ANNEX

PART A

CHEMICAL ACTIVE SUBSTANCES

SECTION 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.

5.1. Studies on absorption, distribution, metabolism and excretion 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.

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[(1)]);

(d) where possible mode of toxic action;

(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 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.

5.2.1. *Oral*
Circumstances in which required

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

5.2.2. *Dermal*
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}[(2)] 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.

5.2.3. *Inhalation*
Circumstances in which required

The acute 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 powder containing a significant proportion of particles of a diameter < 50 μm (> 1 % on 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.

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 study 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 level of 2 000 mg/kg body weight shall be used to waive the need for any dermal irritation studies.

5.2.5. *Eye irritation*

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 likely that severe effects on the eyes may be produced based on criteria listed in the test methods.

5.2.6. *Skin sensitisation*

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

5.2.7. *Phototoxicity*

The study shall provide information on the potential of certain active substances to induce cytotoxicity in combination with light, for example active substances that are phototoxic *in vivo* after systemic exposure and distribution to the skin, as well as active substances that act as photoirritants after dermal application. A positive result shall be taken into account when considering potential human exposure.

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 $L \times mol^{-1} \times cm^{-1}$, no toxicity testing is required.

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 adverse effects under the conditions of the study and to elucidate health hazards occurring at higher dose levels. Such studies provide useful data on the risks for those handling and using plant protection products containing the active substance, among other possible exposed 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 adverse effects observed, following discontinuation of dosing;

(g) where possible, the mode of toxic action;

(h) the relative hazard associated with the different routes of exposure;

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

Toxicokinetic data (that is to say blood concentration) 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).

5.3.1. *Oral 28-day study*
Circumstances in which required

Where available, 28-day studies shall be reported.

5.3.2. *Oral 90-day study*
Circumstances in which required

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

5.3.3. *Other routes*
Circumstances in which required

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

For volatile active substances (vapour pressure $>10^{-2}$ Pascal) expert judgement (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.

5.4. **Genotoxicity testing**

The aim of genotoxicity testing shall be to:
— predict genotoxic 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 $L \times mol^{-1} \times cm^{-1}$, photomutagenicity testing is not required.

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.

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.

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.

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 shall be sufficient:

(a) to identify carcinogenic effects resulting from long-term exposure to the active substance;

(b) to establish the species, sex, and organ specificity of tumours induced;

(c) to establish the dose-response relationship;

(d) where possible, to identify the maximum dose eliciting no carcinogenic effect;

(e) where possible, to determine the mode of action and human relevance of any identified carcinogenic response.

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 shall 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 rat as test species; where possible these studies shall 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 shall include:

(a) identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location;

(b) name of the laboratory and the dates when the study was performed;

(c) description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed;

(d) approximate age, in days, and weight of the control animals at the beginning of the study and at the time of killing or death;

(e) description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (such as diseases, infections);

(f) name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study;

(g) 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.

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 shall be from contemporaneous studies.

The information on historical control data provided shall include:

(a) identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location;

(b) name of the laboratory and the dates when the study was performed;

(c) description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed;

(d) approximate age, in days, and weight of the control animals at the beginning of the study and at the time of killing or death;

(e) description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (such as diseases, infections);

(f) name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study.

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.

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 shall 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.

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 the assessment of effects on embryonic and foetal development, following repeated exposure to the active substance, and in particular shall be sufficient:

(a) to identify direct and indirect effects on embryonic and foetal development resulting from exposure to the active substance;

(b) to identify any maternal toxicity;

(c) to establish the relationship between observed responses and dose in both dam and offspring;

(d) to establish NOAELs for maternal toxicity and pup development;

(e) to provide additional information on adverse effects in pregnant as compared with non-pregnant females;

(f) to provide additional information on any enhancement of general toxic effects of pregnant animals.

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.

5.7. **Neurotoxicity studies**

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.

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.

5.8. **Other toxicological studies**

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.

5.8.2. *Supplementary studies on the active substance*

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

(a) studies on absorption, distribution, excretion and metabolism, in a second species;

(b) studies on the immunotoxicological potential;

(c) a targeted single dose study to derive appropriate acute reference values (ARfD, 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.

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.

5.9. **Medical data**

Where available and without prejudice to Article 10 of Council Directive 98/24/EC[(3)], 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.

5.9.1. *Medical surveillance on manufacturing plant personnel and monitoring studies*

Reports of occupational health surveillance programs 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.

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.

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.

Where supported with the necessary level of detail, such documentation shall be used to confirm the validity of extrapolations from animal data to man and to identify unexpected adverse effects which are specific to humans.

5.9.4. *Epidemiological studies*

Relevant epidemiological studies shall be submitted, where available.

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 including full details of the time courses involved relevant to the ingestion, dermal exposure or inhalation of varying amounts of the active substance.

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, shall be described.

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**) aAOEL, abbreviation for 'Acute AOEL'.

(**2**) LD_{50}, abbreviation for 'Lethal Dose, 50 %', that is to say the dose required to kill half the members of a tested population after a specified test duration.

(**3**) OJ L 131, 5.5.1998, p. 11.

Status:
Point in time view as at 31/01/2020.

Changes to legislation:
There are outstanding changes not yet made to Commission Regulation (EU) No 283/2013. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.