Evaluation Manual for the Authorisation of plant protection products according to Regulation (EC) No 1107/2009

EU part

Plant Protection Products

Chapter 4 Human toxicology; mammalian toxicity dossier

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Chapter 4 Human toxicology; mammalian toxicity dossier

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Changes in the Evaluation Manual

Evaluation manual PPP EU part Chapter 4 Human toxicology; mammalian toxicity dossier			
Version	**Date**	**Paragraph**	**Changes**
2.0	Janaury 2014		
2.1	October 2016		No major changes. Some additional information were added to certain data requirements to reflect the outcome of the Pesticide Peer Review meeting 137.
2.2	March 2017	1.3.5	Updated to take into account the revised Guidance document on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (SANTE-10832-2015 rev. 1.7)

GENERAL INTRODUCTION

This chapter describes the data requirements for estimation of the human toxicological effects of a plant protection product and the active substance, and how reference values are derived within the EU framework (§1 - §1.5) Regulation (EC) No 1107/2009.

1. EU FRAMEWORK

In this document, the procedures for the evaluation and re-evaluation of active substances as laid down in the EU are described. This document aims to give procedures for the approval of active substances and inclusion in Commission Implementing Regulation (EU) No 540/2011.

1.1. Introduction

The use of plant protection products may result in human exposure. Such exposure may occur via different routes: oral, dermal and respiratory. It is therefore important that the intrinsic human toxicological properties of each active substance and product can be evaluated and established.

The information on the toxic effects and kinetics of a substance is mainly based on the results of experimental toxicological research performed with different laboratory animal species. Besides toxicity data on the active substance, data on metabolites may also be required if human exposure to such metabolites occurs.

Each study is summarised separately in the toxicological summary and, if possible, the relevant endpoint is derived such as, e.g., the 'No Observed Adverse Effect Level' (NOAEL), LD_{50}, irritating yes/no, etc. This evaluation results for each study and for each sub-aspect in a toxicologically based endpoint, and finally in the toxicological profile of a substance.

The toxicological endpoints derived from the submitted research, then form the basis of the risk evaluation for operator, worker, bystander and resident (see Chapter 4 Human toxicology; risk operator, worker, bystander and resident), and for consumers (see Chapter 5, Residues; risk to consumers).

The EU uses the so-called list of endpoints. This list is also used for national evaluations. SANCO/12483/2014 - rev. 3 provides the template to be used for the List of Endpoints.

1.2. Data requirements

In order to qualify for inclusion of an active substance in Commission Implementing Regulation (EU) No 540/2011 a dossier that meets the provisions laid down in Commission Regulation (EU) No 283/2013 and Commission Regulation (EU) No 284/2013 of Regulation (EC) No 1107/2009, must be submitted for the active substance as well as for the representative product.

Generally, EU and OECD guidelines for the performance of toxicological studies are mentioned in Commission Communications 2013/C 95/01.

Where the applicant holds the view that a certain study is not necessary, a relevant scientific justification can be provided for the non-submission of the particular study.

Experiments performed after 25 July 1993 must have been performed in accordance with GLP.

The identity of the tested substance and the tested product, and the purity of the tested substance should be clearly stated for each study.

1.2.1. Data requirements for the active substance

The data requirements regarding the mammalian toxicity dossier of the active substance are described in part A of Commission Regulation (EU) No 283/2013, section 5.

The following point stated in part A should specifically be taken into account when submitting an active substance dossier:

1. The relevance of generating toxicity data in animal models with dissimilar metabolic profiles to those found in humans shall be addressed, if such metabolic information is available, and taken into consideration for study design and risk assessment.

2. All potentially adverse effects found during toxicological investigations (including effects on organs/systems such as the immune system, the nervous system, or the endocrine system) shall be reported. Additional studies may be necessary to investigate the mechanisms underlying effects that could be critical to hazard identification or risk assessment.

   All available biological data and information relevant to the assessment of the toxicological profile of the active substance tested, including modelling, shall be reported.

3. Where available, historical control data shall be provided routinely. The data submitted shall be for endpoints that could represent critical adverse effects, and shall be strain-specific and from the laboratory which carried out the index study. They shall cover a five-year period, centred as closely as possible on the date of the index study.

4. When preparing a study plan, available data on the test substance, such as its physico-chemical properties (such as volatility), purity, reactivity (such as rate of hydrolysis, electrophilicity) and structure-activity relationships of chemical analogues, shall be taken into account.

5. For all studies actual achieved dose in mg/kg body weight, as well as in other convenient units (such as mg/L inhalation, mg/cm 2 dermal), shall be reported.

6. The analytical methods to be used in toxicity studies shall be specific for the entity to be measured and shall be adequately validated. The LOQ shall be adequate for the measurement of the range of concentration anticipated to occur in the generation of the toxicokinetic data. Information on the validation of the analytical methods should be submitted under section 4 Analytical methods.

7. Where, as a result of metabolism or other processes in or on treated plants, in livestock, in soil, in ground water, open air, or as a result of processing of treated products, the terminal residue to which humans will be exposed contains a substance which is not the active substance itself and is not identified as a significant metabolite in mammals, toxicity studies shall, where technically possible, be carried out on that substance unless it can be demonstrated that human exposure to that substance does not constitute a relevant risk to health.

   Toxicokinetic and metabolism studies relating to metabolites and breakdown products

shall only be required if toxicity findings of the metabolite cannot be evaluated by the available results relating to the active substance.

8. The oral route shall always be used if it is practical. In cases where exposure of humans is mainly by the gas phase, it can be more appropriate to perform some of the studies via inhalation.

9. For dose selection, toxicokinetic data such as saturation of absorption measured by systemic availability of substance and/or metabolites shall be taken into consideration.

Studies on absorption, distribution, excretion and metabolism in mammals

The specific data requirements on absorption, distribution, metabolism and excretion are provided in Commission Regulation (EU) No 283/2013 section 5.1. The research provides insight into metabolism and kinetics of a substance and, if several exposure routes have been studied, in the possible differences between the exposure routes. The studies provide insight into possible sex differences and accumulation as well.

A comparative *in vitro* metabolism study is also required which provides information on the relevance of the toxicological animal data for humans.

Acute toxicity

The specific data requirements on acute toxicity are provided in Commission Regulation (EU) No 283/2013 section 5.2.

Oral

The acute oral toxicity of the active substance must always be reported.

Percutaneous

The acute dermal toxicity study may be waived if scientifically justifies (e.g. where the oral LD50 is >2000 mg/kg). Both local and systemic effects must also be investigated. When severe skin irritation is observed (grade 4 erythema or oedema) than this will be used for classification and labelling purposes instead of performing a specific irritation study.

Inhalation

The inhalation toxicity of the active substance shall be reported where any of the following apply:

- the active substance has a vapour pressure > 1 × 10-2 Pa at 20 °C;
- the active substance is a powders containing a significant proportion of particles of diameter < 50 µm (> 1% on a weight basis);
- the active substance is included in products that are powders or are applied by spraying.

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

Skin irritation

Before undertaking *in vivo* studies for corrosion/irritation of the active substance, a weight-of-evidence analysis shall be performed on the existing relevant data. Where insufficient data are available, they can be developed through application of sequential testing.

The testing strategy shall follow a tiered approach:

(1) the assessment of dermal corrosivity using a validated *in vitro* test method;

(2) the assessment of dermal irritation using a validated *in vitro* test method (such as

human reconstituted skin models);
(3) an initial *in vivo* dermal irritation study using one animal, and where no adverse effects are noted;
(4) confirmatory testing using one or two additional animals.

The skin irritancy of the active substance shall always be provided. Where available, a dermal toxicity study shown not to produce irritation of the skin at the limit test dose of 2000 mg/kg body weight shall be used to waive the need for any dermal irritation studies.

Eye irritation

Before undertaking *in vivo* studies for eye corrosion/irritation of the active substance, a weight-of-evidence analysis shall be performed on the existing relevant data. Where available data are considered insufficient, further data may be developed through application of sequential testing.

The testing strategy shall follow a tiered approach:
(1) the use of an *in vitro* dermal irritation/corrosion test to predict eye irritation/corrosion;
(2) the performance of a validated or accepted *in vitro* eye irritation study to identify severe eye irritants/corrosives (such as Bovine Corneal Opacity and Permeability (BCOP) assay, Isolated Chicken Eye (ICE) assay, Isolated Rabbit Eye (IRE) assay, Hen's Egg Test - Chorio-Allantoic Membrane assay (HET-CAM)), and where negative results are obtained, the assessment of eye irritation using an *in vitro* test method for identification of non- irritants or irritants, and where not available;
(3) an initial *in vivo* eye irritation study using one animal, and where no adverse effects are noted;
(4) confirmatory testing using one or two additional animals.

The eye irritancy of the active substance shall always be tested, except where it is likely that severe effects on the eyes may be produced based on criteria listed in the test methods.

Skin sensitisation

The study shall always be carried out, except where the active substance is a known sensitiser. The local lymph node assay (LLNA) shall be used, including where appropriate the reduced variant of the assay. In case the LLNA cannot be conducted, a justification shall be provided and the Guinea Pig Maximisation Test shall be performed. Where a guinea pig assay (Maximisation or Buehler), meeting OECD guidelines and providing a clear result, is available, further testing shall not be carried out for animal welfare reasons.

Since an active substance identified as a skin sensitiser can potentially induce hypersensitivity reaction, potential respiratory sensitisation should be taken into account when appropriate tests are available or when there are indications of respiratory sensitisation effects.

Phototoxicity

The study shall 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 after dermal application. A positive result shall be taken into account when considering potential human exposure.

The data requirement states that the *in vitro* study shall be required 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. However, the *in vitro* 3T3 NRU phototoxicity test (OECD guideline 432) is not completely applicable to evaluate phototoxicity of test substance at these UVB-wavelengths because irradiation of cells *in vitro* at wavelengths below 320 nm results in massive cytotoxicity even in the absence of test substance. During Pesticide Peer Review Meeting 137 it was agreed that the phototoxicity test should not be performed with a light source emitting wavelengths lower than 313 nm in the absence of further guidance.

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

Short-term toxicity

The specific data requirements on short-term toxicity are provided in Commission Regulation (EU) No 283/2013 section 5.3.

Toxicokinetic data (that is to say blood concentration) shall be included in short term studies. 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 short term studies at dose levels not producing marked toxicity, supplementary studies, including functional testing, shall be carried out (see point 5.8.2).

Oral 28 day study

Where available, 28-day studies shall be reported.

Oral 90-day study

The short-term oral toxicity of the active substance to rodent (90-day), usually the rat, a different rodent shall be justified, and non-rodents (90-day toxicity studies in dogs), shall always be reported.

In the 90-day study, potential neurotoxic and immunotoxic effects, genotoxicity by way of micronuclei formation and effects potentially related to changes in the hormonal system shall be carefully addressed.

Other routes

For human risk assessment additional percutaneous studies shall be considered on a case by case basis, unless the active substance is a severe irritant.

For volatile substances (vapour pressure >10^{-2} Pascal) expert judgment (for example based on route-specific kinetic data) shall be required to decide whether the short term studies have to be performed by inhalation exposure.

Genotoxicity testing

The specific data requirements on genotoxicity are provided in Commission Regulation (EU) No 283/2013 section 5.4.

For the testing strategy a tiered approach shall be adopted, with selection of higher tier tests being dependent upon interpretation of results at each stage.

Special testing requirements in relation to photomutagenicity may be indicated by the structure of a molecule. If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance and its major metabolites is less than 1 000 L × mol –1 × cm –1 ,

photomutagenicity testing is not required. At the moment no specific guideline is available on how to conduct an photomutagenicity study. The lack of a specific guideline was discussed during Pesticide Peer Review Meeting 137. It was agreed that if a positive result was obtained in the phototoxicity study that the lack of a specific study guideline for photomutagenicity would be raised to the risk managers in the conclusion of the peer review. For the time being it was concluded by the experts that photomutagenicity testing is not required, unless further guidance is provided.

In vitro studies

A bacterial assay for gene mutation, combined test for structural and numerical chromosome aberrations in mammalian cells and test for gene mutation in mammalian cells shall be submitted.

However, 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 shall 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 shall be tested in two different *in vitro* mammalian cell tests for gene mutation. Non performance of the Ames test shall 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 additional study or study plan modifications depends on the chemical nature, the known reactivity and the metabolism data on the structurally alerting active substance.

In vivo studies in somatic cells

If all the results of the *in vitro* studies are negative, at least one *in vivo* study shall be done with demonstration of exposure to the test tissue (such as cell toxicity or toxicokinetic data), unless valid *in vivo* micronucleus data are generated within a repeat dose study and the *in vivo* micronucleus test is the appropriate test to be conducted to address this information requirement.

A negative result in the first *in vivo* test in somatic cells shall provide sufficient reassurance for active substances that are negative in the three *in vitro* tests.

For active substances for which an equivocal or a positive test result is obtained in any *in vitro* test, the nature of additional testing needed shall be considered on a case-by-case basis taking into account all relevant information using the same endpoint as in the *in vitro* test.

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 shall be conducted.

If the *in vitro* micronucleus test for numerical chromosome aberrations on mammalian cells is positive or the *in vitro* mammalian chromosome test is positive for numerical chromosome changes, an *in vivo* micronucleus test shall be conducted. In case of positive result in the *in vivo* micronucleus assay, appropriate staining procedure such as fluorescence in-situ hybridisation (FISH) shall 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 shall be conducted, such as the Transgenic Rodent Somatic and Germ Cell Gene Mutation Assay. During Pesticide Peer Review Meeting 137 it was concluded that an *in vivo* Comet assay would also be acceptable.

When conducting *in vivo* genotoxicity studies, only relevant exposure routes and methods (*such as* admixture to diet, drinking water, skin application, inhalation and gavage) shall be used. There shall 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 shall be justified.

Consideration shall be given to conducting an *in vivo* test as part of one of the short-term toxicity studies described under point 5.3.

In vivo studies in germ cells

The necessity for conducting these tests shall be considered on a case by case basis, taking into account information regarding toxicokinetics, use and anticipated exposure.

For most of the active substances recognised as *in vivo* somatic cell mutagens no further genotoxicity testing shall be necessary since they will be considered to be potential genotoxic carcinogens and potential germ cell mutagens.

However, in some specific cases germ cells studies may be undertaken to demonstrate whether a somatic cell mutagen is or is not a germ cell mutagen.

The type of mutation produced in earlier studies namely gene, numerical chromosome or structural chromosome changes, shall be considered when selecting the appropriate assay.

A study for the presence of DNA adducts in gonad cells may also be considered.

Long-term toxicity and carcinogenicity

The specific data requirements on long-term toxicity and carcinogenicity are provided in Commission Regulation (EU) No 283/2013 section 5.5.

The long-term toxicity and carcinogenicity of all active substances shall be determined. If in exceptional circumstances it is claimed that such testing is unnecessary, that claim must be fully justified

A long-term oral toxicity study and a long-term carcinogenicity study (two years) of the active substance shall be conducted using the rat as test species; where possible these studies can be combined.

A second carcinogenicity study of the active substance shall be conducted using mouse as test species, unless it can be scientifically justified that this is not necessary. In such cases,

scientifically validated alternative carcinogenicity models may be used instead of a second carcinogenicity study.

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

Experimental data, including the elucidation of the possible mode of action involved and relevance to humans, shall be provided where the mode of action for carcinogenicity is considered to be non-genotoxic.
Where submitted, historical control data shall be from the same species and strain, maintained under similar conditions in the same laboratory and shall 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 must 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, of the control animals at the beginning of the study and at the time of killing or death,
(e) description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (e.g. diseases, infections),
(f) name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study;
(g) a statement of the nature of the tumours that may have been combined to produce any of the incidence data.

The historical control data shall 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 shall contain information on the range of values, the mean, median and, if applicable, standard deviation.

The doses tested, including the highest dose tested, shall 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 shall not be considered relevant to evaluations to be made. Determination of blood concentration of the active substance (for example around T max) shall be considered in long-term studies.
In the collection of data and compilation of reports, incidence of benign and malignant tumours shall not be combined. Dissimilar, un-associated tumours, whether benign or malignant, occurring in the same organ, shall not be combined, for reporting purposes.

In the interests of avoiding confusion, conventional histopathological terminology commonly used when the study is conducted such as that published by the International Agency for Research on Cancer shall be used in the nomenclature and reporting of tumours. The system used shall be identified.

Biological material selected for histopathological examination shall include material selected to provide further information on lesions identified during gross pathological examination. Where relevant to the elucidation of mechanism of action and available, special histological (staining) techniques, histochemical techniques and electron microscopic examinations, might be of value, and when conducted, shall be reported.

Reproductive toxicity

The specific data requirements on reproductive toxicity are provided in Commission Regulation (EU) No 283/2013 section 5.6.

The active substance and its relevant metabolites shall be measured in milk 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 shall be carefully addressed and reported.

Investigations shall 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.

While the standard reference point for treatment responses shall be concurrent control data, historical control data may be helpful in the interpretation of particular reproductive studies. Where submitted, historical control data shall be from the same species and strain, maintained under similar conditions in the same laboratory and should be from contemporaneous studies.

The information on historical control data provided must 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, of the control animals at the beginning of the study and at the time of killing or death,
(e) description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (e.g. diseases, infections);
(f) name of the laboratory and the examining scientist responsible for gathering and interpreting the pathological data from the study.

The historical control data shall 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 shall contain information on the range of values, the mean, median and, if applicable, standard deviation.

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.

Generational studies

A reproduction toxicity study in rats over at least two generations shall be reported.

The OECD extended one-generation reproductive toxicity study may be considered as an alternative approach to the multi-generation study.

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.

Developmental toxicity studies

Developmental toxicity studies shall always be carried out.

Developmental toxicity shall be determined for rat and rabbit by the oral route; the rat study shall not be conducted if developmental toxicity has been adequately assessed as part of an extended one-generation reproductive toxicity study.

Additional routes may be useful in human risk assessment. Malformations and variations shall 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 shall be given in the report. The glossary of terminology under development by the International Federation of Teratology Societies shall be considered where possible.

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.

Neurotoxicity studies

The specific data requirements on neurotoxicity are provided in Commission Regulation (EU) No 283/2013 section 5.7.

Neurotoxicity studies in rodents

Such studies shall 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 shall also be considered for substances with a neurotoxic mode of pesticidal action.

Consideration shall be given to including neurotoxicity investigations in routine toxicology studies.

Delayed polyneurotoxicity

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

Other toxicological studies

The specific data requirements are provided in Commission Regulation (EU) No 283/2013 section 5.8.

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 shall 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 shall 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.

Depending on the residue definition, additional toxicity research may be required with regard to the metabolites that are formed in plants or livestock (see Chapter 5 Residues; residue dossier). The data requirements for these metabolites are not elaborated in Regulation (EC) 283/2013. A guidance has recently been adopted on this issue (Guidance on the establishment of the residue definition for dietary risk assessment).

In the EU framework this has been elaborated for metabolites that leach to groundwater (Chapter 6 Behaviour and fate in the environment, behaviour in soil: leaching). A Guidance on the assessment of the relevance of metabolites in groundwater is available (Sanco/221/2000 rev 10. Final). The Guidance states that for metabolites above 0.75 µg/L a refined consumer risk assessment is required. Please note that this risk assessment should include both adults (60 kg, 2 L/day), children (10 kg, 1 L/day) as well as bottle-fed infants (5 kg, 0.75 L/day).

Supplementary studies on the active substance

Supplementary studies shall 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: studies on absorption, distribution, excretion and metabolism, in a second species;

- studies on the immunotoxicological potential,
- a targeted single dose study to derive appropriate acute reference values (ARfD, aAOEL);
- studies on other routes of administration;
- studies on the carcinogenic potential;
- studies on mixture effects

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

Endocrine disrupting properties

If there is evidence that the active substance may have endocrine disrupting properties, additional information or specific studies shall be required:

— to elucidate the mode/mechanism of action,
— to provide sufficient evidence for relevant adverse effects.

Studies required shall 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.

More information on the criteria on endocrine disruption is provided in Chapter 1 general introduction and generic aspects.

Medical data
The specific data requirements are provided in Commission Regulation (EU) No 283/2013 section 5.9.

1.2.2. Data requirements for the product
The data requirements regarding the mammalian toxicity dossier of the plant protection product are described in Commission Regulation No 284/2013, point 7.

Generally, EU and OECD guidelines for the performance of toxicological studies are mentioned in Commission Communications 2013/C 95/02.

Acute toxicity
The specific data requirements are provided in Commission Regulation (EU) No. 284/2013 section 7.1.

Oral toxicity
A test for acute oral toxicity shall be carried out, unless the applicant can justify an alternative approach under Regulation (EC) No 1272/2008. In the latter case, acute oral toxicity of all components shall be provided or reliably predicted with a validated method. Consideration shall be given to the possible effects of components on the toxic potential of the total mixture.

Dermal toxicity
A test for dermal toxicity shall be carried out on a case by case basis, unless the applicant can justify an alternative approach under Regulation (EC). In the latter case, acute dermal toxicity of all components shall be provided or reliably predicted with a validated method. Consideration shall be given to the possible effects of components on the toxic potential of the total mixture.

Findings of severe skin irritation or corrosion in the dermal study may be used instead of performing a specific irritation study.

Inhalation toxicity
The study shall be carried out where the plant protection product:
(a) is a gas or liquified gas;
(b) is a smoke generating plant protection product or fumigant;
(c) is used with fogging/misting equipment;
(d) is a vapour releasing plant protection product;
(e) is supplied in an aerosol dispenser;
(f) is in a form of a powder or granules containing a significant proportion of particles of diameter <50 µm (> 1% on a weight basis),
(g) is to be applied from aircraft in cases where inhalation exposure is relevant;
(h) contains an active substance with a vapour pressure > 1×10^{-2} Pa and is to be used in enclosed spaces such as warehouses or glasshouses;
(i) is to be applied by spraying.

A study shall not be required if the applicant can justify an alternative approach under Regulation (EC) No 1272/2008, where applicable. For this purpose, acute inhalation toxicity of all components shall be provided or reliably predicted with a validated method. Consideration shall be given to the possible effects of components on the toxic potential of the total mixture.

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

Skin irritation

Before undertaking *in vivo* studies for corrosion/irritation of the plant protection product, a weight-of-evidence analysis shall be performed on the existing relevant data. Where insufficient data are available, they can be developed through application of sequential testing.

The testing strategy shall follow a tiered approach:

(1) the assessment of dermal corrosivity using a validated *in vitro* test method;
(2) the assessment of dermal irritation using a validated *in vitro* test method (such as human reconstituted skin models);
(3) an initial *in vivo* dermal irritation study using one animal, and where no adverse effects are noted;
(4) confirmatory testing using one or two additional animals.

Consideration shall be given to use the dermal toxicity study to provide irritancy information.

Findings of severe skin irritation or corrosion in the dermal study may be used instead of performing a specific irritation study.

The skin irritancy of the plant protection product shall be reported based on the tiered approach, unless the applicant can justify an alternative approach under Regulation (EC) No 1272/2008. In the latter case, skin irritation properties of all components shall be provided or reliably predicted with a validated method. Consideration shall be given to the possible effects of components on the irritant potential of the total mixture.

An *in vivo* test may only be performed if the extent of irritation or corrosivity cannot be established on the basis of an analysis of existing relevant data.

Eye irritation

Before undertaking *in vivo* studies for eye corrosion/irritation of the plant protection product, a weight-of- evidence analysis shall be performed on the existing relevant data. Where available data are considered insufficient, further data may be developed through application of sequential testing.

The testing strategy shall follow a tiered approach:

(1) the use of an *in vitro* dermal irritation/corrosion test to predict eye irritation/corrosion;
(2) the performance of a validated or accepted *in vitro* eye irritation study to identify severe eye irritants/ corrosives (such as BCOP, ICE, IRE, HET-CAM), and where negative results are obtained;
(3) the assessment of eye irritation using an available, *in vitro* test method validated for plant protection products for identification of non-irritants or irritants, and when not available;
(4) an initial *in vivo* eye irritation study using one animal, and where no adverse effects are noted;
(5) confirmatory testing using one or two additional animals.

An *in vivo* test may only be performed (see for further information method B5 of Regulation (EC) No 440/2008) if the extent of irritation or corrosivity cannot be established on the basis of an analysis of existing relevant data.

Eye irritation tests shall be provided, unless it is likely that severe effects on the eyes may be produced or the applicant can justify an alternative approach under Regulation (EC) No 1272/2008. In the latter case, eye irritation properties of all components shall be provided or reliably predicted with a validated method. Consideration shall be given to the possible effects of components on the irritant potential of the total mixture.

Skin sensitisation

The skin sensitisation test shall be carried out unless the active substances or co-formulants are known to have sensitising properties or the applicant can justify an alternative approach under Regulation (EC) No 1272/2008. In the latter case, skin sensitisation properties of all components shall be provided or reliably predicted with a validated method. Consideration shall be given to the possible effects of components on the sensitising potential of the total mixture.

The local lymph node assay (LLNA) shall be used, including where appropriate the reduced variant of the assay. In case the LLNA cannot be conducted, a justification shall be provided and the Guinea Pig Maximisation Test shall be performed. Where a guinea pig assay (Maximisation or Buehler), meeting OECD guidelines and providing a clear result, is available, further testing shall not be carried out for animal welfare reasons.

The Ctgb prefers, in accordance with EU requirements, a local lymph node assay (LLNA) according to OECD guideline 429. If a Guinea Pig Maximisation Test is performed, a scientific justification must be submitted to explain why the LLNA could not be conducted. Only when scientifically justified will the Guinea Pig Maximisation Test be accepted.

A guinea pig study (Guinea Pig Maximisation Test or a (modified) Buehler test) with the formulated product, however, is not simply rejected. The results of the guinea pig sensitisation study with the substance and the fact whether the formulation contains co-formulants with components with sensitising properties are always taken into account.

For clarification, a number of situations are described below:

- Where the LLNA (or a justified Maximisation study) with the active substance is negative and the formulation contains no co-formulants with sensitising properties, the Ctgb will accept a well performed (modified) Buehler test or a unjustified Maximisation test.
- Where the LLNA (or a justified Maximisation study) with the active substance is negative but the formulation contains co-formulants with sensitising properties, the Ctgb will use mathematical methods to decide on labelling. Possible negative results from a (modified) Buehler test or unjustified Maximisation test with the formulation are not simply accepted. The results of an LLNA with the formulation, if available, overrule a possible calculation.
- Where the LLNA (or a justified Maximisation study) with the active substance is positive, the Ctgb will use the calculation rules to decide on labelling. Possible negative results from a (modified) Buehler test or unjustified Maximisation study with the formulation are not simply accepted.
  The results of an LLNA (or a justified Maximisation study) with the formulation, if available, overrule possible a calculation, and the results of the (modified) Buehler.
- Where a (modified) Buehler test or Maximisation study with the formulation is clearly positive, such a study is in principle acceptable and performance of an LLNA is not required.

Since a skin sensitiser can potentially induce hypersensitivity reaction, potential respiratory sensitisation shall be taken into account when appropriate tests are available or when there are indications of respiratory sensitisation effects.

Supplementary studies on the plant protection product
The need to perform supplementary studies on the plant protection product shall be discussed with the national competent authorities on a case by case basis in the light of the particular parameters to be investigated and the objectives to be achieved (for example for plant protection products containing active substances or other components suspected to have synergistic or additive toxicological effects).

The type of the study shall be adapted to the endpoint of concern.

Supplementary studies for combinations of plant protection products
In cases where the product label includes requirements for use of the plant protection product with other plant protection products or with adjuvants as a tank mix, it may be necessary to carry out studies for a combination of plant protection products or for the plant protection product with adjuvant. The need to perform supplementary studies shall be discussed with the national competent authorities on a case by case basis, taking into account the results of the acute toxicity studies of the individual plant protection products and the toxicological properties of the active substances, the possibility for exposure to the combination of the products concerned, with particular regard to vulnerable groups, and available information or practical experience with the products concerned or similar products.

Data on exposure
The specific data requirements are provided in Commission Regulation (EU) No. 284/2013 section 7.2.

The data requirement specifies in section 7.2 that in cases where the product label includes requirements for use of the plant protection product with other plant protection products or with adjuvants as a tank mix, the exposure assessment shall cover the combined exposure. Cumulative and synergistic effects resulting from the exposure to more than one active substance and toxicologically relevant compounds, including those in the product and tank mix, shall be taken into account and reported in the dossier.

For more information on the Dutch approach on combination toxicity for tank mix products or products containing multiple active substances it is referred to Chapter 4 Human toxicology; risk operator, worker and bystander (NL part).

Operator exposure
Estimation of operator exposure
An estimation of operator exposure shall always be completed. More information on how to carry out the operator risk assessment can be found in Chapter 4 Human toxicology; risk operator, worker, bystander and resident (EU part).

Where relevant, this estimation shall take into account cumulative and synergistic effects resulting from the exposure to more than one active substance and toxicologically relevant compounds, including those in the product and tank mix.

Measurement of operator exposure
Exposure data for the relevant exposure routes shall be reported where there are no representative data in available calculation models or where the model-based risk assessment indicates that the relevant reference value is exceeded.

The study shall be done under realistic exposure conditions taking into account the proposed conditions of use.

Guidelines for the performance of exposure studies are described in an OECD guidance document (OCDE/GD(97)148).

Bystander and resident exposure

Estimation of bystander and resident exposure

An estimation of bystander and resident exposure shall always be performed. More information on how to carry out the bystander and risk assessment can be found in Chapter 4 Human toxicology; risk operator, worker, bystander and resident (EU part).

Measurement of bystander and resident exposure

Exposure data for the relevant exposure routes shall be required where the model based risk assessment indicates that the relevant reference value is exceeded or where there are no representative data in available calculation models.

The study shall be done under realistic exposure conditions taking into account the proposed conditions of use.

Worker exposure

Estimation of worker exposure

The estimation of worker exposure shall be completed when such exposure could arise under the proposed conditions of use. More information on how to carry out the worker risk assessment can be found in Chapter 4 Human toxicology; risk operator, worker, bystander and resident (EU part).

Measurement of worker exposure

Exposure data for the relevant exposure routes shall be reported where the model based risk assessment indicates that the relevant reference value is exceeded.

The study shall be done under realistic exposure conditions taking into account the proposed conditions of use.

Dermal absorption

The study shall be conducted when dermal exposure is a significant exposure route, and no acceptable risk is estimated using default absorption value.
Data from absorption studies shall be reported. To reduce laboratory animal use an *in vitro* human skin study is preferred in the EU.

Studies shall be performed on representative plant protection products at both the maximum in-use dilution (when applicable) as well as the concentrated form.

In case studies do not correspond with the anticipated exposure situation (for example with regard to the type of co-formulant or the concentration), scientific argument shall be provided before such data can be used with confidence.

In 2012 a new 'Guidance on dermal absorption' has been adopted (EFSA Journal 2012 (10)4: 2665). The dermal absorption study must be (re-)evaluated in accordance with the EFSA Guidance on dermal absorption. The Guidance also provides additional information on when extrapolation between formulations is justified. Particular attention should be given to

the following points:

- Tape strips should be included in the absorbed dose in cases where the absorption is below 75% within half of the study duration.
- Adjustment should be made for variability when the variation is above 25%
- If the recovery is below 95% a correction should be made for the low absorption
- If outliers are excluded this should be properly justified.
- Extrapolation between formulation should be justified.

Available toxicological data relating to non-active substances

The specific data requirements are provided in Commission Regulation (EU) No. 284/2013 section 7.4.

1.3. Derivation of endpoints and reference values

Each study is summarised and evaluated separately. The final conclusion and the endpoint per aspect (such as, e.g., mutagenicity, carcinogenicity, reproduction toxicity etc.) are presented in the list of endpoints.

The toxicological endpoints that are derived from the submitted studies then form the basis for derivation of various reference values (ADI, AOEL, AAOEL and ARfD). Subsequently, these reference values form the basis of the risk assessment for the consumer, operator, worker, bystander and resident.

1.3.1. Derivation of the list of endpoints for human toxicology

Each study is summarised separately in the toxicological summary and, where possible, the 'No Observed Adverse Effect Level' (NOAEL) is derived.

The following factors are, among others, taken into account in the derivation of, e.g., a NOAEL [1]:

- toxicological relevance of the effect (adverse versus non-adverse);
- toxicological relevance of the effect for man;
- dose-response relationship;
- statistical significance of the effect;
- relationship between the effect and other effects that occur at higher dose levels.

International developments, as published by WHO, JMPR and OECD, are also taken into account when determining whether certain effects are relevant.

The dose is expressed in mg/kg bw/day. Where food intake is not reported in a study, standard conversion factors are used to convert from ppm to mg/kg bw/day. For rats and mouse the conversion factors are presented in an EFSA guidance document (EFSA Journal 2012; 10(3):2579). For rabbit and dog no conversion factors are mentioned in the EFSA guidance and for these species the dose in ppm is divided by 33 and 40, respectively, in case of young adult laboratory animals [2,3].

If, in the absence of a useful NOAEL, a reference value is derived from the lowest observed adverse effect level (LOAEL), an additional factor can be applied. A factor of 10 is used as a default value. Information in the dossier, particularly concerning the slope of the dose-response curve, the distance to the probable NOAEL etc. can lead to the use of another factor. The choice must be motivated in the decision-making stage.

The NOAEL of the most relevant chronic study with the most relevant animal species is normally used for derivation of the ADI. The AOEL is derived from the most relevant (usually) semi-chronic or chronic study, depending on the expected exposure scenario of operator and worker. Where possible, the (sub)acute NOAEL is used for derivation of an ARfD and an AAOEL.

The toxicological endpoints with the corresponding NOAEL derived from the submitted studies then serve as basis for the risk evaluation for the operator, worker, bystander, and resident, and for consumers. The EU uses the so-called list of endpoints.

The EU framework gives no specific description how a study must be evaluated. An evaluation methodology for dermal absorption is available in the guidance on dermal absorption.

Classification (symbols, risk and safety phrases) of plant protection products is –among other factors- based on the intrinsic human toxicological properties of the active substance. The criteria used for classification and labelling of an active substance are described in Regulation (EC) No 1272/2008.

1.3.2. Derivation of the ADI

The reference value considered acceptable from a health point of view, such as the ADI (Acceptable Daily Intake) is derived from the available toxicological studies.
The toxicological profile (i.e., the toxicological endpoints) of a substance is derived after summarising the results obtained from animal experimental research. The summary lists the endpoints established for the active substance (LOAELs (NOAELs (No Observed Adverse Effect Levels)). The ADI is derived for chronic exposure.

Calculation of the ADI

Consumers may be exposed to residues of plant protection products via food, throughout their life. The corresponding reference value (ADI) must therefore represent the dose that can be ingested over a lifetime via food without adverse health effects. The JECFA (Joint FAO/WHO Expert Committee on Food Additives) has defined the ADI as follows: 'the estimated amount of active substance, expressed per kg body weight, that can be consumed daily over a lifetime without appreciable health risks'.
The ADI is usually derived from laboratory animal research in which the effect of prolonged exposure to the test substance has been studied. This concerns the chronic toxicity research.
The ADI is based on the most sensitive, or most critical effect.
'Effect' is defined as: an effect that is considered adverse.
Usually, data on several species are available (rat and mouse and in most cases also dog). The data of the most relevant animal species for the most critical effect form the basis for derivation of the ADI. The relevance of the observed effect for man is also important. This does not necessarily always have to be the lowest NOAEL found in the most sensitive test animal. The choice of the NOAEL as starting point depends on the total package of available toxicity studies and the mutual relationships in dose regimes. The most suitable NOAEL on which the ADI is based should be selected on a case-by-case basis, for which expert judgement is required.
In the most recent draft version of the Guidance for the setting of Acceptable Operator Exposure Levels (AOELs) (revision 10, July 2006) it is indicated that human volunteer studies should not be used for the derivation of reference values. Therefore, the reference values shall be based on animal studies. However, in Regulation (EU) No 284/2013 it is stated that where appropriate scientifically valid and ethically generated human data are available and show that humans are more sensitive and lead to lower regulatory limit values, these data shall take precedence over animal data. These data may originate from people exposed during production or application of plant protection products, or from volunteer studies performed under ethical criteria (Helsinki Convention 1971) [4].

A safety factor of 100 is usually applied for extrapolation of the NOAEL from laboratory

animal studies to the ADI. This factor is based on a factor of 10 for differences between animal species (interspecies) and a factor of 10 for variation within the population (intraspecies) in view of the heterogeneous nature of the general population (which may be the cause of large differences in sensitivity (YOPIGs = young, old, pregnant, ill and genetically susceptible people) [5]).

The following formula is used:

ADI = NOAEL / 100 (laboratory animal research)

If further data about the kinetics and mode of action of the substance in laboratory animals or humans are available, these data can justify the use of another safety factor.
If the information of the substance is insufficient, this may be a reason to apply an extra safety factor to compensate for the uncertainty. The value of this factor depends on the nature of the effects [6].
Furthermore, it can be decided to apply an additional safety factor if the margin between NOAEL and LOAEL is small and depending on the observed effects at the LOAEL.

1.3.3. Derivation of the AOEL and AEL

Operator exposure considered acceptable from a health point of view is usually referred to as AOEL (Acceptable Operator Exposure Level)[1].
There is a draft Guidance Document and this is currently used for derivation of an AOEL for a substance to be included in Commission Implementing Regulation (EU) 540/2011.
The AOEL is defined as the maximum amount of a substance to which the operator (including workers in treated crops or treated spaces) can be exposed at which no adverse effects on health are expected.
Where relevant, different AOELs can be established for acute, short-term (semi-chronic) or long-term (chronic) exposure. The AOEL is expressed in mg/kg bw/day.

An exposure considered acceptable from a health point of view is also calculated for the non-professional operator using plant protection products, for which the term AEL (Acceptable Exposure Level) is used. The derivation of the AOEL is presented below. The AEL is calculated accordingly.

Systemic AOEL/AEL

In principle, a systemic AOEL is derived. Systemic effects of active substances are caused by the amount of active substance actually absorbed into the body. In practice, exposure to these substances occurs mainly via the dermal and –to a lesser extent- via the respiratory route. For most active substances in plant protection products that are to be evaluated, however, only suitable studies with repeated exposure via the oral route are available. In practice, an AOEL is therefore usually derived on the basis of an oral study. The choice of the systemic AOEL used in the risk assessment should be justified in the decision making.

Choice of data for calculation of the systemic AOEL/AEL

The suitable studies with repeated exposure to the substance are selected from the toxicological dossier for calculation of the systemic AOEL. In addition, the kinetic data on the substance are used to establish the systemic availability (via the oral, dermal or inhalatory route) of the substance.

[1] Other abbreviations such as HBROEL (Health Based Recommended Occupational Exposure Limits) are also used for this reference value.

In the most recent draft version of the Guidance on AOEL setting (SANCO 7531 rev 10, July 2006) it is indicated that human volunteer studies should not be used for the derivation of reference values. Therefore, the reference values shall be based on animal studies. However, in Regulation (EU) No 283/2013 it is stated that where appropriate scientifically valid and ethically generated human data are available and show that humans are more sensitive and lead to lower regulatory limit values, these data shall take precedence over animal data. These data may originate from people exposed during production or application of plant protection products, or from volunteer studies performed under ethical criteria (Helsinki Convention 1971).

In principle it is assumed that the period during which exposure takes place is shorter than or equal to 3 months per year. This means that the AOEL calculation is preferably based on a short-term, i.e., semi-chronic toxicity study.
If exposure during a period longer than 3 months per year cannot be excluded based on the application scenario, a chronic toxicity study is preferred.

Besides duration and frequency of exposure, the choice of the most relevant study can also be determined by the excretion rate of the active substance and its metabolites, and by the rate at which the effects that may be caused by exposure to a substance are reversible.

The most relevant studies are selected from the dossier on the basis of these considerations. The selection must be justified in the decision making.

Selection of the most relevant studies for derivation of the AEL for non-professional uses is also based on the above-mentioned principles.

The study with the most relevant NOAEL, obtained with the most relevant test animal, is selected. This does not necessarily always have to be the lowest NOAEL found in the most sensitive test animal. The choice of the NOAEL as starting point depends on the total package of available toxicity studies and the mutual relationships in dose regimes. The most suitable NOAEL on which the AOEL is based should be selected on a case-by-case basis, for which expert judgement is required.

Local effects are not taken as starting point for derivation of a systemic AOEL.
Generally, the risk of local effects such as inhalatory effects, skin irritation, eye irritation, and skin sensitisation, are included in the risk management process by placing hazard symbols and risk and safety phrases on the label. Exposure can, e.g., be minimised by prescribing suitable personal protection equipment or other exposure-reducing measures.

Safety factor for calculation of the AOEL/AEL

A systemic AOEL is derived from the selected NOAEL by applying a safety factor.
In accordance with the ADI principle (see §1.3.2) the safety factor applied in the EU is 100. The basis for this approach is a factor of 10 for differences within the animal species (intraspecies differences) and a factor of 10 for differences between animal species (interspecies differences). This latter factor compensates for the wider variation in sensitivity in the population of exposed operators and workers in comparison with the relatively small (and relatively homogeneous) group of exposed laboratory animals.

Absorption after oral exposure

Determination of the level of the systemic AOEL after oral exposure requires insight into the extent to which a substance is absorbed by the body after oral administration.
The value for absorption after oral exposure to a relevant amount of substance is the sum of

the amounts of substance and metabolites that are subsequently excreted in the urine and that remain in tissues and carcass. If the absorbed dose is significantly lower (<80%) than the administered dose, this is adjusted by a correction factor equal to the percentage absorption. Because absorption may be dose-dependent, absorption data are required of a dose in the range of the NOAEL.

Research has shown that inclusion of bile excretion in the amount of absorbed substance may result in overestimation of the systemic availability of substances and their metabolites as result of a first pass effect [7].
In the first pass effect, a substance is in the liver totally or largely removed from the blood after absorption from the intestines, either before or after being metabolised, and is excreted via bile without getting into the total circulation. In case the critical effect does not occur in liver or gall bladder but more peripherally, there is a chance of overestimating the AOEL when the total fraction excreted in bile is considered as systemically available. If liver or gall bladder toxicity is the critical effect on the basis of which the AOEL is established, bile excretion studies are, however, useful for establishing the “organ availability” of the administered dose.
Biliary excretion is therefore no longer taken into account for determination of the systemic availability of a substance if the critical effect has not been found in liver or gall bladder (except when the data show that the biliary excretion occurs after a few hours (based on a case-by-case assessment), because then systemic availability can be assumed). In that case the sum of the amount of substance and metabolites that are excreted in the urine and that remain present in tissues and carcass are used as value for absorption after oral exposure. This means that the risk will be overestimated in some cases.
This can be prevented by a comparison of “Areas Under the Curve” after administration via the oral and intravenous routes which gives a much more reliable picture of the systemic availability.

Calculation of the systemic AOEL/AEL on the basis of oral studies
The following formula is used:

$AOEL_{systemic}$ (mg/kg bw/day) = ($NOAEL_{oral}$ x A): 100

A is the fraction of the substance absorbed by the body after oral administration (see above: Absorption after oral exposure. E.g. 60% oral absorption: A = 0.6).

Route-specific effects
If the results of toxicokinetic or mechanistic studies indicate a relevant first pass effect and/or fundamental differences in metabolism for different exposure routes (resulting in a route-specific effect on type or severity of an effect) selection of suitable route-specific studies as basis for the AOEL should be considered. This is performed on the basis of expert judgement.

1.3.4. Derivation of the ARfD
If a substance has acute toxic properties, an ARfD is derived from the available toxicological studies.

Calculation of the ARfD
A national guideline has been developed, in collaboration with RIVM, for derivation of the ARfD [8] and there is a draft Guidance Document of the European Commission (European Commission 7199/VI/99 rev, 5 d.d. 05/07/2001 (with the RIVM report as one of the supporting documents). It is briefly described below in which cases an ARfD must be

derived. The documents mentioned above also attempt to give a guideline on how the ARfD should be derived, which studies can be used as starting point, and which effects are relevant for acute exposure.

Some substances have specific acute toxic properties or may after a short-term (single) (high) exposure induce prolonged effects. In such a situation it is possible that exceeding the ADI for a short period of time entails a health risk. The ARfD is defined as “the amount of a substance in food or drinking water, expressed in mg per kg body weight per day, that can be ingested during a meal or a day, without appreciable health risk for the consumer, on the basis of all available knowledge at the time of evaluation”.

An ARfD is always derived unless the toxicological profile of the substance meets all following conditions:

- The substance induces no effects (including behaviour, clinical symptoms, or pathology) in an acute oral study at a dose level of 2000 mg/kg bw or higher.
- No embryonic, fetotoxic, or developmental effects were found at dose levels that are not maternally toxic.
- There are no indications or triggers from repeated dose studies which indicate toxic effects after acute exposure (e.g. acute neurological behaviour effects or effects on the gastrointestinal, cardiovascular or respiratory system).
- The substance shows no acute neurotoxicity or this is not expected on the basis of the available toxicological information.
- No other toxicological alerts such as hormonal or biochemical changes have been found in repeated dose studies which may also occur after a single dose.

As a general rule, the ARfD should be based on the most sensitive acute toxicological endpoint of human relevance, derived from the most suitable study in the most suitable (animal) species. Selection of the most relevant effect should be based on the complete, available toxicity research.

Knowledge about the mode of action of a substance may be very valuable when selecting the most relevant endpoint for acute exposure. The fact that the current database is not yet geared to the derivation of an ARfD makes it difficult to identify the correct endpoint and the most suitable study. Sound justification of the derivation of an ARfD is therefore important. Some relevant effects for which an ARfD can be derived are: certain clinical effects (tremors, mucus formation/salivation), acetyl cholinesterase inhibition, delayed neuropathy, neurotoxicity, methemoglobin formation, disturbance of oxygen transport or dissociation mitochondria, embryonic or foetotoxic effects, developmental effects, developmental neurotoxicity, direct effects on gastrointestinal tract, pharmacological effects.
When no ARfD is derived, this should also be justified in the evaluation.

In the most recent draft version of the Guidance on AOEL setting (revision 10, July 2006) it is indicated that human volunteer studies should not be used for the derivation of reference values. Therefore, the reference values shall be based on animal studies. However, in Regulation (EU) No 284/2013 it is stated that where appropriate scientifically valid and ethically generated human data are available and show that humans are more sensitive and lead to lower regulatory limit values, these data shall take precedence over animal data. These data may originate from people exposed during production or application of plant protection products, or from volunteer studies performed under ethical criteria (Helsinki Convention 1971).

A safety factor of 100 is usually applied for extrapolation of the NOAEL from laboratory

animal studies to the ARfD. This factor is based on a factor of 10 for differences between animal species (interspecies) and a factor of 10 for variation within the population (intraspecies) in view of the heterogeneous nature of the general population (which may be the cause of large differences in sensitivity (YOPIGs)).

The following formula is used:

ARfD = NOAEL / 100 (laboratory animal research)

If further data about the kinetics and mode of action of the substance in laboratory animals or humans are available, these data can justify the use of another safety factor.
If there is insufficient information on the substance, this may be a reason to apply an extra safety factor to compensate for the uncertainty. The value of this factor depends on the nature of the effects.

Correction of the safety factor for exposure duration is not applicable because the ARfD is preferably based on a study in which a short-term (single) exposure took place.

1.3.5. Derivation of the AAOEL

If a substance has acute toxic properties, an acute (AAOEL) is derived from the available toxicological studies.

The revised Guidance document on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (SANTE-10832-2015 rev. 1.7) provides more information on how an AAOEL should be derived.

1.3.6. Derivation of the dermal absorption value for the list of endpoints

Operators and workers may be dermally exposed during mixing/loading, application and re-entry activities. The expected external dermal exposure is calculated with models. For calculation of the systemic exposure it is important to know the extent to which the skin absorbs a substance and/or formulation after exposure to a relevant level. These dermal absorption data are used to convert the external exposure of operator and worker to systemic exposure and this is then in the risk assessment compared with the systemic AOEL.
Where no data are available, the percentage dermal absorption for human skin can be estimated. In case dermal absorption data are available, these are used for derivation of the dermal absorption percentage.
Both situations are elucidated below. Starting point is the 'EFSA Guidance on dermal absorption'.

No dermal absorption data available

If no suitable (animal) experimental data are available, the following default values can be used.

- A default dermal absorption value of 25% for the concentrate may be applied for products containing > 5% (50 g/kg for solids or 50 g/L for liquids) active substance.
- A default value of 75% should be used for products or in use dilutions containing ≤ 5% active substance.
- If log Pow < -1 or > 4 and MW > 500 a default dermal absorption value of 10% may be applied.

If oral absorption is less than 75% this can be used as a surrogate dermal absorption value

for diluted products/in-use dilutions. If oral absorption is < 25% it can be used instead of the default value for both the concentrated and the "in use" dilution products. There are usually no oral ADME studies for formulations that include co-formulants which are possibly modifying dermal absorption. For these reasons, estimates based on oral absorption should be applicable in only a limited range of circumstances after careful consideration of doses and vehicle used in the ADME studies, where bile cannulation was also performed.

Dermal absorption data available

In vitro and/or *in vivo* research is required if it is expected that the systemic AOEL will be exceeded when using default values for dermal absorption, and dermal exposure is an important exposure route.

In vitro research (with human and/or ratskin) and/or *in vivo* research (usually performed with the rat), performed at a relevant dose level, is used for derivation of the dermal absorption for man. For the interpretation of the experimental data and the subsequent derivation of dermal absorption, see the guidance.

The dermal absorption studies described above must be performed at dose levels that correspond with the exposure expected for operator and worker. The toxicological dossier may also contain dermal toxicity studies, such as, e.g., a 28-day study with dermal administration. Such studies are usually performed at dose levels that are (much) higher than the expected human exposure and they are not suitable for derivation of dermal absorption values for man.

1.4. Approval

The actual decision whether an active substance or a plant protection product can be approved or authorised follows from the risk assessment for operator, worker, bystander, resident and consumer, which is elaborated in Chapter 4 Human toxicology; risk operator, worker, bystander and resident, and Chapter 5 Residues, risk to consumer.

1.5. Developments

The requirements for the toxicological dossier are continuously changing in accordance with the developments in toxicology and risk assessment.

This may lead to new research questions, research questions becoming defunct or amendments of study guidelines that are already part of the toxicological dossier. Developments are expected in areas such as:

- Endocrine disruption
- Combination toxicology.

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