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

EU part

Plant Protection Products

Chapter 4 Human toxicology; mammalian toxicity dossier

version 2.0; January 2014

ctgb

Board
for the authorisation
of plant protection products and biocides

Chapter 4 Human toxicology; mammalian toxicity dossier

Category: Plant protection products

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].

Substances that are approved under Regulation (EC) No 1107/2009 and were approved under Directive 91/414/EEC [2] are included in Commission Implementing Regulation (EU) No 540/2011 [3].

The chapter describes the procedures following the data requirements as laid down in Commission Regulation (EU) No 283/2013 [4] for active substances and in Commission Regulation (EU) No 284/2013 [5] for plant protection products. These data requirements apply for active substances submitted after 31 December 2013 and for plant protection products submitted after 31 December 2015.

A concept guidance is available on the interpretation of the transitional measures for the data requirements for chemical active substances according to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 (SANCO/11509/2013 – rev. 0.1).

For further information on the former data requirement as laid down in Commission Regulation (EU) No 544/2011 for active substances and in Commission Regulation (EU) No 545/2011 we refer to the Evaluation Manual for Authorisation of plant protection products according to Regulation (EC) No 1107/2009 version 1.0

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; the NL procedure for evaluation of a substance is reverted to when no EU procedure has been laid down. The NL-procedure for the evaluation of a substance is described in §2 - §2.5 of part 2 of the Evaluation Manual (plant protection products). This document aims to give procedures for the approval of active substances and inclusion in Commission Implementing Regulation (EU) No 540/2011 [3].

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 (see Appendix 1).

1.2. Data requirements

In order to qualify for inclusion of an active substance in Commission Implementing Regulation (EU) No 540/2011 [3] a dossier that meets the provisions laid down in Commission Regulation (EU) No 283/2013 [4] and Commission Regulation (EU) No 284/2013 of Regulation (EC) No 1107/2009 [5], must be submitted for the active substance as well as for the product.

Generally, EU and OECD guidelines for the performance of toxicological studies are mentioned in Commission Communications 2013/C 95/01 [6].

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.

Generally, open literature does not meet EU/OECD guidelines and is therefore usually considered as supplementary information.

1.2.1. Data requirements for the active substance

The text below in grey frames has been taken from Commission Regulation (EU) No 283/2013. The numbering in these grey frames follows the section numbering in this Commission Regulation. Any necessary additions to the text have been added below the grey frames.

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.

5. Toxicological and metabolism studies

Introduction

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.

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
(283/2013: 5.1)

5.1 Studies on absorption, distribution, excretion and metabolism in mammals

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), shall 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.

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 as outlined in point 5.8.2;

(d) to compare the metabolism of rats with the metabolism in livestock as outlined in point 6.2.4.

5.1.1 *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 shall 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 requirements in all areas, since the exact requirements will depend upon the results obtained for each particular test substance.

The studies shall provide sufficient information about the kinetics of the active substance and its metabolites in relevant species after being exposed to the following:

(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 shall be provided.

The design of the kinetic studies required shall include:

(a) an evaluation of the rate and extent of oral absorption including maximum plasma concentration (C max), AUC, T max 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 shall 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 shall be given or further tests shall be carried out where a metabolite is detected *in vitro* in human material and not in the tested animal species.

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

Data on absorption, distribution, metabolism and excretion (ADME) following exposure by the dermal route shall be provided where toxicity following dermal exposure is of concern compared to that following oral exposure. Before investigating ADME *in vivo* following dermal exposure, an *in vitro* dermal penetration study shall be conducted to assess the likely magnitude and rate of dermal bioavailability.

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

Dermal absorption estimation from data generated in these studies on the active substance shall be critically assessed for relevance to humans. Dermal absorption measurement of the plant protection product is specifically considered under point 7.3 of Part A of the Annex to Regulation (EU) No 284/2013.

For volatile active substances (vapour pressure > 10 –2 Pascal) absorption, distribution, metabolism and excretion after exposure by inhalation may be useful in human risk assessments.

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 above provides insight into possible sex differences and accumulation as well.

Acute toxicity
(283/2013: 5.2)

5.2 Acute toxicity

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

(a) the toxicity of the active substance;
(b) the time course and characteristics of the effects with full details of behavioural changes, clinical signs, where evident, and possible gross pathological findings at post-mortem;
(c) the possible need to consider establishing acute reference doses (such as ARfD, aAOEL)
(d) where possible mode of toxic action;
(e) the relative hazard associated with the different routes of exposure.

While the emphasis must be on estimating the toxicity ranges involved, the information generated shall also permit the active substance to be classified in accordance with Regulation (EC) No 1272/2008. The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

See for Regulation (EC) No 1272/2008 [7] the following website: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:en:PDF

Oral
(283/2013: 5.2.1)

5.2.1 Oral

Circumstances in which required
The acute oral toxicity of the active substance must always be reported.

Percutaneous
(544/2011: 5.2.2)

5.2.2 Percutaneous

Circumstances in which required
The acute dermal toxicity of the active substance shall be reported unless waiving is scientifically justified (for example where oral LD 50 is greater than 2 000 mg/kg). Both local and systemic effects shall be investigated.

Findings of severe skin irritation (Grade 4 erythema or oedema) in the dermal study shall be used instead of performing a specific irritation study.

Inhalation
(283/2013: 5.2.3)

5.2.3 Inhalation

Circumstances in which required
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
(283/2013: 5.2.4)

5.2.4 Skin irritation

The results of the study shall provide information on the potential for skin irritancy of the active substance including, where relevant, the potential reversibility of the effects observed.

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.

Circumstances in which required
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
(283/2013: 5.2.5)

5.2.5 Eye irritation

Aim of test
The results of the study shall provide the potential of eye irritancy of the active substance including, where relevant, the potential reversibility of the effects observed.

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.

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

Skin sensitisation
(283/2013: 5.2.6)

5.2.6 Skin sensitization

The study shall provide sufficient information to assess the potential of the active substance to provoke skin sensitization reactions.

Circumstances in which required
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
(283/2013: 5.2.7)

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.

Circumstances in which required
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.

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
(283/2013: 5.3)

5.3 Short-term toxicity

Short-term toxicity studies shall be designed to provide information as to the amount of the active substance that can be tolerated without toxic 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 plant protection products containing the active substance, among other possible exposure groups. In particular, short-term studies provide an essential insight into possible repeated actions of the active substance and the risks to humans who may be exposed. In addition short-term studies provide information useful in the design of chronic toxicity studies.

The studies, data and information to be provided and evaluated, shall 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 toxic 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) 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
(283/2013: 5.3.1)

5.3.1 Oral 28-day study

Circumstances in which required
Where available, 28-day studies shall be reported.

Oral 90-day study
(283/2013: 5.3.2)

5.3.2 Oral 90-day study

Circumstances in which required
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
(283/2013: 5.3.3)

5.3.3 Other routes

Circumstances in which required
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
(283/2013: 5.4)

5.4 Genotoxicity testing

The aim of genotoxicity testing shall be to:
- predictgenotoxic potential
- identify genotoxic carcinogens at an early stage
- elucidate the mechanism of action of some carcinogens

Appropriate dose levels, depending on the test requirements, shall be used in either *in vitro* or *in vivo* assays. 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.

In vitro studies
(283/2013: 5.4.1)

5.4.1 *In vitro* studies

Circumstances in which required
The following *in vitro* mutagenicity tests shall be performed: bacterial assay for gene mutation, combined test for structural and numerical chromosome aberrations in mammalian cells and test for gene mutation in mammalian cells.

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
(283/2013: 5.4.2)

5.4.2 *In vivo* studies in somatic cells

Circumstances in which required
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.

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
(283/2013: 5.4.3)

5.4.3 *In vivo* studies in germ cells

Circumstances in which required
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
(283/2013: 5.5)

5.5 Long term toxicity and carcinogenicity

The results of the long-term studies conducted and reported, taken together with other relevant data and information on the active substance, shall be sufficient to permit the identification of effects, following repeated exposure to the active substance, and in particular shall 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,
- 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, shall be sufficient to permit the evaluation of hazards for humans, following repeated exposure to the active substance, and in particular must be sufficient:
- to identify carcinogenic effects resulting from long-term exposure to the active substance,
- to establish the species, sex, and organ specificity of tumours induced,
- to establish the dose-response relationship;
- where possible, to identify the maximum dose eliciting no carcinogenic effect.
- where possible, to determine the mode of action and human relevance of any identified carcinogenic response.

Circumstances in which required
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

Test conditions
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
(283/2013: 5.6)

5.6 Reproductive toxicity

Possible effects on reproductive physiology and the development of progeny shall 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.
- Harmful effects on the progeny, for example any effect interfering with normal development, both before and after birth. This includes morphological malformations such as anogenital distance, nipple retention, and functional disturbances (such as reproductive and neurological effects).

Effects accentuated over generations shall be reported.

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
(283/2013: 5.6.1)

5.6.1 Generational studies

The generational studies reported, taken together with other relevant data and information on the active substance, shall be sufficient to permit the identification of effects for reproduction, following repeated exposure to the active substance, and in particular must be sufficient:
(a) to identify direct and indirect effects on reproduction resulting from exposure to the active substance,
(b) to identify any non-reproductive adverse effects occurring at lower doses than in short-term and chronic toxicity testing;
(c) to establish the NOAELs for parental toxicity, reproductive outcome and pup development.

Circumstances in which required
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
(283/2013: 5.6.2)

5.6.2 Developmental toxicity studies

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

Circumstances in which required
Developmental toxicity studies shall always be carried out.

Test conditions
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
(283/2013: 5.7)

Neurotoxicity studies in rodents
(283/2013: 5.7.1)

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

Circumstances in which required
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
(283/2013: 5.7.1)

5.7.2. *Delayed polyneuropathy studies*
Delayed polyneuropathy studies shall 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.

Circumstances in which required
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
(283/2013: 5.8)

Toxicity studies of metabolites
(283/2013: 5.8.1)

5.8.1 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 have not been elaborated in the EU framework.
In the EU framework this has only been elaborated for metabolites that leach to groundwater (Chapter 6 Behaviour and fate in the environment, behaviour in soil: leaching).

Supplementary studies on the active substance
(283/2013: 5.8.2)

5.8.2 Supplementary studies on the active substance

(a) 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;
(b) studies on the immunotoxicological potential,
(c) a targeted single dose study to derive appropriate acute reference values (ARfD, aAOEL);
(d) studies on other routes of administration;
(e) studies on the carcinogenic potential;
(f) 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
(283/2013: 5.8.3)

5.8.3. *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.

Medical data
(283/2013: 5.9)

5.9 Medical data

Where available, and without prejudice to the provisions of Article 10 of Council Directive

98/24/EC, practical data and information relevant to the recognition of the symptoms of poisoning and on the effectiveness of first aid and therapeutic measures shall be submitted. Such data and information shall include reports of any studies investigating antidote pharmacology or safety pharmacology. Where relevant, the effectiveness of potential antagonists to poisoning shall be investigated and reported.

Data and information relevant to the effects of human exposure, where available shall be used to confirm the validity of extrapolations made and conclusions reached with respect to target organs, dose-response relationships, and the reversibility of adverse effects. Such data may be generated following accidental, occupational exposure or incidents of intentional self-poisoning, and shall be reported if available.

Medicinal surveillance on manufacturing plant personnel and monitoring studies
(283/2013: 5.9.1)

5.9.1 Medicinal surveillance on manufacturing plant personnel and monitoring studies

Reports of occupational health surveillance programmes and of monitoring studies shall be submitted, supported with detailed information on the design of the programme, the number of exposed persons included in the programme, the nature of their exposure to the active substance, and their exposure to other potentially hazardous agents. Such reports shall, where feasible, include data relevant to the mechanism of action of the active substance. These reports shall, where available, include data from persons exposed in manufacturing plants or during or after application of the active substance (for example from monitoring studies in operators, workers, residents, bystanders or victims of accidents). Available information on adverse health effects, including allergenic responses in workers and others exposed to the active substance, shall be provided, and include where relevant details of any incident. The information provided shall, where available, include details of frequency, level and duration of exposure, symptoms observed and other relevant clinical information.

Data collected on humans
*(*283/2013*: 5.9.2)*

5.9.2 Data collected on humans

Where available, reports from studies with humans, such as tests on toxicokinetics and metabolism, or tests on skin irritation or skin sensitisation, shall be submitted.

In general, the reference values shall be based on animal studies, but if 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.

Direct observations
(283/2013: 5.9.3)

5.9.3 Direct observations

Available reports from the open literature, relating to clinical cases and poisoning incidents, where they are from refereed journals or official reports, shall be submitted together with reports of any follow-up studies undertaken. Such reports shall, where

available, contain complete descriptions of the nature, level and duration of exposure, as well as the clinical symptoms observed, first aid and therapeutic measures applied and measurements and observations made.

Epidemiological studies
(283/2013: 5.9.4)

5.9.4 Epidemiological studies

Relevant epidemiological studies shall be submitted, where available.

Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests
(283/2013: 5.9.5)

5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Where available, detailed description of the clinical signs and symptoms of poisoning, including the early signs and symptoms and full details of clinical tests useful for diagnostic purposes shall be provided and include full details of the time courses involved relevant to the ingestion, dermal exposure or inhalation of varying amounts of the active substance.

Proposed treatment: first aid measures, antidotes, medical treatment
(283/2013: 5.9.6)

5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

First aid measures to be used in the event of poisoning (actual and suspected) and in the event of contamination of eyes shall be provided. Therapeutic regimes for use in the event of poisoning or contamination of eyes, including where available the use of antidotes, shall be described in full. Information based on practical experience, where it exists and is available, in other cases on theoretical grounds, as to the effectiveness of alternative treatment regimes, where relevant, shall be provided. Contraindications associated with particular regimes, particularly those relating to 'general medical problems' and conditions, must be described.

Expected effects of poisoning
(283/2013: 5.9.7)

5.9.7 Expected effects of poisoning

Where known, the expected effects and the duration of these effects following poisoning shall be described. That description shall include the impact of:
- the type, level and duration of exposure, or ingestion, and
- varying time periods between exposure, or ingestion, and commencement of treatment.

1.2.2. Data requirements for the product

The text below in grey frames has been taken from Commission Regulation (EU) No 284/2013. The numbering in these grey frames follows the section numbering in this

Commission Regulation. Any necessary additions to the text have been added below the grey frames.

The data requirements regarding the mammalian toxicity dossier of the plant protection product are described in this Commission Regulation, point 7.

Generally, EU and OECD guidelines for the performance of toxicological studies are mentioned in Commission Communications 2013/C 95/02 [8].

7. Toxicological Studies

1. For the evaluation of the toxicity of the plant protection product information shall be provided on acute toxicity, irritation and sensitization of the active substance. The relevant calculation methods used for the classification of mixtures as laid down in Regulation (EC) No 1272/2008 shall, where appropriate, be applied in the hazard assessment of the plant protection product. Where available, information on mode of toxic action, toxicological profile and all other known toxicological aspects of the active substance and of substances of concern, shall be submitted.

2. Consideration shall be given to the possible effects of components on the toxic potential of the total mixture.

Acute toxicity
(284/2013: 7.1)

7.1 Acute toxicity

The studies, data and information to be provided and evaluated, shall be sufficient to permit the identification of effects following a single exposure to the plant protection product, to be assessed, and in particular to establish, or indicate:
(a) the toxicity of the plant protection product,
(b) toxicity of the plant protection product relative to the active substance,
(c) the time course and characteristics of the effect with full details of behavioural changes and possible gross pathological findings at post-mortem,
(d) where possible the mode of toxic action, and
(e) the relative hazard associated with the different routes of exposure.

While the emphasis shall be on estimating the toxicity ranges involved, the information generated shall also permit the plant protection product to be classified in accordance with Regulation (EC) No 1272/2008, where applicable.

See for Regulation (EC) No 1272/2008 [7] the following website: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:en:PDF

If, according to the applicant, information with regard to acute oral, dermal and inhalatory toxicity, and skin and eye irritation, and sensitisation of the formulation obtained by calculation is sufficient, the applicant should submit a toxicologically based justification as indicated in Regulation (EC) No. 1272/2008.

Oral toxicity
(284/2013: 7.1.1)

7.1.1 Oral toxicity

Circumstances in which required
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
(284/2013: 7.1.2)

7.1.2 Dermal toxicity

Circumstances in which required
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
(284/2013: 7.1.3)

7.1.3 Inhalation toxicity

Aim of the test
The study shall provide the inhalation toxicity to rats of the plant protection product or of the smoke it generates.

Circumstances in which required
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
(284/2013: 7.1.4)

7.1.4. Skin irritation

The results of the study shall provide the potential for skin irritancy of the plant protection product including the potential reversibility of the effects observed.

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.

Circumstances in which required
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 (see for further information method B4 of Regulation (EC) No 440/2008 [9]) if the extent of irritation or corrosivity cannot be established on the basis of an analysis of existing relevant data.

Eye irritation
(284/2013: 7.1.5)

7.1.5 Eye irritation

The results of the study shall provide the potential for eye irritation of the plant protection product, including the potential reversibility of the effects observed.

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.

Circumstances in which required
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.

An *in vivo* test may only be performed (see for further information method B5 of Regulation (EC) No 440/2008 [9]) if the extent of irritation or corrosivity cannot be established on the basis of an analysis of existing relevant data.

Skin sensitisation
(284/2013: 7.1.6)

7.1.6 Skin sensitisation

The study shall provide information to assess the potential of the plant protection product to provoke skin sensitisation reactions.

Circumstances in which required
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.

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
(284/2013: 7.1.7)

7.1.7 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
(284/2013: 7.1.8)

7.1.8 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
(284/2013: 7.2)

7.2 Data on exposure

For the purpose of this Regulation the following definitions apply:
(a) operators are people who are involved in activities relating to the application of a plant protection product, such as mixing, loading, application, or relating to cleaning and maintenance of equipment containing a plant protection product; operators may be professionals or amateurs;
(b) workers are people who, as part of their employment, enter an area that has previously been treated with a plant protection product or who handle a crop that has been treated with a plant protection product;
(c) bystanders are people who casually are located within or directly adjacent to an area where application of a plant protection product is in process or has taken place, but not for the purpose of working on the treated area or with the treated commodity;
(d) residents are people who live, work or attend any institution near to areas that are treated with plant protection products, but not for the purpose of working on the treated area or with the treated commodity.

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 shall be taken into account and reported in the dossier.

Operator exposure
(284/2013: 7.2.1)

7.2.1 Operator exposure

Information shall be provided to permit an assessment of the extent of exposure to the active substances and toxicologically relevant compounds in the plant protection product likely to occur under the proposed conditions of use. It shall also provide a basis for the selection of the appropriate protective measures including personal protective equipment to be used by operators and to be specified on the label.

Estimation of operator exposure
(284/2013: 7.2.1.1)

7.2.1.1 Estimation of operator exposure

An estimation shall be made, using where available a suitable calculation model, in order to permit an evaluation of the operator exposure likely to arise under the proposed conditions of use. 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.

Circumstances in which required
An estimation of operator exposure shall always be completed.

Estimation conditions
An estimation shall be made for each type of application method and application equipment proposed for use of the plant protection product taking account of the requirements resulting from Regulation (EC) No 1272/2008, where applicable, for handling the undiluted product. The estimation shall address mixing/loading and application, and shall include clean-up activities and routine maintenance of the application equipment. Specific information on local use conditions (types and sizes of containers to be used, application equipment, typical work rates and application rates, spray concentration, field sizes, crop growing climatic conditions) shall be included.
At first an estimation shall be made with the assumption that the operator is not using any personal protective equipment.

Where appropriate, a further estimation shall be made with the assumption that the operator is using effective and readily obtainable protective equipment, which is feasible to be used in practice. Where protective measures are specified on the label, the estimation will take these into account.

Measurement of operator exposure
(284/2013: 7.2.1.2)

7.2.1.2 Measurement of operator exposure

The study shall provide data to permit an evaluation of the operator exposure likely to arise under the proposed conditions of use. The study shall be ethically sound.

Circumstances in which required
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.

This willbe the case, where the results of the estimation of operator exposure in accordance with point 7.2.1.1 indicate that one or both of the following conditions are fulfilled:
- the AOEL established in the context of approval of the active substancemay be exceeded;
- the Limit Values established for the active substance and toxicologically relevant compounds of the plant protection product in accordance with Directives 98/24/EC and 2004/37/EC may be 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 [10].

Bystander and resident exposure
(284/2013: 7.2.2)

7.2.2 Bystander and resident exposure

Information shall be provided to permit an assessment of the extent of exposure to the active substances and toxicologically relevant compounds likely to occur under the proposed conditions of use, taking into account, where relevant, cumulative and synergistic effects. It shall also provide a basis for the selection of appropriate protective measures, including restricted entry intervals, exclusion of residents and bystanders from treatment areas and separation distances.

Estimation of bystander and resident exposure
(284/2013: 7.2.2.1)

7.2.2.1 Estimation of bystander and resident exposure

An estimation shall be made, using where available a suitable calculation model in order to permit evaluation of the bystander and resident exposure likely to arise under the proposed conditions of use. 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.

The applicant shall take into consideration that bystanders can be exposed during or after the application of plant protection products and residents may be exposed to plant protection products, mainly, but not only, by inhalation and dermal route and that infants and toddlers exposure may also occur by the oral route (through hand-mouth transfer).

Circumstances in which required

An estimation of bystander and resident exposure shall always be performed.

Estimation conditions
An estimation of bystander and resident exposure shall be made for each relevant type of application method. Specific information including maximum total dose and spray concentration shall be included. The estimation shall be made with the assumption that bystanders and residents do not use any personal protective equipment.

Measurement of bystander and resident exposure
(284/2013: 7.2.2.2)

7.2.2.2 Measurement of bystander and resident exposure

The study shall provide data to permit an evaluation of the bystander and resident exposure likely to arise under the specific proposed conditions of use. The study shall be ethically sound.

Circumstances in which required
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
(284/2013: 7.2.3)

7.2.3 Worker exposure
Information shall be provided to permit an assessment of the extent of exposure to the active substances and toxicologically relevant compounds in the plant protection product likely to occur under the proposed conditions of use and agricultural practices, taking into account cumulative and synergistic effects. It shall also provide a basis for the selection of appropriate protective measures, including waiting and re-entry periods.

Estimation of worker exposure
(284/2013: 7.2.3.1)

7.2.3.1 Estimation of worker exposure

An estimation shall be made using, where available, a suitable calculation model, in order to permit an evaluation of the worker exposure likely to arise under the proposed conditions of use. 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.

Circumstances in which required
The estimation of worker exposure shall be completed when such exposure could arise under the proposed conditions of use.

Estimation conditions

An estimation of worker exposure shall be made for crop and task to be carried out. Specific information including description of post-applications activities, exposure duration, application rate, number of applications, minimum spray interval and growth stage, shall be provided. If data on the amount of dislodgeable residues under the proposed conditions of use are not available, default assumptions shall be used.

At first the estimation shall be made using available data on the exposure to be expected with the assumption that the worker is not using any personal protective equipment. Where appropriate, a second estimation shall be made with the assumption that the worker is using effective and readily obtainable protective equipment which is feasible to be used and will be worn habitually by workers, for example because it was necessitated by other aspects of task being undertaken.

Measurement of worker exposure
(284/2013: 7.2.3.2)

7.2.3.2 Measurement of worker exposure

The study shall provide data to permit an evaluation of the worker exposure likely to arise under the proposed conditions of use. The study shall be ethically sound.

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

This willbe the case, where the results of the estimation of worker exposure in accordance with point 7.2.3.1 indicate that one or both of the following conditions are fulfilled:
(a) the AOEL established in the context of approval of the active substancemay be exceeded;
(b) the Limit Values established for the active substance and toxicologically relevant compounds of the plant protection product in accordance with Directives 98/24/EC and 2004/37/EC may be exceeded.

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

Dermal absorption
(545/2011: 7.3)

7.3 Dermal absorption

The studies shall provide a measurement of the absorption through the skin of the active substance and toxicologically relevant compounds in the plant protection product to be authorised.

Circumstances in which required
The study shall be conducted when dermal exposure is a significant exposure route, and no acceptable risk is estimated using default absorption value.

Test conditions
Data from absorption studies, preferably using human skin *in vitro*, shall be reported.

Studies shall be performed on representative plant protection products at both 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' [11] has been adopted.

Available toxicological data relating to non-active substances
(284/2013: 7.4)

7.4 Available toxicological data relating to non-active substances

Where relevant, the applicant shall submit the following information:
(a) the registration number in accordance with Article 20(3) of Regulation (EC) No 1907/2006;
(b) the study summaries included in the technical dossier submitted in accordance with Article 10(a)(vi) of Regulation (EC) No 1907/2006; and
(c) the safety data sheet referred to in Article 31 of Regulation (EC) No 1907/2006.
The safety data sheet under point (c) shall also be submitted and assessed for the plant protection product.

All other available information shall be submitted.

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 (see Appendix 1).
The toxicological endpoints that are derived from the submitted studies then form the basis for derivation of various reference values (ADI, AOEL 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
For each study, if possible, the NOAEL is derived. The NOAEL is the highest dose at which the most relevant critical effect (the adverse health effect that occurs first) is not yet observed.
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.

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 (see Appendix 1).

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 [11].
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 [7].

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 on AOEL setting (revision 10, July 2006) [12] 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) [13].

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) [14]).

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 [15].
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 [12] 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.
In the most recent draft version of the Guidance on AOEL setting (revision 10, July 2006) [12] 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

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

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) [13].

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 [12].

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 [16].
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 [17] and there is a draft Guidance Document of the European Commission [18] (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) [12] 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) [13].

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) [14]).

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 [15].

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 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 ‘Guidance on dermal absorption’ [11].

No dermal absorption data available
If no suitable (animal) experimental data are available, the following default values can be used [11].

- 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.
[11].

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 [11].

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:
- Immunotoxicity research
- Sensitive groups and residents.
- The evaluation framework for local effects must still be elaborated.
- Endocrine disruption
- Combination toxicology.

2. Appendices

Appendix 1 List of endpoints toxicology and metabolism

List of Endpoints toxicology and metabolism

Absorption, distribution, excretion and metabolism (toxicokinetics) (284/2013, point 5.1)

Rate and extent of oral absorption ‡	
Distribution ‡	
Potential for accumulation ‡	
Rate and extent of excretion ‡	
Metabolism in animals ‡	
Toxicologically relevant compounds ‡ (animals and plants)	
Toxicologically relevant compounds ‡ (environment)	

Acute toxicity (284/2013, point 5.2)

Rat LD_{50} oral ‡		
Rat LD_{50} dermal ‡		
Rat LC_{50} inhalation ‡		
Skin irritation ‡		
Eye irritation ‡		
Skin sensitisation ‡		

Short term toxicity (284/2013, point 5.3)

Target / critical effect ‡		
Relevant oral NOAEL ‡		
Relevant dermal NOAEL ‡		
Relevant inhalation NOAEL ‡		

Genotoxicity ‡ (284/2013, point 5.4)

Long term toxicity and carcinogenicity (284/2013, point 5.5)

Target/critical effect ‡		
Relevant NOAEL ‡		
Carcinogenicity ‡		

Reproductive toxicity (284/2013, point 5.6)

Reproduction toxicity

Reproduction target / critical effect ‡		
Relevant parental NOAEL ‡		
Relevant reproductive NOAEL ‡		
Relevant offspring NOAEL ‡		

Developmental toxicity

Developmental target / critical effect ‡		
Relevant maternal NOAEL ‡		
Relevant developmental NOAEL ‡		

Neurotoxicity (284/2013, point 5.7)

Acute neurotoxicity ‡		
Repeated neurotoxicity ‡		
Delayed neurotoxicity ‡		

Other toxicological studies (283/2013, point 5.8)

Mechanism studies ‡	
Studies performed on metabolites or impurities ‡	

Medical data ‡ (283/2013, point 5.9)

Summary	Value	Study	Safety factor
ADI ‡			
AOEL ‡			
ARfD ‡			

Dermal absorption ‡ (284/2013, point 7.3)

Formulation (e.g. name 50 % EC)	

Exposure scenarios (284/2013, point 7.2)

Operator	
Workers	
Bystanders	

Classification and proposed labelling with regard to toxicological data

	RMS/peer review proposal
Substance classified (name)	

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