ECHA
EUROPEAN CHEMICALS AGENCY

SUPERSEDED GUIDANCE – NEWER VERSION AVAILABLE

Guidance on the Biocidal Products Regulation

Volume III: Human health
Part A: Information Requirements

Version 1.1
November 2014

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 Biocidal Products Regulation: Volume III: Human health - Part A: Information Requirements

Reference: ECHA-14-G-12-EN
ISBN: 978-92-9247-005-0
Publ.date: November 2014
Language: EN

If you have questions or comments in relation to this document please send them (indicating the document reference, issue date, chapter and/or page of the document to which your comment refers) using the Guidance feedback request form. The feedback form can be accessed via the ECHA Guidance website or directly via the following link: https://comments.echa.europa.eu/comments_cms/FeedbackGuidance.aspx

European Chemicals Agency

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

DOCUMENT HISTORY

Version	Comment	Date
Version 1.0	First edition	June 2013
Version 1.1	Corrigendum: - Division of the guidance in the 4 volumes of the new BPR Guidance structure - Minor editorial changes	November 2014

PREFACE

The Guidance on the Biocidal Products Regulation – Part A (information requirements) 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 BPR lays down rules and procedures for the approval of biocidal active substances and the authorisation of biocidal products.Consequently, applicants should use this document when preparing dossiers according to:

- Articles 4-9 on validation, evaluation and approval of a new active substance,
- Articles 13 and 14 on the renewal of an approval,
- Articles 12-15 on the review of an approval, or
- Articles 19-21 on the authorisation of a biocidal product.

The scientific guidance provides technical scientific advice on how to fulfil the information requirements set by the BPR, how to perform the risk assessment and it explains the guiding principles for the evaluation of the applications to be performed by the authorities. Part A of each Volume of the Guidance on BPR deals with the information requirements on active substances and on biocidal products and provides technical advice on how to fulfil the information requirements set by the BPR. There are four volumes by major areas, namely:

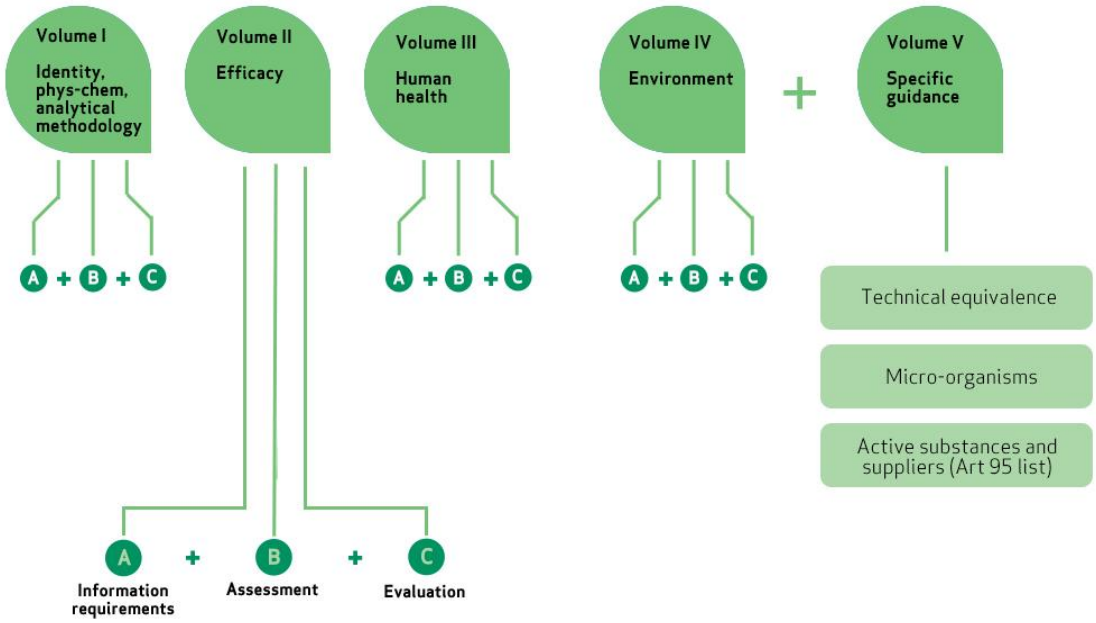
- I. Identity/physico-chemical properties/analytical methodology
- II. Efficacy
- III. Human health (this document)
- IV. Environment

Volume I, also includes (general) information requirements to be included in the dossier as well as the technical advice to fulfil the requirements in the area indicated above. as well as the technical advice to fulfil the requirements in the area indicated above.

To make clear which sections of Annexes II and III of the BPR are covered in each of the four Volumes of Guidance Part A, a finder (see Section 1.9) has been created. The finder contains two tables relating the sections of Annexes II and III of the BPR with the Volume of the guidance where they are covered

The complete guidance series in support of the BPR is shown in the figure below:

Figure 1: BPR guidance structure



The four volumes of the new BPR guidance structure are based on the Technical Notes for Guidance (TNsG) on data requirements under the previous legislation, the Biocidal Products Directive (BPD). However, the information requirements compared to the BPD have changed. Major differences are:

1. The term *information requirement* is used instead of *data requirement.* The new term reflects the fact that applicants do not, in all cases, need to supply data, i.e. information originating from studies but also general information such as addresses and names as well as (quantitative) structure–activity relationship (Q)SAR and so forth.

2. The harmonisation with Guidance from other legal frameworks was a key objective:

    a. When applicable, endpoint sections entail a reference to a relevant REACH (Regulation (EC) No 1907/2006 on Registration, Evaluation, Authorisation and Restriction of Chemicals) Guidance if available;

    b. When applicable, Guidance from the Plant Protection Products Regulation (PPPR, Regulation (EC) No 1107/2009) – Uniform Principles is referred to.

3. The structure has been modified in accordance with the new BPR Annex structure:

    a. The core data set (CDS) and additional data set (ADS) are listed in the same section.

    b. The specific rules for adaptation from standard information requirements (including those given by BPR Annex II and III

column 3) are included in the respective endpoint sections, where available.

4. The core data requirements have been modified and certain long term animal studies are only required when necessary.

5. The BPR also allows for a more systematic approach to the adaptation of information requirements based on exposure as well as the use of techniques such as read-across, (Q)SAR and calculation methods.

6. The principle of proposing and accepting adaptations to the information requirements has been formalised and Member States have to inform and, if possible, assist the applicants with their adaptation requests.

7. It is possible to provide a reduced data package on a case-by-case basis when applying for product authorisation, taking into account the nature of the product and the expected level of exposure.

- When reading this document, please note that the text written in *italics* originates from the BPR or its Annexes. The numbering of the requirements corresponds to the numbering in the BPR Annexes II and III.

Table of Contents

Table of Figures

Table of Tables

List of Abbreviations

Standard term / Abbreviation	Explanation
(Q)SAR	(Quantitative) structure activity relationship
ADME	Administration distribution metabolism and excretion
ADI	Acceptable daily intake
ADS	Additional data set
AEL	overall systemic limit value for the human population
ASTM	American Society for Testing and Materials
BCF	Bioconcentration factor
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
Cat	Category
CDS	Core data set
CEN	European Committee for Normalisation
CIPAC	Collaborative International Pesticides Analytic Council Ltd.
CLP (Regulation)	Classification, Labelling and Packaging Regulation. Regulation (EC) No 1272/2008 of the European Parliament and of the Council on Classification, Labelling and Packaging of substances and mixtures
dw	Dry weight
DG	European Commission Directorate General
DG SANCO	European Commission Directorate-General for Health and Consumers
DNA	Deoxyribonucleic acid
DNT	Developmental Neurotoxicity
DoA	Date of application
DWD	European Drinking Water Directive (Directive 98/83/EC)
EC	European Communities or European Commission
EC method	Test Method as listed in the Test Methods Regulation
ECHA	European Chemicals Agency
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EEC	European Economic Community
EFSA	European Food Safety Agency
EN	European norm
EPA (DK, USA)	Environmental Protection Agency (of Denmark, or the United States of America)
EPPO/OEPP	European and Mediterranean Plant Protection Organization
ESD	Emission Scenario Document, Guidance developed under the BPD tailored for biocides
EU	European Union

Standard term / Abbreviation	Explanation
g	Gram(s)
GC	Gas chromatography
GLP	Good laboratory practice
h	Hour(s)
ha	Hectare(s)
HPLC	High performance (or pressure) liquid chromatography
IPCS	The WHO International Programme on Chemical Safety
ISBN	International standard book number
ISO	International Standards Organisation
ISO (TC, SC, WG)	International Standards Organisation Technical Committee, Scientific Committee, Working Group
ISSN	International standard serial number
ITS	Integrated testing strategy
IUCLID	International Uniform Chemical Information Database
IUPAC	International Union for Pure and Applied Chemistry
JRC	Joint Research Centre
kg	Kilogram(s)
K_{ow}	Octanol-water partition coefficient
Kst	Dust explosion constant
LD_{50}	Lethal dose for 50% of the group of tested animals
LLNA	Murine local lymph node assay
mg	Milligram(s)
MMAD	Mass median aerodynamic diameter
mol	Mole(s)
MOTA	Manual of Technical Agreements of the Biocides Technical Meeting
MRL	Maximum residue limit
MS	Mass spectrometry
MSCA	Member State competent authority
nm	Nanometre(s)
NMR	Nuclear magnetic resonance
NOAEL	No observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
OPPTS	Office of Prevention, Pesticides, and Toxic Substances (U.S.-EPA)
Pa	Pascal(s)
PBPK	Physiologically-based pharmaco(toxico)-kinetics
pH	pH-value, negative decadic logarithm of the hydrogen ion concentration
PPPR	Plant Protection Products Regulation. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of concerning the placing of plant protection products on the market
PT	Product-type
$rate_{a.s.}$	Use rate of active substance [kg /ha]
$rate_{metabolite}$	Application rate at which metabolite should be tested (kg/ha)

Standard term / Abbreviation	Explanation
REACH	Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
s	Second(s)
SMEs	Small and medium-sized enterprises
TC	Technical material In accordance with FAO manual (FAO, 2010), TC is usually the final product from preparation of the active substance prior to being formulated into an end-use product. This may contain a stabiliser and/or anti-caking or anti-static agents (if required) but no other additives. TC is usually ≥900 g/kg with solvent(s) removed during synthesis, with only residual amounts remaining (usually ≤10%) and no solvent added subsequently.
Test Methods Regulation	Regulation (EC) No 440/2008 laying down test methods pursuant to the REACH Regulation
TK	Technical concentrate In accordance with FAO manual (FAO, 2010), TK may also be the final product from preparation of the active substance but it may contain additives (not formulants) in addition to a stabiliser, for example as safety agents. TK may also contain solvent(s) (including water), either deliberately added to a TC or not removed during preparation.
TGD	Technical Guidance Document (EU, 2003)
TNsG	Technical Notes for Guidance
UDS	Unscheduled DNA synthesis
UN	United Nations
UV	Ultraviolet
VDI	Verein Deutscher Ingenieure (The Association of German Engineers)
WHO	World Health Organisation

I. INTRODUCTION TO THE GUIDANCE ON INFORMATION REQUIREMENTS

Regulation (EU) No 528/2012 of the European Parliament and of the Council (Biocidal Products Regulation, the BPR) lays down rules and procedures for approval of the active substances in biocidal products at European Union (EU) level and for the authorisation of biocidal products in both Member States and at EU level[1]. The objective of the BPR is to improve the functioning of the internal market on biocidal products whilst ensuring a high level of environmental and human and animal health protection. In addition, the BPR removes a number of deficiencies that were identified during the implementation of Directive 98/8/EC of the European Parliament and of the Council on the placing on the market of biocidal products (BPD).

Study data and other information must fulfil the minimum requirements whilst being sufficient to conduct a proper risk assessment in order to finally allow for a decision on the suitability of the substance to be approved or, the product to be authorised.

The BPR set out rules on information requirements (especially in Articles 6-8). The information requirements are specified for active substances in Annex II, and for the respective biocidal products in Annex III (in Title 1 of Annex II/III for chemicals and Title 2 of Annex II/III for micro-organisms).

Due to the wide scope of the BPR and the extensive variation of exposure and risks of biocidal products, the general rules provided in the BPR and its Annexes have to be specified in order to ensure efficient and harmonised day-to-day implementation of the regulation. The aim of the Guidance is to provide detailed and practical direction on which study data and other information should be submitted, when applying for approval and authorisation according to the BPR. The requirements outlined in Volume II of the Guidance are also applicable for the simplified authorisation procedure, i.e. those products that fulfil all conditions of the requirements listed in Article 25 of the BPR.

It should be noted that only chemical biocidal products (Title 1 of Annex III to the BPR), including treated articles, and chemical active substances (Title 1 of Annex II to the BPR) are covered by the present document. Guidance on the information requirements for micro-organisms will be available separately in Guidance on micro-organisms (Volume V). Guidance on substances of concern will be available in Part B of Volumes III and IV..

Several documents published by the Commission and ECHA have been used as a basis for the information requirements presented. The most important documents are listed in the Section 1.3.

This Guidance is primarily addressed to applicants, seeking approval of an active substance and for authorisation of a biocidal product, who submit information to the Member State competent authorities (MSCA). The MSCAs task is then to validate and evaluate the application, (adequacy and relevance) of the submitted information.

1.1 Structure of the Guidance on information requirements

1.1.1 Information requirements

The information requirements are two-tiered:

[1] The terms 'EU' or 'Community' used in this document cover the EEA States. The European Economic Area is composed of Iceland, Liechtenstein, Norway and the EU Member States.

I. The core data set (CDS) is mandatory for all product-types. This information always has to be submitted, unless the rules for adaptation of standard information are applicable (see below).

II. The additional data set (ADS) might be required to perform the risk assessment under the following conditions:

   a. ADS information on physical chemical properties, methods of detection and identification and on the toxicological profile is required depending on the intrinsic properties of the active substance or the biocidal product.

   b. ADS information on the ecotoxicological properties and the environmental fate and behaviour of the active substance or biocidal product is required depending on the product-type, i.e. the foreseen use and route of exposure.

   c. ADS information on the ecotoxicological properties and the environmental fate and behaviour might be required to refine the initial risk assessment.

In each of the volumes, the information requirements are divided into two parts:

1) The CDS and ADS for active substances in Chapter II,

2) the CDS and ADS for biocidal products in Chapter III.

The CDS together with the ADS comprise the complete set of information on the basis of which an overall and adequate risk assessment can be carried out.

1.1.2 Comparison BPD-BPR

Figure 2represents a comparison of the structure of the data requirements or information requirements, respectively, under the BPD and under the BPR. In the BPD legal text as well as in the TNsG on data requirements (EU, 2008a), CDS and ADS are listed in separate Annexes. In contrast, the BPR text lists both CDS and ADS in the same Annexes. In addition, '*specific rules for adaptation from standard information concerning some of the information requirements that may require recourse to testing of vertebrates*' represent data waiving possibilities and are listed alongside the respective endpoints in Annexes II and III in the BPR.

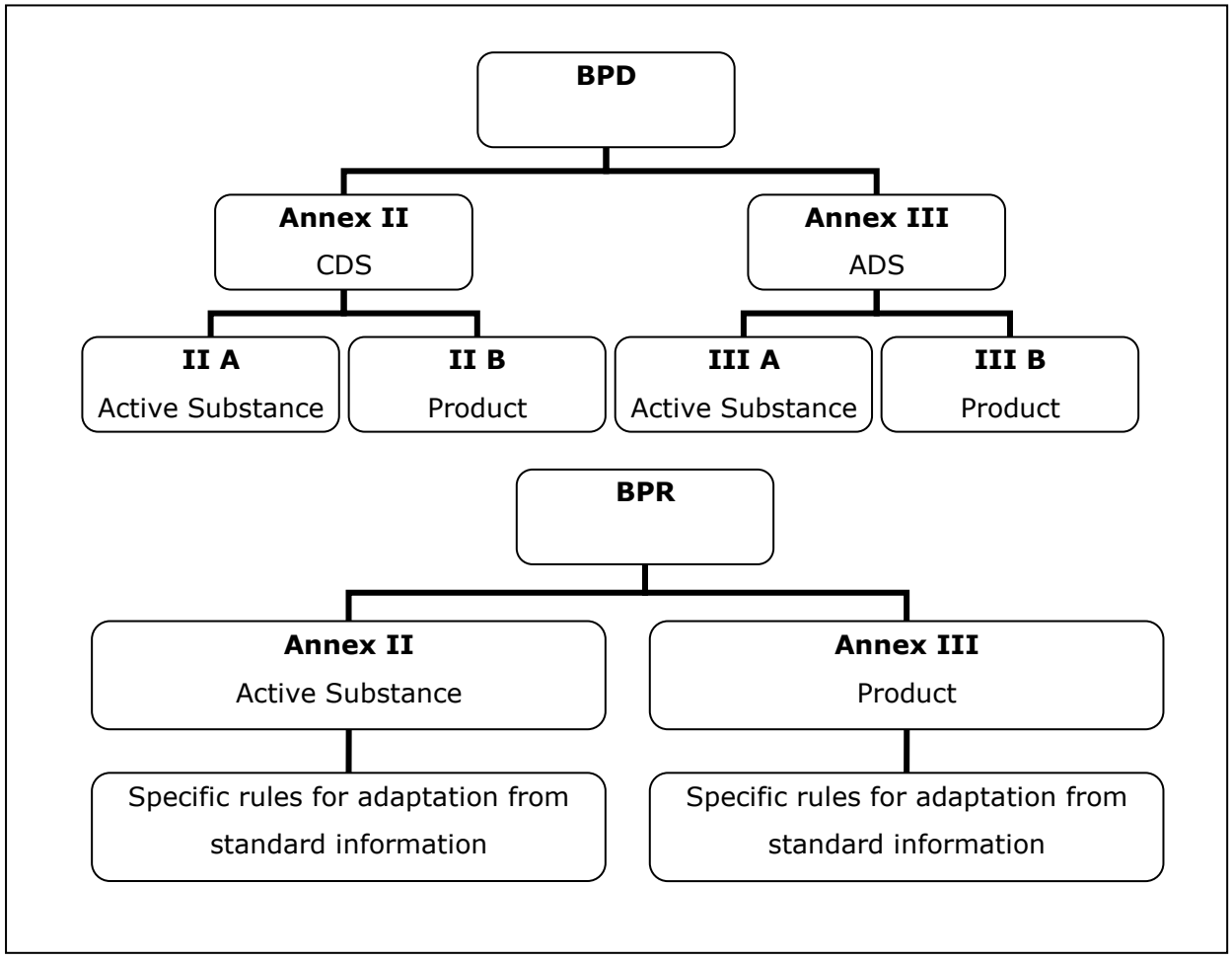


Figure 2 Structure of data/information requirements under the BPD and the BPR.

Unlike under the BPD, the information requirements in Annexes II and III of the BPR are listed in three columns: column 1 contains the actual requirements, column 2 indicates whether it is a CDS or an ADS, column 3 contains waiving statements when applicable (see Table 1). General rules for data waiving can be found in Annex IV of the BPR.

Table 1 Three-column- structure of BPR information requirements in Annexes II and III of the BPR.

COLUMN 1	COLUMN 2	COLUMN 3
Information requirement	ADS label or no label (for CDS)	Specific rules for adaptation from standard information concerning some of the information requirements that may require recourse to testing of vertebrates.

1.1.3 Document structure

As detailed in the preface, the guidance on BPR consists of four volumes and each volume has three Parts. Part A deals with Information Requirements.

This document (Volume III, Part A) covers the specific information requirements for human health :

Chapter I contains general guiding principles for information submission.

Chapter II covers CDS and ADS information requirements as listed in Title 1 of Annex II to the BPR. The chapter explains the BPR requirements for active substances (chemical substances) and contains references to relevant test methods and further guidance. For example, it offers guidance on which test is the most suitable for specific cases. In addition, the chapter contains the *specific rules for adaptation from standard information*, where applicable. These *waiving* rules are generally accepted, scientifically or technically justified exemptions to the information requirements.

Chapter III provides CDS and ADS information requirements as listed in Title 1 of Annex III of the BPR. The chapter explains the BPR requirements for biocidal products (chemical products) and contains references to relevant test methods and further guidance. Similar to Chapter II, it also contains references to relevant test methods and explains the Annex III requirements. It also lists the *specific rules for adaptation from standard information*.

The endpoint-specific sections Chapters II and III are numbered in the same way as the BPR text.

1.2 Guiding principles with regard to information requirements

The following guiding principles reflect the general guidance on information requirements as provided in the BPR.

1. **The common core data set (CDS)** forms the basis of the requirements. In general, it is regarded to be a **minimum set** required for all substances and product-types.

2. **The additional data set (ADS)** includes supplementary information requirements. This information may be required depending on **the characteristics** of the active substance and/or the product-type and on the expected exposure of humans, animals and the environment. The product's use or application method needs to be taken into account under both the proposed normal use and a possible realistic worst case situation (Article 19(2) of the BPR).

3. **The adaptation of information requirements** (i.e. 'data waiving') outlined throughout this Guidance is possible in certain cases for both CDS and ADS. As an example, some of the toxicological information requirements may be adapted occasionally when the exposure is limited or when other product-type-specific factors apply. Sufficient and acceptable justification needs to be provided for the adaptation. In addition, the inherent physical and chemical properties of the substance or the product may justify waiving of some information requirements. The guidance on General Rules for the Adaptation of the Data Requirements is under development by the Commission and will be made available accordingly.

Until then please refer to Chapter 1 Section 1.4 of the TNsG on Data Requirements (EU, 2008a) REACH, Guidance on QSARs and grouping of chemicals (ECHA, Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals) could also be useful.

4. The information requirements have been specified in as much detail as possible. However, in certain cases, **expert judgement** by the applicant and by the competent authority may be necessary in order to assess, for instance, whether an additional study is needed or on which organism or under which conditions a test should be performed. The applicant should propose the initial expert judgement, which is then examined during the evaluation. In making the decision as to whether additional testing is justified, the benefit for the risk assessment, the compatibility with accepted risk assessment rationales, and the feasibility of the required tests may have to be considered. When providing an expert judgement one must, when relevant, take into account both the proposed normal use and a possible realistic worst case situation. Expert judgement decisions should be scientifically justified and transparent. In certain cases, the final decision on information requirements is made by the Biocidal Products Committee (BPC). Special attention is required in cases where there are endpoints of concern and clearly defined or standardised methods are lacking. Here, the applicant is obliged to investigate if relevant methods are applicable. New test methods are continuously being developed and it is the applicant's duty to be up-to-date with the state of science regarding test methods.

5. It is always the **applicant who is responsible** for the submission of the data. All data provided in the application must always be supported by study reports, other data or a letter of access. The information submitted by the applicant on both active substances and biocidal products, and also on substances of concern present in the biocidal product must be sufficient for conducting a risk assessment and decision-making both at EU level and on the level of the individual Member States. The applicant should consult a competent authority to which data should be submitted. This will allow for proper risk mitigation measures to be decided upon if an active substance is likely to fail the criteria for entry into the *Union list of approved active substances* or if a product is likely to fail the criteria to be authorised at national or EU level.

6. The data submitted by the applicant will form the basis for classification and labelling according to the CLP Regulation (harmonised classification in case of active substances and self-classification in case of biocidal products). The active substances may be subject to harmonised classification for the first time or the data can be used to review a previous harmonised classification.

7. The data and test requirements should suit the individual circumstances and thus make it possible to assess the risks under a range of conditions. The following parameters should be taken into account when preparing the application for authorisation:

    a. The characteristics of the application technique,

b. The user type (e.g. professional or non-professional users), and

c. The environment, in which the product is intended to be used or into which the product may be released.

8. Article 62 (1) of the BPR states that *In order to avoid animal testing,* ***testing on vertebrate animals*** *for the purposes of this Regulation shall be undertaken* ***only as a last resort****. Testing on vertebrate animals shall not be repeated for the purposes of this Regulation.* Concerning the latter, further detailed rules are provided in Article 62 (2) of the BPR. The data generated and collected under other legislative regimes, especially under Council Regulation (EU) No 544/2011, Council Regulation (EC) No 1907/2006 and Council Regulation (EC) No 1272/2008 should be used, taking into account the rules on data protection. Sharing of vertebrate data submitted under the BPD or BPR is mandatory.

9. With regard to data sharing, for guidance see the ECHA Biocides Guidance webpages and the reference to the REACH Guidance on data sharing established by ECHA (in accordance with Regulation 1907/2006 (REACH) and the Explanatory Note clarifying which chapters are of relevance to the applicants under Biocidal Products Regulation (EU) No528/2012 (BPR), [http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation].

10. For renewal of a product authorisation the applicant must submit **all relevant data required under Article 20 of the BPR, that it has generated since the initial authorisation**. This requirement corresponds to the obligation to submit any new data after the authorisation has been granted (Article 13(2) of the BPR). This only applies to data that were generated by the applicant and not any other data that may be available. For example, if several reports on similar studies are available to the applicant they should all be submitted to allow a more sound risk assessment with, among others, assessment of inter-species variability. The additional data should be of an acceptable quality (see Annex IV, point 1 of the BPR).

11. Point 8 (a) of Annex VI to the BPR states that for the evaluation of a biocidal product, the evaluating competent authority *shall take into consideration other relevant technical or scientific information which is reasonably available to them with regard to the properties of the biocidal product, its components, metabolites, or residues*. This means that Member States and other stakeholders should also submit relevant data to the evaluating competent authority relevant data, which is reasonably available to them but which has not been available to the applicant. The applicant is not responsible for this additional information. The applicant, however, is responsible to search for data from all sources which he or she may reasonably be expected to have access to.

12. Public literature data can be used in the assessment if the following conditions are fulfilled:

a. The data comply with the BPR Annex II, III introduction points 5-9.

b. The identity, purity and the impurities of the substance have to be defined in the publication and to be comparable with the substance addressed in the application.

c. The reporting of the study allows evaluation of the quality of the study.

If conditions a-c are met the applicant can claim that adequate data is publicly available. Providing that the quality of public data fulfils the criteria, it can be used as key studies.

13. There must be at least one key study or an accepted waiving justification for each CDS endpoint given in the BPR Annexes II and III. The same applies to ADS endpoints in the BPR Annexes II and III, depending on the product-type (in the case of ecotoxicology endpoints and environmental fate and behaviour) and on intrinsic physical-chemical or toxicological properties of the substance or the product, respectively. A key study is the critical study for a certain endpoint and has to be reliable and adequate to use for the risk assessment. For criteria on the selection of key studies and further information, see TNsG on Preparation of Dossiers and Study Evaluation (EU, 2008b). A study with a reliability indicator of 3 or 4 cannot be a key study and can be used only as supportive information.

14. When more than one adequate study is available, expert judgement should be used to decide whether mean or median values should be used instead of the result of a single key study. If there is divergent data from acceptable studies, a study summary should be provided for all these studies. The study summary of each key study must be presented in the IUCLID file.

15. It is always possible to require additional information or studies if this is considered to be necessary for a proper risk assessment and decision making. The need for additional studies may be justified either by the properties of the chemical (i.e. hazard) or by the predicted exposure. In Article 8(2) of the BPR it states that *where it appears that additional information is necessary to carry out the evaluation, the evaluating competent authority shall ask the applicant to submit such information within a specified time limit, and shall inform the Agency accordingly.* In that case, the stop-the-clock rule is applied. Data may also be required for a **substance of concern** present in the biocidal product other than the active substance. General rules and information requirements for substances of concern are is under development by the Commission and will be made available accordingly. . However, the detailed requirements are left mainly to be judged on a case-by-case basis. If the outcome of the applicant's assessment indicates a need for more data, the applicant should already consider further requirements.

16. Point 11 of Annex VI to the BPR states that *During the process of evaluation, applicants and the evaluating bodies shall* ***cooperate*** *in order to resolve quickly any questions on the data requirements, to identify at an early stage any additional studies required, to amend any proposed conditions for the use of the biocidal product, or to modify its nature or its composition in order to ensure full compliance with the requirements of Article 19 and of this Annex. The administrative burden, especially for SMEs, shall be kept to the minimum*

necessary without prejudicing the level of protection afforded to humans, animals and the environment. BPR Specifically SMEs should be allowed extensive guidance from the competent authorities in order to be able to fulfil the obligations laid down in the BPR.

17. For the approval of the active substance a specification of the active substance will need to be derived. This specification must be representative for the manufacturing process as well as for the (eco)toxicological batches tested or, in other words, the reference source would be the source for which the (eco)toxicological data submitted cover the specification. Therefore it needs to be ensured that all impurities in the proposed specification are considered in the environmental fate and (eco)toxicological studies (batches used for the environmental fate and (eco)toxicological studies may contain impurities at levels equal or higher than the proposed specifications or it can be justified why some impurities in the proposed specification are not covered by these studies).

1.3 On the use of additional Guidance documents

1.3.1 Existing biocides Guidance and other relevant documents

Part A in each of the four Volumes of the BPR Guidance replaces the TNsG on Data Requirements in support of the BPD (EU, 2008a). The remaining Guidance and other relevant documents that have been drafted to be used under the BPD, should also still be followed after 1 September 2013 until such a time that all the new Guidance under the BPR is completed and published. Completion of all the new BPR guidance will be published on ECHA's website.

This BPD Guidance and relevant documents should be utilised notwithstanding the references to the BPD and without prejudice to the scientific content. The BPD Guidance and related documents consist of:

- Emission Scenario Documents (ESD) which represent the main guidance to estimate the amount of substances released into the environment.
- Technical Guidance Document (TGD) which forms the basis for the exposure- and risk assessment of both active substances and products.
- Technical Notes for Guidance (TNsG) which deal specifically with biocides and BPD implementation.
- The Manual of Technical Agreements (MOTA) which contains decisions from Biocides Technical Meetings on the technical aspects of the risk assessment (EU, 2011a). The MOTA represents a living document, which is constantly updated. Comments from the MOTA are included in this Guidance where considered appropriate.
- EU Evaluation Manual for the Authorisation of Biocidal Products (EU, 2012a).

The BPD Guidance and MOTA are accessible either from the ECHA website:

http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation.

The Evaluation Manual is available at the Biocides Circa website maintained by DG ENV: https://circabc.europa.eu/w/browse/92668ddd-fd3e-4b7e-9232-b80686747060.[2]

1.3.2 REACH Guidance

In addition, REACH Guidance represents a major guidance source. The REACH Guidance should be taken into account for the evaluation of biocides, where relevant and indicated. The use of REACH Guidance is recommended for a number of endpoints with the intention of facilitating a harmonised approach. ECHA Guidance can be obtained from the ECHA website: http://echa.europa.eu/support.

1.3.3 CLP Guidance

In addition, the Guidance on the Application of the CLP Criteria (ECHA) represents an additional guidance source. This guidance document is a comprehensive technical and scientific document on the application of the CLP Regulation. ECHA Guidance can be obtained from the ECHA website: http://echa.europa.eu/support.

1.4 General guidance on generating the information

If new tests are performed in order to fulfil the data requirements, the following principles have to be followed:

According to point 5 of Annex II and Annex III of the BPR, as a general principle, tests *shall be conducted according to the methods described in Commission Regulation (EC) No 440/2008*. These methods ("EC methods") are based on methods recognised and recommended by international bodies, in particular OECD. In the event of a method being inappropriate or not described, *other methods shall be used which are scientifically appropriate*. Their use needs to be justified. Recommended test methods are listed in the endpoint sections.

According to point 6 of BPR Annexes II and III, tests *'should comply with the relevant requirements of protection of laboratory animals, set out in Directive 2010/63/EU'.*

Furthermore, point 6 of BPR Annexes II and III explains that *'Tests performed should comply with… in the case of ecotoxicological and toxicological tests, good laboratory practice…. **or** other international standards recognised as being equivalent by the Commission or the Agency.'* At the moment there are no "other international standards" considered equivalent to GLP.

In addition point 6 of BPR Annexes II and III declares that '*Tests on physico-chemical properties and safety-relevant substance data should be performed at least according to international standards*.') The test methods for the physico-chemical properties are described in the Test Methods Regulation (EC No 440/2008), whereas preferred tests for the purposes of physical hazard classification are referred to in Part 2 of Annex I to CLP Regulation, via references to the UN Recommendations on the Transport and Dangerous Goods, Manual of Test and Criteria, UN-MTC (UN, 2009). The testing according to international standards should be interpreted as testing carried out by laboratories complying with a relevant recognised standard (e.g. ISO/IEC 17025, ISO 9001).

2

However, most of the methods listed in the Test Methods Regulation '*are developed within the framework of the OECD programme for Testing Guidelines, and should be performed in conformity with the principles of Good Laboratory Practice, in order to ensure as wide as possible 'mutual acceptance of data'*. From 1 January 2014, new tests for physical hazards must be carried out in compliance with a relevant recognised quality system or by laboratories complying with a relevant recognised standard as stipulated by Article 8(5) of the CLP Regulation. Where relevant recognised standards for testing are applicable, the use of the most recent updates is advised, for example the EN and ISO standards.

Where test data exist that have been generated before the DoA of the BPR by methods other than those laid down in the Test Methods Regulation, the adequacy of such data for the purposes of the BPR and the need to conduct new tests according to the Test Methods Regulation must be decided on a case-by-case basis. Amongst other factors, the need to minimise testing on vertebrate animals needs to be taken into account (Article 90(2) of the BPR). Such a decision should first be proposed by the applicant when collecting data for the application and then evaluated by the competent authority when checking the completeness of the application and approving the justification provided for such a case. If a test has been performed, that does not comply with the Test Methods Regulation, the nature of the differences must be indicated and justified. The same applies to deviations from the test protocol used. The test protocol should be provided in full unless there is sufficient detail in the test report.

In certain cases, testing can be replaced by modelling using (Q)SAR, Quantitative Structure Activity Relation. ECHA Guidance on (Q)SARs and grouping of chemicals is available on the ECHA website. The TGD on risk assessment for new notified substances and existing substances (EU, 2003) contains further information.

As a general rule, tests on the active substance should be performed with the substance as manufactured. For some of the physical and chemical properties' tests, a purified form of the substance is being tested, which is indicated by footnote 2 in Annex II column 1 of the BPR, in other cases, the applicant is free to choose between testing on either purified form or the form as manufactured as indicated by footnote 1 in Annex II column 1 of the BPR. The "Active substance as manufactured" is the active substance in its natural state or as obtained by a production process. This includes any additive necessary to preserve the stability of the products and any impurity deriving from the process used. It excludes, however, any solvent which may be separated without affecting the stability of the substance or changing its composition. Furthermore, the identity, purity and the impurities of the substance have to be defined and to be comparable with the substance subject to the application.

In order to implement the three R's, **R**eplacement, **R**efinement and **R**eduction of animals in research, the following should be taken into account when planning new tests: If there is an established EC test method or OECD test guideline for a given purpose, for example testing of acute oral toxicity, and in addition one or more alternative methods which may equivalently be used, the test method that requires a lower number of test animals and/or causes less pain should be used. A number of alternative tests either not using test animals or reducing the number of test animals are under development and when endorsed, these tests are preferred when new tests have to be performed.

A substance which is approved as an active substance (included in the *Union list of approved active substances)* should be related to the active compound in the formulation. This means that a case-by-case decision must be taken by the evaluating competent authority on the name to be given to the active substance. This could be for example simple ions or different molecular structures, precursor/activator, or unstable/breakdown active components, or multiple component products. The specifications of the used material need to be described in detail (point 7 of Annex IIto

the BPR) i.e. a brief description of the composition for all batches used in tests is needed. Where testing is done using an active substance the material used should be of the same specification as that which would be used in the manufacture of preparations to be authorised except where radio labelled material is used. All batches of a substance or a product used for testing should be representative of typical commercial material for which the approval is applied for and within the production concentration range. If for any test the composition of the substance or product is different from that quoted for commercial material, full details must be provided. Certain exceptions on this general rule are provided in this Guidance. When the long term stability is in doubt, the composition should be determined before testing. Where appropriate, details of the stability of the substance in any vehicle used during testing should also be specified. For certain tests (e.g. some physico-chemical tests) there are specific requirements for purity of the active substance.

In addition, the specific guidance provided in the relevant test guidelines should always be followed. For instance, guidance on when the testing of transformation products instead of the active substance is relevant may be found in the test guidelines concerned.

Some active substances may have characteristics that impede testing or limit the methods that can be used. Substances, which are difficult to test, need special attention (OECD, 2000a). The difficulties may arise from the chemical nature of the substance (e.g. insoluble substances, metals, complex mixtures of chemicals, oxidising substances or surface active compounds (surfactants)). Further difficulties may be owing to the activity of the substance.

Where studies are conducted using an active substance produced in the laboratory or in a pilot plant production system, the studies must be repeated using the active substance as manufactured unless it can be justified that the test material used for the purposes of testing and assessment is technically equivalent. In cases of uncertainty, appropriate bridging studies must be submitted to serve as a basis for a decision on the possible need to repeat studies. The test guidelines usually include guidance on the limitations of the method or give detailed guidance on how the method should be modified when testing chemicals with specific characteristics. Separate Guidance documents may be available for specific testing situations. For instance, Guidance on intermediate compounds has been published (ECHA). The Guidance provided in the Technical Guidance Document concerning risk assessment of new and existing substances Part II (EU, 2003) should also be followed when designing the testing strategy for substances that are difficult to test.

The test results must be reported properly and according to the guidelines used. The study summaries and full study reports of all key studies should be included in the data forwarded to the competent authority. Relevant analytical raw data should be provided on request. For example, individual data points should be provided in addition to mean values and calibration equations should be provided to allow a suitable evaluation of the study by an assessor.

1.5 Guidance on non-submission of information

The guidance text to be provided in this section is under development by the Commission and will be made accordingly. Until then please refer to Chapter 1 Section 1.4 of the TNsG on Data Requirements (EU, 2008a).

1.6 Testing of metabolites and transformation products

For the toxicology aspects of metabolites and transformation products, the possibility of the formation of metabolites not investigated by the usual testing must be taken into account. See Chapter II Section 8.8 on metabolism studies in mammals .

For environmental aspects, metabolites relevant for the risk assessment can be distinguished as:

- Major metabolite:
  - formed in amounts of ≥ 10% of the active substance at any time of the degradation studies under consideration, or
  - the metabolite appears at two consecutive sampling points at amounts ≥ 5%, or
  - at the end of the study the maximum of formation is not yet reached but accounts for ≥ 5% of the active substance at the final time point;
- Minor metabolite: all metabolites not meeting the above criteria;
- Ecotoxicologically relevant metabolite: any minor or major metabolite which e.g. poses a comparable or higher hazard than the active substance.

In general, an environmental risk assessment for the relevant compartments needs to be performed for all major metabolites. However, as a first step a semi-quantative assessment of these metabolites using the available data and expert judgement to fill data gaps may be sufficient. A quantitative assessment should be performed on a case-by-case basis.

If there is any reason for concern, a risk assessment also needs to be performed for those ecotoxicologically relevant metabolites which are minor metabolites.

1.7 Background documents

Legal texts

For the detailed legal texts (plus amendments and annexes, when applicable) cited in this guidance document and listed below in this section, please visit the eur-lex bibliographic website: http://eur-lex.europa.eu. or ECHA website: http://echa.europa.eu/regulations/biocidal-products-regulation/legislation.

Regulations

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC*; (REACH)*

Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH); (Test Methods Regulation)

Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006; (CLP Regulation).

Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC; (PPPR).

Commission Regulation (EU) No 1152/2010 of 8 December 2010 amending, for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.

Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances.

Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products; (BPR).

Commission Regulation (EU) No 487/2013 of 8 May 2013 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures.

Directives

Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances; (DSD, Dangerous Substances Directive).

Council Directive 75/440/EEC of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in the Member States.

Council Directive 80/68/EEC of 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances.

Council Directive 88/379/EEC of 7 June 1988 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations.

Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market; (BPD).

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption; (The Drinking Water Directive (DWD)). Consolidated version 2009-08-07.

Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations; (DPD, Dangerous Preparations Directive).

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy; (The EU Water Framework Directive, WFD). Consolidated version 2009-06-25.

Directive 2004/9/EC of the European Parliament and of the Council of 11 February 2004 on the inspection and verification of good laboratory practice; (GLP).

Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances; (GLP).

Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration; The Groundwater Directive.

Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council; The Priority Substances Directive.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Decisions

2000/532/EC: Commission Decision of 3 May 2000 replacing Decision 94/3/EC establishing a list of wastes pursuant to Article 1(a) of Council Directive 75/442/EEC on waste and Council Decision 94/904/EC establishing a list of hazardous waste pursuant to Article 1(4) of Council Directive 91/689/EEC on hazardous waste.

1.8 Sources of test methods and standards

The EC methods are published in the Official Journal of the European Union. The testing methods are described in the Test Methods Regulation (Regulation (EC) No 440/2008). They are regularly updated with new methods introduced as required. More information on the Test Methods Regulation and alternative methods is available at the website of the DG-JRC Institute for Health and Consumer Protection (http://ihcp.jrc.ec.europa.eu/our_activities/alt-animal-testing/test_method_reg).

The OECD test methods can be obtained directly via their internet address (http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals_chem_guide_pkg-en).

The CIPAC methods may be purchased from the Collaborative International Pesticides Analytical Council (http://www.cipac.org).

ASTM Standards may be obtained from the American Society of Testing Methods, West Conshohocken, Pennsylvania, USA (http://www.astm.org).

European Standards (CEN standards), transposed as national standards, can be purchased from National Members and Affiliates of the European Committee for Standardisation (CEN). Contact information for CEN National Members and also draft

European Standards may be obtained from the CEN Central Secretariat, Brussels, Belgium (http://www.cen.eu).

DIN Standards can be purchased from the website of DIN, the German Institute for Standardisation (http://www.din.de).

VDI Guidelines can be obtained from the website of VDI, The Association of German Engineers (http://www.vdi.de).

EPPO Guidelines may be obtained from the Secretary of the European and Mediterranean Plant Protection Organisation (EPPO), Paris, France (http://www.eppo.int/).

Orders for ISO International Standards should be addressed to the ISO member bodies (non-USA users, if subscribing to Internet from a USA-based provider, should consult the ISO member list for ordering ISO standards in their country) which are normally the primary ISO sales agents, or for customers in countries where there is no member body, to the ISO Central Secretariat, Geneva, Switzerland (http://www.iso.org/iso/store.htm).

The US EPA Office of Prevention, Pesticides, and Toxic Substances Test Guidelines can be obtained from the EPA website (http://www.epa.gov/ocspp/pubs/frs/home/testmeth.htm).

1.9 Finder

Please note that the numbering of the sections and sub-sections in this guidance corresponds to the numbering of the BPR Annexes. This means that the numbering of the sections is not always consecutive and that in some Volumes the numbering does not start at 1.

For reference the following table has been added to relate the Annexes sections with the sections in this document or to provide information on the location of the information (i.e the BPR Guidance Volume).

Table 2 lists the sections of Annexes II and III of the BPR and provides either:

- a link to the section of this document giving the technical advice to fulfil the requirements, or
- indicates where the information can be found; i.e which Volume/Part of the Guidance on BPR where the information can be found.

Table 2: Section of Annex II BPR *vs* Section of this document /Volume of the BPR guidance

Annex II BPR section	Section of this document /Volume of the BPR guidance
1. APPLICANT	Volume I Identity/physico-chemical properties/analytical methodology
2. IDENTITY OF THE ACTIVE SUBSTANCE	Volume I Identity/physico-chemical properties/analytical methodology
3. PHYSICAL, CHEMICAL AND TECHNICAL PROPERTIES	Volume I Identity/physico-chemical properties/analytical methodology
4. PHYSICAL HAZARDS AND RESPECTIVE CHARACTERISTICS	Volume I Identity/physico-chemical properties/analytical methodology
5. METHODS OF DETECTION AND IDENTIFICATION	Volume I Identity/physico-chemical properties/analytical methodology
6. EFFECTIVENESS AGAINST TARGET ORGANISMS	Volume II Efficacy
7. INTENDED USES AND EXPOSURE	Volume I Identity/physico-chemical properties/analytical methodology
8. TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMALS	Chapter II Section 8
9. ECOTOXICOLOGICAL STUDIES	Volume IV Environment
10. ENVIRONMENTAL FATE AND BEHAVIOUR	Volume IV Environment
11. MEASURES TO BE ADOPTED TO PROTECT HUMANS, ANIMALS AND THE ENVIRONMENT	Volume I Identity/physico-chemical properties/analytical methodology
12. CLASSIFICATION, LABELLING, AND PACKAGING	Volume I Identity/physico-chemical properties/analytical methodology

Table 3: Section of Annex III BPR *vs* Section of this document / Volume of the BPR guidance

Annex III BPR section	Section of this document /Volume of the BPR guidance
1. APPLICANT	Volume I Identity/physico-chemical properties/analytical methodology
2. IDENTITY OF THE BIOCIDAL PRODUCT	Volume I Identity/physico-chemical properties/analytical methodology
3. PHYSICAL, CHEMICAL AND TECHNICAL PROPERTIES	Volume I Identity/physico-chemical properties/analytical methodology
4. PHYSICAL HAZARDS AND RESPECTIVE CHARACTERISTICS	Volume I Identity/physico-chemical properties/analytical methodology
5. METHODS OF DETECTION AND IDENTIFICATION	Volume I Identity/physico-chemical properties/analytical methodology
6. EFFECTIVENESS AGAINST TARGET ORGANISMS	Volume II Efficacy
7. INTENDED USES AND EXPOSURE	Volume I Identity/physico-chemical properties/analytical methodology
8. TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMALS	Chapter III Section 8
9. ECOTOXICOLOGICAL STUDIES	Volume IV Environment
10. ENVIRONMENTAL FATE AND BEHAVIOUR	Volume IV Environment
11. MEASURES TO BE ADOPTED TO PROTECT HUMANS, ANIMALS AND THE ENVIRONMENT	Volume I Identity/physico-chemical properties/analytical methodology
12. CLASSIFICATION, LABELLING, AND PACKAGING	Volume I Identity/physico-chemical properties/analytical methodology

II. DOSSIER REQUIREMENTS FOR ACTIVE SUBSTANCES

8. Toxicological profile for human and animal including metabolism

Considerations before initiating testing

Before testing is initiated all available information should be scrutinised for evidence that may indicate severe effects, serious specific system or target organ toxicity (e.g. neurotoxicity or immunotoxicity), delayed effects or cumulative toxicity. Consideration should also be given to tests already performed/submitted for the purpose of other regulatory programmes. All available information on toxicity should be taken into account when choosing the dose range for a new study. If there is concern that an effect is not adequately covered by existing OECD Test Guidelines, specialised study protocols may be used. Whenever deviating from OECD Test Guidelines a justification should also be provided. These specialised study protocols should be designed on a case-by-case basis in order to enable an adequate characterisation of these hazards, including the dose-response, threshold for the toxic effect and an understanding of the nature of the toxic effects. Where a need is identified for a modification in the study protocol to cover specific needs, this will be done in consultation with the evaluating Member State.

The endpoints that need to be addressed for the purpose of the BPR are interlinked and therefore in certain cases sequential testing needs to be taken into account to decide which tests need to be performed and in which order. This is due to the impact findings from one study can have on the classification and labelling and the risk management measures, which can make the requirement for testing of other endpoints redundant.

Error! Reference source not found. shows the relationship between this section on information requirements for the toxicological profile of substances and the Hazard Assessment part of the BPR Guidance (guidance under development). For each toxicological endpoint and the respective information requirements described in the following sections steps 1 and 2 need to be considered first to conclude on the need to conduct further testing using integrated testing strategies (ITS) where relevant.

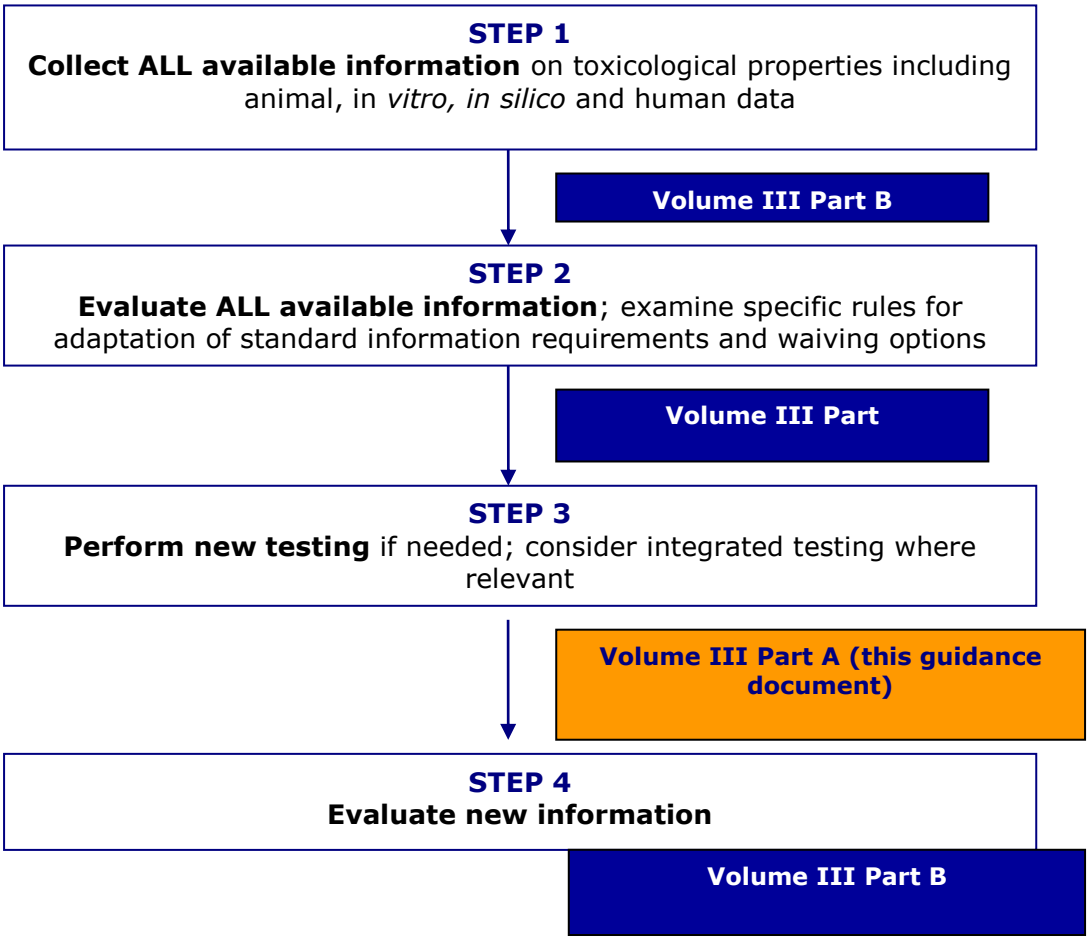


Figure 3 Schematic representation of stepwise approach for fulfilling information requirements for the purpose of the BPR

General considerations for animal data reporting

Where submitted, historical control data should be from the same species and strain, maintained under similar conditions in the same laboratory and should be from contemporaneous studies. Additional historical control data from other laboratories may be reported separately as supplementary information.

The information on historical control data provided should include:

(a) identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location;
(b) name of the laboratory and the dates when the study was performed;
(c) description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed;
(d) approximate age, in days, and weight of the control animals at the beginning of the study and at the time of sacrifice or death;
(e) description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (such as diseases, infections);
(f) name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study;

(g) for carcinogenicity studies: a statement of the nature of the tumours that may have been combined to produce any of the incidence data.

The historical control data should be presented on a study by study basis giving absolute values plus percentage and relative or transformed values where these are helpful in the evaluation. If combined or summary data are submitted, these should contain information on the range of values, the mean, median and, if applicable, standard deviation.

The doses tested, including the highest dose tested, should be selected on the basis of the results of short-term testing and where available at the time of planning the studies concerned, on the basis of metabolism and toxicokinetic data. Dose selection should consider toxicokinetic data such as saturation of absorption measured by systemic availability of active substance and/or metabolites.

Doses causing excessive toxicity should not be considered relevant to evaluations to be made. Determination of blood concentration of the active substance (for example around Tmax) should be considered in long-term repeated dose toxicity studies.

8.1 Skin irritation or skin corrosion

Point 8.1.2 of Annex II to the BPR states that *the assessment of this endpoint shall be carried out according to the sequential testing strategy for dermal irritation and corrosion set out in the Appendix to Test Guideline B.4. Acute Toxicity - Dermal Irritation/Corrosion (Annex B.4. to Regulation (EC)440/2008).*

Steps 1 and 2 Collection and evaluation of available information

Further guidance regarding the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

In principle information requirements for skin irritation/corrosion do not apply in cases when:

1. The available information already indicates that the criteria are met for classification as corrosive to the skin or as a skin irritant.
2. The substance is a strong acid (pH < 2) or base (pH > 11.5).
3. The substance is spontaneously flammable in air at room temperature.
4. The substance is classified as very toxic in contact with skin.
5. An acute toxicity study by the dermal route does not indicate skin irritation up to the limit dose level (2000 mg / kg body weight).

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above further testing is needed to assess the potential for skin irritation or skin corrosion, the following test methods should be used. In addition to the test methods mentioned below, new OECD validated tests for skin irritation/corrosion should be taken into account, once available, in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

The tests will provide information on the degree and nature of skin especially with regard to the reversibility of responses.

1. Testing for skin corrosion (*in vitro* assays)

If after the analysis in steps 1 and 2 above further testing is needed to assess the potential for skin corrosion, one of the following methods should be used.

Test methods for skin corrosion

- EC method B.40 *In vitro* skin corrosion: Transcutaneous Electrical Resistance Test (TER);
- OECD Test Guideline 430: *In vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test;
- EC method B.40 bis *In vitro* skin corrosion: Human Skin Model Test;
- OECD Test Guideline 431: *In vitro* Skin Corrosion: Human Skin Model Test;
- OECD Test Guideline 435: *In vitro* Membrane Barrier Test Method for Skin Corrosion.

Specific limitations that may be described within the Test Guideline protocol should be taken into account before performing a test or during the interpretation of the test results acquired.

If the substance demonstrates corrosive properties following testing according to one of the available OECD and/or EC test guidelines for skin corrosion the Guidance on the Application of the CLP Criteria (ECHA) regarding classification for skin corrosion must be considered.

If the substance does not demonstrate corrosive properties in one of the available OECD and/or EC test guidelines for skin corrosion, proceed to testing for skin irritation as described below.

2. Testing for skin irritation (*in vitro* assays)

To examine the skin irritation potential of an active substance, the following assays should be used.

Test methods for skin irritation

- EC method B.46 *In vitro* skin irritation: reconstructed human epidermis model test;
- OECD Test Guideline 439: *In vitro* Skin Irritation: Reconstructed Human Epidermis Test Method.

Specific limitations that may be described within the Test Guideline protocol should be taken into account before performing a test or during the interpretation of the test results acquired.

3. Testing for skin irritation (*in vivo* assays)

On a case-by-case basis, if specific limitations apply for the conduct of the *in vitro* test to examine skin irritation potential of the substance, as a last resort and with adequate justification *in vivo* testing may be performed with the following test guideline protocol: EC method B.4 Acute Toxicity: Dermal Irritation/Corrosion, OECD Test Guideline 404: Acute Dermal Irritation/Corrosion.

8.2 Eye irritation

Point 8.2 of Annex II to the BPR states that *the assessment of this endpoint shall be carried out according to the sequential testing strategy for eye irritation and corrosion as set down in the Appendix to Test Guideline B.5.Acute Toxicity: Eye Irritation/Corrosion (Annex B.5. to Regulation (EC) No 440/2008).*

Steps 1 and 2 Collection and evaluation of available information

Further guidance regarding the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

In principle information requirements for eye irritation do not apply in cases when:

1. The available information already indicates that the criteria are met for classification of the substance as irritating to eyes or causing serious damage to eyes, or
2. The substance is classified as corrosive to the skin, or
3. The substance is a strong acid (pH<2,0) or base (pH >11,5), or
4. The substance is spontaneously flammable in air at room temperature.

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above further testing is needed to assess the potential for eye irritation, the following test methods should be used. In addition to the test methods mentioned below, new OECD validated tests for eye irritation should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

The tests will provide information on the degree and nature of eye and associated mucous membrane irritation, especially with regard to the reversibility of responses.

1. Testing for eye irritation (*in vitro* assays)

If after the analysis in steps 1 and 2 above further testing is needed to assess the potential for eye irritation, one of the following assays should be used.

Test methods for eye irritation:

- OECD Test Guideline 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants.
- EC method B.47 Bovine corneal opacity and permeability test method for identifying ocular corrosives and severe irritants (Annex of Regulation (EC) No 1152/2010).
- OECD Test Guideline 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants.
- EC method B.48 Isolated chicken eye test method for identifying ocular corrosives and severe irritants (Annex of Regulation (EC) No 1152/2010).

Specific limitations that may be described within the Test Guideline protocol should be taken into account before performing a test or during the interpretation of the test results acquired.

The test methods mentioned above are suitable for the identification of ocular corrosives and severe irritants. Where negative results are obtained, the assessment of eye irritation using an *in vitro* test method suitable also for the identification of non-irritants should follow, if a validated method has become available. If such a method is not available proceed to testing for eye irritation (*in vivo* assays).

2. Testing for eye irritation (*in vivo* assays)

In the case of negative results in *in vitro* assays described above and in the absence of suitable *in vitro* test methods for the identification of ocular non-irritants and non-

corrosives, an acute toxicity eye irritation test should be performed with one of the following test guideline protocols.

Test methods for eye irritation

- EC method B.5 Acute toxicity: eye irritation/corrosion.
- OECD Test Guideline 405: Acute eye irritation/corrosion.

Respiratory Irritation

There are currently no standard tests and no OECD TG available for respiratory irritation and there is no testing requirement for respiratory irritation under the Biocides Regulation. Consequently respiratory irritation is not included in the testing strategies suggested in this Guidance. Nevertheless, account should be taken of any existing and available data that provide evidence of the respiratory irritation potential of a substance. Moreover, the data on local dermal or ocular corrosion/irritation might contain information that is relevant for the respiratory endpoint and this should be considered accordingly. Furthermore, information from cases where symptoms have been described associated with occupational exposures can be used on a case-by-case basis to characterise the respiratory irritation potency of a substance. Information from acute and repeated dose inhalation toxicity studies may also be considered sufficient to show that the substance causes respiratory irritation at a specific concentration level or range. The data need to be carefully evaluated with regard to the exposure conditions (sufficient documentation required). Possible confounding factors should be taken into account.

Additional considerations for the evaluation of all available data with regard to respiratory irritation are provided in Part B (Effects Assessment, BPR guidance under development).

8.3 Skin sensitisation

Point 8.3 of Annex II to the BPR states that *the assessment of this endpoint shall comprise the following consecutive steps:*

1. *an assessment of the available human, animal and alternative data.*
2. *in vivo testing.*

Steps 1 and 2 Collection and evaluation of available information

Assessment of the available human, animal and alternative data.

Further guidance regarding the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

In addition, *in vivo testing does not need to be conducted if:*

- *the available information indicates that the substance should be classified for skin sensitisation or corrosivity, or*
- *the substance is a strong acid (pH < 2,0) or base (pH > 11,5).*

However, the decision on the need to test a substance for skin sensitisation when it fulfils one or both of the above conditions requires expert judgment. This is because the information on skin sensitisation from the active substance will be used for the assessment of this property for products containing the substance, it needs to be taken into account whether sub-corrosive concentrations of a substance may still have sensitising properties (see Chapter III Section 8.3. also). The decision-making process on the testing for a corrosive or strong acid or strong base substance needs to take into account all the available information as specified in steps 1 and 2 above. Any limitation of the additivity concept specified in the Guidance on the Application of the CLP Criteria (ECHA) for sensitisation with regard to addressing sub corrosive concentrations with sensitising potential should also be considered in relation to the use of the data from the active substance for assessing the sensitising potential of the biocidal product.

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above, further testing is needed to assess the potential for skin sensitisation, the following test methods should be used. In addition to the test methods mentioned below, new OECD validated tests for skin sensitisation should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

1. Testing for skin sensitisation (*in vivo* testing)

The Murine Local Lymph Node Assay (LLNA) including, where appropriate, the reduced variant of the assay, is the first-choice method for in vivo testing.

Test methods for skin sensitisation:

- EC method B.42 Skin sensitisation: Local lymph node assay.
- OECD Test Guideline 429: Skin Sensitisation – Local Lymph Node Assay.
- OECD Test Guideline 442A: Skin Sensitisation – Local Lymph Node Assay: DA.
- OECD Test Guideline 442B: Skin Sensitisation – Local Lymph Node Assay: BrdU-ELISA.

The information provided by the LLNA assay should be adequate for the derivation of threshold levels for skin sensitisation. Specific limitations that may be described within the Test Guideline protocol should be taken into account before performing a test or during the interpretation of the test results acquired.

If another skin sensitisation test is used, justification shall be provided.

If the LLNA assay is not considered suitable for a specific class of chemicals other OECD Test Guideline protocols can be used for the assessment of skin sensitisation such as:

- EC method B.6: Skin Sensitisation.
- OECD Test Guideline 406: Skin Sensitisation.

8.4 Respiratory sensitisation (ADS)

There are currently no standard tests and no OECD test guidelines available for respiratory sensitisation. Since an active substance identified as a skin sensitizer can potentially induce a hypersensitivity reaction, potential respiratory sensitisation and respiratory elicitation after dermal sensitisation should be taken into account when appropriate tests are available or when there are indications of respiratory sensitisation effects.

The assessment of the potential of a substance to induce respiratory sensitisation should include assessment of the available existing information (non-human data: physico-chemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data), the outcome of immunotoxicity assessment (see Chapter II Section 8.13.4 in this document), as well as consideration of the Guidance on the Application of the CLP Criteria (ECHA) and Part B (Risk assessment) Volume III).

The following information where available should be provided:

- Information on the sensitisation/allergenicity of workers and others exposed must be provided and included, and where relevant, any incidence of hypersensitivity.
- Reports should include details of frequency, level, duration, symptoms observed, size of exposed population and other relevant data.
- Evidence that the substance can induce specific respiratory hypersensitivity will usually be based on human experience data. The clinical history data including both medical and occupational history, and reports from appropriate lung functions tests related to exposure to the substance should be submitted, if available.
- Reports of other supportive evidence must also be submitted, e.g.
    - A chemical structure related to substances known to cause respiratory hyper-sensitivity;
    - *In vivo* immunological tests;
    - *In vitro* immunological tests;
    - Studies indicating other specific but non-immunological mechanisms of action; and
    - Data from a positive bronchial challenge test.

8.5 Mutagenicity

Point 8.5 of Annex II to the BPR states that *the assessment of this endpoint shall comprise the following consecutive steps:*

- *an assessment of the available in vivo genotoxicity data*
- *an in vitro test for gene mutations in bacteria, an in vitro cytogenicity test in mammalian cells and an in vitro gene mutation test in mammalian cells are required*
- *appropriate in vivo genotoxicity studies shall be considered in case of a positive result in any of the in vitro genotoxicity studies*

The testing of genotoxicity is a screening program to identify substances which might cause permanent transmissible changes in the amount or structure of a single gene or gene segments, a block of genes or chromosomes.

The aim of genotoxicity testing is to:

- predict genotoxic potential;
- identify genotoxic carcinogens at an early stage;
- elucidate the mechanism of action of some carcinogens and reproductive or developmental toxicants inducing germ-line mutations, which may lead to inherited disorders.

Appropriate dose levels, depending on the test requirements, should be used in either in vitro or *in vivo* assays. A tiered approach should be adopted, with selection of higher tier tests being dependent upon interpretation of results at each stage.

At least one *in vitro* test for gene mutations in bacteria, one test for cytogenicity in mammalian cells and one test for gene mutation in mammalian cells are required.

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and

(ECHA, Guidance for Human Health Risk Assessment Description: Volume III Part B)

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above, further testing is needed to assess the potential for genotoxicity *in vitro*, the following test methods should be used. In addition to the test methods mentioned below, new OECD validated tests for genotoxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

(a) Testing for genotoxicity (*in vitro* assays)

The test guideline protocols to follow for the investigation of *in vitro* genotoxicity are listed below (Chapter II Sections 8.5.1-8.5.3). These should be used taking into account some considerations described here but also taking into account the existing information for this endpoint and its assessment (see steps 1 and 2).

If gene mutation and clastogenicity/aneuploidy are detected in a battery of tests consisting of Ames and *in vitro* micronucleus (IVM), no further *in vitro* testing needs to be conducted.

If there are indications of micronucleus formation in an *in vitro* micronucleus assay further testing with appropriate staining procedures should be conducted to clarify if there is an aneugenic or clastogenic response. Further investigation of the aneugenic response may be considered to determine whether there is sufficient evidence for a threshold mechanism and threshold concentration for the aneugenic response (particularly for non-disjunction).

Active substances which display highly bacteriostatic properties as demonstrated in a range finding test should be tested in at least one *in vitro* mammalian cell test for gene mutation, either a Mouse Lymphoma Assay (MLA) or an Hprt gene mutation assay. Non-performance of the Ames test should be justified.

For active substances bearing structural alerts that have given negative results in the standard test battery, additional testing may be required if the standard tests have not been optimised for these alerts. The choice of an additional study or study plan

modifications depends on the chemical nature, the known reactivity and the metabolism data on the structurally alerting active substance.

8.5.1 In vitro gene mutation study in bacteria

Test methods for *in vitro* gene mutation in bacteria:

- EC method B.13/14 Mutagenicity - reverse mutation test using bacteria.
- OECD Test Guideline 471: Bacterial Reverse Mutation Test.
-

8.5.2 In vitro cytogenicity study in mammalian cells

Test methods for *in vitro* cytogenicity in mammalian cells:

- OECD Test Guideline 487. *In vitro* Mammalian Cell Micronucleus Test.[3]
- EC method B.10 Mutagenicity - *In vitro* mammalian chromosome aberration test.
- OECD Test Guideline 473: *In vitro* Mammalian Chromosome Aberration Test.
- *In vitro* Comet assay could be used when justified.
-

The *in vitro* cell micronucleus test can, with the current state of knowledge, be considered as the preferred method for examining *in vitro* cytogenicity in mammalian cells due to its increased sensitivity and ability to identify aneugens.

8.5.3 In vitro gene mutation study in mammalian cells

Test methods for *in vitro* gene mutation in mammalian cells

- EC method B.17 – Mutagenicity – *In vitro* mammalian cell gene mutation test - For this test the mouse lymphoma assay is recommended.
- OECD Test Guideline 476: *In vitro* Mammalian Cell Gene Mutation Test - For this test the mouse lymphoma assay is recommended.
- *In vitro* Comet assay could be used when justified.

8.6 *In vivo* genotoxicity study (ADS)

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development)).

The in vivo genotoxicity study/ies do(es) not generally need to be conducted if:

- *The results are negative for the three in vitro tests and if no metabolites of concern are formed in mammals; or*

[3] http://lysander.sourceoecd.org/vl=17007737/cl=14/nw=1/rpsv/cgi-bin/fulltextew.pl?prpsv=/ij/oecdjournals/1607310x/v1n4/s62/p1.idx

- *Valid in vivo micronucleus data is generated within a repeat dose study and the in vivo micronucleus test is the appropriate test to be conducted to address this information requirement;*
- *The substance is known to be carcinogenic category 1A or 1B or mutagenic category 1A, 1B or 2.*

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above, further testing is needed to assess the potential for genotoxicity *in vivo*, the following test methods should be used. In addition to the test methods mentioned below, new OECD validated tests for genotoxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

(b) Testing for genotoxicity (*in vivo* assays)

***In vivo* studies in somatic cells**

- *If there is a positive result in any of the in vitro genotoxicity studies (in vitro gene mutation study in bacteria, in vitro cytogenicity study in mammalian cells or in vitro gene mutation study in mammalian cells) and there are no results available from an in vivo study already, an appropriate in vivo somatic cell genotoxicity study shall be proposed / conducted by the applicant.*
- *If either of the in vitro gene mutation tests is positive, an in vivo test to investigate unscheduled DNA synthesis shall be conducted.*

However specific considerations on the limitations of the UDS assay should be taken into account before deciding on the most appropriate *in vivo* test to conduct especially with regard to the impact the results will have on potential classification and labelling. Future recommendations from the OECD Test Guideline programme with regard to *in vivo* genotoxicity testing should be followed.

- *A second in vivo somatic cell test may be necessary, depending on the results, quality and relevance of all the available data.*

Before any decisions are made about the need for *in vivo* testing, a review of the *in vitro* test results and all available information on the toxicokinetic and toxicodynamic profile of the test substance is needed. A particular *in vivo* test should be conducted only when it can be reasonably expected from all the properties of the test substance and the proposed test protocol that the specific target tissue will be adequately exposed to the test substance and/or its metabolites. If necessary, a targeted investigation of toxicokinetics should be conducted before progressing to *in vivo* testing (e.g. a preliminary toxicity test to confirm that absorption occurs and that an appropriate dose route is used).

Consideration should be given to conducting an *in vivo* test as part of one of the short-term toxicity studies described under Chapter II Section 8.9.

In the interest of ensuring that the number of animals used in genotoxicity tests is kept to a minimum, both males and females should not automatically be used. In accordance with standard guidelines, testing in one sex only is possible when the substance has

been investigated for general toxicity and no sex-specific differences in toxicity have been observed.

If the *in vitro* mammalian chromosome aberration test or the *in vitro* micronucleus test is positive for clastogenicity, an *in vivo* test for clastogenicity using somatic cells such as metaphase analysis in rodent bone marrow or micronucleus test in rodents should be conducted.

In case of positive result in the *in vivo* micronucleus assay, appropriate staining procedure such as fluorescence in-situ hybridisation (FISH) should be used to identify an aneugenic and/or clastogenic response.

If either of the *in vitro* gene mutation tests is positive, an *in vivo* test to investigate the induction of gene mutation should be conducted, such as the Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay.

When conducting *in vivo* genotoxicity studies, only relevant exposure routes and methods (*such as* admixture to diet, drinking water, skin application, inhalation, gavage) should be used. There should be convincing evidence that the relevant tissue will be reached by the chosen exposure route and application method. Other exposure techniques (*such as* intraperitoneal or subcutaneous injection) that are likely to result in abnormal kinetics, distribution and metabolism should be justified.

The available test guideline protocols for assessing the *in vivo* genotoxic potential of a substance are listed below and reflect current state of knowledge. The choice of the most appropriate test to conduct should reflect the considerations described in this section and future recommendations or changes within the OECD Test Guideline programme for this endpoint.

Test methods for *in vivo* genotoxicity:

- EC method B.12 - Mutagenicity - *In vivo* mammalian erythrocyte micronucleus test EC method
- B.11 - Mutagenicity – *In vivo* mammalian bone-marrow chromosome aberration test
- OECD Test Guideline 474: Mammalian Erythrocyte Micronucleus Test
- OECD Test Guideline 475: Mammalian Bone Marrow Chromosome Aberration Test
- EC method B.39 Unscheduled DNA synthesis (UDS) - Test with mammalian liver cells *in vivo*
- OECD Test Guideline 486: Unscheduled DNA synthesis (UDS) - Test with mammalian liver cells *in vivo*.
- OECD Test Guideline 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays
- *In vivo* Comet assay could be used when justified.

Specific considerations for *in vivo* genotoxicity testing

For substances that are short-lived, reactive, *in vitro* mutagens, or for which no indications of systemic availability have been presented, an alternative strategy involving studies to focus on tissues at initial sites of contact with the body should be considered (e.g. local genotoxicity, photomutagenicity). Expert judgment should be used on a case-by-case basis to decide which tests are the most appropriate. The main options are the *in vivo* Comet assay, gene mutation tests with transgenic rodents, and DNA adduct studies. For any given substance, expert judgment, based on all the available toxicological information, will indicate which of these tests are the most appropriate. The route of exposure should be selected that best allows assessment of the hazard posed to

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

In vivo studies in germ cells

- *If there is a positive result from an in vivo somatic cell study available, the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence to demonstrate that the substance reached the tested organ. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered.*

The potential for substances that give positive results in *in vivo* tests for genotoxic effects in somatic cells to affect germ cells should always be considered. The same is true for substances otherwise classified as category 2 mutagens. The first step is to make an appraisal of all the available toxicokinetic and toxicodynamic properties of the test substance. Expert judgment is needed at this stage to consider whether there is sufficient information to conclude that the substance poses a mutagenic hazard to germ cells. If this is the case, it can be concluded that the substance may cause heritable genetic damage and no further testing is justified. Consequently, the substance is classified as a category 1B mutagen. If the appraisal of mutagenic potential in germ cells is inconclusive, additional investigation will be necessary. In the event that additional information about the toxicokinetics of the substance would resolve the problem, toxicokinetic investigation (i.e. not a full toxicokinetic study) tailored to address this is required. The type of mutation produced in earlier studies namely gene, numerical chromosome or structural chromosome changes, should be considered when selecting the appropriate assay.

A study for the presence of DNA adducts in gonad cells may also be considered. If germ cell testing is to be undertaken, and this should be in exceptional circumstances, expert judgment should be used to select the most appropriate test strategy. Internationally recognised guidelines are available for investigating clastogenicity in rodent spermatogonial cells and for the dominant lethal test. Dominant lethal mutations are believed to be primarily due to structural or numerical chromosome aberrations.

Alternatively, other methods can be used if deemed appropriate by expert judgment. These may include the Comet assay, gene mutation tests with transgenic animals, or DNA adduct analysis.

In order to minimise animal use, the possibility to combine germ cell genotoxicity tests and reproductive toxicity tests should be considered.

The available test guideline protocols for assessing the *in vivo germ cell mutagenicity* of a substance are listed below and reflect current state of knowledge. The choice of the most appropriate test to conduct should reflect the considerations described in this section and future recommendations or changes within the OECD Test Guideline programme for this endpoint.

Test methods for *in vivo* germ cell genotoxicity:

- EC method B.23 Mammalian spermatogonial chromosome aberration test.
- OECD Test Guideline 483: Mammalian Spermatogonial Chromosome Aberration Test**.**
- OECD Test Guideline 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays.

8.7 Acute toxicity

Assessment of the acute toxic potential of a chemical is necessary to determine the adverse health effects that might occur following accidental or deliberate short-term exposure.

Administration via different routes makes an overall assessment of relative acute hazard of exposure in different exposure routes possible.

- *In addition to the oral route of administration (8.7.1), for substances other than gases, the information mentioned under 8.7.2 to 8.7.3 shall be provided for at least one other route of administration.*
- *The choice for the second route will depend on the nature of the substance and the likely route of human exposure.*
- *Gases and volatile liquids should be administered by the inhalation route*
- *If the only route of exposure is the oral route, then information for only that route need be provided. If either the dermal or inhalation route is the only route of exposure to humans then an oral test may be considered. Before a new dermal acute toxicity study is carried out, an in vitro dermal penetration study (OECD 428) should be conducted to assess the likely magnitude and rate of dermal bioavailability*
- *There may be exceptional circumstances where all routes of administration are deemed necessary*

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

The study/ies do(es) not generally need to be conducted if:

- *The substance is classified as corrosive to the skin.*

8.7.1 By oral route

- *The study need not be conducted if the substance is a gas or a highly volatile substance.*

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above, further testing is needed to assess the potential for acute toxicity by the oral route, the following test methods should be used. In addition to the test methods mentioned below, new OECD validated tests for acute toxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

Test methods for Acute toxicity via oral route:

- EC method B.1 tris Acute oral toxicity - Acute toxic class method.

- OECD Test Guideline 423: Acute oral toxicity: acute toxic class method.
- EC method B.1 bis Acute oral toxicity - fixed dose procedure.
- OECD Test Guideline 420: Acute oral toxicity: fixed dose procedure.
- OECD Test Guideline 425: Acute oral toxicity: up-and-down procedure.
- OECD Test Guideline 401: Acute oral toxicity (only acceptable, if performed before December 2002).

The choice of the protocol to follow for this endpoint should take into account animal welfare issues and the OECD TG 420 should be considered as the first choice for testing regarding acute toxicity.

8.7.2 By inhalation

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above, and the considerations listed below, further testing is needed to assess the potential for acute toxicity by inhalation, the following test methods should be used. In addition to the test methods listed in this section, new OECD validated tests for acute inhalation toxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

Testing by the inhalation route is appropriate if exposure of humans via inhalation is likely taking into account:

- *the vapour pressure of the substance (a volatile substance has vapour pressure > 1 x 10^{-2} Pa at 20 °C) and/or*
- *the active substance is a powder containing a significant proportion (e.g. 1 % on a weight basis) of particles with particle size MMAD < 50 micrometers or*
- *the active substance is included in products that are powders or are applied in a manner that generates exposure to aerosols, particles or droplets of an inhalable size (MMAD <50 micrometers)*
- *the Acute Toxic Class Method is the preferred method for the determination of this endpoint*

If there is absence of information on particle/droplet size and where there is potential for exposure via inhalation from the use of biocidal products containing the active substance, an acute inhalation study should be performed.

Test methods for Acute toxicity via inhalation route:

- EC method B.2 Acute toxicity (inhalation).
- OECD Test Guideline 403: Acute Inhalation Toxicity.
- OECD Test Guideline 436: Acute Inhalation Toxicity – Acute Toxic Class Method.
-

The full study using three dose levels may not be necessary if a substance at an exposure concentration equal to the limit concentrations of the test guideline (limit test) or at the maximum attainable concentration produces no compound-related mortalities.

The head/nose only exposure should be used, unless whole body exposure can be justified.

8.7.3 By dermal route

Step 3 Generation of new test data

Testing by the dermal route is necessary only if:

- *inhalation of the substance is unlikely, or*
- *skin contact in production and/or use is likely, and either*
- *the physicochemical and toxicological properties suggest potential for a significant rate of absorption through the skin, or*
- *the results of an in vitro dermal penetration study (OECD 428) demonstrate high dermal absorption and bioavailability.*

Dermal toxicity must be reported for an active substance except for gases.

If after the analysis in steps 1 and 2 above, further testing is needed to assess the potential for acute toxicity by the dermal route, the following test methods should be used. In addition to the test methods mentioned below, new OECD validated tests for acute dermal toxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

Test methods for Acute toxicity via dermal route:

- EC method B.3 Acute toxicity (dermal).
- OECD Test Guideline 402: Acute Dermal Toxicity.

For substances with low acute dermal toxicity a limit test with 2000 mg/kg body weight may be sufficient.

8.8 Toxicokinetics and metabolism studies in mammals

Point 8.8 of Annex II to the BPR states that *the toxicokinetics and metabolism studies should provide basic data about the rate and extent of absorption, the tissue distribution and the relevant metabolic pathway including the degree of metabolism, the routes and rate of excretion and the relevant metabolites.*

The generation of toxicokinetics data should be considered in light of the generation of other toxicity data (i.e. repeated dose toxicity, mutagenicity, and reproductive toxicity) to assist in the estimation of internal exposure to the active substance and/or its metabolites and the correlation of the effects observed with internal dose estimates. The latter is of particular importance for establishing the mode of action of the active substance and whether administered doses caused saturation kinetics resulting in a non-linear dose-response. Such information is valuable for the derivation of assessment factors, route-to-route extrapolation and hazard characterisation.

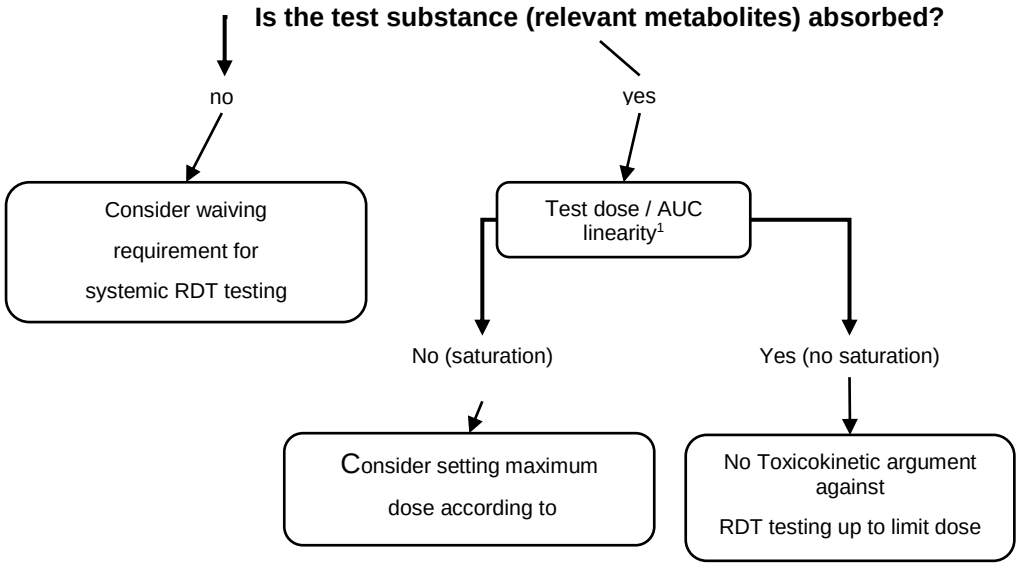
Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within Part B Human Health Effects Assessment (BPR guidance under development)).

Step 3: Generation of new test data

Following the evaluation of all available data, a decision should be made on which type of kinetic data and which test design is most appropriate. It is preferred to generate kinetic data within the toxicity studies such as repeated dose toxicity where possible. The sections below describe the issues to consider when designing new tests for toxicokinetics and the available techniques for the tests suitable for ADME (absorption, distribution, metabolism, elimination) estimation. The importance of the toxicokinetic

data within the design of repeated dose toxicity as well as the refinement of the assessment of the results from toxicity studies is presented in Figure 4 and Figure 5 (adopted from ECHA Guidance R7C, (ECHA)).



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

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

Figure 4: Use of toxicokinetic data in the design of repeated dose toxicity studies

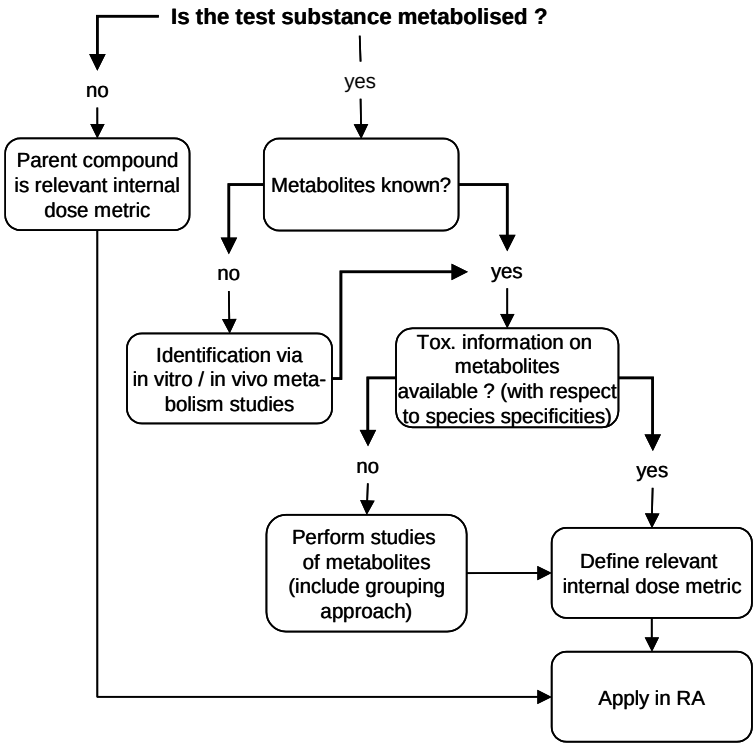


Figure 5 Use of increasing knowledge on substance metabolism

The OECD Test Guideline 417 provides the protocol for the conduct of toxicokinetic studies either as standalone test or in combination with repeated dose toxicity studies.

In vivo studies provide an integrated perspective on the relative importance of different processes in the intact biological system for comparison with the results of the toxicity studies. To ensure a valid set of toxicokinetic data, a toxicokinetic *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, selected experiments (e.g. plasma-kinetics) may be sufficient to provide needed data for further assessments (e.g. bioavailability).

The high dose level administered in an ADME study should be linked to the dose levels that cause adverse effects in toxicity studies. Ideally there should also be a dose without toxic effect, which should be in the range of expected human exposure including consideration of limit of quantification. 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 is 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 areas under the curve (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

of the type of non-linearity involved (e.g. saturable protein binding, saturable metabolism, etc).

Generally *in vitro* studies provide data on specific aspects of pharmacokinetics such as metabolism. A major advantage of *in vitro* studies is that it is possible to carry out parallel tests on samples from the species used in toxicity tests and samples from humans, thus facilitating interspecies comparisons (e.g., metabolite profile, metabolic rate constants). In recent years methods to integrate a number of *in vitro* results into a prediction of ADME *in vivo* by the use of appropriate physiologically based kinetic (PBK) models 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 both to inform development decisions and as part of the risk assessment process. The uncertainty associated with the prediction depends largely on the amount of available data.

Information on blood and tissues concentration of the active substance and relevant metabolites, for example around the time to reach the maximum plasma concentration (T_{max}) or other relevant toxicokinetic parameter, should be generated in short and long-term studies on relevant species to enhance the value of the toxicological data generated in terms of understanding the toxicity studies. If such information is not considered essential for the assessment, full justification should be provided.

The main objective of the toxicokinetic data is to describe the systemic exposure achieved in animals and its relationship to the dose levels and the time course of the toxicity studies.

Other objectives are:

(a) to relate the achieved exposure in toxicity studies to toxicological findings and contribute to the assessment of the relevance of these findings to human health, with a particular regard to vulnerable groups;
(b) to support the design of a toxicity study (choice of species, treatment regimen, selection of dose levels) with respect to kinetics and metabolism;
(c) to provide information which, in relation to the findings of toxicity studies, contributes to the design of supplementary toxicity studies.

Absorption, distribution, metabolism and excretion after exposure by oral route

Limited data restricted to one *in vivo* test species (normally rat) may be all that is required as regards absorption, distribution, metabolism and excretion after exposure by oral route. These data can provide information useful in the design and interpretation of subsequent toxicity tests. However, it should be remembered that information on interspecies differences is crucial in extrapolation of animal data to humans and information on metabolism following administration via other routes may be useful in human risk assessments.

It is not possible to specify detailed data information requirements in all areas, since the exact requirements will depend upon the results obtained for each particular test substance.

Absorption

Absorption is normally investigated by the determination of the test substance and/or its metabolites in excreta, exhaled air and carcass (i.e. radioactivity balance). The biological response between test and reference groups (e.g. oral versus i.v.) is compared and the plasma level of the test substance and/or its metabolites is determined.

Distribution

For determination of the distribution of a substance in the body there are two approaches available at present for analysis of distribution patterns. Quantitative information can be obtained firstly, using whole-body autoradiographic techniques and secondly, by sacrificing animals at different times after exposure and determination of the concentration and amount of the test substance and/or metabolites in tissues and organs (EC method B.36 'Toxicokinetics', OECD TG 417, 'Toxicokinetics').

Accumulative potential

Information derived for the purpose of environmental risk assessment can further inform human health risk assessment and the potential for a substance to accumulate. Bioconcentration refers to the accumulation of a substance dissolved in water by an aquatic organism. The static bioconcentration factor (BCF) is the ratio of the concentration of a substance in an organism to the concentration in water once a steady state has been achieved. Traditionally, bioconcentration potential has been assessed using laboratory experiments that expose fish to the substance dissolved in water (EC method C.13 'Bioconcentration: Flow-Through Fish Test', OECD TG 305 'Bioaccumulation in Fish: Aqueous and Dietary Exposure'). The resulting fish BCF is widely used as a surrogate measure for bioaccumulation potential.

If single dose toxicity and tissue distribution data are not adequate to determine the potential for accumulation, repeated dose administration may be needed to address the potential for accumulation and/or persistence or changes in toxicokinetics.

Accumulating substances can also be measured in milk and therefore additionally allow an estimation of transfer to the breast-fed pup.

Metabolism

In vivo toxicokinetics studies generally only determine the rates of total metabolic clearance (by measurement of radiolabelled products in blood/plasma, bile, and excrements) rather than the contributions of individual tissues. It has to be taken into account that the total metabolic clearance is the sum of the hepatic and potential extrahepatic metabolism.

In vitro tests can be performed using isolated enzymes, microsomes and microsomal fractions, immortalised cell lines, primary cells and organ slices. Most frequently these materials originate from the liver as this is the most relevant organ for metabolism, however, in some cases preparation from other organs are used for investigation of potential organ-specific metabolic pathways.

When using metabolically incompetent cells an exogenous metabolic activation system is usually added into the cultures. For this purpose the post-mitochondrial 9000x g supernatant (S9 fraction) of whole liver tissue homogenate containing a high concentration of metabolising enzymes is most commonly employed - the donor species needs to be considered in the context of the study. In all cases metabolism may either be directly assessed by specific identification of the metabolites or by subtractive calculation of the amount of parent substance lost in the process.

Excretion

The major routes of excretion are in the urine and/or the faeces (via bile and directly from the GI mucosa; see (Rozman, 1986). For this purpose urine, faeces and expired air and, in certain circumstances, bile are collected and the amount of test substance and/or metabolites in these excreta is measured (EC method B.36 'Toxicokinetics', OECD TG 417 'Toxicokinetics').

The excretion of chemicals (metabolites) in other biological fluids such as *saliva*, *milk*, *tears*, and s*weat* is usually negligible compared with renal or biliary excretion. However, in special cases these fluids may be important to study either for monitoring purposes, or in the case of milk allowing an assessment of the exposure of infants.

For volatile substances and metabolites exhaled air may be an important route of elimination. Therefore, exhaled air needs to be examined in respective cases.

The use of *in silico* methods and kinetic modelling (physiologically based pharmacokinetic (PBPK) modelling) should also be considered upfront in the assessment and toxicokinetic data generation. Similarly available data from human biological monitoring and biological marker measurement studies should be part of the assessment. Further guidance on the use of these methods is provided in Part B Effect Assessment (BPR guidance under development).

Aspects to consider in the design of tests for toxicokinetic data generation

The design of the studies is case-by-case dependent and should consider generation of information about the kinetics of the active substance and its metabolites in relevant species after being exposed to the following conditions:

(a) a single oral dose (low and high dose levels);
(b) an intravenous dose preferably or, if available, a single oral dose with assessment of biliary excretion (low dose level); and
(c) a repeated dose.

A key parameter is systemic bioavailability (F), obtained by comparison of the area under the curve (AUC) after oral and intravenous dosing.

When intravenous dosing is not feasible, a justification should be provided. The design of the kinetic studies required should include:

(a) an evaluation of the rate and extent of oral absorption including maximum plasma concentration (Cmax), AUC, Tmax and other appropriate parameters, such as bioavailability;
(b) the potential for bioaccumulation;
(c) plasma half lives;
(d) the distribution in major organs and tissues;
(e) information on the distribution in blood cells;
(f) the chemical structure and the quantification of metabolites in biological fluids and tissues;
(g) the different metabolic pathways;
(h) the route and time course of excretion of active substance and metabolites;
(i) investigations whether and to what extent enterohepatic circulation takes place.

Comparative *in vitro* metabolism studies should be performed on animal species to be used in pivotal studies and on human material (microsomes or intact cell systems) in order to determine the relevance of the toxicological animal data and to guide in the interpretation of findings and in further definition of the testing strategy.

An explanation must be given or further tests should be carried out where a metabolite is detected *in vitro* in human material and not in the tested animal species.

Absorption, distribution, metabolism and excretion after exposure by other routes

Data on absorption, distribution, metabolism and excretion (ADME) following exposure by the dermal route should be provided where toxicity following dermal exposure is of concern compared to that following oral exposure. Before investigating ADME *in vivo* following dermal exposure, default values for estimating dermal uptake and excretion as described in Part B (BPR guidance under development) as well as the need to conduct an *in vitro* dermal penetration study should be considered to assess the likely magnitude and rate of dermal bioavailability.

Absorption, distribution, metabolism and excretion after exposure by the dermal route should be considered on the basis of the above information, unless the active substance causes skin irritation that would compromise the outcome of the study.

For volatile active substances (vapour pressure $>10^{-2}$ Pa at 20 °C) absorption, distribution, metabolism and excretion after exposure by inhalation may be useful in human risk assessments.

Dermal absorption

An appropriate dermal absorption assessment is needed. It is not always mandatory to submit experimental data. If such data are not available, as a first step default values (depending on physicochemical properties of the active substance) can be used (additional guidance provided in Part B of Hazard Identification within the Toxicokinetics chapter (BPR guidance under development)). The OECD Guidance Document on Percutaneous absorption/penetration (OECD, 2004a) and the EFSA Guidance Document on Dermal Absorption (EFSA, 2012) should be followed where applicable for the estimation of dermal absorption both for the active substance and the biocidal product (Chapter III Section 8.6).

The following Test Guidelines are available for the conduct of skin absorption studies:

- EC method B.45 Skin Absorption: *In Vitro* Method
- OECD Test Guideline 428: Skin Absorption: *In Vitro* Method
- EC method B.44 Skin Absorption: *In Vivo* Method
- OECD Test Guideline 427: Skin Absorption: *In Vivo* Method

If testing to assess the likely magnitude and rate of dermal bioavailability is necessary the OECD Test Guideline 428 for *in vitro* skin absorption should be considered first.

In vitro systems allow us to apply to a fixed surface area of the skin an accurate dose of a test chemical in the form, volume and concentration that are likely to be present during human exposure. One of the key parameters in the regulatory guidelines in this field is that sink conditions must always be maintained, which may bias the assay by build-up of the chemical in the reservoir below the skin[4]. A major issue of concern in the *in vitro* procedure turned out to be the presence of test substance in the various skin layers, i.e., absorbed into the skin but not passed into the receptor fluid. It was noted that it is especially difficult to examine very lipophilic substances *in vitro*, because of their low solubility in most receptor fluids. By including the amount retained in the skin *in vitro*, a more acceptable estimation of skin absorption can be obtained. Water-soluble substances can be tested more accurately *in vitro* because they more readily diffuse into the receptor fluid (OECD, 2004a). At present, provided that skin levels are included as absorbed, results from *in vitro* methods seem to adequately reflect those from *in vivo* experiments supporting their use as a replacement test to measure percutaneous absorption.

[4] A build-up of chemical in the reservoir below the skin is not such a problem if a flow through cell is used for *in vitro* testing.

Advantages of the *in vivo* method (EC method B.44 'Skin Absorption: *In Vivo* Method', OECD TG 427 'Skin Absorption: In Vivo Method') are that it uses a physiologically and metabolically intact system, it uses a species common to many toxicity studies and can be modified for use with other species. The disadvantages are the use of animals, the need for radiolabelled material to facilitate reliable results, difficulties in determining the early absorption phase and the differences in permeability of the preferred species (rat) and human skin. Animal skin is generally more permeable and therefore may overestimate human percutaneous absorption (US EPA, Dermal exposure assessment: Principles and Applications. EPA/600/8-91.001B., 1992). The experimental conditions should also be taken into account in interpreting the results. For instance, dermal absorption studies in fur-bearing animals may not accurately reflect dermal absorption in human beings.

If appropriate dermal penetration data are available for rats *in vivo* and for rat and human skin *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*. The latter adjustment may be done because the permeability of human skin is often lower than that of animal skin (Howes, et al., 1996). A generally applicable correction factor for extrapolation to man can, however, not be derived, because the extent of overestimation appears to be dose-, substance- and animal- specific (ECETOC, 1993); (Bronaugh & Maibach, 1987). *In silico* models might also improve the overall knowledge of crucial properties significantly. Mathematical skin permeation models are usually based on uptake from aqueous solution which may not be relevant to the exposure scenario being assessed. In addition, the use of such models for quantitative risk assessment purposes is often limited because these models have generally been validated by *in vitro* data ignoring the fate of the skin residue levels. However, these models may prove useful as a screening tool or for qualitative comparison of skin permeation potential. On a case-by-case basis, and if scientifically justified, the use of (quantitative) structure activity relationships may prove useful, especially within a group of closely related substances.

Considerations for test substances and analytical methodology for toxicokinetic studies

Toxicokinetic and metabolism studies can be carried out using non-labelled compounds, stable isotope-labelled compounds, radioactively labelled compounds or using dual (stable and radio-) labelling. The labels should be placed in metabolically stable positions, the placing of labels such as ^{14}C in positions from which they can enter the carbon pool of the test animal should be avoided. If a metabolic degradation of the test substance may occur, different labelling positions have to be taken into account to be able to determine all relevant degradation pathways. The radiolabelled compound must be of high radiochemical purity and of adequate specific activity to ensure sufficient sensitivity in radio-assay methods.

Separation techniques are used in metabolism studies to purify and separate several radioactive fractions in biota such as urine, plasma, bile and others. These techniques range from relatively simple approaches such as liquid-liquid extraction and column chromatography to more sophisticated techniques such as HPLC (high pressure liquid chromatography). These methods also allow for the establishment of a metabolite profile. Quantitative analytical methods are required to follow concentrations of parent compound and metabolites in the body as a function of time. The most common techniques used are LC/MS (liquid chromatography/ mass spectroscopy) and high performance LC with UV-detection, or if ^{14}C-labelled material is used, radioactivity-detection-HPLC. It is worth mentioning that kinetic parameters generally cannot be calculated from measurement of total radioactivity to receive an overall kinetic estimate. Nevertheless, to generate exact values one has to address parent compound and

metabolites separately. An analytical step is required to define the radioactivity as chemical species. This is usually faster than cold analytical methods. Dual labelling (e.g. ^{13}C and $^{14}C/^{12}C$) is the method of choice for structural elucidation of metabolites (by MS and NMR [nuclear magnetic resonance] spectroscopy). A cold analytical technique, which incorporates stable isotope labelling (for GC/MS [gas chromatography/ mass spectroscopy] or LC/MS), is a useful combination. Unless this latter method has already been developed for the test compound in various matrices (urine, faeces, blood, fat, liver, kidney, etc.), the use of radiolabelled compound may be less costly than other methods.

In any toxicokinetic study, the identity and purity of the chemical used in the test must be assured. Analytical methods capable of detecting undesirable impurities will be required, as well as methods to assure that the substance of interest is of uniform potency from batch to batch. Additional methods will be required to monitor the stability and uniformity of the form in which the test substance is administered to the organisms used in the toxicokinetic studies. Finally, methods suitable to identify and quantify the test substance in toxicokinetic studies must be employed.

In the context of analytical methods, *accuracy* refers to how closely the average value reported for the assay of a sample agrees with the actual amount of substance being assayed in the sample, whereas *precision* refers to the amount of scatter in the measured values around the average result. If the average assay result does not agree with the actual amount in the sample, the assay is said to be *biased*, i.e., lacks specificity; bias can also be due to low recovery.

Assay *specificity* is perhaps the most serious problem encountered. Although *blanks* provide some assurance that no instrument response will be obtained in the absence of the test chemical, a better approach is to select an instrument or bioassay that responds to some biological, chemical, or physical property of the test chemical that is not shared with many other substances.

Besides, it is also necessary that the assay method is usable over a sufficiently wide range of concentrations for the toxic chemical and its metabolites. The lower limit of reliability for an analytical method has been perceived in different ways; frequently, the term sensitivity has been used to indicate the ability of an analytical method to measure small amounts of a substance accurately and with requisite precision. It is unlikely that a single analytical method will be of use for all of these purposes. Indeed, it is highly desirable to use more than one method, at times. If two or more methods yield essentially the same results, confidence in each method is increased.

8.8.1 Further toxicokinetic and metabolism studies in mammals (ADS)

Point 8.8.1 of Annex II to the BPR states that *additional studies might be required based on the outcome of the toxicokinetic and metabolism study conducted in rat. These further studies shall be required if:*

- *there is evidence that metabolism in the rat is not relevant for human exposure*
- *route-to-route extrapolation from oral to dermal/inhalation exposure is not feasible.*
- *Where it is considered appropriate to obtain information on dermal absorption, the assessment of this endpoint shall proceed using a tiered approach for assessment of dermal absorption.*

With the core data set, basic information about the rate and extent of absorption, the tissue distribution and the relevant metabolic pathway including the degree of metabolism, the routes and rate of excretion and the relevant metabolites should be provided by the toxicokinetic and metabolism studies (Annex II Section 8.8). Additional information might be needed based on the outcome of the toxicokinetic and metabolism study conducted in rats (ADS according to Annex II Section 8.8.1) or based on the evaluation of the toxicological and physicochemical profile of the substance.

In some circumstances, e.g. when there are indications for a potential of the active substance to accumulate, to persist or to change the toxicokinetics e.g. by induction of metabolic enzymes, further studies with repeated administration may be necessary. Chapter II Section 8.8 provides guidance on the options available for the toxicokinetics study and its integration with the repeated dose toxicity tests.

8.9 Repeated dose toxicity

Repeated dose toxicity testing provides information on adverse effects as a result of repeated or prolonged exposure.

- *In general, only one route of administration is necessary and the oral route is the preferred route. However, in some cases it may be necessary to evaluate more than one route of exposure.*
- *For the evaluation of the safety of consumers in relation to active substances that may end up in food or feed, it is necessary to conduct toxicity studies by the oral route.*

Justification to replace the oral route by another significant route, or to require testing in addition to the oral route needs to be provided.

- *In order to reduce testing carried out on vertebrates and in particular the need for free-standing, single-endpoint studies, the design of the repeated dose toxicity studies shall take account of the possibility to explore several parameters within the framework of one study*

(e.g. kinetic data generation, micronucleus formation, neurotoxicity, immunotoxicity).

The repeated dose toxicity study (28 or 90 days) does not need to be conducted if:

- *a substance undergoes immediate disintegration and there are sufficient data on the cleavage products for systemic and local effects and no synergistic effects are expected; or*
- *relevant human exposure can be excluded in accordance with section 3 of Annex IV*

8.9.1 Short-term repeated dose toxicity study (28 days), preferred species is rat

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP

criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

In addition to the waiving option for the repeated dose toxicity studies described in Chapter II Section 8.9 *the short-term toxicity study (28 days) does not need to be conducted if:*

- *a reliable sub-chronic (90 days) study is available, provided that the most appropriate species, dosage, solvent and route of administration were used,*
- *the frequency and duration of human exposure indicates that a longer term study is appropriate and one of the following conditions is met:*
    - *other available data indicate that the substance may have a dangerous property that cannot be detected in a short-term toxicity study; or*
    - *appropriately designed toxicokinetic studies reveal accumulation of the substance or its metabolites in certain tissues or organs which would possibly remain undetected in a short term toxicity study but which are liable to result in adverse effects after prolonged exposure.*

In principle, for substances where a 90-day repeated dose toxicity study will need to be performed, an additional 28-day repeated dose toxicity study will not be required.

If a 28-day repeated dose toxicity needs to be performed the considerations described under Chapter II Section 8.9.2 regarding the generation of new test data should also be taken into account.

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above, further testing is needed to assess repeated dose toxicity, the following test methods should be used. In addition to the test methods mentioned below, new OECD validated tests for repeated dose toxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

Repeated Dose toxicity (Oral)

Test methods for repeated dose toxicity via oral route:

- EC method B.7 Repeated dose (28 days) toxicity (oral).
- OECD Test Guideline 407: Repeated dose 28-day oral toxicity study in rodents.

Other routes:

Repeated Dose toxicity (dermal)

Testing by the dermal route shall be considered if:

- *skin contact in production and/or use is likely; and*
- *inhalation of the substance is unlikely; and*
- *one of the following conditions is met:*
  (i) toxicity is observed in an acute dermal toxicity test at lower doses than in the oral toxicity test; or
  (ii) information or test data indicate dermal absorption is comparable or higher than oral absorption; or

(iii) dermal toxicity is recognised for structurally related substances and for example is observed at lower doses than in the oral toxicity test or dermal absorption is comparable or higher than oral absorption.

In addition, if the substance is a severe irritant or corrosive, testing by the dermal route should be avoided unless it can be performed at doses that do not cause irritation or corrosion and such doses are still toxicologically relevant and the outcome can be used in risk assessment.

The following test methods for repeated dose toxicity via dermal route should be used:

- EC method B.9 Repeated dose (28 days) toxicity (dermal)
- OECD Test Guideline 410: Repeated dose dermal toxicity: 21/28-day study.

Repeated Dose toxicity (inhalation)

Testing by the inhalation route shall be considered if:

- *exposure of humans via inhalation is likely taking into account the vapour pressure of the substance (volatile substances and gases have vapour pressure > 1 x 10^{-2} Pa at 20 °C) and/or*
- *there is the possibility of exposure to aerosols, particles or droplets of an inhalable size (MMAD <50 micrometers).*

The following test methods for repeated dose toxicity via inhalation route should be used:

- EC method B.8 Repeated dose (28 days) toxicity (inhalation)
- OECD Test Guideline 412: Subacute inhalation toxicity : 28-day study

8.9.2 Sub-chronic repeated dose toxicity study (90-day), preferred species is rat

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

In addition to the waiving options for the repeated dose toxicity studies described in Chapter II Section 8.9, *the sub-chronic toxicity study (90 days) does not need to be conducted if:*

- *a reliable short-term toxicity study (28 days) is available showing severe toxicity effects according to the criteria for classifying the substance as H372 and H373 (Regulation (EC) No 1272/2008), for which the observed NOAEL-28 days, with the application of an appropriate uncertainty factor allows the extrapolation towards the NOAEL-90 days for the same route of exposure and;*
- *a reliable chronic toxicity study is available, provided that an appropriate species and route of administration were used; or*

- *the substance is unreactive, insoluble, not bioaccumulative and not inhalable and there is no evidence of absorption and no evidence of toxicity in a 28-day "limit test", particularly if such a pattern is coupled with limited human exposure.*

Step 3: Generation of new test data

If after the analysis in steps 1 and 2 above, further testing is needed to assess repeated dose toxicity, the test methods described further below should be used. In addition to the test methods mentioned below, new OECD validated tests for repeated dose toxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

Considerations for the design of the repeated dose subchronic toxicity studies

The study will be performed in a single rodent species, preferably the rat. The oral route will be used unless one of the other routes is more appropriate based on either the most relevant route of human exposure or the physico-chemical properties of the substance. The other routes should be considered especially if route-to-route extrapolation is not appropriate and the predominant human exposure occurs via dermal and/or inhalation route. In the 90-day study, potential neurotoxic and immunotoxic effects (see also Chapter II, Sections 8.13.2 and 8.13.4), genotoxicity by way of micronuclei formation and effects potentially related to changes in the hormonal system (see also Chapter II Section 8.13.3) must be carefully considered during the conduct of the test and reported, taking into account potential limitations when modifying test protocols in order to investigate specific effects.

Information on mode of action from structurally similar substances should also be considered in the design of repeated dose toxicity tests.

Repeated dose toxicity studies should be designed to provide information as to the amount of the active substance that can be tolerated without adverse effects under the conditions of the study and to elucidate health hazards occurring at higher dose levels. Such studies provide useful data on the risks for those handling and using biocidal products containing the active substance, among other possible exposed groups. In particular, repeated dose toxicity studies provide an essential insight into possible repeated actions of the active substance and the risks to humans who may be exposed. In addition repeated dose toxicity studies provide information useful in the design of chronic toxicity studies.

The studies, data and information to be provided and evaluated, should be sufficient to permit the identification of effects following repeated exposure to the active substance, and in particular to further establish, or indicate:

(a) the relationship between dose and adverse effects;
(b) toxicity of the active substance including where possible the No Observed Adverse Effect Level (NOAEL);
(c) target organs, where relevant (including immune, nervous and endocrine systems);
(d) the time course and characteristics of adverse effects with full details of behavioural changes and possible pathological findings at post-mortem;
(e) specific adverse effects and pathological changes produced;
(f) where relevant the persistence and reversibility of certain adverse effects observed, following discontinuation of dosing;
(g) where possible, the mode of toxic action;
(h) the relative hazard associated with the different routes of exposure;

(i) relevant critical endpoints at appropriate time points for setting reference values, where necessary.

Toxicokinetic data (that is to say blood concentration of the active substance and/or the main metabolites) should be included in repeated dose toxicity studies, unless a justification explaining why it is not necessary to do so is provided. In order to avoid increased animal use, the data may be derived in range finding studies.

If nervous system, immune system or endocrine system are specific targets in repeated dose toxicity studies at dose levels not producing marked toxicity, supplementary studies, including functional testing, need to be considered.

Repeated Dose Toxicity (Oral route)

The following test methods should be used.

Test methods for sub-chronic repeated dose toxicity via oral route:

- EC method B.26 Sub-chronic oral toxicity test. Repeated dose 90-day oral toxicity study in rodents.
- EC method B.27 Sub-chronic oral toxicity test. Repeated dose 90-day oral toxicity study in non-rodents.
- OECD Test Guideline 408: Repeated dose 90-day oral toxicity study in rodents.
- OECD Test Guideline 409: Repeated dose 90-day oral toxicity study in non-rodents.

Other routes

Repeated Dose Toxicity (Inhalation route)

Testing by the inhalation route shall be considered if:

- *exposure of humans via inhalation is likely taking into account the vapour pressure of the substance (volatile substances and gases have vapour pressure > 1 x 10^{-2} Pa at 20 °C) and/or*
- *there is the possibility of exposure to aerosols, particles or droplets of an inhalable size (MMAD <50 micrometers).*

The following test methods for sub-chronic repeated dose toxicity via inhalation route should be used:

- EC method B.29 Sub-chronic inhalation toxicity study 90-day repeated inhalation dose study using rodent species.
- OECD Test Guideline 413: Subchronic inhalation toxicity: 90-day study.

Repeated Dose Toxicity (Dermal route)

Testing by the dermal route shall be considered if:

- *skin contact in production and/or use is likely; and*
- *inhalation of the substance is unlikely; and*
- *one of the following conditions is met:*
  (i) toxicity is observed in an acute dermal toxicity test at lower doses than in the oral toxicity test; or
  (ii) information or test data indicate dermal absorption is comparable or higher than oral absorption; or

(iii) dermal toxicity is recognised for structurally related substances and for example is observed at lower doses than in the oral toxicity test or dermal absorption is comparable or higher than oral absorption.

In addition, if the substance is a severe irritant or corrosive, testing by the dermal route should be avoided unless it can be performed at doses that do not cause irritation or corrosion and such doses are still toxicologically relevant and the outcome can be used in risk assessment.

The following test methods for sub-chronic repeated dose toxicity via dermal route should be used:

- EC method B.28 Sub-chronic dermal toxicity test : 90-day repeated dermal dose study using rodent species.
- OECD Test Guideline 411: Subchronic dermal toxicity test: 90-day study.

8.9.3 Long-term repeated dose toxicity (≥ 12 months)

Any new long-term toxicity study and carcinogenicity study (Chapter II Section 8.11) should be combined. This section provides guidance covering both the long-term repeated dose toxicity and the carcinogenicity study. The test is required for one rodent, the rat being the preferred species. In exceptional cases and depending on the results obtained testing in another mammalian species (rodent or non-rodent, see also Chapter II Section 8.9.4 for tests in non-rodent species) may be considered.

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

The long-term toxicity study (≥ 12 months) does not need to be conducted if:

- *long-term exposure can be excluded and no effects have been seen at the limit dose in the 90-day study, or*
- *a combined long-term repeated dose/carcinogenicity study (8.11.1) is undertaken.*

In addition as specified in Annex II of the BPR (8.11) when the combined long-term carcinogenicity study is performed the specific rules for adaptation for carcinogenicity apply:

A carcinogenicity study does not also need to be conducted if:

- *the substance is classified as mutagen category 1A or 1B. The default presumption would be that a genotoxic mechanism for carcinogenicity is likely. In these cases, a carcinogenicity test will normally not be required.*

Step 3: Generation of new test data

If after the analysis in steps 1 and 2 above, further testing is needed to assess long-term repeated dose toxicity, the test methods described further below should be used. In addition to the test methods mentioned below, new OECD validated tests for repeated

dose toxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

The results of the long-term studies conducted and reported, taken together with other relevant data and information on the active substance, should be sufficient to permit the identification of effects, following repeated exposure to the active substance, and in particular should be sufficient to:

- identify adverse effects resulting from long-term exposure to the active substance;
- identify target organs, where relevant;
- establish the dose-response relationship and mode of action;
- establish the NOAEL and, if necessary, other appropriate reference points.

Correspondingly, the results of the carcinogenicity studies taken together with other relevant data and information on the active substance, should be sufficient to permit the evaluation of hazards for humans, following repeated exposure to the active substance, to be assessed, and in particular should be sufficient:

(a) to identify carcinogenic effects resulting from long-term exposure to the active substance;
(b) to establish the species, sex, and organ specificity of tumours induced;
(c) to establish the dose-response relationship and mode of action;
(d) where possible, to identify the maximum dose eliciting no carcinogenic effect;
(e) where possible, to determine the mode of action and human relevance of any identified carcinogenic response.

If comparative metabolism data indicate that either rat or mouse is an inappropriate model for human cancer risk assessment, an alternative species should be considered.

Experimental data, including the elucidation of the possible mode of action involved and relevance to humans, should be provided where the mode of action for carcinogenicity is considered to be non-genotoxic. Suitable mode of action (MOA) studies can be considered to confirm non-relevance of the non-genotoxic MOA to humans.

Investigation of toxicokinetic parameters generated within the combined long term toxicity study should also be considered as described also for short-term toxicity studies in Chapter II Section 8.9.2.

The following test methods should be used.

Test methods for long-term repeated dose toxicity:

- EC method B.30 Chronic toxicity test.
- EC method B.33 Combined chronic toxicity/carcinogenicity test.
- OECD Test Guideline 452: Chronic Toxicity Studies.
- OECD Test Guideline 453: Combined Chronic Toxicity/Carcinogenicity Studies.

8.9.4 Further repeated dose studies (ADS)

When the available data are inadequate for hazard characterisation and risk assessment, further repeated dose studies should be undertaken, including testing on a second species (non-rodent), studies of longer duration than the studies already available or through a different route of administration. However, testing should not be initiated

before the evaluating competent authority has indicated that further testing is necessary. The decision on further testing should be based on expert judgement and on a case-by-case basis.

Requiring further repeated dose toxicity studies

Further repeated dose studies including testing on a second species (non-rodent), studies of longer duration or through a different route of administration shall be undertaken in cases of:

- *no other information on toxicity for a second species (non-rodent) is provided for,*

  When all the toxicological data concern rodent species, an assessment of the data needs to be performed to understand if testing with another species is likely to provide additional information (e.g. potential of different mode of action within different species).

  or

- *failure to identify a no observed adverse effect level (NOAEL) in the 28- or the 90-day study, unless the reason is that no effects have been observed at the limit dose,*

  This trigger is not considered if no effects were observed at the limit dose. Furthermore, failing to identify a NOAEL should not trigger additional studies by default. If the data are sufficient for a robust hazard assessment and for Classification and Labelling, the LOAEL may be used as the starting point.

  or

- *substances bearing positive structural alerts for effects for which the rat or mouse is an inappropriate or insensitive model,*

  A study protocol will be identified that can be reliably performed in a more suitable animal species. It is however possible to conclude that the structural alert concerns an effect that is specific to humans and/or none of the animal models is suitable for studying this specific effect. In this case all the available information, including scientific literature and human data, will be taken into account to judge whether the risk to humans can be concluded. The human data may consist of e.g. records of worker/consumer experience, case reports, consumer tests or epidemiological studies. Whether further testing will be required will depend on a case-by-case expert judgment.

  or

- *toxicity of particular concern (e.g. serious/severe effects),*

  If toxicity of particular concern is already established, the substance will be classified accordingly and the appropriate risk management measures will be implemented, and therefore no further testing is required.

  or

- *indications of an effect for which the available data is inadequate for toxicological and/or risk characterisation. In such cases it may also be more appropriate to perform specific toxicological studies that are designed to investigate these effects (e.g. immunotoxicity, neurotoxicity, hormonal activity),*

  In some cases data derived by protocols designed for other endpoints, as for example the OECD Test Guideline 443 (Extended One-Generation Reproductive

Toxicity Study) may provide valuable information on specific effects such as immunotoxicity, neurotoxicity or endocrine disruption. Furthermore, where a need is identified for a modification in the study protocol to cover specific needs, this will be done in consultation with the evaluating competent authority. Only in exceptional cases should non-standard protocols be used because the scientific value of such results can be questioned.

or

- *concern regarding local effects for which a risk characterisation cannot be performed by route-to-route extrapolation,*

  A new repeated dose toxicity study for the purpose of performing quantitative risk characterisation for local effects should not be performed by default due to the difficulty in deriving threshold levels for local effects that are also relevant for humans. The benefit from the generation of additional data for this purpose should be considered against the effectiveness of qualitative risk characterisation as another option for ensuring safe use.

  or

- *particular concern regarding exposure (e.g. use in biocidal products leading to exposure levels which are close to the toxicologically relevant dose levels),*

  Further studies might be necessary e.g. when the biocidal product is used in one or more consumer products and the (combined) exposure levels are close to toxicologically relevant dose levels where effects on humans may be expected in the relevant time frame. Any exposure-triggered studies proposed or required should be considered on a case-by-case basis.

  or

- *effects shown in substances with a clear relationship in molecular structure with the substance being studied were not detected in the 28- or the 90-day study,*

  The study protocol and the conditions in which the effects were seen in another substance will be examined in detail in order to identify the conditions in which the effect would be expected to occur for the substance to be studied. The study protocol will be selected to repeat and possibly extend the conditions where the effect has been observed. However, where applicable, mechanistic *in vitro* studies examining the specific mechanism of action of the related substances should have preference over further animal studies.

  or

- *the route of administration used in the initial repeated dose study was inappropriate in relation to the expected route of human exposure and route-to-route extrapolation cannot be made.*

  The possibility of route-to-route extrapolation should be carefully considered before concluding that it is not appropriate taking into account the toxicokinetic information available and the use of modelling approaches when performing route-to-route extrapolation.

8.10 Reproductive toxicity

Point 8.10 of Annex II to the BPR states that *for evaluation of consumer safety of active substances that may end up in food or feed, it is necessary to conduct toxicity studies by the oral route*

Possible effects on reproductive physiology and the development of progeny should be investigated and reported concerning the following aspects:

- Impairment of male and female reproductive functions or capacity, for example from effects on oestrus cycle, sexual behaviour, any aspect of spermatogenesis or oogenesis, or hormonal activity or physiological response which would interfere with the capacity to fertilise, fertilisation itself or development of the fertilised ovum up to and including implantation.
- Adverse effects on the progeny, for example any effect interfering with normal development, both before and after birth. This includes morphological anomalies such as changes in anogenital index, nipple retention, and functional disturbances (such as reproductive and neurological effects).

Effects accentuated over generations should be reported.

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

The studies need not be conducted if:

- *the substance is known to be a genotoxic carcinogen and appropriate risk management measures are implemented including measures related to reproductive toxicity; or*
- *the substance is known to be a germ cell mutagen and appropriate risk management measures are implemented including measures related to reproductive toxicity; or*
- *the substance is of low toxicological activity (no evidence of toxicity seen in any of the tests available provided that the dataset is sufficiently comprehensive and informative), it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure, e.g. plasma/blood concentrations below detection limit using a sensitive method and absence of the substance and of metabolites of the substance in urine, bile or exhaled air) and the pattern of use indicates there is no or no significant human exposure*
- *a substance is known to have an adverse effect on fertility, meeting the criteria for classification as Reproductive toxicity Cat 1A or 1B: May damage fertility (H360F), and the available data are adequate to support a robust risk assessment, then no further testing for fertility will be necessary. However, testing for development toxicity must be considered*
- *a substance is known to cause developmental toxicity, meeting the criteria for classification as Reproductive toxicity Cat 1A or 1B: May damage the unborn child*

(H360D), and the available data are adequate to support a robust risk assessment, then no further testing for developmental toxicity will be necessary. However, testing for effects on fertility must be considered

Step 3 Generation of new test data

If after the analysis in steps 1 and 2 above, further testing is needed to assess reproductive toxicity, the test methods described further below (Chapter II Section 8.10.1-8.10.3) should be used. In addition to the test methods mentioned below, new OECD validated tests for reproductive toxicity should be taken into account once available in deciding the test strategy. The OECD Test Guideline programme as well as non-animal test methods that undergo validation available by ECVAM should be regularly consulted for any updates.

8.10.1 Pre-natal developmental toxicity study, preferred species is rabbit; oral route of administration is the preferred route.

Point 8.10.1 of Annex II to the BPR states that *the study shall be initially performed on one species*

The developmental toxicity studies reported, taken together with other relevant data and information on the active substance, should be sufficient to permit the assessment of effects on embryonic and foetal development, following repeated exposure to the active substance, and in particular should be sufficient:

(a) to identify direct and indirect effects on embryonic and foetal development resulting from exposure to the active substance;
(b) to identify any maternal toxicity;
(c) to establish the relationship between observed responses and dose in both dam and offspring;
(d) to establish NOAELs for maternal toxicity and pup development;
(e) to provide additional information on adverse effects in pregnant as compared with non-pregnant females;
(f) to provide additional information on any enhancement of general toxic effects of pregnant animals.

Developmental toxicity should be determined in rabbits by the oral route. The decision on species to be tested primarily depends on consideration of all available information including the type of substance to be tested.

Malformations and variations and external skeletal and visceral anomalies should be reported separately and combined in such a way that all relevant changes which are observed to occur in characteristic patterns in individual foetuses or those that can be considered to represent different grades of severity of the same type of change are reported in a concise manner.

Diagnostic criteria for malformations and variations should be given in the report. The terminology should follow that presented in OECD Guidance Document 43 Appendix I (OECD, 2008b) and via the DevTox project (http://www.devtox.org).

Further guidance on conditions for historical control data is provided in OECD Guidance Document 43 (OECD, 2008b).

When indicated by observations in other studies or the mode of action of the test substance, supplementary studies or information may be required to provide information on the postnatal manifestation of effects such as developmental neurotoxicity.

The following test methods for pre-natal developmental toxicity should be used:

- EC method B.31 Prenatal developmental toxicity study.
- OECD Test Guideline 414: Prenatal developmental toxicity study.
- OECD Test Guideline 426: Developmental neurotoxicity study.

8.10.2 Two-generation reproductive toxicity study, rat, oral route of administration is the preferred route.

Point 8.10.2 of Annex II to the BPR states that *if another reproductive toxicity test is used justification shall be provided. The extended one- generation reproductive toxicity study adopted at OECD level shall be considered as an alternative approach to the multi-generation study*

Investigations should take account of all available and relevant data, including the results of general toxicity studies if relevant parameters (such as semen analysis, oestrous cyclicity, reproductive organ histopathology) are included, as well as knowledge concerning structural analogues to the active substance.

The active substance and its relevant metabolites should be measured in milk, although not required in the OECD test guideline, as a second tier investigation where relevant effects are observed in the offspring or are expected (for example from a range-finding study).

Potential neurotoxic, immunotoxic effects and effects potentially related to changes in the hormonal system should be carefully addressed and reported.

In order to provide useful information in the design and interpretation of developmental toxicity studies, information on blood concentration of the active substance in parents and foetus/offspring may be included in higher tier studies and reported.

The reproductive toxicity studies reported, taken together with other relevant data and information on the active substance, should be sufficient to permit the identification of effects for reproduction, following repeated exposure to the active substance, and in particular should be sufficient to:

(a) identify direct and indirect effects on reproduction resulting from exposure to the active substance;
(b) identify any non-reproductive adverse effects occurring at lower doses than in short-term and chronic toxicity testing;
(c) establish the NOAELs for parental toxicity, reproductive outcome and pup development.

The OECD extended one-generation reproductive toxicity study (OECD TG 443) can be considered as an alternative approach to the multi-generation study. The OECD TG 443 is a modular flexible study design and thus the study design and investigational details should be defined and agreed with the evaluating competent authority to assure that the relevant aspects are taken into consideration.

The decision on whether or not to mate the F1B animals to produce the F2 within the extended one-generation reproductive toxicity study should be made on a case-by-case basis taking into account substance specific properties and remaining uncertainty from the omission of the mating of F1B animals and production of F2 offspring that may have

impact in hazard identification and characterisation. Information from similar substances, use of the substance and the exposure conditions may support the decision making on the assessment of the reproductive performance of the F1 animals and effects in F2 generation.

Similarly the decision on inclusion of the developmental neurotoxicity and the developmental immunotoxicity cohorts within the OECD extended one-generation reproductive toxicity test, should be made taking into account all available information with regard to neurotoxicity and immunotoxicity potential of the substance as derived by existing data (e.g. repeated dose toxicity studies performed with the substance or similar substances), non-test data (e.g. structural alerts by expert systems). In the absence of any existing information or alerts, in order to account for any remaining uncertainty it would be preferred that the two cohorts were performed within the test. In addition the use pattern of the substance and exposure conditions may support the decision on whether one or both of these cohorts should be conducted in order to reduce the remaining uncertainty of detecting potential triggers for (developmental) neurotoxicity and/or (developmental) immunotoxicity.

Where necessary for a better interpretation of the effects on reproduction and as far as this information is not yet available, supplementary studies may be required to provide information on the affected gender and the possible mechanisms.

The following test methods for generation reproductive toxicity should be considered:

- EC method B.35 Two-generation reproduction toxicity study.
- OECD Test Guideline 416: Two-Generation Reproduction Toxicity.
- OECD Test Guideline 443: Extended One-generation Reproduction Toxicity.

8.10.3 Further pre-natal developmental toxicity study, preferred species is rat, oral route of administration (ADS)

Point 8.10.3 of Annex II to the BPR states that *a decision on the need to perform additional studies on a second species or mechanistic studies should be based on the outcome of the first test (8.10.1) and all other relevant available data (in particular rodent reprotox studies).*

The assessment of this endpoint should be carried out according to the EC method B.31 or the corresponding OECD Test Guideline 414 for Prenatal developmental toxicity study. Further guidance is also available in OECD Guidance Document 43 (OECD, 2008b); Guidance on the Application of the CLP Criteria (ECHA).

A decision on the need to perform additional studies on a second species (rat) or mechanistic studies should be based on the outcome of the first test (Chapter II Section 8.10.1) and all other relevant data. The decision on species to be tested primarily depends on consideration of all available information including the type of substance to be tested.

Besides the results from the pre-natal developmental toxicity study all other relevant and available data including indications from repeat dose toxicity studies (28-day and /or 90-day studies), ADME, multigeneration-, developmental neurotoxicity- or the extended one-generation study, further neurotoxicity studies and, if possible, the mode of action of the test substance should be considered when deciding for an additional pre-natal developmental toxicity study on a second species. Knowledge of structural analogues to the active substance should also be included in the assessment. A second pre-natal developmental toxicity study on another species (rat) does not need to be performed if

no prenatal developmental effects are observed in the study conducted in the first species and if no indication of pre- and/or postnatal developmental toxicity are observed in one- or multigeneration reproductive toxicity study (performed in the rat) are observed at the highest dose tested.

According to Janer et al (Janer, et al., 2008) the rat and the rabbit show similar sensitivity with regard to detecting developmental toxicity.

When in specific cases further examination of developmental toxicity is required, in addition to the test performed in the first species (rabbit) this should be done with a focus on elucidating the mode of action of the substance and relevance of the effects for humans. It is more likely that such investigations would require rather mechanistic studies than a new pre-natal developmental toxicity test.

8.11 Carcinogenicity

The carcinogenicity study identifies the carcinogenicity potential of the substance in laboratory animals in order to facilitate the extrapolation of potential risks to humans. The studies should be sufficient to establish the species specificity and organ specificity of tumours induced, to establish the dose-response relationship and for non-genotoxic carcinogens to identify doses eliciting no adverse effects (threshold dose).

See 8.11.1 for new study requirements

Steps 1 and 2

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

A carcinogenicity study does not need to be conducted if:

- *the substance is classified as mutagen category 1A or 1B. The default presumption would be that a genotoxic mechanism for carcinogenicity is likely. In these cases, a carcinogenicity test will normally not be required.*

In addition the study does not need to be conducted if:

- *No genotoxic potential for humans is identified in genotoxicity tests, and*
- *Possible mechanisms of toxicological effects observed in subchronic toxicity studies are without any indications of non-genotoxic carcinogenicity and there are no structural alerts for carcinogenicity, and*
- *The subchronic studies in rodents and/or non-rodents are without indication of substance related adverse effects at the limit dose level.*

8.11.1 Combined carcinogenicity study and long-term repeated dose toxicity

Point 8.11.1 of Annex II to the BPR states that *rat, oral route of administration is the preferred route. If an alternative route is proposed a justification must be provided.*

See Chapter II Section 8.9.3.

8.11.2 Carcinogenicity testing in a second species

Point 8.11.2 of Annex II to the BPR states that:

- *A second carcinogenicity study should normally be conducted using the mouse as test species*
- *For evaluation of consumer safety of active substances that may end up in food or feed, it is necessary to conduct toxicity studies by the oral route*

The rat and the mouse are usually the species used for testing carcinogenic potential, while the rat is used for a combined chronic toxicity/ carcinogenicity testing.

The study is not needed if the conditions specified in 8.11 are fulfilled. In principle a second study in another rodent species is not likely to provide additional information as according to Billington et al (Billington, et al., 2010) the mouse carcinogenicity study does not provide additional information when results from carcinogenicity studies with rat and mice have been compared.

For the purpose of elucidating the mode of action and human relevance when needed further investigation of carcinogenicity after obtaining the results of the combined chronic toxicity study should be considered on a case-by-case basis giving priority to the performance of mechanistic studies.

8.12 Relevant health data, observations and treatments

Point 8.12 of Annex II to the BPR states that *justification should be provided if data is not available.*

When there are no human studies/data already available, new human studies should not be conducted.

Data and information on the effects of human exposure may provide valuable information for confirming the validity of extrapolations made and conclusions reached from animal data and for identifying unexpected adverse effects which are specific to humans.

Available data and information of adequate quality following accidental or occupational exposure have to be submitted.

8.12.1 Medical surveillance data on manufacturing plant personnel

The reports should include detailed information on the design of the programme and exposure to the active substance and to other chemicals.

Data relevant to the mechanism of the action of substance should also be included where feasible. The data may consist of published articles or unpublished medical surveys.

8.12.2 Direct observation, e.g. clinical cases, poisoning incidents

Practical data and information relevant to the recognition of the symptoms of poisoning, on the effectiveness of first aid and therapeutic measures must be included.

The reports should include a complete description of the exposure situation, clinical symptoms observed and therapeutic measures.

Reports of any follow-up studies should be enclosed.

8.12.3 Health records, both from industry and any other available sources

8.12.4 Epidemiological studies on the general population

Information related to occupational exposure or other exposure is available from three main sources: case reports, descriptive epidemiological studies and analytical epidemiological studies, case-control or cohort studies.

Where available, data should be supported with data on levels and duration of exposure.

8.12.5 Diagnosis of poisoning including specific signs of poisoning and clinical tests

A detailed description of clinical signs and details of clinical tests useful for diagnostic purposes (bio-monitoring) must be included.

Symptoms of poisoning including full details of the time courses involved to all exposure routes must be described.

8.12.6 Sensitisation/allergenicity observations

Information on the sensitisation/allergenicity of workers and others exposed must be provided and included, and where relevant, any incidence of hypersensitivity.

Reports should include details of frequency, level, duration, symptoms observed, size of exposure population and other relevant data.

Evidence that the substance can induce specific respiratory hypersensitivity will usually be based on human experience data. The clinical history data including both medical and occupational history, and reports from appropriate lung functions tests related to exposure to the substance should be submitted, if available. Reports of other supportive evidence must also be submitted, e.g.:

(a) a chemical structure related to substances known to cause respiratory hyper-sensitivity,
(b) *in vivo* immunological tests,
(c) *in vitro* immunological tests,
(d) studies indicating other specific but non-immunological mechanisms of action, or
(e) data from a positive bronchial challenge test.

8.12.7 Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known

First aid measures in the event of poisoning and eye contamination must be provided.

Therapeutic regimes and the use of antidotes must be described. Information based on practical experience, where it exists and is available, or in other cases information based on theoretical grounds, as to the effectiveness of alternative treatment regimes, where relevant must be provided. Contraindications associated with particular regimes, particularly those relating to 'general medical problems' and conditions, must be described.

8.12.8 Prognosis following poisoning

The expected effects and the duration of these effects following poisoning must be described.

8.13 Additional studies (ADS)

Point 8.13 of Annex II to the BPR states that *additional data, which may be required depending on the characteristics and intended use of the active substance*

Other available data: Available data from emerging methods and models, including toxicity pathway-based risk assessment, in vitro and 'omic' (genomic, proteomic, metabolomic, etc.) studies, systems biology, computational toxicology, bioinformatics, and high throughput screening shall be submitted in parallel

Toxicity studies of metabolites

Supplementary studies, where they relate to substances other than the active substance, are not a routine requirement. Decisions as to the need for supplementary studies should be made on a case-by-case basis.

Where as a result of metabolism or other processes, metabolites from plants or in animal products, soil, groundwater, open air differ from those in animals used for the toxicology studies or are detected in low proportions in animals, further testing should be carried out on a case-by-case basis, taking into account the amount of metabolite and the chemical structure of the metabolite compared to the parent.

Supplementary studies on the active substance

Supplementary studies should be carried out where they are necessary to further clarify observed effects taking into account the results of the available toxicological and metabolism studies and the most important exposure routes. Such studies may include:

(a) studies on absorption, distribution, excretion and metabolism, in a second species;
(b) studies on the immunotoxicological potential;
(c) a targeted single dose study to derive appropriate acute reference values (ARfD, AEL);
(d) studies on other routes of administration;
(e) studies on the carcinogenic potential;
(f) studies on mixture effects.

Studies required should be designed on an individual basis, in the light of the particular parameters to be investigated and the objectives to be achieved.

8.13.1 Phototoxicity - additional study (ADS)

The study should provide information on the potential of certain active substances to induce cytotoxicity in combination with light, for example active substances that are phototoxic *in vivo* after systemic exposure and distribution to the skin, as well as active substances that act as photoirritants/photosensitisers after dermal application to the skin. A positive result should be taken into account when considering potential human exposure. For photomutagenicity see also Chapter II Section 8.6 also. The *in vitro* study should be required only where the active substance absorbs electromagnetic radiation in

the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution.

If the ultraviolet/visible molar/extinction/absorption coefficient of the active substance is less than 10L x mol^{-1} x cm^{-1}, no toxicity testing is required.

The following test methods should be used.

Test methods for phototoxicity:

- EC method B.41.
- OECD Test Guideline 432: *In vitro* 3T3 NRU phototoxicity test.

8.13.2 Neurotoxicity including developmental neurotoxicity (ADS)

Point 8.13.2 of Annex II to the BPR states that:

- *The preferred test species is the rat unless another test species is justified to be more appropriate*
- *For delayed neurotoxicity tests the preferred species will be the adult hen*
- *If anticholinesterase activity is detected a test for response to reactivating agents should be considered*

If the active substance is an organophosphorus compound or if there is any evidence e.g. knowledge of the mechanism of action or from repeated dose studies that the active substance may have neurotoxic or developmental neurotoxic properties then additional information or specific studies will be required.

For evaluation of consumer safety of active substances that may end up in food or feed, it is necessary to conduct toxicity studies by the oral route

Such studies should be performed for active substances with structures that are similar or related to those capable of inducing neurotoxicity, and for active substances which induce specific indications of potential neurotoxicity, neurological signs or neuropathological lesions in toxicity studies at dose levels not associated with marked general toxicity. Performance of such studies should also be considered for substances with a neurotoxic mode of action. Neurotoxicity studies detect functional changes and/or structural and biochemical changes in the central and peripheral nervous systems. These changes can be morphological, physiological (e.g. electroencephalographic changes), or behavioural nature, or can be changes in biochemical parameters (e.g. neurotransmitter levels).

Indications of neurotoxicity can be acquired from the standard systemic toxicity studies. Further investigation is possible using standard repeated dose toxicity tests (such as 28- and 90 day repeated dose toxicity studies or the extended one generation test) with incorporation of specific neurotoxicity measures.

Neurotoxicity studies in rodents should provide sufficient data to evaluate the potential neurotoxicity of the active substance (neurobehavioural and neuropathological effects) after single and repeated exposure.

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

Step 3 Generation of new test data

When it is considered necessary to conduct a study to investigate specific organ/system toxicity, it is important that the study design is discussed by the contractor/laboratory and the assessor, paying particular attention to the protocol to be used, before initiating the study. The need for (and scope/size of) studies using live animals should be particularly carefully considered.

If further standard 28- or 90-day studies are to be conducted, a number of nervous system endpoints will be examined. These endpoints should be included in the tests irrespective of the administration route. A standard study with additional parameters could be considered. In some cases, it may be necessary to conduct a specific study such as a neurotoxicity test using the OECD Test Guideline 424 (Neurotoxicity Study in Rodents) or corresponding EC method B.43 (Neurotoxicity Study in Rodents) with possible inclusion of a satellite group for assessment of reversibility of effects. The OECD Test Guideline 424 is intended for confirmation or further characterisation of potential neurotoxicity identified in previous studies. The OECD Guideline allows for a flexible approach, in which the number of simple endpoints which duplicate those already examined during standard testing may be minimised, and where more effort is put into in-depth investigation of more specific endpoints by inclusion of more specialised tests. Adjustment of dose levels to avoid confounding by general toxicity should be considered.

If data from standard toxicity studies are clearly indicative of specific neurotoxicity, e.g. neurotoxicity occurring at lower dose levels than systemic toxicity, further specific neurotoxicity testing is required to confirm and extend the findings from the general toxicity studies and to establish an NOAEL for neurotoxicity. Again, the neurotoxicity test according to OECD Test Guideline 424 is considered appropriate for this situation.

Standard exposure conditions may not always be adequate for neurotoxicity studies. The duration of exposure needed to induce specific neurotoxic effects in an animal experiment will depend on the underlying mechanism of action. Short-term peak exposures can be important for certain types of substance/effect. When the test compound is administered as a bolus via the intravenous, subcutaneous or oral route it is essential to determine the time-effect course, and to perform measurements of neurotoxicity parameters preferentially at the time of peak effect.

For example, the neurotoxicity associated with short-term exposure to some volatile organic solvents has largely been identified following human exposure - particularly occupational exposure. Acute inhalation studies, using protocols designed to detect the expected effects, are ideal for such substances/effects. For some neurotoxic substances a long exposure period is necessary to elicit neurotoxicity.

In addition in exceptional cases when relevant triggers are met testing for developmental neurotoxicity effects should be considered. Relevant triggers could be if the substance has been shown to (1) cause structural abnormalities of the central nervous system, (2) cause clear signs of behavioural or functional adverse effects of nervous system involvement in adult studies e.g. repeated-dose toxicity studies or (3) have a mode of action that has been closely linked to neurotoxic or developmental neurotoxicity effects e.g. cholinesterase inhibition or thyroid effects. However, in the case of (3) targeted testing on the specific mode of action in developing animals may provide sufficient information for regulatory purposes.

The DNT test protocol (OECD TG 426, developmental neurotoxicity) is designed to be performed as an independent study. However, observations and measurements described in the protocol can also be added on to a generation reproduction study. However, when the developmental neurotoxicity study is incorporated within or attached to another study, it is imperative to preserve the integrity of both study types. It should also be taken into consideration that by incorporating the developmental neurotoxicity investigations into other studies, it may not be possible to investigate as many parameters with similar statistical power than in an independent study such as the OECD TG 426.

The most appropriate methods for further investigation of neurotoxicity should be determined on a case-by-case basis, guided by the effects seen in the standard systemic toxicity tests and/or from SAR-based predictions. Extensive coverage of methods which may be used is given in (OECD, 2004b), (WHO, 1986) and (ECETOC, 1992), and some are summarised in Table 3, below.

Table 4 Methods for investigation of neurotoxicity

Effect	Methods available	References *
Morphological changes	Neuropathology. Gross anatomical techniques. Immunocytochemistry. Special stains.	Krinke, 1989; O'Donoghue, 1989; Mattsson et al., 1990
Physiological changes	Electrophysiology (e.g. nerve conduction velocity (NCV), Electroencephalogram (EEG), evoked potentials).	Fox et al., 1982; Rebert, 1983; Mattsson and Albee, 1988
Behavioural changes	Functional observations. Sensory function tests. Motor function tests (e.g. locomotor activity). Cognitive function tests.	Robbins, 1977; Tilson et al., 1980; Cabe and Eckerman, 1982; Pryor et al., 1983; Moser and McPhail, 1990; Moser, 1995
Biochemical changes	Neurotransmitter analyses. Enzyme/protein activity. Measures of cell integrity.	Dewar and Moffett, 1977; Damstra and Bondy, 1982; Cooper et al., 1986; Costa, 1998

* Given in full in ECETOC (1992), WHO (1986) or Mitchell (1982) in the References.

If significant acetylcholine esterase inhibition is detected, a test for response to reactivating agents should be considered. Available guidance on the setting of acute reference dose (ARfD) for pesticides from JMPR should also be considered.

If the active substance is an organophosphorus compound or if there is any evidence e.g. knowledge of the mechanism of action or from repeat dose studies that the active substance may have neurotoxic or developmental neurotoxic properties then additional information or specific studies will be required.

Delayed polyneuropathy studies

Delayed polyneuropathy studies should provide sufficient data to evaluate if the active substance may provoke delayed polyneuropathy after acute and repeated exposure. A repeated exposure study may be waived unless there are indications that the compound accumulates and significant inhibition of neuropathy target esterase or clinical/histopathological signs of delayed polyneuropathy occur at around the hen LD_{50} as determined in the single dose test.

These studies should be performed for active substances of similar or related structures to those capable of inducing delayed polyneuropathy such as organophosphorus compounds.

For organophosphorus compounds and carbamates, delayed neurotoxicity tests in the laying hen after acute and repeated exposure (OECD TG 418 and OECD TG 419) should be performed.

Test methods for delayed neuropathy:

- EC method B.43 Neurotoxicity study in rodents
- OECD Test Guideline 424: Neurotoxicity study in rodents. EC method B.37 Delayed neurotoxicity of organophosphorus substances after acute exposure
- EC method B.38 Delayed neurotoxicity of organophosphorus substances 28-day repeated dose study
- OECD Test Guideline 419: Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study
- OECD Test Guideline 418: Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure. Developmental Neurotoxicity
- OECD Test Guideline 426: Developmental Neurotoxicity study
- OECD Test Guideline 443: Extended one generation reproductive study

8.13.3 Endocrine disruption (ADS)

Point 8.13.3 of Annex II to the BPR states that *if there is any evidence from in vitro, repeated dose or reproduction toxicity studies, that the active substance may have endocrine disrupting properties, additional information or specific studies shall be required to:*

- *elucidate the mode/mechanism of action*
- *provide sufficient evidence for relevant adverse effects*

For evaluation of consumer safety of active substances that may end up in food or feed, it is necessary to conduct toxicity studies by the oral route

Information to be generated with regard to elucidating the endocrine mode of action should take into account the design of *in vivo* toxicity studies (repeated dose toxicity, extended one generation toxicity study) to ensure that specific parameters linked to endocrine properties of an active substance are investigated when conducted in *in vivo* animal tests. In addition information derived from the use of expert systems that indicate structural similarities to known endocrine disrupters should be taken into account in deciding the need for additional testing.

Studies required should be designed on an individual basis and taking into account Union or internationally agreed guidelines, in the light of the particular parameters to be investigated and the objectives to be achieved. Expert judgment is needed to decide

whether there is a need to perform additional tests or whether the existing information can be used to conclude that the substance is an endocrine disruptor.

OECD Test Guideline protocols for the examination of endocrine disruption as well as Guidance on this topic by the Commission and OECD should be considered to decide on the design of tests to examine the potential of endocrine disruption for active substances.

8.13.4 Immunotoxicity including developmental immunotoxicity (ADS)

Point 8.13.4 of Annex II to the BPR states that *if there is any evidence, from skin sensitisation, repeated dose or reproduction toxicity studies, that the active substance may have immunotoxic properties then additional information or specific studies shall be required to:*

- *elucidate the mode/mechanism of action*
- *provide sufficient evidence for relevant adverse effects in humans*

For evaluation of consumer safety of active substances that may end up in food or feed, it is necessary to conduct toxicity studies by the oral route

The objectives of investigating immunotoxicity are to investigate:

- *whether the substance of interest has the potential to induce adverse effects involving the immune system; special attention should be paid to the adverse immunotoxic outcome among susceptible and vulnerable groups;*

- *the adverse outcomes caused by exposure to the substance (inflammation, immunosuppression; increased propensity for allergic disease; hypersensitivity reactions directed to the chemical itself; increased risk of autoimmune disease; dysfunctional responses resulting in tissue or organ damage or dysfunction; impact on the developing immune system);*

Steps 1 and 2 Collection and evaluation of available information

For the assessment of existing information (non-human data: physicochemical properties, grouping, (Q)SARs and expert systems, *in vitro* data; human data and animal data) further guidance is available within the Guidance on the Application of the CLP Criteria (ECHA) and Part B Human Health Effects Assessment (BPR guidance under development).

The guidance for the evaluation of all available information before conducting new tests is available in Part B Effects Assessment (BPR guidance under development) and is largely based on the WHO/IPCS Guidance on Immunotoxicity for Risk Assessment (WHO, 2012).

It has also to be noted that current animal studies provide information from an unchallenged immune system which has potential pitfalls in the assessment of immunotoxic potential (WHO/IPCS guidance for Immunotoxicty risk assessment for chemicals (WHO, 2012)).

Step 3 Generation of new test data

If immunotoxicity potential is identified tests consisting of a more specific confirmatory set of studies or in-depth mechanistic studies, is carried out to confirm and further

characterize the endpoint. It is worth noting that further testing to investigate immune function should be conducted only if the outcomes of such studies can be interpreted in relation to the risk assessment for the substance of interest. In addition, the need for further testing to characterise effects of concern for immunotoxicity has to be considered on a case-by-case basis.

It should be considered that the conduct of the repeated dose toxicity tests and the reproductive toxicity tests should be performed in a way that allows evaluation of immunotoxicity potential (e.g. Repeated dose toxicity according to US EPA OPPTS 870.7800 (Health Effects Test Guidelines Immunotoxicity) including parameters for immunotoxicity and OECD TG 443 -extended one generation toxicity test- may be conducted with the immunotoxicity cohort).

The test methods to be used for further immunotoxicity studies will depend also on the triggers from steps 1 and 2 of the weight of evidence analysis. Different test methods can be employed for assessing immune suppression, immune stimulation and autoimmunity as well as developmental immunotoxicity.

Reviews of principles and methods for immunotoxicity are available from WHO/IPCS:

- WHO/IPCS Environmental Health Criteria (EHC) 180, Principles and Methods for Assessing Direct Immunotoxicity Associated with Exposure to Chemicals (WHO, 1996)
- WHO/IPCS Environmental Health Criteria (EHC) 212, Principles and Methods for Assessing Allergic Hypersensitization Associated with Exposure to Chemicals (WHO, 1999)
- WHO/IPCS Environmental Health Criteria (EHC) 236, Principles and Methods for Assessing Autoimmunity Associated with Exposure to Chemicals (WHO, 2007)
- WHO/IPCS Guidance for immunotoxicity risk assessment for chemicals, Harmonisation project document No 10 (WHO, 2012)

Below a list of methods that can be considered for further immunotoxicity testing is provided. This list is not exhaustive but provides the methodological aspects to consider on a case-by-case basis.

Immune Suppression

- US EPA OPPTS 870.7800 Health Effects Test Guidelines Immunotoxicity
- Functional studies as described under Additional Immunotoxicity Studies below

Immune stimulation including hypersensitivity (skin and respiratory sensitisation)

- LLNA assay (see sensitisation section)
- Functional studies as described under Additional Immunotoxicity Studies below

Autoimmunity

- Functional studies as described under Additional Immunotoxicity Studies below

Developmental Immunotoxicity

- OECD Test Guideline 443: Extended One-Generation Reproductive Toxicity Study

Additional Immunotoxicity Studies (adopted from ICH S8)

- T-cell Dependent Antibody Response (TDAR)

- Immunophenotyping
- Natural Killer Cell Activity Assays
- Host Resistance Studies
- Macrophage/Neutrophil Function
- Assays to Measure Cell-Mediated Immunity

8.13.5 Mechanistic data - any studies necessary to clarify effects reported in toxicity studies (ADS)

This data may be relevant on the basis of the toxicological properties of a substance and can clarify the mode of action of the chemical. In addition, this can provide information for refinement in the evaluation process for mixtures.

Studies of the mechanisms of toxicity/mode of action may be necessary when there are indications that active substance may have e.g. a non-genotoxic mechanism for carcinogenicity, species specific effects, adverse effects on reproduction, immunotoxicity or hormone related effects. Such studies are important in confirming that effects observed in experimental animals may be of limited or no relevance to humans.

8.14 Studies related to the exposure of humans to the active substance (ADS)

Toxicity of degradation products, by-products and reaction products related to human exposure.

Information is required on the toxic effects of substances generated from an active substance, other than mammalian metabolites, in normal use of biocidal product.

The decision as to the need for these data should be made on a case-by-case basis by expert judgment. Where human exposure is significant, toxicity testing may be needed.

These data may be relevant for many product-types for example: product-types 1 and 2 (reaction products with water when the substance is used for human hygiene purposes or reaction products with water or other materials released in water or air when the substance is used for the treatment of bathing waters), product-type 5 (substances produced in a reaction with drinking water), product-types 6, 7, 9 and 10 (residuals in treated materials), product-type 8 (irritating and sensitising effects of chemical compounds, such as metal salts, developed on the surface of the treated wood) and product-type 18 (products, which may produce harmful substances with water during gassing).

8.15 Toxic effects on livestock and pets (ADS)

An estimation of toxic effects and exposure via different exposure routes (e.g. inhalation, licking, skin contact and ingestion of poisoned bait) and in relevant, but exceptional cases, toxicity testing in livestock and pets is required. Toxic effects for livestock and pets should be estimated or studied if the substance is to be used in spaces in which animals are housed, kept or transported or exposure is possible via drinking water or feeding stuffs. Information on lethal doses for different species, symptoms of poisoning, details of the time courses in case of poisoning and antidotes should also be submitted, if available.

These data may be relevant e.g. for product-type 3 (substances used for veterinary hygiene purposes), product-type 4 (disinfection of surfaces and equipment), product-type 5 (drinking water) product-types 8 and 10 (treated materials in areas in which animals are housed, kept or transported), product-types 14, 15 and 23 (ingestion of baits), product-types 16 and 17 (contaminated drinking water), product-types 18 and 19 (repellents to be used for veterinary hygiene purposes, residential indoor use).

8.16 Food and feeding stuffs studies including for food producing animals and their products (milk, eggs and honey) (ADS)

Point 8.16 of Annex II to the BPR states that *additional information related to the exposure of humans to the active substance contained in biocidal products.*

Evaluation of residues in food and feed from biocidal uses requires information on the nature of residues as well as quantification of residues, which is covered by data requirements listed under this endpoint in Annex II of the BPR (and the endpoint 8.10 in Annex III of the BPR).

Dietary Risk Assessment (DRA) follows a step-wise approach with each step leading to a more realistic estimate of residue amounts in foods. Lower-level steps generally involve calculation models populated with default values in the first tier with the possibility of including additional data in higher tiers. With few exceptions, data from product- and use-specific residue studies with foods are only necessary if lower tiers fail to exclude a consumer risk. In addition, Maximum Residue Limits (MRLs) must be set when specified threshold amounts in foods are exceeded.

The basic use categories for DRA are "animal husbandry", "biocide-food contact (professional use)" and "biocide-food contact (non-professional use)". Depending on the use category, different calculation models and residue study designs apply. While some required information, e.g. metabolism in livestock and degradation during food processing is related to the active substance itself, other data are connected to the intended use of the respective biocidal product (e.g. supervised residue trials). The former can be submitted at the stage of the evaluation for active substance approval, while the latter must be generated at the product authorisation stage.

Guidance (under development) for dietary risk assessment should be followed.

8.16.1 Proposed acceptable residue levels i.e. maximum residue limits (MRL) and the justification of their acceptability (ADS)

For product-type 5, any relevant regulations relating to acceptable or unacceptable residues in drinking water must be taken into consideration in the justification.

For product-type 21, any directions or restrictions at the Community or national level related to residues in fish and shellfish intended to be used as food or feeding stuffs must be taken into consideration in the justification.

8.16.2 Behaviour of the residue of the active substance, its degradation products and, where relevant, its metabolites on the treated or contaminated food or feeding stuffs including the kinetics of disappearance (ADS)

Residue definitions should be provided where relevant. It is also important to compare residues found in toxicity studies with residues formed in food-producing animals, their product as well as food and feed.

8.16.3 Overall material balance for the active substance (ADS)

Point 8.16.3 of Annex II to the BPR states that *sufficient residue data from supervised trials on food producing species and their products as well as food and feed to demonstrate that residues likely to arise from the proposed use would not be of concern for human or animal health*

8.16.4 Estimation of potential or actual exposure of the active substance to humans through diet and other means (ADS)

Expected exposure via diet taking into account consideration the average consumption of different food types and drinking water should be studied.

8.16.5 If residues of the active substance remain on feeding stuffs for a significant period of time or also residues found in food of animal origin after treatment on or around food producing animals (ADS)

Point 8.16.5 of Annex II to the BPR states that [….] *(e.g. direct treatment on animals or indirect treatment of animal houses or surroundings) then feeding and metabolism studies in livestock shall be required to permit evaluation of residues in food of animal origin*

8.16.6 Effects of industrial processing and/or domestic preparation on the nature and magnitude of residues of the active substance

Provide information as implied by the title.

8.16.7 Any other available information that is relevant (ADS)

Point 8.16.3 of Annex II to the BPR states that *it may be appropriate to include information on migration into food, especially in the case of treatment of food contact materials*

For instance information from other chemical programmes on ADI, MRL or relevant residues

8.16.8 Summary and evaluation of data submitted under 8.16.1. to 8.16.7. (ADS)

Point 8.16.8 of Annex II to the BPR states that *It is important to establish whether the metabolites found in food (from animals or plants) are the same as those tested in toxicity studies. Otherwise values for risk assessment (e.g. ADI) are not valid for the residues found*

Please follow the guidance in Chapter II Section 8.18.

8.17 If the active substance is to be used in products for action against plants including algae then tests to assess toxic effects of metabolites from treated plants, if any, where different from those identified in animals, shall be required (ADS)

This point on action against plants is considered as covered sufficiently by Regulation (EC) No 1107/2009 (PPPR).

8.18 Summary of mammalian toxicology

Provide overall evaluation and conclusion with regard to all toxicological data and any other information concerning the active substances including NOAEL.

III. DOSSIER REQUIREMENTS FOR BIOCIDAL PRODUCTS

8. Toxicological profile for humans and animals

This chapter describes the information requirements for biocidal products for the assessment of the toxicological profile for humans and animals.

8.1 Skin corrosion or skin irritation

Point 8.1 of Annex III to the BPR states that *the assessment of this endpoint shall be carried out according to the sequential testing strategy for dermal irritation and corrosion set out in the Appendix to Test Guideline B.4. Acute Toxicity - Dermal Irritation/Corrosion (Annex B.4. to Regulation (EC) No 440/2008).*

Testing on the product/mixture does not need to be conducted if

- *there are valid data available on each of the components in the mixture sufficient to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected.*

Please follow the guidance in Chapter II Section 8.1.

8.2 Eye irritation

Point 8.2 of Annex III to the BPR states that *the assessment of this endpoint shall be carried out according to the sequential testing strategy for eye irritation and corrosion as set down in the Appendix to Test Guideline B.5.Acute Toxicity: Eye Irritation/Corrosion (Annex B.5. to Regulation (EC) No 440/2008).*

Testing on the product/mixture does not need to be conducted if:

- *there are valid data available on each of the components in the mixture sufficient to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected.*

Please follow the guidance in Chapter II Section 8.2.

8.3 Skin sensitisation

Point 8.3 of Annex III to the BPR states that *the assessment of this endpoint shall comprise the following consecutive steps:*

1. *an assessment of the available human, animal and alternative data*
2. *in vivo testing*

The Murine Local Lymph Node Assay (LLNA) including, where appropriate, the reduced variant of the assay, is the first-choice method for in vivo testing. If another skin sensitisation test is used justification shall be provided.

Testing on the product/mixture does not need to be conducted if:

- *there are valid data available on each of the components in the mixture sufficient to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected;*
- *the available information indicates that the product should be classified for skin sensitisation or corrosivity; or*
- *the substance is a strong acid (pH < 2.0) or base (pH > 11.5)*

Please follow the guidance in Chapter II Section 8.3.

Any limitation of the additivity method specified in the Guidance on the Application of the CLP Criteria (ECHA) in the for sensitisation with regard to addressing sub corrosive concentrations with sensitising potential should also be considered (see also Chapter II Section 8.3).

8.4 Respiratory sensitisation (ADS)

Point 8.4 of Annex III to the BPR states that *testing on the product/mixture does not need to be conducted if:*

- *there are valid data available on each of the components in the mixture sufficient to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected.*

Please follow the guidance in Chapter II Section 8.4.

8.5 Acute toxicity

Point 8.5 of Annex III to the BPR states that:

- *Classification using the tiered approach to classification of mixtures for acute toxicity in Regulation (EC) No 1272/2008 is the default approach*

Testing on the product/mixture does not need to be conducted if:

- *there are valid data available on each of the components in the mixture sufficient to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected.*

8.5.1 By oral route

Please follow guidance in Chapter II Section 8.7.1.

8.5.2 By inhalation

Please follow guidance in Chapter II Section 8.7.2.

8.5.3 By dermal route

Please follow guidance in Chapter II Section 8.7.3.

8.5.4 For biocidal products that are intended to be authorised for use with other biocidal products,

Point 8.5.4 of Annex III to the BPR states that […] *the risks to human health, animal health and the environment arising from the use of these product combinations shall be assessed. As an alternative to acute toxicity studies, calculations can be used. In some cases, for example where there are no valid data available of the kind set out in column 3, this may require a limited number of acute toxicity studies to be carried out using combinations of the products*

Testing on the mixture of products does not need to be conducted if:

- *there are valid data available on each of the components in the mixture sufficient to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected.*

8.6 Information on dermal absorption

Point 8.6 of Annex III to the BPR states that *information on dermal absorption when exposure occurs to the biocidal product. The assessment of this endpoint shall proceed using a tiered approach*

It is not always mandatory to submit experimental data. If such data are not available, as a first step default values (depending on physicochemical properties of the active substance) can be used (additional guidance provided in Part B of Hazard Identification within the Toxicokinetics chapter (BPR guidance under development)). The OECD Guidance Document on Percutaneous absorption/penetration (OECD, 2004a) and the EFSA Guidance Document on Dermal Absorption (EFSA, 2012) should be followed where applicable for the estimation of dermal absorption both for the biocidal product and the active substance (Chapter II Section 8.8).

The following Test Guidelines are available for the conduct of skin absorption studies:

- EC method B.45 Skin Absorption: *In Vitro* Method.
- OECD Test Guideline 428: Skin Absorption: *In Vitro* Method.
- EC method B.44 Skin Absorption: *In Vivo* Method.
- OECD Test Guideline 427: Skin Absorption: *In Vivo* Method.

If testing to assess the likely magnitude and rate of dermal bioavailability is necessary the OECD Test Guideline 428 for *in vitro* skin absorption should be considered first.

Dermal absorption can be estimated on the basis of existing information that comes from other sources. Mostly, this will be extrapolation of experimental data obtained with a

similar formulation, but in this case strict and transparent rules should be followed as to when another formulation or product can be considered similar. Expert judgment will always be needed in these cases as well as justification of less frequently used approaches such as the application of QSARs or a comparison of the results obtained in oral and dermal toxicity studies.

Before new studies are commenced, it should be checked whether the intended use is safe when the appropriate default value is applied. If no experimental data are available, studies with similar formulations should be looked for or further information used that may give at least a rough estimate. If valid studies with the same formulation for which authorisation is to be granted have been performed, their results should be used with a preference to an *in vitro* study on human skin.

Dermal absorption can be measured *in vitro* and/or *in vivo*. If valid studies with the formulation to be regulated are available, their results should be directly used for risk assessment. However, deviations from OECD TG 427 and OECD TG 428 require justification including an assessment of the impact of the deviation. Acceptable studies should be in full compliance with OECD test guidelines 427 (*in vivo*) or 428 (*in vitro*) or at least similar to them in all main aspects, based on expert judgement. The applicant should ensure to provide the necessary relevant information in the study report, e.g. regarding the use of tape stripping. It must be acknowledged that both guidelines leave a certain degree of freedom to modify the study design. Although it is widely accepted that the so-called "triple pack", i.e., a combination of *in vivo* (rat) and *in vitro* (comparison of permeability through human and rat skin) data will provide the most reliable prediction of dermal absorption in man, *in vitro* studies on human skin are considered sufficiently predictive and conservative. Therefore, *in vitro* results obtained on human skin should be normally used for the risk assessment and a complete "triple pack" including testing in living animals will not be required. However, available triple pack data may be used for refinement of the assessment. Likewise, *in vivo* studies on rats or in vitro studies on rat skin as "stand alone" information may also be used but it should be acknowledged that, in the vast majority of cases will result in clear overestimation of dermal absorption in humans.

Other types of studies (e.g., in human volunteers) could be taken into consideration in exceptional cases but in general their use is not recommended.

8.7 Available toxicological data relating to:

- *non-active substance(s) (i.e. substance(s) of concern), or*
- *a mixture that a substance(s) of concern is a component of*

If insufficient data are available for a non-active substance(s) and cannot be inferred through read-across or other accepted non-testing approaches, targeted test(s) described in Annex II, shall be carried out for the […] substance(s) of concern or a mixture that a substance(s) of concern is a component of.

Testing on the product/mixture does not need to be conducted if:

- *there are valid data available on each of the components in the mixture sufficient to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected.*

8.8 Food and feedingstuffs studies (ADS)

8.8.1 If residues of the biocidal product remain on feedingstuffs for a significant period of time, then feeding and metabolism studies in livestock shall be required to permit evaluation of residues in food of animal origin (ADS)

Please follow guidance in Chapter II Section 8.16.

8.9 Effects of industrial processing and/or domestic preparation on the nature and magnitude of residues of the biocidal product (ADS)

The objective of these studies is to establish whether or not breakdown or reaction products arise from residues in the raw products during processing which may require a separate risk assessment.

Depending upon the level and chemical nature of the residue in the raw commodity, a set of representative hydrolysis situations (simulating the relevant processing operations) should be investigated, where appropriate. The effects of process other than hydrolysis may also have to be investigated, where the properties of the active substance or metabolites indicate that toxicologically significant degradation products may occur as a result of these processes. The studies are normally conducted with a radio-labelled form of the active substance.

Please follow guidance in Chapter II Section 8.16.

8.10 Other test(s) related to the exposure to humans (ADS)

Point 8.10 of Annex III to the BPR states that *suitable test(s) and a reasoned case will be required for the biocidal product.*

In addition, for certain biocides which are applied directly or around livestock (including horses) residue studies might be needed.

Please follow guidance in Chapter II Section 8.16.

REFERENCES AND BACKGROUND DOCUMENTS

Billington, R., Lewis, W. R., Mehta, M. J., Dewhurst, I., _, , et al. (2010). The mouse carcinogenicity study is no longer a scientifically justifiable core data requirement for the safety assessment of pesticides. *Critical Reviews in Toxicolog, 40(1)*, 35–49.

Bronaugh, R. L., & Maibach, H. I. (1987). In vitro percutaneous absorption. In F. N. Marzulli, & H. I. Maibach, *Dermatotoxicology* (pp. 121-34). Washington DC: Hemishere Publishing.

ECETOC. (1992). *Evaluation of the neurotoxic potential of chemicals. Monograph No. 18, .* Brussels.

ECETOC. (1993). *Percutaneous Absorption. Monograph 20.* Brussels: ECETOC.

ECHA. (n.d.). *Guidance for Human Health Risk Assessment Description: Volume III Part B.*

ECHA. (n.d.). *Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals.*

ECHA. (n.d.). *Guidance on information requirements and chemical safety assessment: Endpoint specific guidance. R7.*

ECHA. (n.d.). *Guidance on intermediates.*

ECHA. (n.d.). *Guidance on the application of the CLP criteria.*

ECHA. (n.d.). *Guidance on the application of the CLP criteria.*

EFSA. (2012). Guidance on Dermal Absorption. *EFSA Journal 10(4)*, 2665-2695.

EU. (2003). *Technical Guidance Document (TGD) on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the.*

EU. (2008a). *Technical Guidance Document in support of the of Directive 98/8/EC concerning the placing of biocidal products on the market. Guidance on Data Requirements for active substances and biocidal products. Short title: TNsG Data Requirements.*

EU. (2008b). *The Technical Notes for Guidance on Dossier preparation including preparation and evaluation of study summaries under Directive 98/8/EC Concerning the Placing of Biocidal Products on the Market. Short title: TNsG on Dossier Preparation of Dossiers and St.*

EU. (2011a). *Manual of Technical Agreements of the Biocides Technical Meeting (MOTA). Version 4.*

EU. (2012a). *Technical Notes Guidance EU Evaluation Manual for the Authorisation of Biocidal Products. Short title: Evaluation manual.*

FAO. (2010). *Manual on the Development and Use of FAO and WHO Specifications for Pesticides, second revision.*

Howes, D., Guy, R. H., Hadgraft, J., Heylings, J., Hoeck, U., Kemper, F., et al. (1996). *Method for assessing percutaneous absorption – Report and Recommendations of ECVAM Workshop 13. ATLA, 24, 81-106.*

Janer, G., Slob, W., Hakkert, C. B., Vermeire, T., Piersman, H. A., _, et al. (2008). A retrospective analysis of developmental toxicity studies in rat and rabbit: What is the added value of the rabbit as an additional test species? *Regulatory Toxicology and Pharmacology 50*, 206-217.

OECD. (2000a). *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. Series on Testing and Assessment, No 23.*

OECD. (2004a). *Guidance Document for the conduct of skin absorption studies, Series on Testing and Assessment No 28. ENV/JM/MONO(2004)2.*

OECD. (2004b). *Guidance Document for Neurotoxicity Testing. Series on Testing and Assessment No 20, Paris. NV/JM/MONO(2004)25.*

OECD. (2008b). *Guidance Documents on mammalian reproductive toxicity testing and assessment. Series on Testing and Assessment No 43. ENV/JM/MONO(2008)16.*

Rozman, K. K. (1986). Faecal excretion of toxic substances. In K. K. Rozman, & O. Hanninen, *Gastrointestinal Toxicology.* Amsterdam: Elsevier.

UN. (2009). *Recommendations on the Transport of Dangerous Goods. Manual of Tests and Criteria. ST/SG/AC.10/11/Rev.5. (UN-MTC).* New York and Geneva.

US EPA. (1992). *Dermal exposure assessment: Principles and Applications. EPA/600/8-91.001B.* Washigton DC.

WHO. (1986). *WHO/IPCS Environmental Health Criteria (EHC) 60. Principles and Test Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals.*

WHO. (1996). *WHO/IPCS Environmental Health Criteria (EHC) 180, Principles and Methods for Assessing Direct Immunotoxicity Associated with Exposure to Chemicals .*

WHO. (1999). *WHO/IPCS Environmental Health Criteria (EHC) 212, Principles and Methods for Assessing Allergic Hypersensitization Associated with Exposure to Chemicals .*

WHO. (2007). *WHO/IPCS Environmental Health Criteria (EHC) 236, Principles and Methods for Assessing Autoimmunity Associated with Exposure to Chemicals.*

WHO. (2012). *Guidance for immunotoxicity risk assessment for chemicals, Harmonization Project Document No. 10.*

ANNANKATU 18, P.O. BOX 400,
FI-00121 HELSINKI, FINLAND
ECHA.EUROPA.EU