

## **ANNEX II**

### **REQUIREMENTS FOR THE DOSSIER TO BE SUBMITTED FOR THE INCLUSION OF AN ACTIVE SUBSTANCE IN ANNEX I**

#### **INTRODUCTION**

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 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, according to 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 an EEC Method which consists in the transposal of a method developed by an international organization (e.g. OECD), Member States may accept that the required information is generated according to the latest version of that method if at the initiation of the studies the EEC Method 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 Directive 86/609/ EEC.

2.1. Tests and analyses must be conducted in accordance with the principles laid down in Directive 87/18/EEC (1) 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 substances with respect to honey-bees and beneficial arthropods other than bees shall be conducted by official or officially-recognized testing facilities or organizations which satisfy at least the requirements as set out under points 2.2 and 2.3 of the introduction to Annex III.

This derogation applies to trials 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 the provisions of Section 6 "Residues in or on treated products, food and feed" on plant protection products containing active substances already on the market two years after notification of the Directive shall be conducted by official or officially -recognized testing facilities or organizations which satisfy at least the requirements under points 2.2 and 2.3 of the introduction to Annex III.

This derogation applies for supervised residue trials 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 of Annex III.

#### **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 characterize 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 (permanent Community address) 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 inclusion in Annex I 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 inclusion of the active substances in Annex I, 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 I to Directive 67/548/EEC, or, if not included in this Directive, 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, EEC and CIPAC numbers (if available)

Chemical Abstracts, EEC (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 stabilized.

#### 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 stabilized, if production changes result in a changed specification of 1.10. Identity of isomers, impurities and additives (e.g. stabilizers), 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/diastereoisomers, 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 according to IUPAC and CA nomenclature,
- ISO common name or proposed common name if available,
- CAS number, EEC (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 according to IUPAC and CA nomenclature,
- ISO common name or proposed common name if available,
- CAS number, EEC (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 stabilized, 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 according to IUPAC and CA nomenclature, - ISO common name or proposed common name if available,
- CAS number, EEC (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,
- other (specify),
- buffer,
- dispersing agent,
- stabilizer.

#### 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 should 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 analyzed, 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 characterize 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 inclusions in Annex I to be selected,
- and
- appropriate risk and safety phrases 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 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 minimized, taking account of manner of use.

(iii) The extent to which active substances of which inclusion in Annex I 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 according to EEC method A 1. Measurements should be taken up to 360 °C.

2.1.2. Where appropriate the boiling point of purified active substances must be determined and reported according to EEC method A 2. Measurements should 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 according to EEC method A 3.

## 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 according to EEC method A 4. 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  $\text{Pa} \times \text{m}^3 \times \text{mol}^{-1}$ ).

## 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

2.5.1. The following spectra including a table of signal characteristics needed for interpretation must be determined and reported: Ultraviolet/Visible (UV/VIS), infrared (IR), 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 according to EEC method A 6. 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-dichloroethane,
- 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 is unsuitable (e.g. reacts with test material), alternative solvents can be used instead. In such cases, 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 according to EEC method A 8. 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 report according to EEC method C 7. 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 ( $\epsilon$ )  $> 10$  ( $1 \times \text{mol}^{-1} \times \text{cm}^{-1}$ ) 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 solubilizer, must be determined and reported. Sensitizers such as acetone must not be used as a cosolvent or solubilizer. 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  $\geq 10\%$  of the active substance added, a mass balance to account for at least 90 % of the applied radioactivity, as well as photochemical half-life must be reported.

2.9.3. Where necessary to investigate direct phototransformation, 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.

2.9.4. Where dissociation in water occurs, the dissociation constant(s) (pKa values) of the purified active substance must be determined and reported according to 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 according to EEC method A 10, A 11 or A 12 as appropriate.

2.11.2. The auto-flammability of active substances as manufactured must be determined and reported according to EEC method A 15 or A 16 as appropriate, and/or, where necessary according to 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 according to EEC method A 9; only closed cup methods should be used.

2.13. Explosive properties

The explosive properties of active substances as manufactured, must be determined and reported according to EEC method A 14 where necessary.

2.14. Surface tension

The surface tension has to be determined and reported according to EEC method A 5.

2.15 Oxidizing properties

The oxidizing properties of active substances as manufactured, must be determined and reported according to EEC method A 17, 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 oxidizing properties of the 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

(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 should 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
- nematocide
- 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 fertilization etc., must be reported.

### 3.5. Mode of action

#### 3.5.1. To the extent that it 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 according to IUPAC and CA nomenclature,
- ISO common name or proposed common name,
- CAS EEC-number EEC (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 27 of Council Directive 65/548/EEC (1) 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 Directive 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** Any component other than the pure active substance which is present in the active substance as manufactured (including non-active isomers) originating from the manufacturing process or from degradation during storage,

**Relevant impurities** Impurities of toxicological and/or ecotoxicological or environmental concern,

**Significant impurities** Impurities with a content of  $\geq 1$  g/kg in the active substance as manufactured,

**Metabolites** Metabolites include products resulting from degradation or reaction of the active substance,

**Relevant metabolites** Metabolites of toxicological and/or ecotoxicological or environmental concern.

On request the following samples must be provided:

- analytical standards of the pure active substance;
- samples of the active substance as manufactured;
- analytical standards of relevant metabolites and all other components included in the residue definition;
- 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 inclusion in Annex I to Directive 91/414/EEC. 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. stabilizers) 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  $\pm 3\%$  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. Dixon's 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 should 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 Directive 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 according to the provisions of section 6, 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 should 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 according to the requirements of Annex VI.

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 included in Annex I,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I,
- 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 hazard symbols, the indications of danger, and the risk and safety phrases 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 is 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 section 1 point 11 be provided. Tests should be conducted using active substance of that specification to be used in the manufacture of preparations to be authorized, 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 should 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 utilized 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 Annex III, point 7.2.3 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 should 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 dependant upon the results obtained for each particular test substance.

Aim of the test:

The tests should provide sufficient data to permit:

- an evaluation of the rate and extent of absorption,
- 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 results are different after single versus repeated doses, should 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

Commission Directive 87/302/EEC of 18 November 1987 adapting to technical progress for the ninth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (1), part B, 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 Council Directive 67/548/EEC. 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 Commission Directive 92/69/EEC of 31 July 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (2), Method B1 or B1 bis.

##### 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 Directive 92/69/EEC method B3.

##### 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 liquefied 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  $< 50 \mu\text{m}$  ( $> 1\%$  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  $1\%$  on a weight basis).

Test guideline

The test must be carried out in accordance with Directive 92/69/EEC Method B2.

##### 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 Directive 92/69/EEC Method B4.

##### 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 Directive 92/69/EEC Method B5.

#### 5.2.6. Skin sensitization

##### Aim of test

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

##### Circumstances in which required

The test must always be carried out except where the substance is a known sensitizer.

##### Test guideline

The test must be carried out in accordance with Directive 92/69/EEC Method B6.

#### 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 Directive 92/69/EEC Method B7.

##### 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

Directive 87/302/EEC, Part B, sub-chronic oral toxicity test.

##### 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: Directive 92/69/EEC Method B9,
- 90-day dermal: Directive 87/302/EEC, Part B, sub-chronic dermal toxicity study,
- 28-day inhalation: Directive 92/69/EEC Method B8,
- 90-day inhalation: Directive 87/302/EEC, Part B, sub-chronic 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 artifacts of the test system, excessively toxic doses must not be used in either in vitro or in vivo assays for mutagenicity. This approach should be regarded as general guidance. It is important that a flexible approach is adopted, with selection of further tests being dependant 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:

Directive 92/69/EEC Method B14 - Salmonella Typhimurium reverse mutation assay

Directive 92/69/EEC Method B10 - in vitro mammalian cytogenetic test

Directive 87/302/EEC, Part B - 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 resting 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 metabolizing 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

Acceptable test guidelines are:

Directive 92/69/EEC Method B12 - Micronucleus test,

Directive 87/302/EEC Part B - Mouse spot test,

Directive 92/69/EEC Method B11 - In vivo Mammalian Bone-Marrow cytogenetic test, Chromosomal analysis.

#### 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 recognized 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 (two 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 should be from the same species and strain, maintained under similar conditions and should 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),
- 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 should 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 should 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 American Society of Toxicologic Pathologists (3), or the Hannover Tumour Registry (RENI) should 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 Directive 87/302/EEC, part B, Chronic toxicity test, Carcinogenicity test or combined chronic toxicity/carcinogenicity test.

#### 5.6. Reproductive toxicity

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 should be from the same species and strain, maintained under similar conditions and should 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 Directive 87/302/EEC, Part B, two-generation reproduction toxicity test. 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 should 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 Directive 87/302/EEC, Part B, teratogenicity test - rodent and non-rodent.

#### 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 5 of Council Directive 80/1107/EEC of 27 November 1980 on the protection of workers from the risks related to chemical, physical and biological agents at work (4), 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 should be provided. Where relevant, the effectiveness of potential antagonists to poisoning, should 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 should, 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 sensitization 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 should 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 should 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 recognized standards (5), 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. Where relevant, in the light of findings with respect to the analytical profile of batches of the active substance (paragraph 1.11) and any bridging studies conducted (paragraphs 5 (iv)), the relevance of the data as submitted to the assessment of the toxicological profile of the active substance as manufactured, must be argued.

(1) On the basis of an assessment of the data base, and the relevant decision making criteria and guidelines, justifications must be

submitted for the Noels proposed for each relevant study.

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

(3) Standardized System of Nomenclature and Diagnostic Criteria - Guides for Toxicologic Pathology

(4) OJ No L 327, 3. 12. 1980, p. 8.

(5) 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

## **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 included in Annex I,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I.

(ii) A detailed description (specification) of the material used, as provided under Section 1, point 11 must be provided.

(iii) Studies should be performed according to the guidance available on regulatory testing procedures for residues of plant protection products in food.

(iv) Where relevant, data should be analyzed using appropriate statistical methods. Full details of the statistical analysis should 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 (six months in the case of radio-labelled material).

Studies with non-radio-labelled substances should 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 should 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 and 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 feedingstuffs.

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 feedingstuffs 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 Annex II dossier for those uses of plant protection products for which authorization is sought at the moment of introduction of a dossier for inclusion of the active substance in Annex I.

Test conditions

Supervised trials should 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. should be taken into account.

In general, for a comparable set of conditions, trials should be carried out over a minimum of two growing seasons.

All exceptions should 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 should 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 should be submitted.

Where it appears from the metabolism studies submitted in accordance with the provisions of 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) should be investigated, where appropriate. The effects of process other than hydrolysis, may also have to be investigated, where the properties of the active substance or metabolites indicate that toxicologically significant degradation products may occur as a result of these processes. The studies are normally conducted with a radio-labelled form of the active substance.

#### 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 should 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 should be given for the selection made of these representative process(es). The technology to be used in processing studies should always correspond as closely as possible to the actual conditions that are normally used in practice. A balance sheet should 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 recognized 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 Annex II, Section 7, point 7.1 or Annex III, Section 9, point 9.1, 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 should be given to the residue situation. This should 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 should 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 postharvest 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 predictions 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 should be carried out according to the guidance given by the competent authorities of the Member States concerning the format of such summaries and evaluations.

It should 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 should 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, should be sufficient to:

- decide whether, or not, the active substance can be included in Annex I,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I,
- classify the active substance as to hazard;
- specify the hazard symbols, the indications of danger, and relevant risk and safety phrases 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 minimize contamination of the environment and impact on non-target species.

(iii) A detailed description (specification) of the material used, as provided for under Section 1, point 11 must be provided. Where testing is done using active substance the material used should be of that specification that will be used in the manufacture of preparations to be authorized 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 should 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 should be designed and data analysed using appropriate statistical methods.

Full details of the statistical analysis should be reported (e.g. all point estimates should be given with confidence intervals, exact p-values should 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, 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 Community 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 - paragraphs 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 should 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 should not have extreme characteristics with respect to parameters such as particle size distribution, organic carbon content and pH.

Soils should 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 should 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, should 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 according to 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<sub>2</sub>,
- volatile compounds other than CO<sub>2</sub>,
- 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 nonextractable 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 characterized.

The duration of the study is normally 120 days, except where after a shorter period the levels of non-extractable residues and CO<sub>2</sub> 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 (1).

#### 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 paragraph 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 should provide best possible estimates of the time taken for degradation of 50 % and 90 % (DT50lab and DT90lab), 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 paragraph

##### 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 Community 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 DT50 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 according to 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 according to 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 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 DT50 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 should provide estimates of the time taken for dissipation of 50 % and 90 % (DT50f and DT90f), of the active substance under field conditions. Where relevant, information on relevant metabolites, degradation and reaction products must be reported.

##### Circumstances in which required

Where plant protection products containing the active substance are intended to be used in cold climatic conditions, the tests have to be conducted where DT50lab, 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 should provide estimates of the soil residue levels at harvest or at time of cowing or planting succeeding crops.

Circumstances in which required

Soil residue studies must be reported where DT50lab 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 should 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 DT90f > one 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 absorption 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 should 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 absorption and desorption studies provided for under point 7.1.2 it is not possible to obtain reliable absorption 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 should 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 to point 7.1.2 or 7.1.3.1 was performed.

Test conditions

The period(s) of ageing should 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 should 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 should be carried out, taking into account the results of degradation and other mobility studies and the predicted environmental concentrations in groundwater (PECGW), calculated in accordance with the provisions of Annex III, Section 9. The type and conditions of the study to be conducted should 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 should 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 analyzed 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 according to 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 should be 100 cm; their maximal depth should be 130 cm. The soil cross 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 authorization 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 should be given to the number and the location of water collection devices. The placement of these devices in the soil should 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, should 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, should 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 the provisions of Annex VI to Directive 67/548/EEC for the classification of the active substance.

##### Test guideline

EEC method C4.

##### 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)

##### Guidance under development.

#### 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, should be sufficient to:

- decide whether, or not, the active substance can be included in Annex I,

- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I,
  - 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 hazard symbols, the indications of danger, and relevant risk and safety phrases 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 point 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 should be designed and data analysed using appropriate statistical methods. Full details of the statistical analysis should be reported (e. g. all point estimates should 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 should be of that specification that will be used in the manufacture of preparations to be authorized 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 should 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 utilized 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 points 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 should, 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 should provide, where possible, LD50 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 (1).

### 8.1.2. Short-term dietary toxicity

#### Aim of the test

The test should provide the short term dietary toxicity (LC50 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 (five-day) toxicity of the active substance to birds must always be investigated on one species except where a study in accordance with the provisions of point 8.1.3 is reported. Where its acute oral NOEL is  $\leq 500$  mg/kg body weight or where the short-term NOEC  $< 500$  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 should 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 should 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 the provisions of Annex VI to Directive 67/548/EEC for the classification of the active substance.

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 should provide the acute toxicity (LC50), 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 Commission Directive 92/69/EEC (1) adapting to technical progress for the 17th time Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification and labelling of dangerous substances, Method C1.

#### 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 EC50 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 (DT90  $> 100$  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 should 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 should 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 should 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  $\log_{10} K_{ow} \geq 3$  - 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 should provide the 24 and 48-hour acute toxicity of the active substance, expressed as the median effective concentration (EC50) for immobilization, and where possible the highest concentration causing no immobilization.

##### 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 Directive 92/69/EEC, Method C2.

#### 8.2.5. Chronic toxicity to aquatic invertebrates

##### Aim of the test

The test should provide where possible EC50 values for effects such as immobilization 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

##### 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 should provide EC50 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 Directive 92/69/EEC, Method C3.

#### 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 EC50 values and the NOEC values.

Circumstances in which required

Where environmental fate and behaviour data required in point 7 report that an active substance is likely to partition to and persist in aquatic sediments, expert judgement should be used to decide whether an acute or a chronic sediment toxicity test is required. Such expert judgement should take into account whether effects on sediment dwelling invertebrates are likely by comparing the aquatic invertebrate toxicity EC50 data from points 8.2.4 and 8.2.5 with the predicted levels of the active substances in sediment from data in Annex III, point 9.

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 should provide the acute oral and contact LD50 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 should 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 Ruijter 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 should 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*) should be tested. In addition to these, two additional species must also be tested, which should be relevant to the intended use of the substance. Where possible and if appropriate, they should 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 should follow the proposals outlined in Setac - Guidance document on regulatory testing procedures for pesticides with non-target arthropods<sup>1</sup>. Testing must be conducted at rates equivalent to the highest rate of field application to be recommended.

Test guideline

Where relevant, testing should be done according to 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 should provide the LC50 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 Commission Directive 88/302/EEC<sup>2</sup> adapting to technical progress for the ninth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, Part C, Toxicity for earthworms: Artificial soil test.

##### 8.4.2. Sublethal effects

Aim of the test

The test should 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 (DT90 > 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 should provide sufficient data to evaluate the impact of the active substance on soil microbial activity, in terms of nitrogen transformation and carbon mineralization.

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 sterilization, 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 two 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.

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<sup>1</sup> From the Workshop European Standard Characteristics of beneficials Regulatory Testing (Escort), 28 to 30 March 1994, ISBN

<sup>2</sup> OJ No L 133, 30. 5. 1988, p. 1.

#### 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 points 7 and 8

10. Proposals including justification for the proposals for the classification and labelling of the active substance according to Council Directive 67/548/EEC

- Hazard symbol(s)
- Indications of danger
- Risk phrases
- Safety phrases

11. A dossier as referred to in Annex III, part A, for a representative plant protection product

#### **PART B**

##### **Introduction**

(i) Active substances are defined in Article 2(4) and include chemical substances and micro-organisms including viruses.

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

For the purposes of Annex II, Part B, the term "micro-organism" is used and is defined as follows: "A microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material".

The definition 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 microorganism.

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<sup>3</sup>); where appropriate test guidelines as described in Annex II, Part A, should be adapted in such a way that they are appropriate for micro-organisms. Testing should 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, according to the provisions of section 1, point 1.4, must be provided. The material used should 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, as defined in Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms<sup>4</sup>, a copy of the evaluation of the data concerning the assessment of risk to the environment, as stated in Article 1(3) of Directive 91/414/EEC, has to be submitted.

(vi) Where relevant, data should be analysed using appropriate statistical methods. Full details of the statistical analysis should be reported (e.g. all point estimates should 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 should 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 Annex II, Part A.

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<sup>3</sup> USEPA Microbial Pesticide Test Guidelines, OPPTS Series 885, February 1996(  
<http://www.epa.gov/oppbppd1/biopesticides/guidelines/series885.htm> )

<sup>4</sup> OJ L 117, 8.5.1990, p. 15

## **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 (permanent community address) 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 inclusion in Annex I 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 inclusion of the micro-organism in Annex I, 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 should 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 Annex IA, Part 2, and Annex IB to Directive 90/220/EEC.
- 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 should 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 should 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 microorganisms), if possible. The level and nature of acceptable contaminants should 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 Annex II, Part B, section 1, point 1.3.

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 should be identified and characterised at different states or growth stages of the micro-organism (see Annex IIB, Introduction, (viii)).

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 Annex II, Part A, section 1, point 1.10.

#### 1.4.3. Analytical profile of batches

Where relevant, the same data as outlined in Annex II, Part A, section 1, point 1.11, 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, should 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 should 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 (as defined in Annex IA, Part 2, and Annex IB to Directive 90/220/EEC), 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 should be indicated. In connection with the mode of action it should 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 should be described.

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

It should 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 should 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 should 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) should be provided.

Any available information on the influence of the produced metabolites on the micro-organism's mode of action should 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 should be monitored. The quality assurance criteria for the production should 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 should 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 are to 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 similar to that required for chemical active substances in Article 27 of Directive 67/548/EEC<sup>5</sup> 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 Directive 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 Annex II, Part A, points 4.1 and 4.2, are also required for methods used to analyse micro-organisms and their residues.

For this section the following applies:

Impurities            Any component (including contaminating micro-organisms and/or chemical substances) other

<sup>5</sup> See doc. 6853/VI/98, Concise outline report of the first peer review meeting on micro-organisms.

than the specified micro-organism, originating from the manufacturing process or from degradation during storage

**Relevant impurities** Impurities, as defined above, that are of concern for human or animal health and/or the environment

**Metabolites** Metabolites include products resulting from degradative and biosynthetic reactions taking place within the micro-organism or other organisms used to produce the micro-organism of interest

**Relevant metabolites** Metabolites that are of concern for human or animal health and/or the environment

**Residues** Viable micro-organisms and substances produced in significant quantities by these micro-organisms which persist after the disappearance of the micro-organisms and 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 should 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 to 3), including health and medical reports may be sufficient for a decision whether the microorganism 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 microorganism, 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 included in Annex I,

- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I,

- 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 should 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 should 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 micro-organism's potential 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 5 of Council Directive 80/1107/EEC of 27 November 1980 on the protection of workers from the risks related to chemical, physical and biological agents at work<sup>6</sup> and Articles 5 to 17 of Council Directive 90/679/EEC of 26 November 1990 on the protection of workers from the risks related to biological agents at work<sup>7</sup>, 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, should be investigated and reported. Where relevant, methods to kill or render the micro-organism uninfected must be indicated (see section 3, 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 should 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 should include details of frequency, level and duration of exposure, symptoms observed and other relevant clinical observation. Information should 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 should 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 most of the OECD guidelines, to extend the studies concerned with

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<sup>6</sup> OJ L 327, 3.12.1980, p. 8.

<sup>7</sup> OJ L 374, 31.12.1990, p. 1.

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<sup>8</sup>

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<sup>9</sup>

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 should 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<sup>10</sup>, 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 according to 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

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<sup>8</sup> 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).

<sup>9</sup> 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.

<sup>10</sup> An inhalation study may be replaced by an intratracheal study.

should 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 should 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<sup>11</sup>

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 should 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 to 3 clearly demonstrates that the microorganism does not replicate in warm-blooded organisms. A cell culture study should 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 should 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.

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<sup>11</sup> 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.

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 formulants in the plant protection product can influence the toxicity and infectiveness of a microorganism.

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 are 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.

Circumstances in which required

When any result of an in vivo study in somatic cells is positive, in vitro 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 microorganism, 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 included in Annex I to Directive 91/414/EEC,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I to Directive 91/414/EEC,
- 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 through 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 section 2, points 2.4 and 2.5, full experimental residue data as provided for in Annex II, Part A, section 6, 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 agreement with Directive 91/414/EEC, 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 according to point 6.1 suggests persistence of relevant amounts of the microorganism 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 agreement with Directive 91/414/EEC, 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 sections 2.3, 2.5 or 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 through 6.

The function of the micro-organism in environmental processes (as defined in section 2, point 2.1.2) 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 should be sufficient to:

- decide whether, or not, the micro-organism can be included in Annex I,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I,
- specify the hazard symbols (once introduced), the indications of danger, and relevant risk and safety phrases 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 should be characterised. If relevant metabolites are present in or produced by the micro-organism, data as outlined under Annex II, Part A, point 7 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 should 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 should be reported in several cultivated and uncultivated soils representative of soils typical of the various Community regions where use exists or is anticipated. The provisions on choice of soil and its collection and handling, as referred to in Part A, point 7.1, Introduction, 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 to 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 should 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 microorganism, 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, should be sufficient to:

- decide whether, or not, the micro-organism can be included in Annex I,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I,
- 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 hazard symbols (once introduced), the indications of danger, and relevant risk and safety phrases 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 is 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 should, 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) should be reported. Expert judgement is required to decide whether additional studies are necessary. Such decision will take into consideration the available information in this 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 should 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, should be carried out according to the guidance given by the competent authorities of the Member States concerning the format of such summaries and evaluations. It should 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 should 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.