ANNEX

DATA REQUIREMENTS FOR ACTIVE SUBSTANCES, AS PROVIDED FOR IN ARTICLE 8(1)(b) OF REGULATION (EC) No 1107/2009

INTRODUCTION

1. The information required shall:

1.1. include a technical dossier supplying the information necessary for evaluating the foreseeable risks, whether immediate or delayed, which the active substance may entail for humans, animals and the environment and containing at least the information and results of the studies referred to below;

1.2. where relevant, be generated using test guidelines, in accordance with the latest adopted version, referred to or described in this Annex; in the case of studies initiated before the entry into force of the modification of this Annex, the information shall be generated using suitable internationally or nationally validated test guidelines or, in the absence thereof, test guidelines accepted by the competent authority;

1.3. in the event of a test guideline being inappropriate or not described, or where another one than those referred to in this Annex has been used, include a justification, which is acceptable to the competent authority for the guidelines used. In particular, when reference is made in this Annex to a method laid down in Commission Regulation (EC) No 440/2008[(1)] which consists in the transposition of a method developed by an international organisation (e.g. OECD), Member States may accept that the required information is generated in accordance with the latest version of that method if at the initiation of the studies the method under Regulation (EC) No 440/2008 has not yet been updated;

1.4. include, when required by the competent authority, a full description of test guidelines used, except if they are referred to or described in this Annex, and a full description of any deviations from them including a justification, which is acceptable to the competent authority, for these deviations;

1.5. include a full and unbiased report of the studies conducted as well as full description of them or a justification, which is acceptable to the competent authority where:
- particular data and information which would not be necessary owing to the nature of the product or its proposed uses, are not provided, or
- it is not scientifically necessary, or technically possible to supply information and data;

1.6. where relevant, have been generated in accordance with the requirements of Council Directive 86/609/EEC[(2)].

2. **Test and analyses**

2.1. Tests and analyses must be conducted in accordance with the principles laid down in Directive 2004/10/EC of the European Parliament and of the Council[(3)] where testing is done to obtain data on the properties and/or safety with respect to human or animal health or the environment.

2.2. By way of derogation from point 2.1, Member States may provide that tests and analyses, performed on their territory in order to obtain data on the properties and/or safety of the active substances with respect to honey-bees and beneficial arthropods other than bees shall be conducted by official or officially-recognised testing facilities

or organisations which satisfy at least the requirements as set out under points 2.2 and 2.3 of the introduction to the Annex to Commission Regulation (EU) No 545/2011[(4)].

This derogation applies to trials which actually started on or before 31 December 1999.

2.3. By way of derogation from point 2.1, Member States may provide that supervised residue trials performed on their territory in accordance with Section 6 'Residues in or on treated products, food and feed' on plant protection products containing active substances already on the market 2 years after notification of the Directive 91/414/EEC shall be conducted by official or officially-recognised testing facilities or organisations which satisfy at least the requirements under points 2.2 and 2.3 of the introduction to the Annex to Regulation (EU) No 545/2011.

This derogation applies for supervised residue trials which actually started on or before 31 December 1997.

2.4. By way of derogation from point 2.1, for active substances consisting of micro-organisms or viruses, tests and analyses done to obtain data on the properties and/or safety with respect to other aspects than human health, may have been conducted by official or officially recognised testing facilities or organisations which satisfy at least the requirements under points 2.2 and 2.3 of the introduction to the Annex to Regulation (EU) No 545/2011.

PART A

CHEMICAL SUBSTANCES

1. Identity of the active substance

The information provided must be sufficient to identify with precision each active substance, to define it in terms of its specification and to characterise it as to its nature. The information and data referred to, unless otherwise specified, are required for all active substances.

1.1. *Applicant (name, address, etc.)*

The name and address of the applicant must be provided as must the name, position, telephone and telefax number of the appropriate person to contact.

Where, in addition, the applicant has an office, agent or representative in the Member State to which the application for approval is submitted, and if different, in the rapporteur Member State appointed by the Commission, the name and address of the local office, agent or representative must be provided, as must the name, position, telephone and telefax number of the appropriate person to contact.

1.2. *Manufacturer (name, address, including location of plant)*

The name and address of the manufacturer or manufacturers of the active substance must be provided as must the name and address of each manufacturing plant in which the active substance is manufactured. A contact point (preferably a central contact point, to include name, telephone and telefax number) must be provided, with a view to providing updating information and responding to queries arising, regarding manufacturing technology, processes and the quality of product (including where relevant, individual batches). Where following approval of the active substances, there are changes in the location or number of manufacturers, the information required must again be notified to the Commission and the Member States.

1.3. *Common name proposed or ISO-accepted, and synonyms*

The ISO common name, or proposed ISO common name and where relevant, other proposed or accepted common names (synonyms), including the name (title) of the nomenclature authority concerned, must be provided.

1.4. *Chemical name (IUPAC and CA nomenclature)*

The chemical name as given in Annex VI to Regulation (EC) No 1272/2008 of the European Parliament and of the Council[5], or, if not included in that Regulation, in accordance with both IUPAC and CA nomenclature, must be provided.

1.5. *Manufacturer's development code number(s)*

Code numbers used to identify the active substance, and where available, formulations containing the active substance, during development work, must be reported. For each code number reported, the material to which it relates, the period for which it was used, and the Member States or other countries in which it was used and is being used, must be stated.

1.6. *CAS, EC and CIPAC numbers (if available)*

Chemical Abstracts, EC (Einecs or ELINCS), and CIPAC numbers, where they exist, must be reported.

1.7. *Molecular and structural formula, molecular mass*

The molecular formula, molecular mass and structural formula of the active substance, and where relevant, the structural formula of each stereo and optical isomer present in the active substance, must be provided.

1.8. *Method of manufacture (synthesis pathway) of the active substance*

The method of manufacture, in terms of the identity of the starting materials, the chemical pathways involved, and the identity of by-products and impurities present in the final product, must be provided, for each manufacturing plant. Generally process engineering information is not required.

Where the information provided relates to a pilot plant production system, the information required must again be provided once industrial scale production methods and procedures have stabilised.

1.9. *Specification of purity of the active substance in g/kg*

The minimum content in g/kg of pure active substance (excluding inactive isomers) in the manufactured material used for production of formulated products, must be reported.

Where the information provided relates to a pilot plant production system, the information required must again be provided to the Commission and the Member States once industrial scale production methods and procedures have stabilised, if production changes result in a changed specification of purity.

1.10. *Identity of isomers, impurities and additives (e.g. stabilisers), together with the structural formula and the content expressed as g/kg*

The maximum content in g/kg of inactive isomers as well as the ratio of the content of isomers/ diastereo-isomers, where relevant, must be provided. In addition, the maximum content in g/kg of each further component other than additives, including by-products, and impurities, must be provided. In the case of additives the content in g/kg must be provided.

For each component, present in quantities of 1 g/kg or more, the following information, where relevant, must be provided:

— chemical name in accordance with IUPAC and CA nomenclature,
— ISO common name or proposed common name if available,
— CAS number, EC (Einecs or ELINCS) number, and CIPAC number if available,
— molecular and structural formula,
— molecular mass, and
— maximum content in g/kg.

Where the manufacturing process is such that impurities and by-products which are particularly undesirable because of their toxicological, ecotoxicological or environmental properties could be present in the active substance, the content of each such compound must be determined and reported. In such cases, the analytical methods used and the limits of determination, which must be sufficiently low, for each compound of concern, must be reported. Additionally the following information, where relevant, must be provided:

— chemical name in accordance with IUPAC and CA nomenclature,
— ISO common name or proposed common name if available,
— CAS number, EC (Einecs or ELINCS) number, and CIPAC number if available,
— molecular and structural formula,
— molecular mass, and
— maximum content in g/kg.

Where the information provided relates to a pilot plant production system, the information required must again be provided once industrial scale production methods and procedures have stabilised, if production changes result in a changed specification of purity.

Where the information provided does not fully identify a component, viz. condensates, detailed information on the composition must be provided for each such component.

The trade name of components added to the active substance, prior to manufacture of formulated product, to preserve stability and facilitate ease of handling, where they are used, must also be provided. Additionally the following information, where relevant, must be provided for such additives:

— chemical name in accordance with IUPAC and CA nomenclature,
— ISO common name or proposed common name if available,
— CAS number, EC (Einecs or ELINCS) number, and CIPAC number if available,
— molecular and structural formula,
— molecular mass, and
— maximum content in g/kg.

For added components, other than active substance and other than impurities resulting from the manufacturing process, the function of the component (additive) must be given:

— antifoaming agent,
— antifreeze,
— binder,
— stabiliser,
— buffer,
— dispersing agent,
— other (specify).

1.11. *Analytical profile of batches*

Representative samples of the active substance must be analysed for content of pure active substance, inactive isomers, impurities and additives, as appropriate. The analytical results reported must include quantitative data, in terms of g/kg content, for all components present in quantities of more than 1 g/kg and typically shall account for at least 98 % of the material analysed. The actual content of components which are particularly undesirable because of their toxicological, ecotoxicological or environmental properties, must be determined and reported. Data reported must include the results of the analysis of individual samples and a summary of that data, to show the minimum or maximum and typical content of each relevant component, as appropriate.

Where an active substance is produced in different plants this information must be provided for each of the plants separately.

In addition, where available and relevant, samples of the active substance produced in laboratory scale or pilot production systems, must be analysed, if such material was used in generating toxicological or ecotoxicological data.

2. **Physical and chemical properties of the active substance**

(i) The information provided, must describe the physical and chemical properties of active substances and together with relevant information, must serve to characterise them. In particular, the information provided must permit:

— physical, chemical, and technical hazards associated with active substances, to be identified,

— classification of active substance as to hazard,

— appropriate restrictions and conditions to be associated with approvals, and

— appropriate hazard and precautionary statements to be specified.

The information and data referred to are required for all active substances, except where otherwise specified.

(ii) The information provided, taken together with that provided for relevant preparations, must permit the physical, chemical and technical hazards associated with preparations, to be identified, permit preparations to be classified, and permit establishment that preparations can be used without unnecessary difficulty, and be such that exposure of man, animals, and the environment is minimised, taking account of manner of use.

(iii) The extent to which active substances of which approval is sought, comply with relevant FAO specifications, must be stated. Divergences from FAO specifications must be described in detail, and justified.

(iv) In certain specified instances, tests must be conducted using purified active substance of stated specification. In such cases the principles of the method(s) of purification must be reported. The purity of such test material, which must be as high as can be achieved using the best available technology, must be reported. A reasoned justification must be provided in cases where the degree of purity achieved is less than 980 g/kg.

Such justification must demonstrate that all technically feasible and reasonable possibilities for the production of the pure active substance have been exhausted.

2.1. *Melting point and boiling point*

2.1.1. The melting point or where appropriate the freezing or solidification point of purified active substance must be determined and reported in accordance with method A 1 of Regulation (EC) No 440/2008. Measurements shall be taken up to 360 °C.

2.1.2. Where appropriate the boiling point of purified active substances must be determined and reported in accordance with method A 2 of Regulation (EC) No 440/2008. Measurements shall be taken up to 360 °C.

2.1.3. Where melting point and/or boiling point cannot be determined because of decomposition or sublimation, the temperature at which decomposition or sublimation occurs, must be reported.

2.2. *Relative density*

In the case of active substances which are liquids or solids, the relative density of the purified active substance must be determined and reported in accordance with method A 3 of Regulation (EC) No 440/2008.

2.3. *Vapour pressure (in Pa), volatility (e.g. Henry's law constant)*

2.3.1. The vapour pressure of purified active substance must be reported in accordance with method A 4 of Regulation (EC) No 440/2008. Where vapour pressure is less than 10^{-5} Pa, the vapour pressure at 20 or 25 °C may be estimated by a vapour pressure curve.

2.3.2. In the case of active substances which are solids or liquids, volatility (Henry's law constant) of purified active substance must be determined or calculated from its water solubility and vapour pressure and be reported (in $Pa \times m^3 \times mol^{-1}$).

2.4. *Appearance (physical state, colour and odour; if known)*

2.4.1. A description of both the colour, if any, and the physical state of both the active substance as manufactured and purified active substance, must be provided.

2.4.2. A description of any odour associated with the active substance as manufactured and purified active substance, noted when handling the materials in laboratories or production plants, must be reported.

2.5. *Spectra (UV/VIS, IR, NMR, MS), molecular extinction at relevant wavelengths*

2.5.1. The following spectra including a table of signal characteristica needed for interpretation must be determined and reported: Ultraviolet/Visible (UV/VIS), infrared (R), nuclear magnetic resonance (NMR), and mass spectra (MS) of purified active substance and molecular extinction at relevant wavelengths, must be determined and reported.

The wavelengths at which UV/visible molecular extinction occurs are to be determined and reported and must include where appropriate a wavelength at the highest absorption value above 290 nm.

In the case of active substances which are resolved optical isomers their optical purity must be measured and reported.

2.5.2. The UV/visible absorption spectra, IR, NMR and MS spectra, where necessary for the identification of the impurities considered to be of toxicological, ecotoxicological or environmental significance must be determined and reported.

2.6. *Solubility in water including effect of pH (4 to 10) on solubility*

The water solubility of purified active substances under atmospheric pressure must be determined and reported in accordance with method A 6 of Regulation (EC) No 440/2008. These water solubility determinations must be made in the neutral range (i.e. in distilled water in

equilibrium with atmospheric carbon dioxide). Where the active substance is capable of forming ions, determinations must also be made in the acidic range (pH 4 to 6) and in the alkaline range (pH 8 to 10), and be reported. Where the stability of the active substance in aqueous media is such that water solubility cannot be determined, a justification based on test data must be provided.

2.7. *Solubility in organic solvents*

The solubility of the active substances as manufactured in the following organic solvents at 15 to 25 °C must be determined and reported if less than 250 g/kg; the temperature applied must be specified:

— Aliphatic hydrocarbon: preferably n-heptane,
— Aromatic hydrocarbon: preferably xylene,
— Halogenated hydrocarbon: preferably 1,2-dichlorethane,
— Alcohol: preferably methanol or isopropyl alcohol,
— Ketone: preferably acetone,
— Ester: preferably ethyl acetate.

If for a particular active substance, one or more of these solvents are unsuitable (e.g. reacts with test material), alternative solvents can be used instead. In such cases, the choices made must be justified in terms of their structure and polarity.

2.8. *Partition coefficient n-octanol/water including effect of pH (4 to 10)*

The n-octanol/water partition coefficient of purified active substance must be determined and reported in accordance with method A 8 of Regulation (EC) No 440/2008. The effect of pH (4 to 10) must be investigated when the substance is acidic or basic as defined by its pKa value (< 12 for acids, > 2 for bases).

2.9. *Stability in water, hydrolysis rate, photochemical degradation, quantum yield and identity of breakdown product(s), dissociation constant including effect of pH (4 to 9)*

2.9.1. The hydrolysis rate of purified active substances (usually radiolabelled active substance, > 95 % purity), for each of the pH values 4, 7 and 9, under sterile conditions, in the absence of light, must be determined and reported in accordance with method C 7 of Regulation (EC) No 440/2008. For substances with a low rate of hydrolysis, the rate can be determined at 50 °C, or another appropriate temperature.

If degradation is observed at 50 °C, degradation rate at another temperature must be determined, and an Arrhenius plot must be constructed to permit an estimate to be made of hydrolysis at 20 °C. The identity of hydrolysis products formed and the rate constantly observed, must be reported. The estimated DT_{50} value must also be reported.

2.9.2. For compounds with a molar (decadic) absorption coefficient (ε) > 10 ($1 \times mol^{-1} \times cm^{-1}$) at a wavelength $\lambda \geq 290$ nm, direct phototransformation in purified (e.g. distilled) water at 20 to 25 °C, of purified active substance usually radio labelled using artificial light under sterile conditions, if necessary using a solubiliser, must be determined and reported. Sensitisers such as acetone must not be used as a cosolvent or solubiliser. The light source must simulate sunlight and be equipped with filters to exclude radiation at wavelengths $\lambda < 290$ nm. The identity of breakdown products formed which at any time during the study are present in quantities ≥ 10 % of the active substance added, a mass balance to account for at least 90 % of the applied radioactivity, as well as photochemical halflife must be reported.

2.9.3. Where necessary to investigate direct phototrans-formation, the *quantum yield of direct photodegradation in water* must be determined and reported, together with calculations to estimate theoretical lifetime of the active substance in the top layer of aqueous systems and the real lifetime of the substance.

The method is described in the FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides[6].

2.9.4. Where dissociation in water occurs, the dissociation constant(s) (pKa values) of the purified active substance must be determined and reported in accordance with OECD Test Guideline 112. The identity of the dissociated species formed, based on theoretical considerations, must be reported. If the active substance is a salt, the pKa value of the active principle must be given.

2.10. *Stability in air, photochemical degradation, identity of breakdown product(s)*

An estimation of the photochemical oxidative degradation (indirect phototransformation) of the active substance, must be submitted.

2.11. *Flammability including auto-flammability*

2.11.1. The flammability of active substances as manufactured, which are solids, gases, or are substances which evolve highly flammable gases, must be determined and reported in accordance with method A 10, A 11 or A 12 of Regulation (EC) No 440/2008 as appropriate.

2.11.2. The auto-flammability of active substances as manufactured must be determined and reported in accordance with method A 15 or A 16 of Regulation (EC) No 440/2008 as appropriate, and/or, where necessary in accordance with the UN-Bowes-Cameron-Cage-Test (UN-Recommendations on the Transport of Dangerous Goods, Chapter 14, No 14.3.4).

2.12. *Flash point*

The flash point of active substances as manufactured with a melting point below 40 °C, must be determined and reported in accordance with method A 9 of Regulation (EC) No 440/2008; only closed cup methods shall be used.

2.13. *Explosive properties*

The explosive properties of active substances as manufactured, must be determined and reported in accordance with method A 14 of Regulation (EC) No 440/2008 where necessary.

2.14. *Surface tension*

The surface tension has to be determined and reported in accordance with method A 5 of Regulation (EC) No 440/2008.

2.15. *Oxidising properties*

The oxidising properties of active substances as manufactured, must be determined and reported in accordance with method A 17 of Regulation (EC) No 440/2008, except where examination of its structural formula, establishes beyond reasonable doubt that the active substance is incapable of reacting exothermically with a combustible material. In such cases, it is sufficient to provide that information as justification for not determining the oxidising properties of the active substance.

3. **Further information on the active substance**

(i) The information provided must describe the intended purposes for which preparations containing the active substance are used, or are to be used and the dose and manner of their use or proposed use.

(ii) The information provided must specify the normal methods and precautions to be followed, in the handling, storage and transport of the active substance.

(iii) The studies, data and information submitted, together with other relevant studies, data and information, must both specify and justify the methods and precautions to be followed in the event of fire. The possible products of combustion in the event of fire shall be estimated, based on the chemical structure and the chemical and physical properties of the active substance.

(iv) The studies, data and information submitted, together with other relevant studies, data and information, must demonstrate the suitability of measures proposed for use in emergency situations.

(v) The information and data referred to are required for all active substances, except where otherwise specified.

3.1. *Function, e.g. fungicide, herbicide, insecticide, repellent, growth regulator*

The function must be specified from among the following:
— acaricide
— bactericide
— fungicide
— herbicide
— insecticide
— molluscicide
— nematicide
— plant growth regulator
— repellent
— rodenticide
— semio-chemicals
— talpicide
— viricide
— other (must be specified).

3.2. *Effects on harmful organisms, e.g. contact poison, inhalation poison, stomach poison, fungitoxic, etc. systematic or not in plants*

3.2.1. The nature of the effects on harmful organisms must be stated:
— contact action
— stomach action
— inhalation action
— fungitoxic action
— fungistatic action
— desiccant
— reproduction inhibitor

— other (must be specified).

3.2.2. It must be stated whether or not the active substance is translocated in plants and where relevant whether such translocation is apoplastic, symplastic or both.

3.3. *Field of use envisaged, e.g. field, protected crops, storage of plant products, home gardening*

The field(s) of use, existing and proposed, for preparations containing the active substance must be specified from among the following:

— Field use, such as agriculture, horticulture, forestry and viticulture
— Protected crops
— Amenity
— Weed control on non-cultivated areas
— Home gardening
— House plants
— Plant products storage practice
— Other (specify).

3.4. *Harmful organisms controlled and crops or products protected or treated*

3.4.1. Details of existing and the intended use in terms of crops, groups of crops, plants, or plant products treated and where relevant protected, must be provided.

3.4.2. Where relevant, details of harmful organisms against which protection is afforded, must be provided.

3.4.3. Where relevant, effects achieved e.g. sprout suppression, retardation of ripening, reduction in stem length, enhanced fertilisation, etc. must be reported.

3.5. *Mode of action*

3.5.1. To the extent that is has elucidated, a statement must be provided as to the mode of action of the active substance in terms, where relevant, of the biochemical and physiological mechanism(s) and biochemical pathway(s) involved. Where available, the results of relevant experimental studies must be reported.

3.5.2. Where it is known that to exert its intended effect, the active substance must be converted to a metabolite or degradation product following application or use of preparations containing it, the following information, cross referenced to and drawing on information provided in the context of paragraphs 5.6, 5.11, 6.1, 6.2, 6.7, 7.1, 7.2 and 9, where relevant, must be provided for active metabolite or degradation product:

— chemical name in accordance with IUPAC and CA nomenclature,
— ISO common name or proposed common name,
— CAS EC-number EC (Einecs or ELINCS) number, and CIPAC number if available,
— empirical and structural formula, and
— molecular mass.

3.5.3. Available information relating to the formation of active metabolites and degradation products must be provided, to include:

— the processes, mechanisms and reactions involved,
— kinetic and other data concerning the rate of conversion and if known the rate limiting step,
— environmental and other factors effecting the rate and extent of conversion.

3.6. *Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies*

Where available, information on possible occurrence of the development of resistance or cross-resistance must be provided.

3.7. *Recommended methods and precautions concerning handling, storage, transport or fire*

A safety data sheet pursuant to Article 31 of Regulation (EC) No 1907/2006 of the European Parliament and of the Council[7] must be provided for all active substances.

3.8. *Procedures for destruction or decontamination*

3.8.1. *Controlled incineration*

In many cases the preferred or sole means to safely dispose of active substances, contaminated materials, or contaminated packaging, is through controlled incineration in a licensed incinerator.

Where the content of halogens of the active substance is greater than 60 %, the pyrolytic behaviour of the active substance under controlled conditions (including where relevant supply of oxygen and defined residence time), at 800 °C and the content of polyhalogenated dibenzo-p-dioxins and dibenzo-furans in the products of pyrolysis must be reported. The application must provide detailed instructions for safe disposal.

3.8.2. *Others*

Other methods to dispose of the active substance, contaminated packaging and contaminated materials, where proposed, must be fully described. Data must be provided for such methods, to establish their effectiveness and safety.

3.9. *Emergency measures in case of an accident*

Procedures for the decontamination of water in case of an accident must be provided.

4. **Analytical methods**

Introduction

The provisions of this Section only cover analytical methods required for post-registration control and monitoring purposes.

For analytical methods used for generation of data as required in this Regulation or for other purposes the applicant has to provide a justification for the method used; where necessary separate guidance will be developed for such methods on the basis of the same requirements as defined for methods for post-registration control and monitoring purposes.

Descriptions of methods must be provided and include details of equipment, materials and conditions used.

As far as practicable these methods must employ the simplest approach, involve the minimum cost, and require commonly available equipment.

For this Section the following applies:

Impurities, metabolites, relevant metabolites	as defined in Regulation (EC) No 1107/2009

Relevant impurities	Impurities of toxicological and/or ecotoxicological or environmental concern
Significant impurities	Impurities with a content of ≥ 1 g/kg in the active substance as manufactured

On request the following samples must be provided:

(i) analytical standards of the pure active substance;

(ii) samples of the active substance as manufactured;

(iii) analytical standards of relevant metabolites and all other components included in the residue definition;

(iv) if available, samples of reference substances for the relevant impurities.

4.1. *Methods for the analysis of the active substance as manufactured*

For this point the following definitions apply:

(i) *Specificity*

Specificity is the ability of a method to distinguish between the analyte being measured and other substances.

(ii) *Linearity*

Linearity is defined as the ability of the method, within a given range, to obtain an acceptable linear correlation between the results and the concentration of analyte in samples.

(iii) *Accuracy*

The accuracy of a method is defined as the degree to which the determined value of analyte in a sample corresponds to the accepted reference value (for example ISO 5725).

(iv) *Precision*

Precision is defined as the closeness of agreement between independent test results obtained under prescribed conditions.

Repeatability: Precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

The reproducibility is not required for the active substance as manufactured (for definition of reproducibility see ISO 5725).

4.1.1. Methods, which must be described in full, must be provided for the determination of pure active substance in the active substance as manufactured as specified in the dossier submitted in support of approval. The applicability of existing Cipac methods must be reported.

4.1.2. Methods must also be provided for the determination of significant and/or relevant impurities and additives (e.g. stabilisers) in the active substance as manufactured.

4.1.3. *Specificity, linearity, accuracy and repeatability*

4.1.3.1. Specificity of methods submitted must be demonstrated and reported. In addition, the extent of interference by other substances present in the active substance as manufactured (e.g. isomers, impurities or additives), must be determined.

While interferences due to other components may be identified as systematic errors in the assessment of the accuracy of methods proposed for the determination of pure active substance in the active substance as manufactured, an explanation must be provided for any interference occurring which contributes more than ± 3 % to the total quantity determined. The degree of interference for methods for the determination of impurities must also be demonstrated.

4.1.3.2. The linearity of proposed methods over an appropriate range must be determined and reported. For the determination of pure active substance, the calibration range must extend (by at least 20 %) the highest and lowest nominal content of the analyte in relevant analytical solutions. Duplicate calibration determinations must be made at three or more concentrations. Alternatively, five concentrations, each as single measurements, are acceptable. Reports submitted must include the equation of the calibration line and the correlation coefficient and representative and properly labelled documentation from the analysis, e.g. chromatograms.

4.1.3.3. Accuracy is required for methods for the determination of pure active substance and significant and/or relevant impurities in the active substance as manufactured.

4.1.3.4. For the repeatability in the determination of the pure active substance in principle a minimum of five determinations must be made. The relative standard deviation (% RSD) must be reported. Outliers identified through an appropriate method (e.g. Dixons or Grubbs test), may be discarded. Where outliers have been discarded, that fact must be clearly indicated. An explanation as to the reason for the occurrence of individual outliers must be attempted.

4.2. *Methods for the determination of residues*

The methods must be capable of determining the active substance and/or relevant metabolites. For each method and for each relevant representative matrix, the specificity, precision, recovery, and limit of determination must be experimentally determined and reported.

In principle, residue methods proposed shall be multi-residue methods; a standard multi-residue method must be assessed and reported as to its suitability for residue determination. Where residue methods proposed are not multi-residue methods, or are not compatible with such methods, an alternative method must be proposed. Where this requirement results in an excessive number of methods for individual compounds, a ‘common moiety method’ may be acceptable.

For this Section the following definitions apply:

(i) *Specificity*

Specificity is the ability of a method to distinguish between the analyte being measured and other substances.

(ii) *Precision*

Precision is defined as the closeness of agreement between independent test results obtained under prescribed conditions.

Repeatability : Precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on identical

test material in the same laboratory by the same operator using the same equipment within short intervals of time.

Reproducibility : As the definition of reproducibility in relevant publications (for example, in ISO 5725) is in general not practicable for residue analytical methods, reproducibility in the context of this Regulation is defined as a validation of repeatability of recovery, from representative matrices and at representative levels, by at least one laboratory which is independent from that which initially validated the study (this independent laboratory may be within the same company) (independent laboratory validation).

(iii) *Recovery*

The percentage of the amount of active substance or relevant metabolite originally added to a sample of the appropriate matrix which contains no detectable level of the analyte.

(iv) *Limit of determination*

The limit of determination (often referred to as limit of quantification) is defined as the lowest concentration tested, at which an acceptable mean recovery is obtained (normally 70 to 110 % with a relative standard deviation of preferably ≤ 20 %; in certain justified cases lower or higher mean recovery rates as well as higher relative standard deviations may be acceptable).

4.2.1. *Residues in and/or on plants, plant products, foodstuffs (of plant and animal origin), feedingstuffs*

Methods submitted must be suitable for the determination of all components included in the residue definition as submitted in accordance with points 6.1 and 6.2 in order to enable Member States to determine compliance with established MRL's or to determine dislodgeable residues.

The specificity of the methods must enable all components included in the residue definition to be determined, using an additional confirmatory method if appropriate.

The repeatability must be determined and reported. The replicate analytical portions for test can be prepared from a common field treated sample, containing incurred residues. Alternatively the replicate analytical portions for test can be prepared from a common untreated sample with aliquots fortified at the required level(s).

The results from an independent laboratory validation must be reported.

The limit of determination including the individual and mean recovery must be determined and reported. The overall relative standard deviation, as well as the relative standard deviation for each fortification level must be experimentally determined and reported.

4.2.2. *Residues in soil*

Methods for analysis of soil for parent compound and/or relevant metabolites must be submitted.

The specificity of the methods must enable the parent compound and/or relevant metabolites to be determined, using an additional confirmatory method if appropriate.

The repeatability, recovery and the limit of determination including the individual and mean recovery must be determined and reported. The overall relative standard deviation, as well as the relative standard deviation for each fortification level must be experimentally determined and reported.

The proposed limit of determination must not exceed a concentration which is of concern with regard to exposure of non-target organisms or because of phytotoxic effects. Normally the proposed limit of determination shall not exceed 0,05 mg/kg.

4.2.3. *Residues in water (including drinking water, ground water and surface water)*

Methods for analysis in water for parent compound and/or relevant metabolites must be submitted.

The specificity of the methods must enable the parent compound and/or relevant metabolites to be determined, using an additional confirmatory method if appropriate.

The repeatability, recovery and the limit of determination including the individual and mean recovery must be determined and reported. The overall relative standard deviation, as well as the relative standard deviation for each fortification level must be experimentally determined and reported.

For drinking water the proposed limit of determination must not exceed 0,1 μg/l. For surface water the proposed limit of determination must not exceed a concentration which has an impact on non-target organisms deemed to be unacceptable in accordance with the requirements of the Annex to Commission Regulation (EU) No 546/2011[8].

4.2.4. *Residues in air*

Methods for the analysis in air of the active substance and/or relevant metabolites formed during or shortly after application must be submitted unless it can be justified that exposure of operators, workers or bystanders is not likely to occur.

The specificity of the methods must enable the parent compound and/or relevant metabolites to be determined, using an additional confirmatory method if appropriate.

The repeatability, recovery and the limit of determination including the individual and mean recovery must be determined and reported. The overall relative standard deviation, as well as the relative standard deviation for each fortification level must be experimentally determined and reported.

The proposed limit of determination must take into account relevant health based limit values or relevant exposure levels.

4.2.5. *Residues in body fluids and tissues*

Where an active substance is classified as toxic or highly toxic appropriate analytical methods must be submitted.

The specificity of the methods must enable the parent compound and/or relevant metabolites to be determined, using an additional confirmatory method if appropriate.

The repeatability, recovery and the limit of determination including the individual and mean recovery must be determined and reported. The overall relative standard deviation, as well as the relative standard deviation for each fortification level must be experimentally determined and reported.

5. Toxicological and metabolism studies

Introduction

(i) The information provided, taken together with that provided for one or more preparations containing the active substance, must be sufficient to permit an evaluation to be made as to the risks for man, associated with the handling and use of plant protection products containing the active substance, and the risk for man arising from residual traces remaining in food and water. In addition, the information provided must be sufficient to:

— permit a decision to be made as to whether, or not, the active substance can be approved,
— specify appropriate conditions or restrictions to be associated with any approval,
— classify the active substance as to hazard,
— establish a relevant acceptable daily intake (ADI) level for man,
— establish acceptable operator exposure level(s) (AOEL),
— specify the pictograms, signal words, and relevant hazard and precautionary statements for the protection of man, animals and the environment to be included in packaging (containers),
— identify relevant first aid measures as well as appropriate diagnostic and therapeutic measures to be followed in the event of poisoning in man, and
— permit an evaluation to be made as to the nature and extent of the risks for man, animals (species normally fed and kept or consumed by man) and of the risks for other non-target vertebrate species.

(ii) There is a need to investigate and report all potentially adverse effects found during routine toxicological investigations (including effects on organs and special systems such as immunotoxicity and neurotoxicity) and to undertake and report such additional studies which may be necessary to investigate the probable mechanism involved, to establish Noaels (no observed adverse effect levels), and to assess the significance of these effects. All available biological data and information which are relevant to the assessment of the toxicological profile of the substance tested, must be reported.

(iii) In the context of the influence that impurities can have on toxicological behaviour, it is essential that for each study submitted, a detailed description (specification) of the material used, as mentioned under point 1.11 of Part A be provided. Tests shall be conducted using active substance of that specification to be used in the manufacture of preparations to be authorised, except where radiolabelled material is required or permitted.

(iv) Where studies are conducted using an active substance produced in the laboratory or in a pilot plant production system, the studies must be repeated using the active substance as manufactured, unless it can be justified that the test material used is essentially the same, for the purposes of toxicological testing and assessment. In cases of uncertainty, appropriate bridging studies must be submitted to serve as a basis for a decision as to the possible need for repetition of the studies.

(v) In the case of studies in which dosing extends over a period, dosing shall preferably be done using a single batch of active substance if stability permits.

(vi) For all studies actual achieved dose in mg/kg body weight, as well as in other convenient units, must be reported. Where dosing via the diet is utilised the test compound must be distributed uniformly in the diet.

(vii) Where, as a result of metabolism or other processes in or on treated plants, or as a result of processing of treated products, the terminal residue (to which consumers or workers as defined in the Annex to Regulation (EU) No 545/2011 point 7.2.3 of Part A will be exposed) contains a substance which is not the active substance itself and is not identified as a metabolite in mammals, it will be necessary to carry out toxicity studies on these components of the terminal residue unless it can be demonstrated that consumer or worker exposure to these substances does not constitute a relevant risk to health. Toxicokinetic and metabolism studies relating to metabolites and degradation products shall only be conducted if toxicity findings of the metabolite cannot be evaluated by the available results relating to the active substance.

(viii) The way of administration of the test substance depends on the main exposure routes. In cases where exposure is mainly by the gas phase, it can be more appropriate to perform inhalation studies instead of oral studies.

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

Quite limited data, as described below and restricted to one test species (normally the rat) may be all that is required in this area. These data can provide information useful in the design and interpretation of subsequent toxicity tests. However, it must be remembered that information on interspecies differences may be crucial in extrapolation of animal data to man and information on percutaneous penetration, absorption, distribution, excretion and metabolism may be useful in operator risk assessments. It is not possible to specify detailed data requirements in all areas, since the exact requirements will be dependent upon the results obtained for each particular test substance.

Aim of the test:

The tests shall provide sufficient data to permit:

— an evaluation of the rate and extent of absorption,

— an evaluation of the tissue distribution and the rate and extent of excretion of the test substance and the relevant metabolites,

— the identification of metabolites and the metabolic pathway.

The effect of dose level on these parameters and whether the results are different after single versus repeated doses, shall also be investigated.
Circumstances in which required

A single dose toxicokinetic study in rats (oral route of administration) in at least two dose levels as well as a repeated dose toxicokinetic study in rats (oral route of administration) at a single dose level, must be conducted and reported. It may be necessary in some cases to perform additional studies on another species (such as goat or chicken).
Test guideline

Regulation (EC) No 440/2008, Method B 36, Toxicokinetics.

5.2. *Acute toxicity*

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

— the toxicity of the active substance,

— the time course and characteristics of the effects with full details of behavioural changes and possible gross pathological findings at post-mortem,

— where possible mode of toxic action, and

— the relative hazard associated with the different routes of exposure.

While the emphasis must be on estimating the toxicity ranges involved, the information generated must 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 must always be reported.

Test guideline

The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method B 1 bis or B 1 ter.

5.2.2. *Percutaneous*
Circumstances in which required

The acute percutaneous toxicity of the active substance must always be reported.
Test guideline

Both local and systemic effects must be investigated. The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, method B 3.

5.2.3. *Inhalation*
Circumstances in which required

The inhalation toxicity of the active substance must be reported where the active substance is:

— a gas or liquified gas,
— is to be used as a fumigant,
— is to be included in a smoke generating, aerosol or vapour releasing preparation,
— is to be used with fogging equipment,
— has a vapour pressure $> 1 \times 10^{-2}$ Pa and is to be included in preparations to be used in enclosed spaces such as warehouses or glasshouses,
— is to be included in preparations which are powders containing a significant proportion of particles of diameter < 50 μm (> 1 % on a weight basis), or
— is to be included in preparations to be applied in a manner which generates a significant proportion of particles or droplets of diameter < 50 μm (> 1 % on a weight basis).

Test guideline

The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method B 2.

5.2.4. *Skin irritation*
Aim of the test

The test will provide the potential of skin irritancy of the active substance including the potential reversibility of the effects observed.
Circumstances in which required

The skin irritancy of the active substance must be determined except where it is likely, as indicated in the test guideline, that severe skin effects may be produced or that effects can be excluded.
Test guideline

The acute skin irritation must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method B 4.

5.2.5. *Eye irritation*
Aim of test

The test will provide the potential of eye irritancy of the active substance including the potential reversibility of the effects observed.
Circumstances in which required

Eye irritation tests must be conducted except where it is likely, as indicated in the test guideline, that severe effects on the eyes may be produced.
Test guidelines

The acute eye irritation must be determined in accordance with the Annex to Regulation (EC) No 440/2008, Method B 5.

5.2.6. *Skin sensitisation*
Aim of test

The test will provide sufficient information to assess the potential of the active substance to provoke skin sensitisation reactions.
Circumstances in which required

The test must always be carried out except where the substance is a known sensitiser.
Test guideline

The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method B 6.

5.3. *Short-term toxicity*

Short-term toxicity studies must 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. Such studies provide useful data on the risks for those handling and using preparations containing the active substance. In particular, short-term studies provide an essential insight into possible cumulative actions of the active substance and the risks to workers who may be intensively 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, must be sufficient to permit the identification of effects following repeated exposure to the active substance, and in particular to further establish, or indicate:

— the relationship between dose and adverse effects,
— toxicity of the active substance including where possible the Noael,
— target organs, where relevant,
— the time course and characteristics of poisoning with full details of behavioural changes and possible pathological findings at post-mortem,
— specific toxic effects and pathological changes produced,
— where relevant the persistence and reversibility of certain toxic effects observed, following discontinuation of dosing,
— where possible, the mode of toxic action, and
— the relative hazard associated with the different routes of exposure.

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

Although it is not mandatory to perform 28-day short-term studies, they can be useful as range finding tests. Where conducted, they must be reported, since the results could be of particular value in the identification of adaptive responses which can be masked in chronic toxicity studies.
Test guideline

The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method B 7.

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

The short-term oral toxicity (90 day) of the active substance to both rat and dog, must always be reported. Where there is evidence that the dog is significantly more sensitive and where such data are likely to be of value in extrapolating results obtained to man, a 12-month toxicity study in dogs must be conducted and reported.
Test guidelines

The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Methods B 26 and B 27, sub-chronic oral toxicity test repeated dose 90.

5.3.3. *Other routes*
Circumstances in which required

For the assessment of operator exposure additional percutaneous studies may be useful.

For volatile substances (vapour pressure > 10^{-2} Pascal) expert judgment is required to decide whether the short-term studies have to be performed by oral or inhalation exposure.
Test guidelines

— 28-day dermal: the Annex to Regulation (EC) No 440/2008, Method B 9, repeated dose toxicity (dermal),
— 90-day dermal: the Annex to Regulation (EC) No 440/2008 Method B 28, sub-chronic dermal toxicity study,
— 28-day inhalation: the Annex to Regulation (EC) No 440/2008, Method B 8, repeated dose toxicity (inhalation),
— 90-day inhalation: the Annex to Regulation (EC) No 440/2008, Method B 29, sub-chronique inhalation toxicity study.

5.4. *Genotoxicity testing*
Aim of the test

These studies are of value in:
— the prediction of genotoxic potential
— the early identification of genotoxic carcinogens
— the elucidation of the mechanism of action of some carcinogens.

To avoid responses that are artefacts of the test system, excessively toxic doses must not be used in either *in vitro* or *in vivo* assays for mutagenicity. This approach shall be regarded as general guidance. It is important that a flexible approach is adopted, with selection of further tests which are dependent upon interpretation of results at each stage.

5.4.1. *In vitro studies*
Circumstances in which required

In vitro mutagenicity tests (bacterial assay for gene mutation, test for clastogenicity in mammalian cells and test for gene mutation in mammalian cells) must always be performed.
Test guidelines

Acceptable test guidelines are:
— Annex to Regulation (EC) No 440/2008, Method B 13/14 — reverse mutation test using bacteria,
— Annex to Regulation (EC) No 440/2008, Method B 10 — *in vitro* mammalian chromosome aberration test,

— Annex to Regulation (EC) No 440/2008, Method B 17 — *in vitro* mammalian cell gene mutation test.

5.4.2. *In vivo studies in somatic cells*
Circumstances in which required

If all the results of the *in vitro* studies are negative further testing must be done with consideration of other relevant information available (including toxicokinetic, toxicodynamic and physico-chemical data and data on analogous substances). The test can be an *in vivo* study or an *in vitro* study using a different metabolising system from that/those previously used.

If the *in vitro* cytogenetic test is positive, an *in vivo* test using somatic cells (metaphase analysis in rodent bone marrow or micronucleus test in rodents) must be conducted.

If either of the *in vitro* gene mutation tests are positive, an *in vivo* test to investigate unscheduled DNA synthesis or a mouse spot test must be conducted.
Test guidelines

The following test guidelines are acceptable:
— Annex to Regulation (EC) No 440/2008, Method B 12 — *In vivo* mammalian erythrocyte micronucleus test,
— Annex to Regulation (EC) No 440/2008, Method B 24— Mouse spot test,
— Annex to Regulation (EC) No 440/2008, Method B 11 — *In vivo* Mammalian Bone-Marrow chromosome aberration test.

5.4.3. *In vivo studies in germ cells*
Circumstances in which required

When any result of an *in vivo* study in somatic cells is positive, *in vivo* testing for germ cell effects may be justified. The necessity for conducting these tests will have to be considered on a case by case basis, taking into account information regarding toxicokinetics, use and anticipated exposure. Suitable tests would need to examine interaction with DNA (such as the dominant lethal assay), to look at the potential for inherited effects and possibly make a quantitative assessment of heritable effects. It is recognised that in view of their complexity, the use of quantitative studies would require strong justification.

5.5. *Long-term toxicity and carcinogenicity*
Aim of the test

The long-term studies conducted and reported, taken together with other relevant data and information on the active substance, must be sufficient to permit the identification of effects, following repeated exposure to the active substance, and in particular must be sufficient to:
— identify adverse effects resulting from exposure to the active substance,
— identify target organs, where relevant,
— establish the dose-response relationship,
— identify changes in toxic signs and manifestations observed, and
— establish the Noael.

Similarly, the carcinogenicity studies taken together with other relevant data and information on the active substance, must be sufficient to permit the hazards for humans, following repeated exposure to the active substance, to be assessed, and in particular must be sufficient:
— to identify carcinogenic effects resulting from exposure to the active substance,
— to establish the species and organ specificity of tumours induced,
— to establish the dose-response relationship, and

— for non-genotoxic carcinogens, to identify the maximum dose eliciting no adverse effect (threshold dose).

Circumstances in which required

The long-term toxicity and carcinogenicity of all active substances must be determined. If in exceptional circumstances, it is claimed that such testing is unnecessary, that claim must be fully justified, viz. toxicokinetic data demonstrates that absorption of the active substance does not occur from the gut, through the skin or via the pulmonary system.

Test conditions

A long-term oral toxicity and carcinogenicity study (2 years) of the active substance must be conducted using the rat as test species; these studies can be combined.

A carcinogenicity study of the active substance must be conducted using the mouse as test species.

Where a non-genotoxic mechanism for carcinogenicity is suggested, a well argued case, supported with relevant experimental data, including that necessary to elucidate the possible mechanism involved, must be provided.

While the standard reference points for treatment responses are concurrent control data, historical control data, may be helpful in the interpretation of particular carcinogenicity studies. Where submitted, historical control data shall be from the same species and strain, maintained under similar conditions and shall be from contemporaneous studies. The information on historical control data provided must include:

— identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location,
— name of the laboratory and the dates when the study was performed,
— description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed,
— approximate age, in days, of the control animals at the beginning of the study and at the time of killing or death,
— description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (e.g. diseases, infections),
— name of the laboratory and the examining scientists responsible for gathering and interpreting the pathological data from the study, and
— a statement of the nature of the tumours that may have been combined to produce any of the incidence data.

The doses tested, including the highest dose tested, must 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. The highest dose level in the carcinogenicity study shall elicit signs of minimal toxicity such as slight depression in body-weight gain (less than 10 %), without causing tissue necrosis or metabolic saturation and without substantially altering normal lifespan due to effects other than tumours. If the long-term toxicity study is carried out separately, the highest dose level shall elicit definite signs of toxicity without causing excessive lethality. Higher doses, causing excessive toxicity are not considered relevant to evaluations to be made.

In the collection of data and compilation of reports, incidence of benign and malignant tumours must not be combined, unless there is clear evidence of benign tumours becoming malignant with time. Similarly, dissimilar, un-associated tumours, whether benign or malignant, occurring in the same organ, must not be combined, for reporting purposes. In the interests of avoiding confusion, terminology such as that developed by the American Society of Toxicologic

Pathologists[9], or the Hannover Tumour Registry (RENI) shall be used in the nomenclature and reporting of tumours. The system used must be identified.

It is essential that biological material selected for histopathological examination includes 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, must be conducted and reported.
Test guideline

The studies must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method B 30 Chronic toxicity test, Method B 32 Carcinogenicity test or Method B 33 combined chronic toxicity/carcinogenicity test.

5.6. *Reproductive toxicity*

The adverse reproductive effects are of two main types:
— impairment of male or female fertility, and
— impacts on the normal development of progeny (developmental toxicity).

Possible effects on all aspects of reproductive physiology in both males and females, as well as possible effects on pre-natal and post-natal development, must be investigated and reported. If in exceptional circumstances, it is claimed that such testing is unnecessary, that claim must be fully justified.

While the standard reference point for treatment responses are 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 and shall be from contemporaneous studies. The information on historical control data provided must include:
— identification of species and strain, name of the supplier, and specific colony identification, if the supplier has more than one geographical location,
— name of the laboratory and the dates when the study was performed,
— description of the general conditions under which animals were maintained, including the type or brand of diet and, where possible, the amount consumed,
— approximate age, in days, of the control animals at the beginning of the study and at the time of killing or death,
— description of the control group mortality pattern observed during or at the end of the study, and other pertinent observations (e.g. diseases, infections), and
— name of the laboratory and the examining scientist responsible for gathering and interpreting the toxicological data from the study.

5.6.1. *Multi-generation studies*
Aim of the test

The studies reported, taken together with other relevant data and information on the active substance, must be sufficient to permit the identification of effects for reproduction, following repeated exposure to the active substance, and in particular must be sufficient:
— to identify direct and indirect effects on reproduction resulting from exposure to the active substance,
— to identify any enhancement of general toxic effects (noted during short-term and chronic toxicity testing),
— to establish the dose-response relationship,

— to identify changes in toxic signs and manifestations observed, and
— to establish the Noael.

Circumstances in which required

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

Test guideline

The tests must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method B 35, two-generation reproduction toxicity study. In addition organ weight of reproductive organs must be reported.

Supplementary studies

Where necessary for a better interpretation of the effects on reproduction and as far as this information is not yet available it could be necessary to perform supplementary studies in order to provide the following information:

— separate male and female studies,
— three segment designs,
— dominant lethal assay for male fertility,
— cross-matings of treated males with untreated females and vice versa,
— effects on spermatogenesis,
— effects on oogenesis,
— sperm motility, mobility and morphology, and
— investigation of hormonal activity.

5.6.2. *Developmental toxicity studies*

Aim of the test

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

— to identify direct and indirect effects on embryonic and foetal development resulting from exposure to the active substance,
— to identify any maternal toxicity,
— to establish the relationship between observed responses and dose in both dam and offspring,
— to identify changes in toxic signs and manifestations observed, and
— to establish the Noael.

Furthermore, the tests will give additional information on any enhancement of general toxic effects of pregnant animals.

Circumstances in which required

The tests must always be carried out.

Test conditions

Developmental toxicity must be determined both to rat and rabbit by the oral route. Malformations and variations shall be reported separately. A glossary of terminology and diagnostic principles for malformations and variations must be given in the report.

Test guideline

The tests must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method B 31, prenatal developmental toxicity study.

5.7. *Delayed neurotoxicity studies*

Aim of the test

The test shall provide sufficient data to evaluate if the active substance could provoke delayed neurotoxicity after acute exposure.

Circumstances in which required

These studies have to be performed for substances of similar or related structures to those capable of inducing delayed neurotoxicity such as organophosphates.

Test guidelines

The test must be carried out in accordance with OECD Guideline 418.

5.8. *Other toxicological studies*

5.8.1. *Toxicity studies of metabolites as referred to in the introduction, point (vii)*

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 must be made on a case by case basis.

5.8.2. *Supplementary studies on the active substance*

In certain cases it can be necessary to carry out supplementary studies to further clarify observed effects. These studies could include:

— studies on absorption, distribution, excretion and metabolism,
— studies on the neurotoxic potential,
— studies on the immunotoxicological potential,
— studies on other routes of administration.

Decisions as to the need for supplementary studies must be made on a case by case basis, taking into account the results of the available toxicological and metabolism studies and the most important exposure routes.

Studies required must be designed on an individual basis, 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 the provisions of Article 10 of Council Directive 98/24/EC[10], practical data and information relevant to the recognition of the symptoms of poisoning, and on the effectiveness of first aid and therapeutic measures have to be submitted. More specific references to the investigation for antidotal pharmacology or safety pharmacology using animals shall be provided. 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 and of the necessary quality, are of particular value, in confirming the validity of extrapolations made and conclusions reached with respect to target organs, dose-response relationships, and the reversibility of toxic effects. Such data can be generated following accidental or occupational exposure.

5.9.1. *Medicinal surveillance on manufacturing plant personnel*

Reports of occupational health surveillance programmes, supported with detailed information on the design of the programme, on exposure to the active substance and exposure to other chemicals, must be submitted. 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 after application of the active substance (e.g. in efficacy trials).

Available information on the sensitisation including allergenic response of workers and others exposed to the active substance, must be provided, and include where relevant details of any incidence of hypersensitivity. The information provided shall include details of frequency, level and duration of exposure, symptoms observed and other relevant clinical information.

5.9.2. *Direct observation, e.g. clinical cases and poisoning incidents*

Available reports from the open literature, relating to clinical cases and poisoning incidents, where they are from refereed journals or official reports, must be submitted together with reports of any follow-up studies undertaken. Such reports shall 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. Summary and abstract information is not of value.

Where supported with the necessary level of detail, such documentation can be of particular value in confirming the validity of extrapolations from animal data to man and in identifying unexpected adverse effects which are specific to humans.

5.9.3. *Observations on exposure of the general population and epidemiological studies if appropriate*

Where available, and supported with data on levels and duration of exposure, and conducted in accordance with recognised standards[11], epidemiological studies are of particular value and must be submitted.

5.9.4. *Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests*

A 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, where available, must 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.

5.9.5. *Proposed treatment: first aid measures, antidotes, medical treatment*

The first aid measures to be used in the event of poisoning (actual and suspected) and in the event of contamination of eyes must be provided.

Therapeutic regimes for use in the event of poisoning or contamination of eyes, including where available the use of antidotes, must 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, must be provided. Contraindications associated with particular regimes, particularly those relating to ‘general medical problems’ and conditions, must be described.

5.9.6. *Expected effects of poisoning*

Where known, the expected effects and the duration of these effects following poisoning must be described and 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.

5.10. *Summary of mammalian toxicity and overall evaluation*

A summary of all data and information provided under paragraphs 5.1 through 5.10, must be submitted, and include a detailed and critical assessment of those data in the context of relevant evaluative and decision making criteria and guidelines, with particular reference to the risks for man and animals that may or do arise, and the extent, quality and reliability of the data base.

Where relevant, in the light of findings with respect to the analytical profile of batches of the active substance (point 1.11) and any bridging studies conducted (point (iv) of the introduction to Section 5), the relevance of the data as submitted to the assessment of the toxicological profile of the active substance as manufactured, must be argued.

On the basis of an assessment of the data base, and the relevant decision making criteria and guidelines, justifications must be submitted for the Noaels proposed for each relevant study.

On the basis of these data scientifically reasoned proposals for the establishment of ADI and AOEL(s) for the active substance must be submitted.

6. Residues in or on treated products, food and feed

Introduction

(i) The information provided, taken together with that provided for one or more preparations containing the active substance, must be sufficient to permit an evaluation to be made as to the risks for man, arising from residues of the active substance and relevant metabolites, degradation and reaction products remaining in food. In addition, the information provided must be sufficient to:

— permit a decision to be made as to whether, or not, the active substance can be approved,

— specify appropriate conditions or restrictions to be associated with any approval.

(ii) A detailed description (specification) of the material used, as provided under point 1.11 must be provided.

(iii) Studies shall be performed in accordance with the EU Guidelines for the generation of data concerning residues[12].

(iv) Where relevant, data shall be analysed using appropriate statistical methods. Full details of the statistical analysis shall be reported.

(v) Stability of residues during storage.

If may be necessary to perform studies on the stability of residues during storage. Provided samples are frozen within generally 24 hours after sampling and unless a compound is otherwise known to be volatile or labile, data are not normally required for samples extracted and analysed within 30 days from sampling (6 months in the case of radio-labelled material).

Studies with non-radio-labelled substances shall be carried out with representative substrates and preferably on samples from treated crops or animals with incurred residues. Alternatively, if this is not possible, aliquots of prepared control samples shall be spiked with a known amount of chemical before storage under normal storage conditions.

Where the degradation during storage is significant (more than 30 %) it may be necessary to change the storage conditions or not to store the samples prior to analysis and repeat any studies where the unsatisfactory storage conditions were used.

Detailed information with respect to the sample preparation and storage conditions (temperature and duration) of samples and extracts must be submitted. Storage stability data using sample extracts will also be required unless samples are analysed within 24 hours of extraction.

6.1. *Metabolism, distribution and expression of residue in plants*
Aim of the tests

The objectives of these studies are:

— to provide an estimate of total terminal residues in the relevant portion of crops at harvest following treatment as proposed,

— to identify the major components of the total terminal residue,

— to indicate the distribution of residues between relevant crops parts,

— to quantify the major components of the residue and to establish the efficiency of extraction procedures for these components,

— to decide on the definition and expression of a residue.

Circumstances in which required

These studies must always be performed unless it can be justified that no residues will remain on plants/plant products which are used as food or feeding stuffs.
Test conditions

Metabolism studies have to involve crops or categories of crops in which plant protection products containing the active substance in question would be used. If a wide range of uses in different crop categories or in the category fruits is envisaged, studies have to be carried out on at least three crops unless it can be justified that a different metabolism is unlikely to occur. In cases where use is envisaged in different categories of crops, the studies must be representative for the relevant categories. For this purpose crops can be considered as falling into one of five categories: root vegetables, leafy crops, fruits, pulses and oilseeds, cereals. If studies are available for crops from three of these categories and the results indicate that the route of degradation is similar in all three categories then it is unlikely that any more studies will be needed unless it could be expected that a different metabolism will occur. The metabolism studies have also to take into account the different properties of the active substance and the intended method of application.

An evaluation of the results from different studies has to be submitted on the point and path of uptake (e.g. via leaves or roots), and on the distribution of residues between relevant parts of the crop at harvest (with particular emphasis on edible parts for man or animals). If the active substance or relevant metabolites are not taken up by the crop, this must be explained. Information on the mode of action and the physico-chemical properties of the active substance may be helpful in assessing trial data.

6.2. *Metabolism, distribution and expression of residue in livestock*
Aim of tests

The objectives of these studies are:

— to identify the major components of the total terminal residue in edible animal products,

— to quantify the rate of degradation and excretion of the total residue in certain animal products (milk or eggs) and excreta,

— to indicate the distribution of residues between relevant edible animal products,

— to quantify the major components of the residue and to show the efficiency of extraction procedures for these components,

— to generate data from which a decision on the need for livestock feeding studies as provided for in point 6.4 can be made,

— to decide on the definition and expression of a residue.

Circumstances in which required

Metabolism studies on animals, such as lactating ruminants (e.g. goat or cow) or laying poultry, are only required when pesticide use may lead to significant residues in livestock feed (≥ 0,1 mg/kg of the total diet as received, except special cases e.g. active substances which accumulate). Where it becomes apparent that metabolic pathways differ significantly in the rat as compared to ruminants a pig study must be conducted unless the expected intake by pigs is not significant.

6.3. *Residue trials*

Aim of the tests

The objectives of these studies are:

— to quantify the highest likely residue levels in treated crops at harvest or outloading from store following the proposed good agricultural practice (GAP), and

— to determine, when appropriate, the rate of decline of plant protection product deposits.

Circumstances in which required

These studies must always be performed where the plant protection product will be applied to plants/plant products which are used as food or feeding stuffs or where residues from soil or other substrates can be taken up by such plants except where extrapolation from adequate data on another crop is possible.

Residue trial data shall be submitted in the dossier for those uses of plant protection products for which authorisation is sought at the moment of the submission of a dossier for approval of the active substance.

Test conditions

Supervised trials shall correspond to proposed critical GAP. The test conditions must take into account the highest residues which may reasonably arise (e.g. maximum number of proposed applications, use of the maximum envisaged quantity, shortest pre-harvest intervals, withholding periods or storage periods) but which remain representative of the realistic worst case conditions in which the active substance would be used.

Sufficient data must be generated and submitted to confirm that patterns determined hold for the regions and the range of conditions, likely to be encountered in the regions concerned for which its use is to be recommended.

When establishing a supervised trial programme, normally factors such as climatic differences existing between production areas, differences in production methods (e.g. outdoor versus glasshouse uses), seasons of production, type of formulations, etc. shall be taken into account.

In general, for a comparable set of conditions, trials shall be carried out over a minimum of two growing seasons. All exceptions shall be fully justified.

The precise number of trials necessary is difficult to determine in advance of a preliminary evaluation of the trial results. Minimum data requirements only apply where comparability can be established between production areas, e.g. concerning climate, methods and growing seasons of production, etc. Assuming all other variables (climate, etc.) are comparable, a minimum of eight trials representative of the proposed growing area is required for major crops. For minor crops normally four trials representative of the proposed growing area are required.

Due to the inherently higher level of homogeneity in residues arising from post-harvest treatments or protected crops, trials from one growing season will be acceptable. For post-

harvest treatments, in principle a minimum of four trials are required, carried out preferably at different locations with different cultivars. A set of trials has to be carried out for each application method and store type unless the worst case residue situation can be clearly identified.

The number of studies per growing season to be performed can be reduced if it can be justified that the residue levels in plants/plant products will be lower than the limit of determination.

Where a significant part of the consumable crop is present at the time of application, half of the supervised residue trials reported shall include data to show the effect of time on the level of residue present (residue decline studies) unless it can be justified that the consumable crop is not affected by the application of the plant protection product under the proposed conditions of use.

6.4. *Livestock feeding studies*
Aim of the tests

The objective of these studies is to determine the residue in products of animal origin which will result from residues in feedingstuffs or fodder crops.
Circumstances in which required

Feeding studies are only required:

— when significant residues (≥ 0,1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crop (e.g. trimmings, waste) fed to animals, and

— when metabolism studies indicate that significant residues (0,01 mg/kg or above the limit of determination if this would be higher than 0,01 mg/kg) may occur in any edible animal tissue taking into account the residue levels in potential feedingstuffs obtained at the 1 × dose rate.

Where appropriate separate feeding studies for lactating ruminant and/or laying poultry shall be submitted. Where it appears from the metabolism studies submitted in accordance with point 6.2 that metabolic pathways differ significantly in the pig as compared to ruminants, a pig feeding study must be conducted unless the expected intake by pigs is not significant.
Test conditions

In general, the feed is administered in three dosages (expected residue level, three to five times, and 10 times the expected residue level). When setting the 1 × dose, a theoretical feed ration must be compiled.

6.5. *Effects of industrial processing and/or household preparations*
Circumstances in which required

The decision as to whether it is necessary to carry out processing studies will depend on:

— the importance of a processed product in the human or animal diet,

— the level of residue in the plant or plant product to be processed,

— the physico-chemical properties of the active substance or relevant metabolites, and

— the possibility that degradation products of toxicological significance may be found after processing of the plant or plant product.

Processing studies are not normally necessary if no significant or no analytically determinable residues occur in the plant or plant product which would be processed, or if the total theoretical maximum daily intake (TMDI) is less than 10 % of the ADI. In addition, processing studies are not normally required for plants or plant products mostly eaten raw except for those with inedible portions such as citrus, banana or kiwi fruit where data on the distribution of the residue in peel/pulp may be required.

'Significant residues' generally refer to residues above 0,1 mg/kg. If the pesticide concerned has a high acute toxicity and/or a low ADI, consideration must be given to conducting processing studies for determinable residues below 0,1 mg/kg.

Studies on the effects on the nature of the residue are not normally required where only simple physical operations, not involving a change in temperature of the plant or the plant product, are involved such as washing, trimming or pressing.

6.5.1. *Effects on the nature of the residue*
Aim of the tests

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

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

6.5.2. *Effects on the residue levels*
Aim of the tests

The main objectives of these studies are:

— to determine the quantitative distribution of residues in the various intermediate and end products, and to estimate transfer factors,

— to enable a more realistic estimate to be made of dietary intake of residues.

Test conditions

Processing studies shall represent household processing and/or actual industrial processes.

In the first instance it is usually only necessary to carry out a core set of 'balance studies' representative of the common processes relevant to the plants or plant products containing significant residues. Justification shall be given for the selection made of these representative process(es). The technology to be used in processing studies shall always correspond as closely as possible to the actual conditions that are normally used in practice. A balance sheet shall be made in which the mass balance of residues in all intermediate and end products is investigated. In drawing up such a balance sheet any concentrations or reductions in residues in individual products can be recognised and the corresponding transfer factors can also be determined.

If the processed plant products play an important part in the diet, and if the 'balance study' indicates that a significant transfer of residue into the processed products could occur, then three 'follow-up studies' to determine residue concentration or dilution factors must be carried out.

6.6. *Residues in succeeding crops*
Aim of the test

The objective of these studies is to permit an evaluation of possible residues in succeeding crops.
Circumstances in which required

Where data generated in accordance with point 7.1 of this Annex or point 9.1 of the Annex to Regulation (EU) No 545/2011, shows that significant residues (> 10 % of the applied active substance as a total of unchanged active substance and its relevant metabolites or degradation

products) remain in soil or in plant materials, such as straw or organic material up to sowing or planting time of possible succeeding crops, and which could lead to residues above the limit of determination in succeeding crops at harvest, consideration shall be given to the residue situation. This shall include consideration of the nature of the residue in the succeeding crops and involve at least a theoretical estimation of the level of these residues. If the likelihood of residues in succeeding crops can not be excluded, metabolism and distribution studies shall be carried out, if necessary followed by field trials.

Test conditions

If a theoretical estimation of residues in succeeding crops is done, full details and a justification shall be given.

Metabolism and distribution studies and field trials, if necessary, shall be carried out on representative crops chosen to represent normal agricultural practice.

6.7. *Proposed maximum residue levels (MRLs) and residue definition*

A full justification for the proposed MRLs must be provided, including, where relevant, full details of the statistical analysis used.

When judging which compounds are to be included in the residue definition, account has to be taken of the toxicological significance of the compounds, amounts likely to be present and the practicality of the analytical methods proposed for post-registration control and monitoring purposes.

6.8. *Proposed pre-harvest intervals for envisaged uses, or withholding periods or storage periods, in the case of post-harvest uses*

A full justification for the proposals must be provided.

6.9. *Estimation of the potential and actual exposure through diet and other means*

Consideration will be given to the calculation of a realistic prediction of dietary intake. This may be done in a step-wise fashion leading to an increasingly realistic prediction of intake. Where relevant, other sources of exposure such as residues arising from the use of medicines or veterinary drugs have to be taken into account.

6.10. *Summary and evaluation of residue behaviour*

A summary and evaluation of all data presented in this Section shall be carried out in accordance with the guidance given by the competent authorities of the Member States concerning the format of such summaries and evaluations. It shall include a detailed and critical assessment of those data in the context of relevant evaluative and decision-making criteria and guidelines, with particular reference to the risks for man and animals that may or do arise, and the extent, quality and reliability of the data base.

In particular, the toxicological significance of any non-mammalian metabolites must be addressed.

A schematic diagram shall be prepared of the metabolic pathway in plants and animals with a brief explanation of the distribution and chemical changes involved.

7. Fate and behaviour in the environment

Introduction

(i) The information provided, taken together with that for one or more preparations containing the active substance, must be sufficient to permit an assessment of the fate and behaviour of the active substance in the environment, and of the non-target species

likely to be at risk from exposure to the active substance, its metabolites, degradation and reaction products, where they are of toxicological or environmental significance.

(ii) In particular, the information provided for the active substance, together with other relevant information, and that provided for one or more preparations containing it shall be sufficient to:

— decide whether, or not, the active substance can be approved,

— specify appropriate conditions or restrictions to be associated with any approval,

— classify the active substance as to hazard,

— specify the pictograms, signal words and relevant hazard and precautionary statements for the protection of the environment, which are to be included on packaging (containers),

— predict the distribution, fate, and behaviour in the environment of the active substance and relevant metabolites, degradation and reaction products as well as the times courses involved,

— identify non-target species and populations for which hazards arise because of potential exposure, and

— identify measures necessary to minimise contamination of the environment and impact on non-target species.

(iii) A detailed description (specification) of the material used, as provided for under point 1.11 must be provided. Where testing is done using active substance the material used shall be of that specification that will be used in the manufacture of preparations to be authorised except where radio-labelled material is used.

Where studies are conducted using active substance produced in the laboratory or in a pilot plant production system, the studies must be repeated using active substance as manufactured, unless it can be justified that the test material used is essentially the same for the purposes of environmental testing and assessment.

(iv) Where radio-labelled test material is used, radio-labels shall be positioned at sites (one or more as necessary), to facilitate elucidation of metabolic and degradative pathways and to facilitate investigation of the distribution of the active substance and of its metabolite, reaction and degradation products in the environment.

(v) It may be necessary to conduct separate studies for metabolites, degradation or reaction products, where these products can constitute a relevant risk to non-target organisms or to the quality of water, soil and air and where their effects cannot be evaluated by the available results relating to the active substance. Before such studies are performed the information from the Sections 5 and 6 has to be taken into account.

(vi) Where relevant, tests shall be designed and data analysed using appropriate statistical methods.

Full details of the statistical analysis shall be reported (e.g. all point estimates shall be given with confidence intervals, exact p-values shall be given rather than stating significant/non-significant).

7.1. *Fate and behaviour in soil*

All relevant information on the type and the properties of the soil used in the studies, including pH, organic carbon content, cation exchange capacity, particle size distribution and water holding capacity, at pF = 0 and pF = 2,5 has to be reported in accordance with relevant ISO or other international standards.

The microbial biomass of soils used for laboratory degradation studies must be determined just prior to the commencement and at the end of the study.

It is recommended to use as much as possible the same soils throughout all laboratory soil studies.

The soils used for degradation or mobility studies must be selected such that they are representative of the range of soils typical of the various EU regions where use exists or is anticipated, and be such that:

— they cover a range of organic carbon content, particle size distribution and pH values, and
— where on the basis of other information, degradation or mobility are expected to be pH dependent (e.g. solubility and hydrolysis rate — points 2.7 and 2.8), they cover the following pH ranges:
  — 4,5 to 5,5,
  — 6 to 7, and
  — 8 (approximately).

Soils used must, wherever possible, be freshly sampled. If use of stored soils is unavoidable, storage shall be properly carried out for a limited time under defined and reported conditions. Soils stored for longer periods of time can only be used for adsorption/desorption studies.

The soil chosen to begin studying shall not have extreme characteristics with respect to parameters such as particle size distribution, organic carbon content and pH.

Soils shall be collected and handled in accordance with ISO 10381-6 *(Soil quality — Sampling — Guidance on the collection, handling and storage of soil for the assessment of microbial processes in the laboratory)*. Any deviations must be reported and justified.

Field studies shall be carried out in conditions as close to normal agricultural practice as possible on a range of soil types and climatic conditions representative of the area(s) of use. Weather conditions shall be reported in cases where field studies are conducted.

7.1.1. *Route and rate of degradation*

7.1.1.1. *Route of degradation*
Aim of the tests

The data and information provided, together with other relevant data and information, shall be sufficient to:

— identify, where feasible, the relative importance of the types of process involved (balance between chemical and biological degradation),
— identify the individual components present which at any time account for more than 10 % of the amount of active substance added, including, where feasible, non-extractable residues,
— identify where possible also individual components present which account for less than 10 % of the amount of active substance added,
— establish the relative proportions of the components present (mass balance), and
— permit the soil residue of concern and to which non-target species are or may be exposed, to be defined.

Where a reference is made to non-extractable residues these are defined as chemical species originating from pesticides used in accordance with good agricultural practice that cannot be extracted by methods which do not significantly change the chemical nature of these residues.

These non-extractable residues are not considered to include fragments through metabolic pathways leading to natural products.

7.1.1.1.1. Aerobic degradation
Circumstances in which required

The degradation pathway or pathways must always be reported except where the nature and manner of use of preparations containing the active substance, preclude soil contamination such as uses on stored products or wound healing treatments for trees.
Test conditions

The degradation pathway or pathways must be reported for one soil.

Results obtained must be presented in the form of schematic drawings showing the pathways involved, and in the form of balance sheets which show the distribution of radio-label as a function of time, as between:

— active substance,
— CO_2,
— volatile compounds other than CO_2,
— individual identified transformation products,
— extractable substances not identified, and
— non-extractable residues in soil.

The investigation of degradation pathways must include all feasible steps to characterise and quantify non-extractable residues formed after 100 days when exceeding 70 % of the applied dose of the active substance. The techniques and methodologies applied are best selected on a case-by-case basis. A justification must be provided where the compounds involved are not characterised.

The duration of the study is normally 120 days, except where after a shorter period the levels of non-extractable residues and CO_2 are such that they can be extrapolated in a reliable way to 100 days.
Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides[13].

7.1.1.1.2. Supplementary studies

— *Anaerobic degradation*
Circumstances in which required

An anaerobic degradation study must be reported unless it can be justified that exposure of the plant protection products containing the active substance to anaerobic conditions is unlikely to occur.
Test conditions and test guideline

The same provisions as provided for under the corresponding paragraphs of point 7.1.1.1.1 apply.

— *Soil photolysis*
Circumstances in which required

A soil photolysis study must be reported unless it can be justified that deposition of the active substance at the soil surface is unlikely to occur.
Test guideline

SETAC — Procedures for assessing the Environmental fate and ecotoxicity of pesticides.

7.1.1.2. *Rate of degradation*

7.1.1.2.1. Laboratory studies
Aim of the tests

The soil degradation studies shall provide best possible estimates of the time taken for degradation of 50 % and 90 % (DT_{50lab} and DT_{90lab}), of the active substance, and of relevant metabolites, degradation and reaction products under laboratory conditions.

— *Aerobic degradation*
Circumstances in which required

The rate of degradation in soil must always be reported, except where the nature and manner of use of plant protection products containing the active substance preclude soil contamination such as uses on stored products or wound healing treatments for trees.
Test conditions

The rate of aerobic degradation of the active substance in three soil types additional to that referred to in point 7.1.1.1.1 must be reported.

In order to investigate the influence of temperature on degradation, one additional study at 10 °C has to be performed on one of the soils used for the investigation of degradation at 20 °C until a validated EU calculation model for the extrapolation of degradation rates at low temperatures is available.

The duration of the study is normally 120 days except if more than 90 % of the active substance is degraded before that period expires.

Similar studies for three soil types must be reported for all relevant metabolites, degradation and reaction products which occur in soil and which at any time during the studies account for more than 10 % of the amount of active substance added, except where their DT_{50} values were able to be determined from the results of the degradation studies with the active substance.
Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

— *Anaerobic degradation*
Circumstances in which required

The rate of anaerobic degradation of the active substance must be reported where an anaerobic study has to be performed in accordance with point 7.1.1.1.2.
Test conditions

The rate of anaerobic degradation of the active substance must be carried out in the soil used in the anaerobic study performed in accordance with point 7.1.1.1.2.

The duration of the study is normally 120 days except if more than 90 % of the active substance is degraded before that period expires.

Similar studies for one soil must be reported for all relevant metobolites, degradation and reaction products which occur in soil and which at any time during the studies account for more than 10 % of the amount of active substance added, except where their DT_{50} values were able to be determined from the results of the degradation studies with the active substance.
Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

7.1.1.2.2. Field studies

— *Soil dissipation studies*

Aim of the test

The soil dissipation studies shall provide estimates of the time taken for dissipation of 50 % and 90 % (DT_{50f} and DT_{90f}), of the active substance under field conditions. Where relevant, information on relevant metabolites, degradation and reaction products must be reported.

Circumstances in which required

The tests have to be conducted in those conditions where DT_{50lab} determined at 20 °C and at a moisture content of the soil related to a pF value of 2 to 2,5 (suction pressure) is greater than 60 days.

Where plant protection products containing the active substance are intended to be used in cold climatic conditions, the tests have to be conducted where DT_{50lab}, determined at 10 °C and at a moisture content of the soil related to a pF value of 2 to 2,5 (suction pressure) is greater than 90 days.

Test conditions

Individual studies on a range of representative soils (normally four different types) must be continued until > 90 % of the amount applied have dissipated. The maximum duration of the studies is 24 months.

Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

— *Soil residue studies*

Aim of the test

Soil residue studies shall provide estimates of the soil residue levels at harvest or at time of sowing or planting succeeding crops.

Circumstances in which required

Soil residue studies must be reported where DT_{50lab} is greater than one-third of the period between the application and harvest and where absorption by the succeeding crop is possible, except where soil residues at sowing or planting of a succeeding crop can be reliably estimated from the data of the soil dissipation studies or where it can be justified that these residues can not be phytotoxic to or leave unacceptable residues in rotational crops.

Test conditions

Individual studies must be continued until harvest or time of sowing or planting succeeding crops, unless > 90 % of the amount applied have dissipated.

Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

— *Soil accumulation studies*

Aim of the tests

The tests shall provide sufficient data to evaluate the possibility of accumulation of residues of the active substance and of relevant metabolites, degradation and reaction products.

Circumstances in which required

Where on the basis of soil dissipation studies it is established that DT_{90f} > 1 year and where repeated application is envisaged, whether in the same growing season or in succeeding years,

the possibility of accumulation of residues in soil and the level at which a plateau concentration is achieved must be investigated except where reliable information can be provided by a model calculation or another appropriate assessment.
Test conditions

Long-term field studies must be done on two relevant soils and involve multiple applications.

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type of study to be performed.

7.1.2. *Adsorption and desorption*
Aim of the test

The data and information provided, together with other relevant data and information, should be sufficient to establish the adsorption coefficient of the active substance and of relevant metabolites, degradation and reaction products.
Circumstances in which required

The studies must always be reported except where the nature and manner of use of preparations containing the active substance, preclude soil contamination such as uses on stored products or wound healing trees.
Test conditions

Studies on the active substance must be reported for four soil types.

Similar studies, for at least three soil types, must be reported for all relevant metabolites, degradation and reaction products which in soil degradation studies account at any time for more than 10 % of the amount of active substance added.
Test guideline

OECD method 106.

7.1.3. *Mobility in the soil*

7.1.3.1. *Column leaching studies*
Aim of the test

The test shall provide sufficient data to evaluate the mobility and leaching potential of the active substance and if possible of relevant metabolites, degradation and reaction products.
Circumstances in which required

Studies in four soils must be carried out where in the adsorption and desorption studies provided for under point 7.1.2 it is not possible to obtain reliable adsorption coefficient values.
Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

7.1.3.2. *Aged residue column leaching*
Aim of the test

The test shall provide sufficient data to estimate the mobility and leaching potential of relevant metabolites, degradation and reaction products.
Circumstances in which required

The studies must be performed except:

— where the nature and manner of use of preparations containing the active substance, preclude soil contamination such as uses on stored products or wound healing treatments for trees, or

— where a separate study for the metabolite, degradation or reaction product in accordance with point 7.1.2 or point 7.1.3.1 was performed.

Test conditions

The period(s) of ageing shall be determined from inspection of the degradation patterns of active substance and metabolites to ensure that a relevant spectrum of metabolites is present at the time of leaching.

Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

7.1.3.3. *Lysimeter studies or field leaching studies*

Aim of the tests

The test shall provide data on:

— the mobility in soil,

— the potential for leaching to ground water,

— the potential distribution in soil.

Circumstances in which required

Expert judgement will be necessary to decide whether lysimeter studies or field leaching studies shall be carried out, taking into account the results of degradation and other mobility studies and the predicted environmental concentrations in groundwater (PEC_{GW}), calculated in accordance with Section 9 of the Annex to Regulation (EU) No 545/2011. The type and conditions of the study to be conducted shall be discussed with the competent authorities.

Test conditions

Great care is necessary in design of both experimental installations and individual studies, to ensure that results obtained can be used for assessment purposes. Studies shall cover the realistic worst case situation, taking into account the soil type, climatic conditions, the application rate and the frequency and period of application.

Water percolating from soil columns must be analysed at suitable intervals, while residues in plant material must be determined at harvest. Residues in the soil profile in at least five layers must be determined on termination of experimental work. Intermediate sampling must be avoided, since removal of plants (except for harvesting in accordance with normal agricultural practice) and soil cross influences the leaching process.

Precipitation, soil and air temperatures have to be recorded at regular intervals (at least on a weekly base).

— *Lysimeter studies*

Test conditions

The minimal depth of the lysimeters shall be 100 cm; their maximal depth shall be 130 cm. The soil monolith must be undisturbed. Soil temperatures must be similar to those pertaining in the field. Where necessary, supplementary irrigation must be provided to ensure optimal plant growth and to ensure that the quantity of infiltration water is similar to that in the regions for which authorisation is sought. When during the study the soil has to be disturbed for agricultural reasons it must not be disturbed deeper than 25 cm.

— *Field leaching studies*

Test conditions

Information on the groundwater table in the experimental fields must be submitted. If soil cracking is observed during the study this must be fully described.

Great attention shall be given to the number and the location of water collection devices. The placement of these devices in the soil shall not result in preferential flow paths.
Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

7.2. *Fate and behaviour in water and air*
Aim of the tests

The information and data provided, taken together with that provided for one or more preparations containing the active substance, and other relevant information, shall be sufficient to establish, or permit estimation of:

— persistence in water systems (bottom sediment and water, including suspended particles),
— the extent to which water, sediment organisms and air are at risk,
— potential for contamination of surface water and groundwater.

7.2.1. *Route and rate of degradation in aquatic systems (as far as not covered by point 2.9)*
Aim of the tests

The data and information provided, together with other relevant data and information, shall be sufficient to:

— identify the relative importance of the types of processes involved (balance between chemical and biological degradation),
— where possible, identify the individual components present,
— establish the relative proportions of the components present and their distribution as between water, including suspended particles, and sediment, and
— permit the residue of concern and to which non-target species are or may be exposed, to be defined.

7.2.1.1. *Hydrolytic degradation*
Circumstances in which required

The test must always be performed for relevant metabolites, degradation and reaction products which account at any time for more than 10 % of the amount of active substance added unless sufficient information on their degradation is available from the test performed in accordance with point 2.9.1.
Test conditions and test guideline

The same provisions as provided under the corresponding paragraphs of point 2.9.1 apply.

7.2.1.2. *Photochemical degradation*
Circumstances in which required

The test must always be performed for relevant metabolites, degradation and reaction products which account at any time for more than 10 % of the amount of active substance added unless sufficient information on their degradation is available from the test performed in accordance with points 2.9.2 and 2.9.3.
Test conditions and test guideline

The same provisions as provided under the corresponding paragraphs of points 2.9.2 and 2.9.3 apply.

7.2.1.3. *Biological degradation*

7.2.1.3.1. ‘Ready biodegradability’

Circumstances in which required

The test must always be performed unless it is not required under Part 4 of Annex I to Regulation (EC) No 1272/2008.
Test guideline

Method C 4 of Regulation (EC) No 440/2008.

7.2.1.3.2. Water/sediment study
Circumstances in which required

The test must be reported unless it can be justified that contamination of surface water will not occur.
Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

7.2.1.4. *Degradation in the saturated zone*
Circumstances in which required

Transformation rates in the saturated zone of active substances and of relevant metabolites, degradation and reaction products can provide useful information on the fate of these substances in the groundwater.
Test conditions

Expert judgement is required to decide whether this information is necessary. Before performing these studies the applicant shall seek the agreement of the competent authorities on the type of study to be performed.

7.2.2. *Route and rate of degradation in air (as far as not covered by point 2.10)*

Appropriate guidelines are included in the report prepared by the FOCUS[(14)] Working Group on Pesticides in Air: 'PESTICIDES IN AIR: CONSIDERATIONS FOR EXPOSURE ASSESSMENT (2008)'.

7.3. *Definition of the residue*

In the light of the chemical composition of residues occurring in soil, water or air, resulting from use, or proposed use, of a plant protection product containing the active substance a proposal for the definition of the residue must be submitted, taking account of both the levels found and their toxicological and environmental significance.

7.4. *Monitoring data*

Available monitoring data concerning fate and behaviour of the active substance and relevant metabolites, degradation and reaction products must be reported.

8. **Ecotoxicological studies**
Introduction

(i) The information provided, taken together with that for one or more preparations containing the active substance, must be sufficient to permit an assessment of the impact on non-target species (flora and fauna), likely to be at risk from exposure to the active substance, its metabolites, degradation and reaction products, where they are of environmental significance. Impact can result from single, prolonged or repeated exposure and can be reversible or irreversible.

(ii) In particular, the information provided for the active substance, together with other relevant information, and that provided for one or more preparations containing it, shall be sufficient to:

— decide whether, or not, the active substance can be approved,

— specify appropriate conditions or restrictions to be associated with any approval,

— permit an evaluation of short- and long-term risks for non-target species — populations, communities, and processes — as appropriate,

— classify the active substance as to hazard,

— specify the precautions necessary for the protection of non-target species, and

— specify the pictograms, signal words and relevant hazard and precautionary statements for the protection of the environment, to be mentioned on packaging (containers).

(iii) There is a need to report all potentially adverse effects found during routine ecotoxicological investigations and to undertake and report, where required by the competent authorities, such additional studies which may be necessary to investigate the probable mechanisms involved and assess the significance of these effects. All available biological data and information which is relevant to the assessment of the ecotoxicological profile of the active substance must be reported.

(iv) The information on fate and behaviour in the environment, generated and submitted in accordance with points 7.1 to 7.4, and on residue levels in plants generated and submitted in accordance with Section 6 is central to the assessment of impact on non-target species, in that together with information on the nature of the preparation and its manner of use, it defines the nature and extent of potential exposure. The toxicokinetic and toxicological studies and information submitted in accordance with points 5.1 to 5.8 provide essential information as to toxicity to vertebrate species and the mechanisms involved.

(v) Where relevant, tests shall be designed and data analysed using appropriate statistical methods. Full details of the statistical analysis shall be reported (e. g. all point estimates shall be given with confidence intervals, exact p-values should be given rather than stating significant/non-significant).

Test substance

(vi) A detailed description (specification) of the material used, as provided for under point 1.11 must be provided. Where testing is done using active substance the material used shall be of that specification that will be used in the manufacture of preparations to be authorised except where radiolabelled material is used.

(vii) Where studies are conducted using active substance produced in the laboratory or in a pilot plant production system, the studies must be repeated using active substance as manufactured, unless it can be justified that the test material used is essentially the same, for the purposes of ecotoxicological testing and assessment. In cases of uncertainty, appropriate bridging studies must be submitted to serve as a basis for a decision as to the possible need for repetition of the studies.

(viii) In the case of studies in which dosing extends over a period, dosing shall preferably be done using a single batch of active substance if stability permits.

Whenever a study implies the use of different doses, the relationship between dose and adverse effect must be reported.

(ix) For all feeding studies, average achieved dose must be reported, including where possible the dose in mg/kg body weight. Where dosing via the diet is utilised the test compound must be distributed uniformly in the diet.

(x) It may be necessary to conduct separate studies for metabolites, degradation or reaction products, where these products can constitute a relevant risk to non-target organisms and where their effects cannot be evaluated by the available results relating to the active substance. Before such studies are performed the information from Sections 5, 6 and 7 has to be taken into account.

Test organisms

(xi) In order to facilitate the assessment of the significance of test results obtained, including the estimation of intrinsic toxicity and the factors affecting toxicity, the same strain (or recorded origin) of each relevant species shall, where possible, be used in the various toxicity tests specified.

8.1. *Effects on birds*

8.1.1. *Acute oral toxicity*
Aim of the test

The test shall provide, where possible, LD_{50} values, the lethal threshold dose, time courses of response and recovery and the NOEL, and must include relevant gross pathological findings.
Circumstances in which required

The possible effects of the active substance on birds must be investigated except where the active substance is intended solely to be included in preparations for exclusive use in enclosed spaces (e.g. in glasshouses or in food storage practice).
Test conditions

The acute oral toxicity of active substance to a quail species (Japanese quail *(Coturnix coturnix japonica)* or Bobwhite quail *(Colinus virginianus)*) or to mallard duck *(Anas platyrhynchos)* must be determined. The highest dose used in tests need not exceed 2 000 mg/kg body weight.
Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

8.1.2. *Short-term dietary toxicity*
Aim of the test

The test shall provide the short-term dietary toxicity (LC_{50} values, lowest lethal concentration (LLC), where possible no observed effect concentrations (NOEC), time courses of response and recovery) and include relevant gross pathological findings.
Circumstances in which required

The dietary (5-day) toxicity of the active substance to birds must always be investigated on one species except where a study in accordance with point 8.1.3 is reported. Where its acute oral NOEL is $\leq$ 500 mg/kg body weight or where the short-term NOEC is < 500 mg/kg food the test must be performed on a second species.
Test conditions

The first species to be studied must be either a quail species or mallard duck. If a second species must be tested it shall not be related to the first species tested.
Test guideline

The test must be carried out in accordance with OECD Method 205.

8.1.3. *Subchronic toxicity and reproduction*
Aim of the test

The test shall provide the subchronic toxicity and reproductive toxicity of the active substance to birds.
Circumstances in which required

The subchronic and reproductive toxicity of the active substance to birds must be investigated, unless it can be justified that continued or repeated exposure of adults, or exposure of nest sites during the breeding season is unlikely to occur.
Test guideline

The test must be carried out in accordance with OECD Method 206.

8.2. *Effects on aquatic organisms*

The data of the tests referred to in points 8.2.1, 8.2.4 and 8.2.6 have to be submitted for every active substance even when it is not expected that plant protection products containing it could reach surface water following the proposed conditions of use. These data are required under Part 4 of Annex I to Regulation (EC) No 1272/2008.

Data reported must be supported with analytical data on concentrations of the test substance in the test media.

8.2.1. *Acute toxicity to fish*
Aim of the test

The test shall provide the acute toxicity (LC_{50}), and details of observed effects.
Circumstances in which required

The test must always be carried out.
Test conditions

The acute toxicity of the active substance must be determined for rainbow trout *(Oncorhynchus mykiss)* and for a warm water fish species. Where tests with metabolites, degradation or reaction products have to be performed the species used must be the more sensitive of the two species tested with the active substance.
Test guideline

The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method C 1.

8.2.2. *Chronic toxicity to fish*
Circumstances in which required

A chronic toxicity study must be carried out unless it can be justified that continued or repeated exposure of fish is unlikely to occur or unless a suitable microcosm or mesocosm study is available.

Expert judgment is required to decide which test has to be performed. In particular for active substance for which there are indications of particular concerns (related to the toxicity of the active substance for fish or the potential exposure) the applicant shall seek the agreement of the competent authorities on the type of test to be performed.

A fish early life stage toxicity test might be appropriate where bioconcentration factors (BCF) are between 100 and 1 000 or where EC_{50} of the active substance < 0,1 mg/l.

A fish life cycle test might be appropriate in cases where:

— the bioconcentration factor is greater than 1 000 and the elimination of the active substance during a depuration phase of 14 days is lower than 95 %, or

— the substance is stable in water or sediment (DT_{90} > 100 days).

It is not necessary to perform a chronic toxicity test on juvenile fish when a fish early life stage toxicity test or a fish life cycle test has been performed; it is likewise not necessary to perform a fish early life stage toxicity test when a fish life cycle test has been performed.

8.2.2.1. *Chronic toxicity test on juvenile fish*
Aim of the test

The test shall provide effects on growth, the threshold level for lethal effects and for observed effects, the NOEC and details of observed effects.
Test conditions

The test must be conducted on juvenile rainbow trout, following exposure of 28 days to the active substance. Data on the effects on growth and behaviour must be generated.

8.2.2.2. *Fish early life stage toxicity test*
Aim of the test

The test shall provide effects on development, growth and behaviour, the NOEC and details of observed effects on fish early life stages.
Test guideline

The test must be carried out in accordance with OECD Method 210.

8.2.2.3. *Fish life cycle test*
Aim of the test

The test will provide effects on reproduction of the parental and the viability of the filial generation.
Test conditions

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.2.3. *Bioconcentration in fish*
Aim of the test

The test shall provide the steady-state bioconcentration factors, uptake rate constants and depuration rate constants, calculated for each test compound, as well as relevant confidence limits.
Circumstances in which required

The bioconcentration potential of active substances, of metabolites and of degradation and reaction products, likely to partition into fatty tissues (such as log $p_{ow} \geq 3$ — see point 2.8 or other relevant indications of bioconcentration), must be investigated and be reported, unless it can be justified that exposure leading to bioconcentration is not likely to occur.
Test guideline

The test must be carried out in accordance with OECD Method 305E.

8.2.4. *Acute toxicity to aquatic invertebrates*
Aim of the test

The test shall provide the 24 and 48-hour acute toxicity of the active substance, expressed as the median effective concentration (EC_{50}) for immobilisation, and where possible the highest concentration causing no immobilisation.
Circumstances in which required

The acute toxicity must always be determined for *Daphnia* (preferably *Daphnia magna*). Where plant protection products containing the active substance are intended to be used directly on surface water additional data have to be reported on at least one representative species from each of the following groups: aquatic insects, aquatic crustaceans (on a species not related to *Daphnia*) and aquatic gastropod molluscs.
Test guideline

The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method C 2.

8.2.5. *Chronic toxicity to aquatic invertebrates*
Aim of the test

The test shall provide where possible EC_{50} values for effects such as immobilisation and reproduction and the highest concentration at which no effect such as on mortality or reproduction occurs (NOEC) and details of observed effects.
Circumstances in which required

A test on *Daphnia* and on at least one representative aquatic insect species and an aquatic gastropod mollusc species must be carried out unless it can be justified that continued or repeated exposure is not likely to occur.
Test conditions

The test with *Daphnia* must be continued for 21 days.
Test guideline

The test must be carried out in accordance with OECD Method 202, Part II.

8.2.6. *Effects on algal growth*
Aim of the test

The test shall provide EC_{50} values for growth and growth rate, NOEC values, and details of observed effects.
Circumstances in which required

Possible effects on algal growth of active substances must always be reported.

For herbicides a test on a second species from a different taxonomic group has to be performed.
Test guideline

The test must be carried out in accordance with the Annex to Regulation (EC) No 440/2008, Method C 3.

8.2.7. *Effects on sediment dwelling organisms*
Aim of test

The test will measure effects on survival and development (including effects on emergence of adults for *Chironomus*), the relevant EC_{50} values and the NOEC values.
Circumstances in which required

Where environmental fate and behaviour data required in Section 7 report that an active substance is likely to partition to and persist in aquatic sediments, expert judgement shall be

used to decide whether an acute or a chronic sediment toxicity test in required. Such expert judgement shall take into account whether effects on sediment dwelling invertebrates are likely by comparing the aquatic invertebrate toxicity EC_{50} data from points 8.2.4 and 8.2.5 with the predicted levels of the active substances in sediment from data in Section 9 of the Annex to Regulation (EU) No 545/2011.
Test conditions

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.2.8. *Aquatic plants*

A test on aquatic plants has to be performed for herbicides.

Before performing these studies the applicant shall seek the agreement of the competent authorities on the type and conditions of the study to be performed.

8.3. *Effect on arthropods*

8.3.1. *Bees*

8.3.1.1. *Acute toxicity*
Aim of the test

The test shall provide the acute oral and contact LD_{50} value of the active substance.
Circumstances in which required

Potential impact on bees must be investigated, except where preparations containing the active substance are for exclusive use in situations where bees are not likely to be exposed such as:
— food storage in enclosed spaces,
— non-systemic seed dressings,
— non-systemic preparations for application to soil,
— non-systemic dipping treatments for transplanted crops and bulbs,
— wound sealing and healing treatments,
— rodenticidal baits,
— use in glasshouses without pollinators.
Test guideline

The test must be carried out in accordance with EPPO Guideline 170.

8.3.1.2. *Bee brood feeding test*
Aim of the test

The test shall provide sufficient information to evaluate possible risks from the plant protection product on honeybee larvae.
Circumstances in which required

The test must be carried out when the active substance may act as an insect growth regulator unless it can be justified that it is not likely that bee brood would be exposed to it.
Test guideline

The test must be carried out in accordance with ICPBR Method (e.g. P. A. Oomen, A. de Riujter and J. van der Steen. Method for honeybee brood feeding tests with insect growth-regulating insecticides. *EPPO Bulletin*, Volume 22, pp. 613 to 616, 1992.)

8.3.2. *Other arthropods*

Aim of the test

The test shall provide sufficient information to evaluate the toxicity (mortality and sublethal effects) of the active substance to selected arthropod species.
Circumstances in which required

Effects on non-target terrestrial arthropods (e.g. predators or parasitoids of harmful organisms) must be investigated. The information obtained for these species can also be used to indicate the potential for toxicity to other non-target species inhabiting the same environment. This information is required for all active substances except where preparations containing the active substance are for exclusive use in situations where non-target arthropods are not exposed such as:

— food storage in enclosed spaces,

— wound sealing and healing treatments,

— rodenticidal baits.

Test conditions

The test must be performed initially in the laboratory on an artificial substrate (i.e. glass plate or quartz sand, as appropriate) unless adverse effects can be clearly predicted from other studies. In these cases, more realistic substrates may be used.

Two sensitive standard species, a parasitoid and predatory mite (e.g. *Aphidius rhopalosiphi* and *Typhlodromus pyri*) shall be tested. In addition to these, two additional species must also be tested, which shall be relevant to the intended use of the substance. Where possible and if appropriate, they shall represent the other two major functional groups, ground dwelling predators and foliage dwelling predators. Where effects are observed with species relevant to the proposed use of the product, further testing may be carried out at the extended laboratory/ semi-field level. Selection of the relevant test species shall follow the proposals outlined in SETAC — Guidance document on regulatory testing procedures for pesticides with non-target arthropods[(15)]. Testing must be conducted at rates equivalent to the highest rate of field application to be recommended.
Test guideline

Where relevant, testing shall be done in accordance with appropriate guidelines which satisfy at least the requirements for testing as included in SETAC — Guidance document on regulatory testing procedures for pesticides with non-target arthropods.

8.4. *Effects on earthworms*

8.4.1. *Acute toxicity*
Aim of the test

The test shall provide the LC_{50} value of the active substance to earthworms, where possible the highest concentration causing no mortality and the lowest concentration causing 100 % mortality, and must include observed morphological and behavioural effects.
Circumstances in which required

Effects on earthworms must be investigated, where preparations containing the active substance are applied to soil, or can contaminate soil.
Test guideline

The test must be carried out in accordance with Annex to Regulation (EC) No 440/2008, Method C 8, Toxicity for earthworms: Artificial soil test.

8.4.2. *Sublethal effects*
Aim of the test

The test shall provide the NOEC and the effects on growth, reproduction and behaviour.
Circumstances in which required

Where on the basis of the proposed manner of use of preparations containing the active substance or on the basis of its fate and behaviour in soil (DT_{90} > 100 days), continued or repeated exposure of earthworms to the active substance, or to significant quantities of metabolites, degradation or reaction products, can be anticipated expert judgement is required to decide whether a sublethal test can be useful.
Test conditions

The test must be carried out on *Eisenia foetida*.

8.5. *Effects on soil non-target micro-organisms*
Aim of the test

The test shall provide sufficient data to evaluate the impact of the active substance on soil microbial activity, in terms of nitrogen transformation and carbon mineralisation.
Circumstances in which required

The test must be carried out where preparations containing the active substance are applied to soil or can contaminate soil under practical conditions of use. In the case of active substances intended for use in preparations for soil sterilisation, the studies must be designed to measure rates of recovery following treatment.
Test conditions

Soils used must be freshly sampled agricultural soils. The sites from which soil is taken must not have been treated during the previous 2 years with any substance that could substantially alter the diversity and levels of microbial populations present, other than in a transitory manner.
Test guideline

SETAC — Procedures for assessing the environmental fate and ecotoxicity of pesticides.

8.6. *Effects on other non-target organisms (flora and fauna) believed to be at risk*

A summary of available data from preliminary tests used to assess the biological activity and dose range finding, whether positive or negative, which may provide information with respect to possible impact on other non-target species, both flora and fauna, must be provided, together with a critical assessment as to its relevance to potential impact on non-target species.

8.7. *Effects on biological methods for sewage treatment*

Effects on biological methods for sewage treatment must be reported where the use of plant protection products containing the active substance can give rise to adverse effects on sewage treatment plants.

9. **Summary and evaluation of Sections 7 and 8**

10. **Proposals including justification for the proposals for the classification and labelling of the active substance in accordance with Regulation (EC) No 1272/2008**

— Pictogram(s)
— Signal words
— Hazard statements
— Precautionary Statements.

11. **A dossier as referred to in Part A of the Annex to Regulation (EU) No 545/2011, for a representative plant protection product**

PART B

MICRO-ORGANISMS INCLUDING VIRUSES

Introduction

(i) Active substances are defined in Article 2(2) of Regulation (EC) No 1107/2009 and include chemical substances and micro-organisms including viruses.

This Part provides data requirements for active substances consisting of micro-organisms, including viruses.

The term 'micro-organism' as defined in Article 3 of Regulation (EC) No 1107/2009 applies to, but is not limited to, bacteria, fungi, protozoa, viruses and viroids.

(ii) For all micro-organisms that are subject to application all available relevant knowledge and information in literature should be provided.

The most important and informative information is obtained by the characterisation and identification of a micro-organism. Such information is found in Sections 1 to 3 (identity, biological properties and further information) which form the basis for an assessment of human health and environmental effects.

Newly generated data from conventional toxicological and/or pathological experiments on laboratory animals are normally required unless the applicant can justify, on the basis of the previous information, that the use of the micro-organism, under the proposed conditions of use, does not have any harmful effects on human and animal health or on groundwater or any unacceptable influence on the environment.

(iii) Pending the acceptance of specific guidelines at international level, the information required shall be generated using available test guidelines accepted by the competent authority (e.g. USEPA guideline[16]; where appropriate, test guidelines as described in Part A of this Annex should be adapted in such a way that they are appropriate for micro-organisms. Testing shall include viable and, if appropriate, non-viable micro-organisms, and a blank control.

(iv) Where testing is done, a detailed description (specification) of the material used and its impurities, in accordance with point 1.4, must be provided. The material used shall be of that specification that will be used in the manufacture of preparations to be authorised.

Where studies are conducted using micro-organisms produced in the laboratory or in a pilot plant production system, the studies must be repeated using micro-organisms as manufactured, unless it can be demonstrated that the test material used is essentially the same for the purposes of the testing and assessment.

(v) Where the micro-organism has been genetically modified, a copy of the evaluation of the data concerning the assessment of risk to the environment, as stated in Article 48 to Regulation (EC) No 1107/2009, has to be submitted.

(vi) Where relevant, data shall be analysed using appropriate statistical methods. Full details of the statistical analysis shall be reported (e.g. all point estimates shall be given with confidence intervals, exact p-values should be given rather than stating significant/non-significant).

(vii) In the case of studies in which dosing extends over a period, dosing shall preferably be done using a single batch of the micro-organism, if stability permits.

If the studies are not performed using a single batch of the micro-organism, the similarity of the different batches has to be stated.

Whenever a study implies the use of different doses, the relationship between dose and adverse effect must be reported.

(viii) If the plant protection action is known to be due to the residual effect of a toxin/metabolite or if significant residues of toxins/metabolites are to be expected not related to the effect of the active substance, a dossier for the toxin/metabolite has to be submitted in accordance with the requirements of Part A of this Annex,.

1. **Identity of the micro-organism**

The identification together with the characterisation of the micro-organism provides the most important information and is a key point for decision-making.

1.1. *Applicant*

The name and address of the applicant must be provided, as must the name, position, telephone and fax number of the appropriate person to contact.

Where, in addition, the applicant has an office, agent or representative in the Member State to which the application for approval is submitted, and if different, in the rapporteur Member State appointed by the Commission, the name and address of the local office, agent or representative must be provided, as must the name, position, telephone and fax number of the appropriate person to contact.

1.2. *Producer*

The name and address of the producer or producers of the micro-organism must be provided as must the name and address of each plant in which the micro-organism is produced. A contact point (preferably a central contact point, to include name, telephone and fax number) must be provided, with a view to providing updating information and responding to queries arising, regarding production technology, processes and the quality of product (including where relevant, individual batches). Where, following approval of the micro-organism, there are changes in the location or number of producers, the information required must again be notified to the Commission and the Member States.

1.3. *Name and species description, strain characterisation*

(i) The micro-organism should be deposited at an internationally recognised culture collection and given an accession number and these details must be submitted.

(ii) Each micro-organism that is subject to the application shall be identified and named at the species level. The scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism, must be stated.

It must be indicated whether the micro-organism:

— is indigenous or non-indigenous at the species level to the intended area of application,

— is a wild type,

— is a spontaneous or induced mutant,

— has been modified, using techniques described in Part 2 of Annex IA and in Annex IB to Directive 2001/18/EC of the European Parliament and of the Council[17].

In the latter two cases, all known differences between the modified micro-organism and the parent wild strain must be provided.

(iii) Best available technology should be used to identify and characterise the micro-organism at the strain level. The appropriate test procedures and criteria used for identification (e.g. morphology, biochemistry, serology, molecular identification) must be provided.

(iv) Common name or alternative and superseded names and code names used during the development, if any, must be provided.

(v) Relationships to known pathogens shall be indicated.

1.4. *Specification of the material used for manufacturing of formulated products*

1.4.1. *Content of the micro-organism*

The minimum and maximum content of the micro-organism in the material used for manufacturing of formulated products, must be reported. The content shall be expressed in appropriate terms, such as number of active units per volume or weight or any other manner that is relevant to the micro-organism.

Where the information provided relates to a pilot plant production system, the information required must again be provided to the Commission and the Member States once industrial scale production methods and procedures have stabilised, if production changes result in a changed specification of purity.

1.4.2. *Identity and content of impurities, additives, contaminating micro-organisms*

It is desirable to have a plant protection product without contaminants (including contaminating micro-organisms), if possible. The level and nature of acceptable contaminants shall be judged from a risk assessment point of view, by the competent authority.

If possible and appropriate, the identity and maximum content of all contaminating micro-organisms, expressed in the appropriate unit, must be reported. The information on identity must be provided where possible as outlined in point 1.3 of Part B of this Annex.

Relevant metabolites (i.e. if expected to be of concern to human health and/or the environment) known to be formed by the micro-organism shall be identified and characterised at different states or growth stages of the micro-organism (see point (viii) of the introduction to this Part).

Where relevant detailed information on all components such as condensates, culture medium, etc. must be provided.

In the case of chemical impurities that are relevant for human health and/or the environment, the identity and maximum content, expressed in appropriate terms, must be provided.

In the case of additives, the identity and content in g/kg must be provided.

The information on identity of chemical substances such as additives must be provided as outlined in point 1.10 of Part A of this Annex.

1.4.3. *Analytical profile of batches*

Where relevant, the same data as outlined in point 1.11 of Part A of this Annex have to be reported, using the appropriate units.

2. Biological properties of the micro-organism

2.1. *History of the micro-organism and its uses. Natural occurrence and geographical distribution*

Familiarity, interpreted as the availability of relevant knowledge of the micro-organism, shall be presented.

2.1.1. *Historical background*

The historical background of the micro-organism and its use (tests/research projects or commercial use) must be provided.

2.1.2. *Origin and natural occurrence*

The geographical region and the place in the ecosystem (e.g. host plant, host animal, or soil from which the micro-organism was isolated) must be stated. The method of isolation of the micro-organism shall be reported. The natural occurrence of the micro-organism in the relevant environment shall be given if possible at strain level.

In the case of a mutant, or a genetically modified micro-organism, detailed information should be provided on its production and isolation and on the means by which it can be clearly distinguished from the parent wild strain.

2.2. *Information on target organism(s)*

2.2.1. *Description of the target organism(s)*

Where relevant, details of harmful organisms against which protection is afforded, must be provided.

2.2.2. *Mode of action*

The principal mode of action shall be indicated. In connection with the mode of action it shall also be stated if the micro-organism produces a toxin with a residual effect on the target organism. In that case, the mode of action of this toxin shall be described.

If relevant, information on the site of infection and mode of entry into the target organism and its susceptible stages shall be given. The results of any experimental studies must be reported.

It shall be stated by which way an uptake of the micro-organism, or its metabolites (especially toxins) may occur (e.g. contact, stomach, inhalation). It must also be stated whether or not the micro-organism or its metabolites are translocated in plants and, where relevant, how this translocation takes place.

In case of pathogenic effect on the target organism, infective dose (the dose needed to cause infection with the intended effect on a target species) and transmissibility (possibility of spread of the micro-organism in the target population, but also from one target species to another (target) species) after application under the proposed condition of use shall be indicated.

2.3. *Host specificity range and effects on species other than the target harmful organism*

Any available information on the effects on non-target organisms within the area to which the micro-organism may spread shall be given. The occurrence of non-target organisms being either closely related to the target species or being especially exposed shall be indicated.

Any experience of the toxic effect of the active substance or its metabolic products on humans or animals, of whether the organism is capable of colonising or invading humans or animals (including immunosuppressed individuals) and whether it is pathogenic shall be stated. Any experience of whether the active substance or its products may irritate skin, eyes or respiratory organs of humans or animals and whether it is allergenic in contact with skin or when inhaled shall be stated.

2.4. *Development stages/life cycle of the micro-organism*

Information on the life cycle of the micro-organism, described symbiosis, parasitism, competitors, predators, etc. including host organisms, as well as vectors for viruses, must be presented.

The generation time and the type of reproduction of the micro-organism must be stated.

Information on the occurrence of resting stages and their survival time, their virulence and infection potential must be provided.

The potential of the micro-organism to produce metabolites, including toxins that are of concern for human health and/or the environment, in its different development stages after the release, must be indicated.

2.5. *Infectiveness, dispersal and colonisation ability*

The persistence of the micro-organism and information on its life cycle under the typical environmental conditions of use must be indicated. In addition, any particular sensitivity of the micro-organism to certain compartments of the environment (e.g. UV light, soil, water) must be stated.

The environmental requirements (temperature, pH, humidity, nutrition requirements, etc.) for survival, reproduction, colonisation, damage (including human tissues) and effectiveness of the micro-organism must be stated. The presence of specific virulence factors shall be indicated.

The temperature range at which the micro-organism grows must be determined, including minimum, maximum and optimum temperatures. This information is of particular value as a trigger for studies of effects on human health (Section 5).

The possible effect of factors such as temperature, UV light, pH, and the presence of certain substances on the stability of relevant toxins must also be stated.

Information on possible dispersal routes of the micro-organism (via air as dust particles or aerosols, with host organisms as vectors, etc.), under typical environmental conditions relevant to the use, must be provided.

2.6. *Relationships to known plant or animal or human pathogens*

The possible existence of one or more species of the genus of the active and/or, where relevant, contaminating micro-organisms known to be pathogenic to humans, animals, crops or other non-target species and the type of disease caused by them shall be indicated. It shall be stated whether it is possible, and if so, by which means to clearly distinguish the active micro-organism from the pathogenic species.

2.7. *Genetic stability and factors affecting it*

Where appropriate, information on genetic stability (e.g. mutation rate of traits related to the mode of action or uptake of exogenous genetic material) under the environmental conditions of proposed use must be provided.

Information must also be provided on the micro-organism's capacity to transfer genetic material to other organisms as well as its capacity to being pathogenic for plants, animals or man. If the micro-organism carries relevant additional genetic elements, the stability of the encoded traits shall be indicated.

2.8. *Information on the production of metabolites (especially toxins)*

If other strains belonging to the same microbial species as the strain subject to the application are known to produce metabolites (especially toxins) with unacceptable effects on human health and/or the environment during or after application, the nature and structure of this substance, its presence inside or outside the cell and its stability, its mode of action (including external and internal factors of the micro-organism necessary to action) as well as its effect on humans, animals or other non-target species shall be provided.

The conditions under which the micro-organism produces the metabolite(s) (especially toxin(s)) must be described.

Any available information on the mechanism by which the micro-organisms regulate the production of the(se) metabolite(s) shall be provided.

Any available information on the influence of the produced metabolites on the micro-organism's mode of action shall be provided.

2.9. *Antibiotics and other anti-microbial agents*

Many micro-organisms produce some antibiotic substances. Interference with the use of antibiotics in human or veterinary medicine must be avoided at any stage of the development of a microbial plant protection product.

Information on the micro-organism's resistance or sensitivity to antibiotics or other anti-microbial agents must be provided, in particular the stability of the genes coding for antibiotic resistance, unless it can be justified that the micro-organism has no harmful effects on human or animal health, or that it can not transfer its resistance to antibiotics or other anti-microbial agents.

3. Further information on the micro-organism

Introduction

(i) The information provided must describe the intended purposes for which preparations containing the micro-organism are used, or are to be used and the dose and manner of their use or proposed use.

(ii) The information provided must specify the normal methods and precautions to be followed in the handling, storage and transport of the micro-organism.

(iii) The studies, data and information submitted, must demonstrate the suitability of measures proposed for use in emergency situations.

(iv) The information and data referred to are required for each micro-organism, except where otherwise specified.

3.1. *Function*

The biological function must be specified from among the following:

— control of bacteria,
— control of fungi,
— control of insects,
— control of mites,
— control of molluscs,
— control of nematodes,
— control of weeds,
— other (must be specified).

3.2. *Field of use envisaged*

The field(s) of use, existing and proposed, for preparations containing the micro-organism must be specified from among the following:

— field use, such as agriculture, horticulture, forestry, and viticulture,
— protected crops (e.g. in greenhouses),
— amenity,
— weed control on non-cultivated areas,
— home gardening,
— house plants,
— stored products,
— other (specify).

3.3. *Crops or products protected or treated*

Details of existing and intended use in terms of crops, groups of crops, plants, or plant products protected, must be provided.

3.4. *Method of production and quality control*

Full information on how the micro-organism is produced in bulk must be provided.

Both production method/process and product must be subject to a continuous quality control by the applicant. In particular, the occurrence of spontaneous changing of major characteristics of the micro-organism and of the absence/presence of significant contaminants shall be monitored. The quality assurance criteria for the production shall be submitted.

The techniques used to ensure a uniform product, and the assay methods for its standardisation, maintenance and purity of the micro-organism must be described and specified (e.g. HACCP).

3.5. *Information on the occurrence or possible occurrence of the development of resistance of the target organism(s)*

Available information on the possible occurrence of the development of resistance or cross-resistance of the target organism(s) must be provided. Where possible, appropriate management strategies shall be described.

3.6. *Methods to prevent loss of virulence of seed stock of the micro-organism*

Methods to prevent loss of virulence of starting cultures shall be provided.

In addition, any method, if available, that could prevent the micro-organism from losing its effects on the target species must be described.

3.7. *Recommended methods and precautions concerning handling, storage, transport or fire*

A safety data sheet pursuant to Article 31 of Regulation (EC) No 1907/2006 must be provided for each micro-organism.

3.8. *Procedures for destruction or decontamination*

In many cases the preferred or sole means of safe disposal of micro-organisms, contaminated materials, or contaminated packaging, is through controlled incineration in a licensed incinerator.

Methods to dispose safely of the micro-organism or, where necessary, to kill it prior to disposal, and methods to dispose of contaminated packaging and contaminated materials, must be fully described. Data must be provided for such methods to establish their effectiveness and safety.

3.9. *Measures in case of an accident*

Information on procedures for rendering the micro-organism harmless in the environment (e.g. water or soil) in case of an accident must be provided.

4. Analytical methods

Introduction

The provisions of this Section only cover analytical methods required for post-registration control and monitoring purposes.

Post-approval monitoring might be considered for all areas of risk assessment. This is particularly the case when (strains of) micro-organisms that are non-indigenous to the intended area of application are considered for approval. For analytical methods used for generation of data as required in this Regulation or for other purposes the applicant has to provide a justification for the method used; where necessary separate guidance will be developed for such methods on the basis of the same requirements as defined for methods for post-registration control and monitoring purposes.

Descriptions of methods must be provided and include details of equipment, materials and conditions used. The applicability of any internationally recognised method must be reported.

As far as practicable these methods must employ the simplest approach, involve the minimum cost, and require commonly available equipment.

Data on specificity, linearity, accuracy and repeatability, as defined in points 4.1 and 4.2 of Part A of this Annex, are also required for methods used to analyse micro-organisms and their residues.

For this Section the following applies:

Impurities, Metabolites, Relevant metabolites, Residues	As defined in Regulation (EC) No 1107/2009
Relevant impurities	Impurities, as defined above, that are of concern for human or animal health and/or the environment

On request the following samples must be provided:

(i) samples of the micro-organism as manufactured;

(ii) analytical standards of relevant metabolites (especially toxins) and all other components included in the residue definition;

(iii) if available, samples of reference substances for the relevant impurities.

4.1. *Methods for the analysis of the micro-organism as manufactured*

— Methods for the identification of the micro-organism,

— Methods for providing information on possible variability of seed stock/active micro-organism,

— Methods to differentiate a mutant of the micro-organism from the parent wild strain,

— Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity,
— Methods to determine the content of the micro-organism in the manufactured material used for the production of formulated products and methods to show that contaminating micro-organisms are controlled to an acceptable level,
— Methods for the determination of relevant impurities in the manufactured material,
— Methods to control the absence and to quantify (with appropriate limits of determination) the possible presence of any human and mammalian pathogens,
— Methods to determine storage stability, shelf-life of the micro-organism, if appropriate.

4.2. *Methods to determine and quantify residues (viable or non-viable)*

of:
— the active micro-organism(s),
— relevant metabolites (especially toxins).

on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant.

Analytical methods for amount or activity of proteinaceous products shall also be included, e.g. by testing exponential cultures and culture supernatants in an animal cell bioassay.

5. **Effects on human health**

Introduction

(i) Available information based on the properties of the micro-organism and corresponding organisms (Sections 1, 2 and 3), including health and medical reports may be sufficient for a decision whether the micro-organism would cause health effects (infectious/pathogenic/toxic) in humans or not.

(ii) The information provided, taken together with that provided for one or more preparations containing the micro-organism, must be sufficient to permit an evaluation to be made as to the risks for man, directly and/or indirectly associated with the handling and use of plant protection products containing the micro-organism, and the risk for man handling treated products, and the risk for man arising from residual traces or contaminants remaining in food and water. In addition, the information provided must be sufficient to:
— permit a decision to be made as to whether, or not, the micro-organism can be approved,
— specify appropriate conditions or restrictions to be associated with any approval,
— specify risk and safety phrases (once introduced) for the protection of man, animals and the environment to be included on packaging (containers),
— identify relevant first aid measures as well as appropriate diagnostic and therapeutic measures to be followed in the event of infection or another adverse effect in man.

(iii) All effects found during investigations shall be reported. Investigations which may be necessary in order to evaluate the probable mechanism involved, and to assess the significance of these effects, must also be performed.

(iv) For all studies actual achieved dose in colony forming units per kg body weight (cfu/kg), as well as in other appropriate units, must be reported.

(v) Evaluation of the micro-organism shall be carried out in a tier-wise manner.

The first tier (Tier I) includes available basic information and basic studies, which have to be performed for all micro-organisms. Expert judgment will be necessary to decide about the appropriate test programme on a case-by-case basis. Newly generated data from conventional toxicological and/or pathological experiments on laboratory animals are normally required unless the applicant can justify, on the basis of the previous information, that the use of the micro-organism, under the proposed conditions of use, does not have any harmful effects on human and animal health. Pending the acceptance of specific guidelines at international level, the information required shall be generated using available test guidelines (e.g. USEPA OPPTS Guidelines).

Tier II studies must be conducted if tests under Tier I have shown adverse health effects. The type of study to be performed depends on the effects observed in the Tier I studies. Before performing such studies, the applicant shall seek agreement of the competent authorities on the type of study to be performed.
TIER I

5.1. *Basic information*

Basic information is required about the potential of the micro-organism to cause adverse effects such as ability to colonise, to cause damage and to produce toxins and other relevant metabolites.

5.1.1. *Medical data*

Where available, and without prejudice to the provisions of Article 10 of Directive 98/24/EC, practical data and information relevant to the recognition of the symptoms of infection or pathogenicity and on the effectiveness of first aid and therapeutic measures have to be submitted. Where relevant, the effectiveness of potential antagonists, shall be investigated and reported. Where relevant, methods to kill or render the micro-organism uninfective must be indicated (see point 3.8).

Data and information relevant to the effects of human exposure, where available and of the necessary quality, are of particular value, in confirming the validity of extrapolations made and conclusions reached with respect to target organs, virulence, and the reversibility of adverse effects. Such data can be generated following accidental or occupational exposure.

5.1.2. *Medical surveillance on manufacturing plant personnel*

Available reports of occupational health surveillance programmes, supported with detailed information on the design of the programme and on exposure to the micro-organism must be submitted. Such reports should, where feasible, include data relevant to the mechanism of action of the micro-organism. These reports shall, where available, include data from persons exposed in manufacturing plants or after application of the micro-organism (e.g. in efficacy trials).

Special attention shall be devoted to those whose susceptibility may be affected, e.g. pre-existing disease, medication, compromised immunity, pregnancy or breast feeding.

5.1.3. *Sensitisation/allergenicity observations, if appropriate*

Available information on the sensitisation and allergenic response of workers, including workers in manufacturing plants, agricultural and research workers and others exposed to the micro-organism must be provided, and include, where relevant, details of any incidences of hypersensitivity and chronic sensitisation. The information provided shall include details of frequency, level and duration of exposure, symptoms observed and other relevant clinical observation. Information shall be given about whether workers have been subjected to any allergy tests or interviewed about allergenic symptoms.

5.1.4. *Direct observation, e.g. clinical cases*

Available reports from the open literature on the micro-organism or closely related members of the taxonomic group (relating to clinical cases), where they are from reference journals or official reports, must be submitted together with reports of any follow-up studies undertaken. Such reports are of particular value and shall 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. Summary and abstract information is of limited value.

If there are animal studies performed, reports relating to clinical cases can be of particular value in confirming the validity of interpretations from animal data to man and in identifying unexpected adverse effects which are specific to humans.

5.2. *Basic studies*

In order to make it possible to correctly interpret the obtained results, it is of greatest importance that the suggested test methods are relevant regarding species sensitivity, administration route, etc. and relevant from a biological and toxicological point of view. The way of administration of the test micro-organism depends on the main exposure routes to humans.

To evaluate medium- and long-term effects after acute, sub-acute or semi-chronic exposure to micro-organisms, it is necessary to use the options provided in the OECD guidelines, to extend the studies concerned with a recovery period (after which full macroscopic and microscopic pathology is to be performed, including an exploration for micro-organisms in the tissues and organs). This facilitates the interpretation of certain effects and provides the possibility to recognise infectiveness and/or pathogenicity, which in turn helps taking decisions on other issues such as the necessity to perform long-term studies (carcinogenicity etc. see point 5.3), and whether or not to perform residue studies (see point 6.2).

5.2.1. *Sensitisation*[18]
Aim of the test

The test will provide sufficient information to assess the potential of the micro-organism to provoke sensitisation reactions by inhalation as well as with dermal exposure. A maximised test has to be performed.
Circumstances in which required[19]

Information on sensitisation must be reported.

5.2.2. *Acute toxicity, pathogenicity and infectiveness*

The studies, data and information to be provided and evaluated must be sufficient to permit the identification of effects following a single exposure to the micro-organism, and in particular to establish, or indicate:

— the toxicity, pathogenicity and infectiveness of the micro-organism,
— the time course and characteristics of the effects with full details of behavioural changes and possible gross pathological findings at post-mortem,
— where possible mode of toxic action,
— the relative hazards associated with the different routes of exposure, and
— blood analyses throughout the studies in order to evaluate the clearance of the micro-organism.

Acute toxic/pathogenic effects may be accompanied by infectiveness and/or more long-term effects which cannot be observed immediately. With a view to health evaluation, it is therefore necessary to carry out studies on the ability to infect in connection with oral intake, inhalation and intraperitoneal/subcutaneous injection by test mammals.

During the acute toxicity, pathogenicity and infectiveness studies, an estimation of the micro-organism and/or the active toxin clearance in the organs deemed to be relevant for microbial examination (e.g. liver, kidneys, spleen, lungs, brain, blood and site of administration) must be performed.

The observations to be made shall reflect expert scientific judgement and may include the micro-organism numeration in all the tissues likely to be affected (e.g. showing lesions) and in the main organs: kidneys, brain, liver, lungs, spleen, bladder, blood, lymphatic ganglia, gastrointestinal tract, thymus gland and lesions at the inoculation site in the dead or moribund animals and at interim and final sacrifice.

The information generated through acute toxicity, pathogenicity and infectiveness testing is of particular value in assessing hazards likely to arise in accident situations and consumer risks due to exposure to possible residues.

5.2.2.1. *Acute oral toxicity, pathogenicity and infectiveness*
Circumstances in which required

The acute oral toxicity, pathogenicity and infectiveness of the micro-organism must be reported.

5.2.2.2. *Acute inhalation toxicity, pathogenicity and infectiveness*
Circumstances in which required

The inhalation toxicity[(20)], pathogenicity and infectiveness of the micro-organism must be reported.

5.2.2.3. *Intraperitoneal/subcutaneous single dose*

The intraperitoneal/subcutaneous test is considered a highly sensitive assay to elicit in particular infectiveness.
Circumstances in which required

The intraperitoneal injection is always required for all micro-organisms, however, expert judgement may be exercised to evaluate whether subcutaneous injection is preferred instead of intraperitoneal injection if the maximum temperature for growth and multiplication is lower than 37 °C.

5.2.3. *Genotoxicity testing*
Circumstances in which required

If the micro-organism produces exotoxins in accordance with point 2.8, then these toxins and any other relevant metabolites in the culture medium must also be tested for genotoxicity. Such tests on toxins and metabolites shall be performed using the purified chemical if possible.

If basic studies do not indicate that toxic metabolites are formed, studies on the micro-organism itself shall be considered depending on expert judgement on the relevance and validity of the basic data. In the case of a virus the risk of insertional mutagenesis in mammal cells or the risk of carcinogenicity has to be discussed.
Aim of the test

These studies are of value in:
— the prediction of genotoxic potential,
— the early identification of genotoxic carcinogens,
— the elucidation of the mechanism of action of some carcinogens.

It is important that a flexible approach is adopted, with selection of further tests being dependent upon interpretation of results at each stage.

Test conditions[21]

Genotoxicity of cellular micro-organisms will be studied after breaking of the cells, wherever possible. Justification should be provided on the method of sample preparation used.

Genotoxicity of viruses shall be studied on infectious isolates.

5.2.3.1. *In vitro studies*
Circumstances in which required

Results of *in vitro* mutagenicity tests (bacterial assay for gene mutation, test for clastogenicity in mammalian cells and test for gene mutation in mammalian cells) must be provided.

5.2.4. *Cell culture study*

This information must be reported for intracellular replicating micro-organisms, such as viruses, viroids or specific bacteria and protozoa, unless the information from Sections 1, 2 and 3 clearly demonstrates that the micro-organism does not replicate in warm-blooded organisms. A cell culture study shall be performed in human cell or tissue cultures of different organs. Selection can be based on expected target organs after infection. If human cell or tissue cultures of specific organs are not available, other mammal cell and tissue cultures can be used. For viruses, the ability to interact with the human genome is a key consideration.

5.2.5. *Information on short-term toxicity and pathogenicity*
Aim of the test

Short-term toxicity studies must be designed to provide information as to the amount of the micro-organism that can be tolerated without toxic effects under the conditions of the study. Such studies provide useful data on the risks for those handling and using preparations containing the micro-organism. In particular, short-term studies provide an essential insight into possible cumulative actions of the micro-organism, and the risks to workers who may be intensively 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, must be sufficient to permit the identification of effects following repeated exposure to the micro-organism, and in particular to further establish, or indicate:

— the relationship between dose and adverse effects,
— toxicity of the micro-organism including where necessary the NOAEL for toxins,
— target organs, where relevant,
— the time course and characteristics of the effects with full details of behavioural changes and possible gross pathological findings at post-mortem,
— specific toxic effects and pathological changes produced,
— where relevant the persistence and reversibility of certain toxic effects observed, following discontinuation of dosing,
— where possible, the mode of toxic action, and
— the relative hazard associated with the different routes of exposure.

During the short-term toxicity study, an estimation of the micro-organism clearance in the main organs must be performed.

Investigations shall be included for pathogenicity and infectiveness end points.
Circumstances in which required

The short-term toxicity (minimum 28 days) of the micro-organism must be reported.

The choice of test species has to be justified. The choice of study length depends on acute toxicity and clearance data.

Expert judgement is required to decide what route of administration is preferable.

5.2.5.1. *Health effects after repeated inhalatory exposure*

Information on the health effects after repeated inhalatory exposure is considered necessary, particularly for the risk assessment of the occupational setting. Repeated exposure might influence the clearance capacity (e.g. resistance) of the host (human). Furthermore, for proper risk assessment the toxicity after repeated exposure to contaminants, growth medium, co-formulants and the micro-organism needs to be addressed. It should be kept in mind that the co-formulants in the plant protection product can influence the toxicity and infectiveness of a micro-organism.
Circumstances in which required

Information on the short-term infectiveness, pathogenicity and toxicity (respiratory route) of a micro-organism is required, unless the information already provided is sufficient to assess human health effects. This can be the case if it is demonstrated that the test material has no inhalable fraction and/or repeated exposure is not expected.

5.2.6. *Proposed treatment: first aid measures, medical treatment*

The first aid measures to be used in the event of infection and in the event of contamination of eyes must be provided.

Therapeutic regimes for use in the event of ingestion or contamination of eyes and skin must 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, must be provided.

Information on resistance to antibiotics must be provided.

(END OF TIER I)
TIER II

5.3. *Specific toxicity, pathogenicity and infectiveness studies*

In certain cases, it can be necessary to carry out supplementary studies to further clarify the adverse human effects.

In particular, if results from earlier studies indicate that the micro-organism may cause long-term health effects, studies on chronic toxicity, pathogenicity and infectiveness, carcinogenicity and reproductive toxicity must be carried out. Furthermore, where a toxin is produced, kinetic studies must be performed.

Studies required must be designed on an individual basis, in the light of the particular parameters to be investigated and the objectives to be achieved. Before performing such studies, the applicant shall seek agreement of the competent authorities on the type of study to be performed.

5.4. In vivo *studies in somatic cells*
Circumstances in which required

If all the results of the *in vitro* studies are negative further testing must be done with consideration of other relevant information available. The test can be an *in vivo* study or an *in vitro* study using a different metabolising system from that/those previously used.

If the *in vitro* cytogenetic test is positive, an *in vivo* test using somatic cells (metaphase analysis in rodent bone marrow or micronucleus test in rodents) must be conducted.

If either of the *in vitro* gene mutation tests is positive, an *in vivo* test to investigate unscheduled DNA synthesis or a mouse spot test must be conducted.

5.5. *Genotoxicity* — In vivo *studies in germ cells*
Aim of the test and test conditions

See point 5.4 of Part A.
Circumstances in which required

When any result of an *in vivo* study in somatic cells is positive, *in vivo* testing for germ cell effects may be justified. The necessity for conducting these tests will have to be considered on a case-by-case basis, taking into account other relevant information available including use and expected exposure. Suitable tests would need to examine interaction with DNA (such as the dominant lethal assay), to look at the potential for inherited effects and possibly make a quantitative assessment of heritable effects. It is recognised that in view of their complexity, the use of quantitative studies would require strong justification.

(END OF TIER II)

5.6. *Summary of mammalian toxicity, pathogenicity and infectiveness and overall evaluation*

A summary of all data and information provided under points 5.1 through 5.5, must be submitted, and include a detailed and critical assessment of those data in the context of relevant evaluative and decision making criteria and guidelines, with particular reference to the risks for man and animals that may or do arise, and the extent, quality and reliability of the data base.

It must be explained whether exposure of animals or humans has any implications for vaccination or serological monitoring.

6. Residues in or on treated products, food and feed

Introduction

(i) The information provided, taken together with that for one or more preparations containing the micro-organism, must be sufficient to permit an evaluation to be made as to the risk for man and/or animals, arising from exposure to the micro-organism and its residual traces and metabolites (toxins) remaining in or on plants or plant products.

(ii) In addition, the information provided must be sufficient to:

— permit a decision to be made as to whether or not the micro-organism can be approved,

— specify appropriate conditions or restrictions to be associated with any approval,

— where relevant, set maximum residue levels, preharvest intervals to protect consumers and waiting periods, to protect workers handling the treated crops and products.

(iii) For the evaluation of risk arising from residues, experimental data on levels of exposure to the residue may not be required where it can be justified, that the micro-organism and its metabolites are not hazardous to humans in the concentrations that could occur as a result of authorised use. This justification can be based on open literature, on practical experience and on information submitted in Sections 1, 2 and 3 and Section 5.

6.1. *Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs*

A substantiated estimation of persistence/competitiveness of the micro-organism and relevant secondary metabolites (especially toxins) in or on the crop under the environmental conditions prevailing at and after the intended use, taking into account in particular the information provided in Section 2, has to be delivered.

Moreover, the application shall state to which extent and on which basis it is considered that the micro-organism can (or cannot) multiply in or on the plant or plant product or during processing of raw products.

6.2. *Further information required*

Consumers may be exposed to micro-organisms for a considerable time as a result of the consumption of treated food commodities; potential effects on the consumers must, therefore, be derived from chronic or semi-chronic studies, so that a toxicological end point, such as the ADI, can be established for risk management.

6.2.1. *Non-viable residues*

A non-viable micro-organism is a micro-organism that is not capable of replication or of transferring genetic material.

If relevant quantities of the micro-organism or of produced metabolites, especially toxins, have been found to be persistent in points 2.4 and 2.5, full experimental residue data as provided for in Section 6 of Part A of this Annex is required, if concentrations of the micro-organism and/or its toxins in or on the treated foodstuffs or feedingstuffs are expected to occur in concentrations higher than under natural conditions or in a different phenotypic state.

In accordance with Regulation (EC) No 1107/2009, the conclusion concerning the difference between natural concentrations and an elevated concentration due to treatment with the micro-organism, is to be based on experimentally obtained data, and not on extrapolations or calculations using models.

Before performing such studies, the applicant shall seek agreement of the competent authorities on the type of study to be performed.

6.2.2. *Viable residues*

If the information submitted in accordance with point 6.1 suggests persistence of relevant amounts of the micro-organism in or on treated products, food or feed, possible effects on humans and/or animals must be investigated, unless it can be justified from Section 5, that the micro-organism and its metabolites and/or degradation products are not hazardous to humans in the concentrations and of the nature that could occur as a result of authorised use.

In accordance with Regulation (EC) No 1107/2009, the conclusion concerning the difference between natural concentrations and an elevated concentration due to treatment with the micro-organism, is to be based on experimentally obtained data, and not on extrapolations or calculations using models.

The persistence of viable residues needs special attention if infectiveness or pathogenicity to mammals have been found in points 2.3 and 2.5 or in Section 5 and/or if any other information suggests a hazard to consumers and/or workers. In this case the competent authorities may require studies similar to those provided for in Part A.

Before performing such studies, the applicant shall seek agreement of the competent authorities on the type of study to be performed.

6.3. *Summary and evaluation of residue behaviour resulting from data submitted under points 6.1 and 6.2*

7. Fate and behaviour in the environment

Introduction

(i) Information on the origin, the properties, and the survival of the micro-organism and its residual metabolites as well as its intended use form the basis for an assessment of environmental fate and behaviour.

Experimental data are normally required unless it can be justified that an assessment of its fate and behaviour in the environment can be performed with the information already available. This justification can be based on open literature, on practical experience and on information submitted in Sections 1 to 6. The function of the micro-organism in environmental processes is of particular interest.

(ii) The information provided, taken together with other relevant information, and that for one or more preparations containing the micro-organism, must be sufficient to permit an assessment of its fate and behaviour as well as that of its residual traces and toxins, where they are of significance for human health and/or the environment.

(iii) In particular, the information provided shall be sufficient to:

— decide whether, or not, the micro-organism can be approved,

— specify appropriate conditions or restrictions to be associated with any approval,

— specify the pictograms (once introduced), signal words, and relevant hazard and precautionary statements for the protection of the environment, which are to be included on packaging (containers),

— predict the distribution, fate, and behaviour in the environment of the micro-organism and its metabolites as well as the time courses involved,

— identify measures necessary to minimise contamination of the environment and impact on non-target species.

(iv) Any relevant metabolites (i.e. of concern for human health and/or the environment) formed by the test organism under any relevant environmental conditions shall be characterised. If relevant metabolites are present in or produced by the micro-organism, data as outlined under Section 7 of Part A of this Annex may be required, if all of the following conditions are met:

— the relevant metabolite is stable outside the micro-organism, see point 2.8, and

— a toxic effect of the relevant metabolite is independent of the presence of the micro-organism, and

— the relevant metabolite is expected to occur in the environment in concentrations considerably higher than under natural conditions.

(v) Available information on the relationship with naturally occurring wild type relatives shall be taken into account.

(vi) Before performing studies as referred to below, the applicant shall seek agreement of the competent authorities on whether studies need to be performed and, if so, the type of study to be conducted. The information from the other Sections has, also, to be taken into account.

7.1. *Persistence and multiplication*

Where relevant, appropriate information on the persistence and multiplication of the micro-organism, in all environmental compartments has to be given, unless it can be justified that exposure of the particular environmental compartment to the micro-organism is unlikely to occur. Special attention shall be given to:

— competitiveness under the environmental conditions prevailing at and after the intended use, and

— population dynamics in seasonally or regionally extreme climates (particularly hot summer, cold winter and rainfall) and to agricultural practices applied after intended use.

Estimated levels of the specified micro-organism in a time course after use of the product under the proposed conditions of use shall be given.

7.1.1. *Soil*

Information on viability/population dynamics shall be reported in several cultivated and uncultivated soils representative of soils typical of the various EU regions where use exists or is anticipated. The provisions on choice of soil and its collection and handling, as referred to in the introduction to point 7.1 of Part A, have to be followed. If the test organism is to be used in association with other media, e.g. rockwool, this must be included in the test range.

7.1.2. *Water*

Information should be reported on viability/population dynamics in natural sediment/water systems under both dark and illuminated conditions.

7.1.3. *Air*

In case of particular concerns for operator, worker or bystander exposure, information on the concentrations in air might be necessary.

7.2. *Mobility*

The possible spread of the micro-organism and its degradation products in relevant environmental compartments has to be evaluated, unless it can be justified that exposure of the particular environmental compartments to the micro-organism is unlikely to occur. In this context, the intended use (e.g. field or greenhouse, application to soil or to crops), life cycle stages, including occurrence of vectors, persistence and the ability of the organism to colonise adjacent habitats are of particular interest.

The spread, the persistence and probable transport ranges need special attention if toxicity, infectiveness or pathogenicity have been reported or if any other information suggests possible hazard to humans, animals or to the environment. In this case the competent authorities may require studies similar to those provided for in Part A. Before performing such studies, the applicant shall seek agreement of the competent authorities on the type of study to be performed.

8. **Effects on non-target organisms**

Introduction

(i) The information on identity, biological properties and further information in Sections 1, 2, 3 and 7 is central to the assessment of impact on non-target species. Additional useful information may be found on fate and behaviour in the environment in Section 7 and on residue levels in plants in Section 6 which, together with information on the nature of the preparation and its manner of use, defines the nature and extent of potential exposure. The information submitted in accordance with Section 5 will provide essential information as to effects to mammals and the mechanisms involved.

Experimental data are normally required, unless it can be justified that an assessment of effects on non-target organisms can be performed with the information already available.

(ii) The choice of the appropriate non-target organisms for testing of environmental effects shall be based on the identity of the micro-organism (including the host specificity, mode of action and ecology of the organism). From such knowledge it would be possible to choose the appropriate test-organisms, such as organisms closely related to the target organism.

(iii) The information provided, taken together with that for one or more preparations containing the micro-organism, must be sufficient to permit an assessment of the impact on non-target species (flora and fauna), likely to be at risk from exposure to the micro-organism, where they are of environmental significance. Impact can result from single, prolonged or repeated exposure and can be reversible or irreversible.

(iv) In particular, the information provided for the micro-organism, together with other relevant information, and that provided for one or more preparations containing it, shall be sufficient to:

— decide whether, or not, the micro-organism can be approved,

— specify appropriate conditions or restrictions to be associated with any approval,

— permit an evaluation of short- and long-term risks for non-target species — populations, communities, and processes — as appropriate,

— classify the micro-organism as to biological hazard,

— specify the precautions necessary for the protection of non-target species, and

— specify the pictograms (once introduced), signal words, and relevant hazard and precautionary statements for the protection of the environment, to be mentioned on packaging (containers).

(v) There is a need to report all potentially adverse effects found during routine investigations on environmental effects, to undertake and report, where required by the competent authorities, such additional studies which may be necessary to investigate the probable mechanisms involved and to assess the significance of these effects. All available biological data and information which are relevant to the assessment of the ecology profile of the micro-organism must be reported.

(vi) For all studies, average achieved dose in cfu/kg body weight as well as in other appropriate units must be reported.

(vii) It may be necessary to conduct separate studies for relevant metabolites (especially toxins), where these products can constitute a relevant risk to non-target organisms and where their effects cannot be evaluated by the available results relating to the micro-organism. Before such studies are performed, the applicant shall seek agreement of the competent authorities on whether such studies need to be performed and, if so, the type of study to be conducted. The information from Sections 5, 6 and 7 has to be taken into account.

(viii) In order to facilitate the assessment of the significance of test results obtained, the same strain (or recorded origin) of each relevant species shall, where possible, be used in the various tests specified.

(ix) Tests must be performed unless it can be justified that the non-target organism will not be exposed to the micro-organism. If it is justified that the micro-organism does not cause toxic effects or is not pathogenic or infective to vertebrates or plants, only reaction to appropriate non-target organisms must be investigated.

8.1. *Effects on birds*
Aim of the test

Information on toxicity, infectiveness and pathogenicity to birds must be reported.

8.2. *Effects on aquatic organisms*
Aim of the test

Information on toxicity, infectiveness and pathogenicity to aquatic organisms must be reported.

8.2.1. *Effects on fish*
Aim of the test

Information on toxicity, infectiveness and pathogenicity to fish must be reported.

8.2.2. *Effects on freshwater invertebrates*
Aim of the test

Information on toxicity, infectiveness and pathogenicity to freshwater invertebrates must be reported.

8.2.3. *Effects on algae growth*
Aim of the test

Information on effects on algal growth, growth rate and capacity to recover must be reported.

8.2.4. *Effects on plants other than algae*
Aim of the test

Information on effects on plants other than algae must be reported.

8.3. *Effects on bees*
Aim of the test

Information on toxicity, infectiveness and pathogenicity to bees must be reported.

8.4. *Effects on arthropods other than bees*
Aim of the test

Information on toxicity, infectiveness and pathogenicity to arthropods other than bees must be reported. The selection of the test species should be related to the potential use of the plant protection products (e.g. foliar or soil application). Special attention should be given to organisms used for biological control and organisms playing an important role in integrated pest management.

8.5. *Effects on earthworms*
Aim of the test

Information on toxicity, infectiveness and pathogenicity to earthworms must be reported.

8.6. *Effects on non-target soil micro-organisms*

Impact on relevant non-target micro-organisms and on their predators (e.g. protozoa for bacterial inoculants) shall be reported. Expert judgement is required to decide whether additional studies are necessary. Such decision will take into consideration the available information in this Section and other Sections, in particular data on the specificity of the micro-organism, and the expected exposure. Useful information may also be available from the observations carried out in efficacy testing. Special attention shall be given to organisms used in integrated crop management (ICM).

8.7. *Additional studies*

The additional studies might include further acute studies on additional species or processes (such as sewage systems) or higher tier studies such as chronic, sub-lethal or reproductive studies on selected non-target organisms.

Before performing such studies, the applicant shall seek agreement of the competent authorities on the type of study to be performed.

9. Summary and evaluation of environmental impact

A summary and evaluation of all data relevant to the environmental impact, shall be carried out in accordance with the guidance given by the competent authorities of the Member States concerning the format of such summaries and evaluations. It shall include a detailed and critical assessment of those data in the context of relevant evaluative and decision making criteria and guidelines, with particular reference to the risks for the environment and non-target species that may or do arise, and the extent, quality and reliability of the data base. In particular the following issues shall be addressed:

— distribution and fate in the environment, and the time courses involved,

— identification of non-target species and populations at risk, and the extent of their potential exposure,

— identification of precautions necessary to avoid or minimise contamination of the environment, and for the protection of non-target species.

(**1**) OJ L 142, 31.5.2008, p. 1.

(**2**) OJ L 358, 18.12.1986, p. 1.

(**3**) OJ L 50, 20.2.2004, p. 44.

(**4**) See page 67 of this Official Journal.

(**5**) OJ L 353, 31.12.2008, p. 1.

(**6**) Food and Agriculture Organisation of the United Nations Rome — December 1989. http://www.fao.org/ag/AGP/AGPP/Pesticid/Code/Download/ENVICRI.pdf

(**7**) OJ L 396, 30.12.2006, p. 1.

(**8**) See page 127 of this Official Journal.

(**9**) *Standardised System of Nomenclature and Diagnostic Criteria — Guides for Toxicologic Pathology.*

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

(**11**) *Guidelines for Good Epidemiology Practices for Occupational and Environmental Research, developed by the Chemical Manufacturers Association's Epidemiology Task Group, as part of the Epidemiology Resource and Information Centre (ERIC),* Pilot Project, 1991.

(**12**) http://ec.europa.eu/food/plant/protection/resources/publications_en.htm#residues

(**13**) Society of Environmental Toxicology and Chemistry (SETAC), 1995. *Procedures for assessing the environmental fate and ecotoxicity of pesticides*, ISBN 90-5607-002-9.

(**14**) FOrum for the Co-ordination of pesticide fate models and their USe.

(**15**) From the Workshop European Standard Characteristics of beneficials Regulatory Testing (Escort), 28 to 30 March 1994, ISBN 0-95-22535-2-6.

(**16**) *USEPA Microbial Pesticide Test Guidelines*, OPPTS Series 885, February 1996.

(**17**) OJ L 106, 17.4.2001, p. 1.

(**18**) The available methods for testing dermal sensitisation are not suitable for testing micro-organisms. Sensitisation by inhalation is most probably a greater problem compared with dermal exposure to micro-organisms but so far, there are no validated test methods. Development of these kinds of methods is therefore of great importance. Until then, all micro-organisms should be regarded as potential sensitisers. This approach also takes into consideration immuno-compromised or other sensitive individuals in the population (e.g. pregnant women, new-born children or elderly).

(**19**) As a consequence of the absence of proper test methods all micro-organisms will be labelled as potential sensitisers, unless the applicant wants to demonstrate the non-sensitising potential by submitting data. Therefore, this data requirement should be regarded as not obligatory but optional, on a provisional base.

(**20**) An inhalation study may be replaced by an intratracheal study.

(**21**) As the present test methods are designed to be performed on soluble chemicals, it is necessary that the methods are developed so as to become relevant for micro-organisms.