account the nature (quantitative or qualitative) and severity of the hazard classification of the SoC, the concentration/percentage of the SoC in the biocidal product, the relative toxicity of the SoC compared to that of the active substance and the relative ratio between the active substance concentration and the SoC concentration in the biocidal product. The approach has been developed to be initially applicable to those (toxicological) SoCs clearly defined in Art 3(f) of the BPR, i.e. classified co-formulants present in a biocidal product at concentrations leading or contributing to the classification of the product according to Directive 1999/45/EC or the CLP Regulation. Ecotoxicological SoCs identified in accordance with Art 3(f) of the BPR because of their POP, PBT and/or vPvB properties are outside the scope of this paper and are to be addressed by the environmental risk assessment of the biocidal product.

The proposal requires that for each SoC, the classification of the product triggered by the classified SoC is determined first. The SoC is then assigned to one of four possible product hazard classification bands (from A to D) of increasing evaluation and risk management requirements. If the SoC can be assigned to more than one band, the evaluation/risk management requirements of the higher band will apply. Less severe hazards and/or hazards for which, normally, the available dose-response information tends to be qualitative or semi-quantitative are assigned to the lower bands; more severe hazards and/or hazards for which, normally, the available dose-response information tends to be quantitative are assigned to the higher bands.

It should not be forgotten that products classified as Toxic, Very Toxic or C (Carcinogenic), M (Mutagenic) or R (Toxic for Reproduction) Cat 1 or 2 under Directive 1999/45/EC or classified for Acute Toxicity Category 1, 2 or 3 or as C or M or R category 1A or 1B under the CLP Regulation cannot be used by the general public (Art 19(4) of the BPR), regardless of whether the classification is triggered by the active substance or by one or more SoCs in the product. It should also not be forgotten that if, for the general public, the wearing of personal protective equipment would be the only possible method for reducing exposure to an acceptable level, the product shall not normally be authorised (Annex VI, para 63 of BPR).

Regardless of band, for SoCs classified for the same endpoint, the potential exists that they act additively with other SoCs and/or with the active substance(s) and that a combined risk assessment would be required. However, as there is little experience of applying such methodology at present, it has been proposed that for the time being a combined risk assessment should only be applied to multiple (2 or more) active substances (including those identified as SoCs under criterion (2)) within a product, and not to SoCs. When sufficient experience has been gained, the combined risk assessment methodology could be extended to include SoCs.

The proposed scheme utilises both the classification and labelling elements of Directive 67/548/EEC and the CLP Regulation. The table below outlines the main features of the proposed banding scheme.

Banding evaluation scheme for classified SoCs leading to the classification of the biocidal product

Band	Classification of biocidal product according to Directive 67/548/EEC due to classified SoC	Classification of biocidal product according to CLP Regulation due to classified SoC	Associated evaluation/risk management requirements
A	R20, R21, R22	Acute Tox 4 (H332, H312, H302)	Application of S-phrases/P-statements normally associated with concerned R-phrases/H statements
	R68/20, 21, 22	STOT SE 2 (H371)	
	R65, R66, R67	Asp Tox 1 (H304), EUH066, STOT SE 3 (H336)	
	R36, R37, R38	Eye Irrit 2 (H319), STOT SE 3 (H335), Skin Irrit 2 (H315)	
B	R23, R24, R25	Acute Tox 3 (H331, H311, H301)	Qualitative exposure and risk assessment to determine whether S-phrases/P-statements normally associated with concerned R-phrases/H statements are sufficient or whether other risk mitigation measures should be applied
	R39/23, /24, /25	STOT SE 1 (H370)	
	R26, R27, R28	Acute Tox 2 (H330, H310, H300), Acute Tox 1 (H330, H310, H300)	
	R39/26, /27, /28	STOT SE 1 (H370)	
	R34, R35, R41	Skin Corr 1A, 1B, 1C (H314), Eye Dam 1 (H318)	
	R43, R42	Skin Sens 1 (H317), Resp Sens 1(H334)	
C	R48/20, /21, /22	STOT RE 2 (H373)	Fully quantitative risk assessment by using EU IOELVs (when available), DNELs or

Band	Classification of biocidal product according to Directive 67/548/EEC due to classified SoC	Classification of biocidal product according to CLP Regulation due to classified SoC	Associated evaluation/risk management requirements
	R48/23, /24, /25	STOT RE 1 (H372)	other reference values (e.g. AELs, AECs)
	Carc Cat 3 (R40)	Carc 2 (H351)	
	Repr Cat 3 (R62, R63)	Repr 2 (H361f, d)	
	Muta Cat 3 (R68) with threshold	Muta 2 (H341) with threshold	
	R64	Lact (H362)	
D	Carc Cat 1, 2 (R45, R49)	Carc 1A, 1B (H350)	Use of such SoCs to be discouraged; however, if essential and no safer alternatives available, a full risk assessment should be conducted against EU IOELVs (when available), DNELs, DMELs, other references values (e.g. AELs and AECs) or in qualitative manner
	Repr Cat 1, 2 (R60, R61)	Repr 1A, 1B (H360F, D)	
	Muta Cat 3 (R68) with no threshold	Muta 2 (H341) with no threshold	
	Muta Cat 1, 2 (R46)	Muta 1A, 1B (H340)	

BAND A – This band includes SoCs which trigger products to be classified for moderate acute toxicity, including narcosis, and/or mild irritation. It should be noted that for these hazards, a fully quantitative risk assessment is not usually performed because only qualitative or semi-quantitative dose-response information is normally available. It is proposed that for these SoCs, appropriate risk mitigation measures, in the form of the safety (S)-phrases triggered by the relevant risk (R)-phrases under Directive 67/548/EEC or the precautionary (P)-statements normally associated with the concerned hazard (H)-statements under the CLP Regulation, should be applied. If acute toxicity and/or irritation studies on the formulation are available, these should be considered to verify whether the predicted hazard(s) (by the calculation method of Directive 1999/45/EC and CLP Regulation) are confirmed. If the predicted hazards are not confirmed by the formulation test data, then the product is no longer classified for acute toxicity/irritation and there is no need to perform an evaluation of the initial SoC. If the predicted hazards are confirmed, then the evaluation/risk management requirements associated with this band should be applied.

BAND B - This band includes SoCs which trigger products to be classified for severe or very severe acute toxicity, corrosion and/or sensitisation. As for the hazards in band A, a fully quantitative risk assessment is not usually performed for these properties because only qualitative or semi-quantitative dose-response information is normally available. It

is proposed that for these SoCs only a qualitative risk assessment is performed. This should consider the potential for exposure to the SoC, by taking into account the physical-chemical properties of the SoC (e.g. dustiness, volatility), the concentration of the substance in the product and the use pattern of the product. If exposure is regarded to be significant, in addition to the S-phrases/P-statements normally associated with the concerned R-phrases/H-statements, further risk mitigation measures, as appropriate, should be considered.

Similarly to the SoCs in band A, if acute toxicity, irritation and/or sensitisation studies on the formulation are available, these should be considered to verify whether the predicted hazard(s) (by the calculation method of Directive 1999/45/EC and CLP Regulation) are confirmed. If the predicted hazards are not confirmed by the formulation test data, then the product is no longer classified for acute toxicity/irritation/sensitisation and there is no need to perform an evaluation of the initial SoC. If the predicted hazards are confirmed, then the evaluation/risk management requirements associated with this band should be applied.

BAND C - This band includes SoCs which trigger products to be classified for repeated dose toxicity, lactation effects and/or carcinogenicity, mutagenicity (when a threshold approach can be taken) or reprotoxicity in the lowest category. Since for these hazards quantitative dose-response information is normally available, it is proposed that a fully quantitative risk assessment is performed according to ECHA BPR Guidance for Human Health Risk Assessment (Volume III, Part B). This will entail for each SoC a comparison of the exposure estimates with appropriate toxicological reference dose levels. Wherever possible, no additional animal testing on the SoC should be conducted simply for the purposes of establishing a reference dose value for the SoC within the scope of the BPR. Every effort should be made by the applicant to avoid further vertebrate testing and to gain access to available data/information. It can be reasonably assumed that as a minimum the information that has triggered the classification of the SoC must exist. If the applicant is unable to obtain access to the available data, other evaluation options should be discussed with the regulatory authority.

For SoCs for which Community workplace exposure limits (IOELVs – Indicative Occupational Exposure Limit Values) have been set, a quantitative inhalation risk assessment for the professional operator against the IOELV should always be conducted. If the IOELV is associated with a "skin notation" and is driven by systemic effects (rather than local effects), then route-to-route extrapolation should be performed (using standard parameters for body weight and ventilation rate) to derive a dermal or a systemic IOELV. This should then be used to conduct a dermal quantitative risk assessment for the professional operator. If the extent of dermal absorption for the SoC is not known, a default, worst-case dermal absorption value should be used (according to Guidance on Dermal Absorption; EFSA Journal 2012; 10(4):2665). If a risk assessment for members of the general public is also required, it should be considered whether the IOELV is appropriate for such use or whether it should be lowered by the application of an assessment factor to take account of vulnerable groups.

For SoCs for which IOELVs have not been set or are not appropriate (e.g. for non-professional users), the existence of other possible reference values should be explored. As it is most likely that the majority of co-formulants used in biocidal products fall within the scope of REACH registration, it is proposed that DNELs (Derived No Effect Levels) stipulated within the Chemical Safety Assessment (CSA) of registration dossiers are used as reference values. DNELs for specific substances can be retrieved from the dissemination website of ECHA, but also from the extended SDSs (Safety Data Sheets) accompanying such substances. DNELs for different routes of exposure and for different populations (workers and general public) should normally be available. Relevant DNELs for different SoC exposure scenarios should be selected as appropriate. It is noted that DNELs are derived by registrants and actors in the chemical supply chain. At present,

only a very small minority of these DNELs has been validated by the regulatory system (ECHA or Member States). Therefore, if a national authority has concerns about the validity of any such DNELs for a specific SoC, alternative and more stringent reference values could be set, as appropriate.

With regard to the exposure assessment of the SoC, a Tier II evaluation should be undertaken only if unacceptable risks are identified at Tier I.

BAND D – This band includes SoCs which trigger products to be classified for carcinogenicity and reprotoxicity in the highest two categories and for mutagenicity in all three categories, including the lowest category (the latter only when a non-threshold approach is taken). These are serious hazards which, with the exception of mutagenicity in the lowest category, meet the exclusion criteria for approval (Art (5) of the BPR) when expressed by the active substance. It is therefore proposed that the use of such co-formulants/SoCs in biocidal products should be discouraged. This approach is in accordance with Annex III of Regulation (EC) No 1107/2009 (unacceptable co-formulants). However, if an applicant shows that they are essential in the formulation and there are no safer alternatives, then a full risk assessment should be performed to determine whether or not they pose an unacceptable risk. Similarly to the SoCs in Band C, the risk assessment of these SoCs should be performed against an IOELV, when available, or a DNEL (for threshold effects), DMEL –Derived Minimal Effect Level (for non-threshold genotoxic carcinogens) or in a more qualitative manner (for non-threshold effects for which suitable quantitative dose-response information is not available).

As explained above, the proposed banding evaluation scheme applies to those (toxicological) SoCs clearly defined in Art 3(f) of the BPR, i.e. classified co-formulants present in a biocidal product at concentrations leading to the classification of the product according to Directive 1999/45/EC or the CLP Regulation.

With regard to the additional SoCs identified through the "other grounds for concern" route, the principles of the scheme can be adapted to be applicable to these substances as well. Therefore, the following is proposed in terms of evaluation/risk management requirements:

- SoCs meeting criterion (1) – This criterion will ultimately capture, over and above the clearly-defined SoCs specified in Art 3(f) of the BPR, co-formulants classified for acute toxicity and/or irritation/corrosion, which are present in the biocidal product at concentrations insufficient to trigger the classification of the product by themselves, but that together with other co-formulants/active substance(s) contribute to the classification of the product. Depending on the severity of the hazard classification, the requirements of band A or B (appropriate risk mitigation measures/qualitative risk assessment) should apply to these SoCs.
- SoCs meeting criterion (2) – This criterion identifies other active substances in the biocidal product that act as co-formulants (e.g. in-can preservatives). Reference values (i.e. AELs – Acceptable Exposure Levels and AECs – Acceptable Exposure Concentrations) will normally be available for these SoCs. Therefore, the requirements of band C (i.e. a fully quantitative risk assessment) should apply to these SoCs.
- SoCs meeting criterion (3) – This criterion identifies synergists. For these substances, an appropriate evaluation of the risks posed by the active substance in the presence of the synergist rather than an evaluation of the risks posed by the synergist/SoC itself should be undertaken. The principles of band C (i.e. a fully quantitative risk assessment) should apply to this scenario.
- SoCs meeting criterion (4) – This criterion will ultimately capture, over and above the clearly-defined SoCs specified in Art 3(f) of the BPR, endocrine disruptors (EDs) and substances with hazards of equivalent concern to CMR 1A or 1B (under

CLP Regulation). These are serious hazards, and in the case of EDs, they meet the exclusion criteria for approval (Art (5) of the BPR) when expressed by the active substance. Therefore, the requirements of band D (full risk assessment) should apply to these SoCs.

- SoCs meeting criterion (5) – This criterion identifies substances for which there are EU IOELVs. The requirements of band C should apply to these SoCs.

EUROPEAN CHEMICALS AGENCY
ANNANKATU 18, P.O. BOX 400,
FI-00121 HELSINKI, FINLAND
ECHA.EUROPA.EU