

# **Technical Notes for Guidance on data requirements for micro-organisms including viruses and fungi**

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**EUROPEAN COMMISSION**  
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## **TECHNICAL NOTES FOR GUIDANCE ON DATA REQUIREMENTS FOR MICRO-ORGANISMS INCLUDING VIRUSES AND FUNGI**

This document has been conceived as a working document of the Commission Services, which was elaborated in co-operation with the Member States. It does not intend to produce legally binding effects and by its nature does not prejudice any measure taken by a Member State within the implementation prerogatives under Directive 98/8/EC, nor any case law developed with regard to this provision. This document also does not preclude the possibility that the European Court of Justice may give one or another provision direct effect in Member States.

## **GUIDANCE ON DATA SET FOR ACTIVE SUBSTANCES THAT ARE MICRO-ORGANISMS INCLUDING VIRUSES AND FUNGI**

1. For the purposes of Annex IV the term “micro-organism” is understood to mean the following: “Any 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.”<sup>1</sup>
2. Dossiers on active micro-organisms are required to address at least all the points listed under ‘Dossier requirements’ in Annex IVA to Directive 98/8/EC. For all micro-organisms that are subject to application, all available relevant knowledge and information in literature should be provided. The most important information is related to the characterisation and identification of a micro-organism including mode of action. Such information has to be entered Sections I to IV of the data requirements which defines the basis for an assessment of possible impacts on human health and the environment.
3. Information which is not necessary owing to the nature of the micro-organisms contained in a biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information, as provided for in Article 8(5) of Directive 98/8/EC. In such cases, a justification, acceptable to the competent authority must be submitted. Such a justification may be the existence of a frame-formulation to which the applicant has the right of access.
4. A Dossier has to be prepared on strain level of the micro-organism, unless information is submitted that shows that the species is known to be sufficiently homogeneous, or the applicant provides other arguments in accordance with Article 8(5) of this Directive.
5. Where the micro-organism has been genetically modified, as defined in Council Directive 2001/18/EC of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC<sup>2</sup>, a copy of the evaluation of the data concerning the assessment of the risks to the environment as established in Article 4(2) of that Directive, shall also be submitted.
6. If the biocidal product action is known to be partly or entirely due to the 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 Annexes IIA and, where specified, the relevant parts of Annex IIIA.

### **Dossier requirements**

#### **SECTIONS:**

- I. Identity of the micro-organism
- II. Biological properties of the micro-organism
- III. Further information on the micro-organism
- IV. Analytical methods
- V. Effects on human health
- VI. Residues in or on treated materials, food and feed
- VII. Fate and behaviour in the environment
- VIII. Effects on non-target organisms
- IX. Classification and labelling
- X. Summary and evaluation of sections I to IX including conclusions of the risk assessment and recommendations

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1 Article 2(a) Council Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro-organisms, OJ L 117,8.5.1990, p.1.

2 OJ L 106, 17.4.2001, p.1.

The following data will be required to support submissions on the above points.

## **I. IDENTITY OF THE MICRO-ORGANISM**

### *1.1. Applicant*

The name and address of the applicant (permanent community address) must be provided, as must the name, position, telephone, fax number and email 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, IA or IB is submitted, and if different, the name and address of the local office, agent or representative must be provided, as must the name, position, telephone, fax number and e-mail of the appropriate person to contact.

Where, an agent has submitted the dossier on behalf of the applicant, a power of attorney has to be presented to confirm that the agent is acting on the participant's behalf.

### *1.2. Manufacturer*

The name and address of the manufacturer(s) 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, fax number and email 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, IA or IB, 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. Name and species description, strain characterisation*

The taxonomic identification of a subject micro-organism is a key element in any risk assessment for a biotechnology product. The uses of taxonomy in risk assessment may be seen as having two components:

1. providing a common frame of reference; and
2. use in predictive analysis. In order that predictive analyses can take place, good identification of both the subject and a comparison micro-organism is needed.

By having a well supported name for the organism one may confidently select appropriate related micro-organisms for comparison and have confidence that the use of data from such related organisms will be meaningful in support of assessing the potential risk of the subject micro-organism.

For the taxonomic purposes there is an official nomenclature. Since 1980 there has been an Approved List of Bacterial Names that is maintained by the International Committee on Systematics of Prokaryotes and updated with each publication of the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM). Names of bacteria not previously listed in the "Approved List" in 1980, have been added through publication, either published directly in the IJSEM (or its predecessor the *International Journal of Systematic Bacteriology*, IJSB), or through valid publication in other journals, with subsequent listing through validation lists in IJSB/IJSEM.

Similar validations have also taken place for other micro-organisms such as viruses by the International Committee on Taxonomy of Viruses (ICTV) with the task of developing, refining and maintaining universal virus taxonomy. During a 38-year period the goal has been to categorise the multitude of known viruses into a single classification system that reflects their evolutionary relationships and their individual phylogenies. In the publication *Virus Taxonomy* (Elsevier Academic Press, 2005) and in the periodic updates publication *Virology Division News* (VDN), sections of *Archives of Virology* are posted on the ICTV website: <http://www.danforthcenter.org/iltab/ictvnes/asp/> the concepts of virus taxonomy and updates on newly discovered and described viruses are presented.

Non-members can start with <http://www.danforthcenter.org/iltab/ictvnet/asp/MainPage.asp> and become a member to find the publication.

Methods to generate characterization data range from traditional culture-based phenotypic and biochemical tests to more elaborate molecular techniques. The basic approaches to classification and identification have evolved as the science of microbiology has become more sophisticated, and the methods used to identify and characterised micro-organisms have evolved with these approaches.

### *1.3.1. Common name of the micro-organism (including alternative and superseded names)*

Common name or alternative and superseded names and code names used during the development, if any, must be provided. Further, scientific name of the micro-organism to species level or a level sufficient has to be submitted to show taxonomic relation to known micro-organisms, in particular pathogens.

### *1.3.2. Taxonomic name and strain indicating whether it is a stock variant, a mutant strain or a GMO; for viruses, taxonomic designation of the agent, serotype, strain or mutant*

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. (Guidance Document on the Use of Taxonomy in Risk Assessment of micro-organisms: Bacteria, *OECD Environment, Health and Safety Publications, Series on Harmonisation of Regulatory Oversight in Biotechnology, No.29, Paris 2003.*)

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 I, Part 2, and Annex IB to Directive 2001/18/EC.

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

### *1.3.3. Collection and culture reference number where the culture is deposited*

The micro-organism shall be deposited at an internationally recognised culture collection. This may be one of the Affiliated Members (e.g. DSMZ or ATCC) of the World Federation of Culture Collections (WFCC) who publish the World Directory of Collections of Cultures of Micro-organisms, The accession number given and other details must be submitted.

### *1.3.4. Methods, procedures and criteria used to establish the presence and identity of the micro-organism (e.g. morphology, biochemistry, serology, etc.)*

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, and molecular identification) must be provided. (Guidance Document on Methods for Detection of Micro-organisms introduced into the Environment: Bacteria, *OECD Environment, Health and Safety Publications Series on Harmonisation of Regulatory Oversight in Biotechnology No. 30, Paris 2004* and *Guidance Document on the Use of Taxonomy in Risk Assessment of micro-organisms: Bacteria. OECD Environment, Health and Safety Publications, Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 29, Paris 2003*)

Following methods could be used to provide information about the identity of the micro-organism:

#### *3. Phenotypic methods*

- morphological methods (e.g. colony shape, cell stain, light and electron microscopy)
- physiological methods (e.g. growth temperature, pH of growth range, oxygen and carbon dioxide tolerance, salt tolerance)

- metabolic methods (e.g. nutritional profiles based on utilisation and/or degradation, enzymatic activity)
4. Chemotaxonomic methods
- Typing (e.g. protein, lipopolysaccharide and fatty acid profiles and serotyping in particular serology profiling)
5. Genotypic methods
- genotypic methods (in particular gene probing)
  - DNA base ratios and DNA hybridization
  - DNA-based typing methods (e.g. DNA fingerprinting)
  - RNA-based typing methods (e.g. RNA fingerprinting)
  - sequencing of house keeping genes (in particular amplification of taxon specific DNA/RNA sequences)

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

The information on the identity of the chemical substances such as additives must be provided as outlined in Annex IIA, point 2.8.

##### 1.4.1. *Content of the micro-organism*

The following information must be submitted:

- the content of the micro-organism(s) in the material used for manufacturing of the active substance. These must include the maximum, minimum and nominal content of the viable and non-viable material,
- the content of other chemical substances used in the manufacturing process,
- the content of all other components (such as by-products, condensates, culture medium, etc.) and contaminating micro-organisms, derived from production process.

The contents of the chemical components should be expressed in terms as provided for in Directive 1967/548/EC<sup>3</sup> for chemicals and appropriate terms should be used for micro-organisms (number of active units per volume or weight or any other manner that is relevant to the micro-organism).

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

It is desirable to have a biocidal product without contaminants (including contaminating micro-organisms), if possible. The level and nature of acceptable contaminants should be judged from a risk assessment point of view.

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 IVA, section 1. Relevant metabolites/toxins (i.e. if expected to be of concern to human and animal health and/or the environment) known to be formed by the micro-organism and/or be involved in the mode of action should be identified and characterised at different states or growth stages of the micro-organism.

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 and animal health and/or the environment, the identity and maximum content, expressed in appropriate terms, must be provided. In the case of chemical additives, the identity and the content in g/kg or g/l, as appropriate, must be provided.

The information on the identity of the chemical substances such as additives must be provided as outlined in Annex IIA, point 2.8.

##### 1.4.3. *Analytical profile of batches*

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3 OJ L 196, 16.8.1967, p. 1.

Quality is important in all aspects of the stock biocidal product. The quality of the materials used, e.g. media and other substances, will affect the quality of the cultures and products derived from them. The main source of contamination is substances and materials used to grow the stock.

Information submitted shall lay out the laboratory practice and methods intended to ensure a minimized risk of contamination both during the manufacturing phase of the process and the biocidal product of the seed stock batches.

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).

## **II. BIOLOGICAL PROPERTIES OF THE MICRO-ORGANISM**

Due to their metabolic versatility, micro-organisms are present everywhere in the earth's biosphere, playing many vital functions in environmental metabolism. The deliberate introduction of micro-organisms into the environment requires risk assessments based on detailed information about the source of the micro-organism in question. (Guidance Document on Methods for Detection of Micro-organisms introduced into the Environment: Bacteria, OECD Environment, Health and Safety Publications Series on Harmonisation of Regulatory Oversight in Biotechnology No. 30, Paris 2004 and Guidance Document on the Use of Taxonomy in Risk Assessment of micro-organisms: Bacteria. OECD Environment, Health and Safety Publications, Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 29, Paris 2003)

### *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.

A description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts, organisms with which transfer of genetic material is known to occur under natural conditions etc. should be presented. This information will contribute to the classification and identification of the micro-organism.

In the case of a mutant, or a genetically modified micro-organism (as defined in Annex IA, Part 2, and Annex IB to Directive 2001/18/EC), 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.

#### *2.1.1. Historical background*

The historical background of the wild type micro-organism and its uses (tests/research projects or commercial uses) including other information such as phylogeny and phenotypic and genotypic evolution, should be submitted.

#### *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.

### *2.2. Information on target organism(s)*

The nature of an infection can vary widely with respect to severity, location and number of organisms involved. An infection may or may not result in overt disease. Any parasitic organism or agent that produces an infectious disease is a pathogen. Its ability to cause disease is pathogenicity.

The term virulence refers to the degree or intensity of pathogenicity. It is determined by three characteristics of the pathogen: invasiveness, infectivity and pathogenic potential. Invasiveness is the

ability of the organism to spread to adjacent or other tissues. Infectivity is the ability of the organism to establish a focal point of infection. Pathogenic potential refers to the degree that the pathogen causes morbid symptoms.

Following definitions are used for the purpose of the data requirements as established in Annex IV:

Infectivity/Infectiveness: the ability of a micro-organism to invade and persist in a viable state or to multiply within or on an organism, with or without disease manifestation.

Pathogenicity: the ability of a micro-organism to inflict injury and damage in the host after infection, and depends on host resistance or susceptibility.

Toxicity: the injury or damage in a host caused by a toxin including metabolites; infection, replication or viability of the microorganism is not necessarily required.

It is important to have information on the mode of action of the micro-organism to be able to assess the adverse effects it can exert on humans, animals or the environment. In order to be able to assess possible adverse effects, it is eminent to have information on the target organism(s).

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

Details on the target organisms(s) 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 the case of a relevant metabolite/toxin, the mode of action of this relevant metabolite / 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 relevant metabolites (in particular toxins) may occur (e.g. contact, stomach, inhalation). It must also be stated whether or not the micro-organism or its relevant metabolites/toxins are translocated in other organisms or 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 organism(s)*

Information on the pathogenicity of the micro-organism to the target organism(s) and other non-target organism(s). More details on the host interaction should be presented under the section dealing with mode of action.

Where relevant, details of target organism(s) against which protection is afforded, must be provided. Any available information on the effects on non-target organisms within the area to which the micro-organism(s) may spread shall be given. The occurrence of non-target organism(s) 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 on humans or animals, of whether the organism is capable of colonising or invading humans or animals or the toxic effect of its metabolic products on humans or animals 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.

Information on any other effect ascertained by the micro-organism than infectiousness. The information is more related to ecological effects such as competition or biochemical properties such as toxins or degradation of material which are beneficial to non-target organism(s).



#### 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*

An infectious disease results partly from the pathogen's growth and reproduction (or invasiveness) that often result in tissue alterations. Information on the various pathways which the micro-organism may infect and establish itself in both target organisms and other non-target organisms.

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 environmental conditions (e.g. UV light or acidic waters or soils) 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. 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, plants 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.

If possible, information regarding the mutation frequency and other related data shall be submitted.

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

Metabolites and breakdown products including toxins produced or secreted by the micro-organism may occur in many environmental compartments (in particular in soil, surface waters, groundwater and air), in animal feed or in food for human consumers. These substances could vary in structure, some consist of simple organic molecules such as antimicrobial agents produced by fungi and some are peptides or proteins.

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

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, IA or IB,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I, IA or IB, and
- where relevant, set maximum residue levels to protect consumers, workers handling the treated material and products.

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 and on information submitted in accordance with the data requirements established in this Directive.

## 2.9. *Antibiotics and other anti-microbial agents*

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

Information on the micro-organism's resistance or sensitivity to antibiotics or other antimicrobial 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 the environment, or that it can not transfer its resistance to antibiotics or other anti-microbial agents. Information on the location of the antimicrobial substance resistance gene within the organism has to be provided *e.g.* plasmids, transposons, incorporation sites etc.

## 2.10 *Robustness to Environmental Factors*

Information on the tenacity of the micro-organism, in particular resistance to radiation including UV, dryness, temperature, moisture, salt content or non-antimicrobial chemicals etc. shall be provided.

## 2.11 *Effects on materials, substances and products*

Information on effects, in particular on the unintended effects on non-target materials, substances or products, shall be submitted.

# III. FURTHER INFORMATION ON THE MICRO-ORGANISM

## 3.1. *Function*

The information provided must describe the intended purposes for which biocidal products containing the micro-organism are used, or are to be used and the dose and manner of their use or proposed use. If the micro-organism in itself is not directly affecting the target organism, then the identity of the toxin(s) or metabolite(s) that give rise to the effect shall be provided.

## 3.2. *Field of use envisaged*

The field(s) of use, existing or proposed, for the biocidal products containing the micro-organism must be specified. This information shall also include the indicated fields of use as specified in the Directive. In addition, give a detailed description of the overall use patterns. Information on the fields of

use envisaged should be sufficient to allow for an approximate but realistic estimation of human and environmental exposure to the active substance.

### 3.3. *Product type(s) and category of users for which the micro-organism should be listed in Annex I, IA or IB*

The product type, which covers the intended use, shall also be specified. The product types are established in Annex V to this Directive.

### 3.4. *Method of production and quality control*

Full information on how the micro-organism is produced in bulk must be provided. For mutant strains and GMO's detailed information should be provided on production and isolation, together with all known differences between the mutant strains and parent and naturally occurring strains.

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).

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.

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

Resistance to antimicrobial agents is a relatively common feature in natural microbial communities for a range of different habitats such as soils, aquatic systems and animal- and human-associated habitats. The mechanism(s) conferring resistance to these agents in micro-organisms can vary, including options such as enzymatic inactivation or modification of the microbial agent, modification of host targets to prevent antimicrobial agents binding, failure of the antimicrobial agent to be transported into and/or to be maintained in the micro-organism.

In addition to the presence of antimicrobial substance resistance genes in the antimicrobial agent manufacturer organisms, these genes also occur in natural bacterial assemblages in the so-called "horizontal gene pool", *i.e.* the fraction of genes in the bacterial population that is carried on mobile genetic elements such as plasmids and conjugative transposons. The horizontal gene pool provides flexibility to natural bacterial communities by protecting against the effect of antimicrobial agents at times when antimicrobial agent selective pressure is common in the local habitat.

Information regarding the horizontal gene transfers and other information related to the possibility of the micro-organism to either act as a donor or a recipient to receive antimicrobial substance resistance genes shall be provided. Other information on the wild type population of the micro-organism and occurrence of antimicrobial substance resistance genes shall be provided, in particular if there has been observed cases of antimicrobial substance resistance genes in the population which are of medical or veterinary medicinal importance.

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. In case such genes has been observed in the micro-organism or related members of the species, information on risk management and strategies to deal with the transfer of the genetic material has to be provided.

### 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>4</sup> must be provided for the biocidal product containing micro-organisms, when applicable.

### 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. The methods shall take into account the information submitted in point 3.10.

### 3.9. *Measures in case of an accident*

Information on indications or assumptions that the use of the micro-organism in certain circumstances or environmental conditions might pose a risk to the humans, animals or the environment shall be submitted.

Information on procedures and methods used for rendering the micro-organism harmless in the environment (*e.g.* water or soil) must be provided.

### 3.10. *Procedures for waste management*

Procedures for destruction and decontamination must be developed for both small quantities (user level) and large quantities (warehouse level). The procedures must be consistent with provisions in place relating to the disposal of waste and of toxic waste (Directive 75/442/EEC<sup>5</sup>, as last amended by Regulation (EC) No 1882/2003<sup>6</sup>). The means of disposal proposed should be without unacceptable influence on the environment and be the most cost effective and practical means of disposal feasible. Information has to be submitted in regard to whether the presence of the micro-organisms in the waste makes it hazardous pursuant to Annex III to Directive 91/689/EEC<sup>7</sup>, and in particular if it displays one of the hazard-properties listed in that Annex, in particular infectiousness (H9).

### 3.11. *Monitoring plan to be used for the active micro-organism including handling, storage, transport and use*

The objective of a monitoring plan is to confirm and identify any assumptions regarding the occurrence and impact of potential adverse effects of the micro-organism or its use in the risk assessment is correct. Monitoring should, where relevant, take place after the placing on the market of a biocidal product containing micro-organisms as active substances.

The interpretation of the data collected by monitoring should be considered in the light of other existing environmental conditions and activities.

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4 See doc. 6853/VI/98, Concise outline report of the first peer review meeting on micro-organisms.  
5 OJ L 194, 25.7.1975, p. 39-41.  
6 OJ L 284, 31.10.2003, p. 1.  
7 OJ L 37, 31.12.1991, p. 20-27.

Where changes in the environment are observed, further assessment should be considered to establish whether the changes are due to the micro-organism released or its use, or if such changes may be the result of environmental factors other than the placing of the product on the market.

Experience and data gained through the monitoring of experimental releases of the micro-organism may assist in designing the post marketing monitoring regime required for the placing on the market of biocidal products containing micro-organisms as active substances.

The design of the monitoring plan should be detailed on a case by case basis taking into account the outcome of the risk assessment and also take into account the characteristics of the micro-organism, the characteristics and scale of its intended use and the range of relevant environmental conditions where the micro-organism is expected to be released.

Further, the plan shall incorporate general surveillance measures for unanticipated adverse effects and, if necessary, (case-) specific monitoring focusing on adverse effects identified in the risk assessment:

- whereas case-specific monitoring should be carried out for a sufficient time period to detect immediate and direct as well as, where appropriate, delayed or indirect effects which have been identified in the risk assessment,
- whereas surveillance could, if appropriate, make use of already established routine surveillance practices such as the monitoring of the biocidal, or veterinary and medical products.

It is important that the plan identifies who will carry out the various tasks the monitoring plan requires and who is responsible for ensuring that the monitoring plan is set into place and carried out appropriately, and ensure that there is a route by which the authorisation holder of the biocidal product and the competent authority will be informed on any observed adverse effects on human health and the environment. (Time points and intervals for reports on the results of the monitoring shall be indicated).

Finally, the monitoring plan shall give consideration to the mechanisms for identifying and confirming any observed adverse effects on human health and environment and enable the notifier or the competent authority, where appropriate, to take the measures necessary to protect human health and the environment. The monitoring plan shall also include information on emergency measures in case of accident.

#### **IV. ANALYTICAL METHODS**

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 IIA, points 4.1 and 4.2, are also required for methods used to analyse micro-organisms and their residues.

On request the following samples must be provided:

- samples of the micro-organism as manufactured;
- analytical standards of relevant metabolites (especially toxins) and all other components included in the residue definition;
- if available, samples of reference substances for the relevant impurities.

For this section the following applies:

Impurities: Any component (including contaminating micro-organisms and/or chemical substances) other than the specified micro-organism, originating from the manufacturing process or from degradation of the biocidal product 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 the degradation 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.

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

The information on the methods for the analysis of the micro-organism as manufactured must be provided as outlined in Annex IVA, section 1.3.4. This information shall also include methods used for the analysis of the chemical substances such as additives as outlined in Annex IIA, section 4.

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

Methods to determine and quantify residues (viable or non-viable) of the micro-organism and significant or relevant metabolites (in particular exotoxins/endotoxins), on and/or in a biocidal product, in crops, 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 should be submitted.

Detection of toxins and pathogens requires a combinational methodology from three different fields: biochemical methods, immunological methods, molecular and analytical methods. All these methods alone have their advantages and limitations.

Biochemical methods are applied to purify and separate molecules and study them in vitro. The information which is gained is mainly about the physical properties of the isolated molecule. These methods have their limitations such as the molecule must be assayable, the molecule and its function have to be known and these methods do not reflect the in vivo function of the molecule. Examples of these analytical methods are HPLC, NMR, GC and MS.

Immunological methods could be used to ascertain that putative virulence factors are being produced in the host during infection. These methods determine whether antibodies to bacteria product are protective in infected hosts. The limitation in these methods is usually that the antibodies do cross-react with other antigenic areas and might not be site specific to the molecule. Examples of these methods are ELISA, generation of monoclonal antibodies, Immunoblotting, Liposomes, MIC susceptibility tests (for detection of antimicrobial substance resistance phenotype).

Molecular methods are mainly relying on DNA- and RNA-sequence data of the micro-organisms. The information gained by these methods is very useful to identify the existence of genotypic traits of different organisms. Pathogenic regulations and in vivo simulations could be studied. The main limitation with these methods is that the genetic existence of a virulence factor does not mean that the factor is actually produced by the organism. Examples of these methods are PCR, RT-PCR, Phage display, DNA fingerprinting, DNA hybridization, Recombination, Cloning.

Toxicological 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.

The information on characterisation and identity must be provided as outlined in Annex IVA, section 2.

## V. EFFECTS ON HUMAN HEALTH

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

Available information on the intended use/uses of the micro-organism together with the mode of action must be taken into account. Since micro-organisms are living (except for viruses) organisms the exposure for humans and animals must be assessed together with the mode of action.

The information provided, taken together with that provided for one or more biocidal products 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 biocidal products containing the micro-organism, and the risk for man handling treated products, and the risk for man arising from residual traces or contaminants remaining in food and water.

In addition, the information provided must be sufficient to:

- permit a decision to be made as to whether, or not, the micro-organism can be included in Annex I, IA or IB,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I, IA or IB,
- 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.

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.

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.

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.

## **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 10 of Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual Directive within the meaning of Article 16(1) of Directive 89/391/EEC)<sup>8</sup> and Articles 5 to 17 of Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work

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8 OJ L 131, 5.5.1998, p.11.

(seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC)<sup>9</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 attenuate the micro-organism must be indicated.

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*

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 or epidemiological 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 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-

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9 OJ L 262, 17.10.2000, p. 21.



term studies (carcinogenicity etc., see point 5.3), and whether or not to perform residue studies (see point 3.2).

### 5.2.1. *Sensitisation*<sup>10</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 the test is required

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.

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
- -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 proper test organ has to be decided on a case-by-case basis.

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

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<sup>10</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, unless the applicant submits contradictory data. This approach also takes into consideration immuno-compromised or other sensitive individuals in the population (*e.g.* pregnant women, new-born children or elderly).

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>11</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. *In vitro 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.

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

##### 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 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 insertion mutagenesis in mammal cells or the risk of carcinogenicity has to be discussed.

##### Test conditions<sup>12</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 biocidal product used. Genotoxicity of viruses should be studied on infectious isolates.

##### In vitro mutagenicity tests

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

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11 An inhalation study may be replaced by an intratracheal study.

12 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.

#### 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 micro-organism 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 and the potential integration or interaction with the human genome has to be assessed.

#### 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 biocidal products 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,
- 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. 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 metabolites or toxins needs to be addressed. It should be kept in mind that the formulants in the biocidal product can influence the toxicity and infectiveness of a micro-organism.

##### Circumstances in which required

Information on the short-term infectiveness, pathogenicity and toxicity (respiratory route) of a micro-organism is required, unless the information already provided is sufficient to assess human health effects.

This can be the case if it is demonstrated that the test material has no inhalable fraction and/or repeated exposure is not expected.

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

The first aid measures to be used in the event of infection and in the event of contamination of eyes must be provided. Therapeutic regimes for use in the event of ingestion or contamination of eyes and skin must be described in full. Information based on practical experience, where it exists and is available, in other cases on theoretical grounds, as to the effectiveness of alternative treatment regimes, where relevant, must be provided.

Information on resistance to antimicrobial agents must be provided.

#### 5.2.7. *Any pathogenicity and infectiveness to humans and other mammals under conditions of immunosuppression*

If the micro-organism is suspected to be pathogenic to humans who are immunocompromised, the following points have to be in particular addressed:

- diseases caused and mechanism of pathogenicity including invasiveness and virulence,
- communicability,
- infective dose,
- host range, possibility of alteration,
- possibility of survival outside of human host,
- presence of vectors or means of dissemination,
- biological stability,
- antimicrobial substance resistance patterns,
- allergenicity,
- availability of appropriate therapies.

## **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 adverse effects in short-term studies or may point to 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; this can also include studies on a second species. Before performing such studies, the applicant shall seek agreement of the competent authorities on the type of study to be performed

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

(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 section 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.

## **VI. RESIDUES IN OR ON TREATED MATERIALS, FOOD AND FEED**

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

A substantiated estimation of persistence/competitiveness of the micro-organism and relevant secondary metabolites (especially toxins) in or on the organic material under the environmental conditions prevailing at and after the intended use, taking into account in particular the information provided in sections 1 to 6, 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 material treated with biocidal products or food-/feedingstuffs or during processing of raw products.

### 6.2. *Further information required*

Humans may be exposed to micro-organisms and or its residues for a considerable time as a result of the consumption of or contact with treated food commodities or contact with the material treated with it; potential effects on the humans must, therefore, be derived from chronic or semi-chronic studies, so that a toxicological reference dose, such as the ADI, can be established for risk management.

#### 6.2.1. *Non-viable residues*

A non-viable residue is a residue 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, full experimental residue data as provided for in Annex IIIA, section 6.4-6.7, 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.

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 micro-organism in or on treated products, food or feed, possible effects on humans and/or animals must be investigated, unless it can be justified from section 5, that the micro-organism and its metabolites and/or degradation products are not hazardous to humans in the concentrations and of the nature that could occur as a result of authorised use.

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 has been found in Sections 1 to 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 Annex IIA and IIIA.

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 residues in or on materials, food and feed*

A summary of all data and information provided under section 6, 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 and the environment that may or do arise, and the extent, quality and reliability of the data base.

## **VII. FATE AND BEHAVIOUR IN THE ENVIRONMENT**

Information on the origin, the properties, and the survival of the micro-organism and its residues 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 Annex IV. The interactions of the micro-organism with the environment (as defined in section 2) is of particular interest.

The information provided, taken together with other relevant information, and that for one or more biocidal products 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.

In particular, the information provided should be sufficient to:

- decide whether, or not, the micro-organism can be included in Annex I, IA or IB,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I, IA or IB,
- 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.

Any relevant metabolites / toxins (*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 / toxins are present in or produced by the micro-organism, data as outlined under Annex IIA, section 6 and 7, may be required, if all of the following conditions are met:

- the relevant metabolite / toxin is stable outside the micro-organism, see point 2.7, and
- a toxic effect of the relevant metabolite / toxin is independent of the presence of the micro-organism, and
- the relevant metabolite / toxin is expected to occur in the environment in concentrations higher than under natural conditions.

Available information on the relationship with naturally occurring wild type relatives should be taken into account.

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*

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).

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. If the test organisms to be used in association with other media, e.g. rockwool, this must be included in the test range.

### 7.1.2. *Water*

Where applicable or in case of concern, information should be reported on viability / population dynamics in natural sediment/water systems under both dark and illuminated conditions.

### 7.1.3. *Air*

Where applicable or in case of concern, information should be submitted on viability and concentration of the micro-organism in air, taking into account epidemiological data. This information should in particular address concerns for operator, worker, or bystander exposure.

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

### 7.3. *Summary and evaluation of fate and behaviour in the environment*

A summary of all data and information provided under section 7, 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 and the environment that may or do arise, and the extent, quality and reliability of the data base.

## **VIII. EFFECTS ON NON-TARGET ORGANISMS**

The information on identity, biological properties and further information in sections 1 to 4 is central to the assessment of impact on the environment and non-target organisms. Additional useful information may be found on fate and behaviour in the section 7 and on residue levels in section 4 which, together with information on the nature of the biocidal product 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.

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.

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

In particular, the information provided for the micro-organism, together with other relevant information, and that provided for one or more biocidal products containing it, should be sufficient to:

- decide whether, or not, the micro-organism can be included in Annex I, IA or IB of this Directive,
- specify appropriate conditions or restrictions to be associated with any inclusion in Annex I, IA or IB,
- 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 biohazard,
- 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).

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.

For all studies, average achieved dose in cfu/kg body weight as well as in other appropriate units must be reported.

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 the previous sections has to be taken into account.

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.

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. Useful information may also be available from the observations carried out in efficacy testing.

### 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, infectivity 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 biocidal products. (

#### 8.5. *Effects on earthworms*

##### Aim of the test

Information on toxicity, infectiveness and pathogenicity to earthworms must be reported.

#### 8.6. *Effects on 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.

#### 8.7. *Further 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.

##### 8.7.1 *Terrestrial plants*

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

##### 8.7.2 *Mammals*

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

##### 8.7.3 *Other relevant species and processes*

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

#### 8.8. *Summary and evaluation of effects on non-target organisms*

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, limiting and favouring environmental factors 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.

## **IX. CLASSIFICATION AND LABELLING**

In addition to a proposal for allocation of the micro-organism to one of the risk groups outlined in Article 2 of Directive 2000/54/EC<sup>13</sup>, the applicant should provide a proposal for an appropriate classification and labelling of the micro-organism. The provisions of Directive 67/548/EEC should be used, as far as possible, in order to ensure maximum level of harmonisation with regard to the classification and labelling of the micro-organism.

## **X. SUMMARY AND EVALUATION OF SECTIONS I TO IX INCLUDING CONCLUSIONS OF THE RISK ASSESSMENT AND RECOMMENDATION**

A summary and evaluation of all submitted data relevant to the risk assessment 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 human and animal health for the environment that may or do arise, and the extent, quality and reliability of the data base.

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13 OJ L 262, 17.10.2000, p. 21.

## **GUIDANCE ON DATA SET FOR BIOCIDAL PRODUCTS CONTAINING ACTIVE SUBSTANCES THAT ARE MICRO-ORGANISMS INCLUDING VIRUSES AND FUNGI**

1. This Part provides guidance on the data requirements for the authorisation of a biocidal product based on biocidal products of micro-organisms including viruses or fungi as specified in Annex IV B to Directive 98/8/EC. For the purposes of Annex IV the term “micro-organism” is understood to mean the following: “Any 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.”<sup>14</sup>
2. For all biocidal products based on biocidal products containing micro-organisms that are subject to application, all available relevant knowledge and information in literature should be provided. The most important information is related to characterisation and identification of all the components in a biocidal product. Such information has to be entered Sections II to V of the data requirements which defines the basis for an assessment of possible impacts on human health and the environment.
3. Information which is not necessary owing to the nature of the biocidal product or of its proposed uses need not be supplied. The same applies where it is not scientifically necessary or technically possible to supply the information, as provided for in Article 8(5) of this Directive. In such cases, a justification, acceptable to the competent authority must be submitted. Such a justification may be the existence of a frame-formulation to which the applicant has the right of access.
4. Information may be derived from existing data where a justification acceptable to the competent authority is provided. In particular, the provisions of Council Directive 67/548/EEC<sup>15</sup> of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances and Directive 1999/45/EC<sup>16</sup> of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous biocidal products should be used wherever possible to minimise animal testing.
5. Where testing is done, a detailed description (specification) of the material used and its impurities, according to the provisions of Section II, must be provided. Where necessary, data as established in Annexes IIB, IIIB shall be required for all the toxicologically / eco-toxicologically relevant chemical components of the biocidal product, in particular if the components are substances of concern as defined in Article 2(1)(e) of this Directive.
6. In cases where a new biocidal product is to be dealt with, extrapolation from Annex IV A, could be acceptable, provided that all the possible effects of the components, especially on pathogenicity and infectiveness, are evaluated.

### **Dossier requirements**

#### **SECTIONS:**

- I. Identity of the biocidal product
- II. Physical, chemical and technical properties of the biocidal product
- III. Data on application
- IV. Further information on the biocidal product
- V. Analytical methods
- VI. Efficacy data

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14 Article 2(a) Council Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro-organisms, OJ L 117,8.5.1990, p.1.

15 OJ L 196, 16.8.1967, p. 1.

16 OJ L 200, 30.7.1999, p.1.

- VII. Effects on human health
- VIII. Residues in or on treated materials, food and feed
- IX. Fate and behaviour in the environment
- X. Effects on non-target organisms
- XI. Classification, packaging and labelling of the biocidal product
- XII. Summary and evaluation of sections I to XI including conclusions of the risk assessment and recommendations

The following data will be required to support submissions on the above points.

## **I. IDENTITY OF THE BIOCIDAL PRODUCT**

In order to characterize the formulants in a biocidal product, it is necessary to be able to determine their composition and chemical and physical properties. The parameters critically related to identity and qualities of formulants in a biocidal product are set by the Annexes IIA, IIB, IIIA and IIIB and in case contaminating micro-organisms are present, the necessary data to establish identity are set by Annex IVA.

A specification of the formulants should be brief but it must be supported by appropriate test data. Since a formulant might by its nature be a substance of concern as defined by Article 2(1)(e), the dossier must contain sufficient information on the identity and the quantity of the formulants. The information required in this section must be submitted for all the sources of the biocidal product.

### *1.1. Applicant*

The name and address of the applicant (permanent community address) must be provided, as must the name, position, telephone, fax number and e-mail 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, IA or IB is submitted, and if different, the name and address of the local office, agent or representative must be provided, as must the name, position, telephone, fax number and e-mail of the appropriate person to contact.

Where, an agent has submitted the dossier on behalf of the applicant, a power of attorney has to be presented to confirm that the agent is acting on the participant's behalf.

### *1.2. Manufacturer of the biocidal product and the micro-organism(s)*

The name and address of the manufacturer or manufacturers of biocidal product containing the micro-organism must be provided as must the name and address of each plant in which the biocidal product 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, IA or IB, 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. Trade name or proposed trade name, and manufacturer's development code number of the biocidal product*

All former and current trade names and proposed trade names and development code numbers of the biocidal product referred to in the dossier as well as the current names and numbers must be provided. Full detail of any differences must be provided. The proposed trade name must not give rise to confusion with the trade name of already authorised biocidal products.

#### 1.4. *Detailed quantitative and qualitative information on the composition of the biocidal product*

Each micro-organism that is subject to the application should be identified and named at the species level as set in Annex IVA, sections 1 and 2. In addition, the development phase of the micro-organism (*e.g.* spores, mycelium) in the marketed product shall be stated.

For biocidal products the following information must be submitted:

- the content of the micro-organism(s) in the biocidal product and the content of the micro-organism in the material used for manufacturing of the biocidal product. These must include the maximum, minimum and nominal content of the viable and non-viable material,
- the content of other formulants in the biocidal product,
- the content of all other components (such as by-products, condensates, culture medium, etc.) and contaminating micro-organisms, derived from production process.

The contents should be expressed in terms as provided for in Directive 1999/45/EC<sup>17</sup> for chemicals and appropriate terms should be used for micro-organisms (number of active units per volume or weight or any other manner that is relevant to the micro-organism).

Formulants shall where it is technically and scientifically possible, be identified either by their 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. Their structure or structural formula must be provided. For each component of the formulants the relevant EC (EINECS or ELINCS) number and CAS number where they exist, must be provided. Where the information provided does not fully identify a formulant, an appropriate specification must be provided. The trade name of formulants, where they exist, must also be provided.

For formulants the function must be given:

- adhesive (sticker)
- antifoaming agent
- antifreeze
- binder
- buffer
- carrier
- deodorant
- dispersing agent
- dye
- emetic
- emulsifier
- fertiliser
- odorant
- perfume
- preservative
- propellant
- repellent
- safener
- solvent
- stabiliser
- synergist
- thickener
- wetting agent
- any other substance present in the biocidal product (specify)

Identification of contaminating micro-organisms and other components derived from production process.

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17 OJ L 200, 30.7.1999, p. 1.

Contaminating micro-organisms must be identified as outlined in Annex IVA, sections 1 and 2.

Chemicals (inert components, by-products, etc.) must be identified as outlined in Annex IIA, section 2.

Where the information provided does not fully identify a component, such as condensate, culture medium, etc., detailed information on the composition must be provided for each such component.

### 1.5. *Physical state and nature of the biocidal product*

A full description of the physical nature and state of the biocidal product must be provided, together with a proposal for a suitable description of the type of biocidal product and a proposal for its definition, e.g. emulsifiable concentrate, wettable powder, solution etc.

### 1.6. *Function*

The biological function of the biocidal product shall be specified as set in Annex V. Where applicable the function and the area of use of the biocidal product shall be further specified and elaborated to reflect sub functions within a given product type, such as control of certain bacteria, fungi, viruses or control of specific insects etc.

## **II. PHYSICAL, CHEMICAL AND TECHNICAL PROPERTIES OF THE BIOCIDAL PRODUCT**

The information shall provide direct input parameters for assessing physical, chemical and technical hazards, prerequisites for performing and guidance information for optimising other tests.

The data requirements established in this section are expected to correspond to the same type of information as specified in Annex IIB, Section III.

### 2.1. *Appearance (colour and odour)*

A description of both the colour and odour, if any, and the physical state of the biocidal product, must be provided.

For substances with intense odour or taste in water, a description of the substance(s) in question must be given, together with a threshold concentration for air or water, if available.

### 2.2. *Storage stability and shelf-life*

#### 2.2.1. *Effects of light, temperature and humidity on technical characteristics of the biocidal product*

The physical and biological stability of the biocidal product at the recommended storage temperature including information on the growth of contaminating micro-organisms must be determined and reported. The conditions under which the test has been performed must be justified.

Additionally in the case of liquid biocidal products, the effect of low temperatures on physical stability, must be determined and reported according to CIPAC<sup>18</sup> Methods MT 39, MT 48, MT 51 or MT 54 as appropriate.

The shelf life of the biocidal product at the recommended storage temperature must be reported. Where shelf life is less than two years, the shelf life in months, with appropriate temperature specifications, must be reported. Useful information could be found in GIFAP<sup>19</sup> Monograph No 17.

#### 2.2.2. *Other factors affecting stability*

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18 Collaborative International

19 International Group of National Pesticide Manufacturers' Associations.

Effect of exposure to air, packaging, etc., on the product stability must be explored.

### 2.3 *Explosivity and oxidising properties*

Explosive properties shall be determined as defined in Annex IIB, point 3.2. EC method A.14 could be used to establish this end point.

Oxidising properties will be determined as defined in Annex IIB, point 3.3. Oxidising properties do not have to be determined if it can be shown without reasonable doubt on the basis of thermodynamic information that the biocidal product is incapable of reacting exothermically with combustible materials. EC method A.17 could be used to establish this end point.

An acceptable justification for non-performance of a test for explosive and oxidising properties is where none of the components are classified as explosive or oxidising, and where available thermodynamic information establishes beyond reasonable doubt that the product is incapable of exothermic reaction.

### 2.4 *Flash point and other indications of flammability or spontaneous ignition*

Flash point and flammability must be determined, as defined in Annex IIB, point 3.4, unless it can be justified that it is technically or scientifically not necessary to perform such studies.

An acceptable justification for non-performance of a test for flammability properties is where none of the components are classified as flammable and where available thermodynamic information establishes beyond reasonable doubt that the product is incapable of exothermic reaction.

The flash-point of liquids must be determined and reported according to EC method A.9 and the flammable properties of solids and gases according to EC methods A.10 (solids), A.11 (gases), and A.12 (contact with water), as appropriate. The auto-flammability of biocidal products must be determined and reported according to A.15 (liquids and gases) or A.16 (solids), as appropriate.

### 2.5 *Acidity, alkalinity and pH value*

Acidity, alkalinity and pH will be determined as defined in Annex IIB, point 3.5, unless it can be justified that it is technically or scientifically not necessary to perform such studies.

The product pH should be determined and if found to be acidic or alkaline, the quoted test method used. In cases where biocidal products are acidic (pH<4), the acidity and pH must be determined and reported e.g. according to CIPAC method MT31 (MT is Material Test) and where biocidal products are alkaline (pH>10) the alkalinity must be determined and reported e.g. according to CIPAC method MT 75. The pH of a 1% aqueous dilution, emulsion or dispersion of biocidal product must be determined e.g. according to CIPAC method MT 75, where relevant.

### 2.6 *Viscosity and surface tension*

In the case of liquid biocidal products for Ultra Low Volume use (ULV) the kinematic viscosity must be determined and reported according to OECD Test Guideline 114.

For non newtonian liquids the viscosity must be determined and reported together with the test conditions. In the case of liquid biocidal products the surface tension has to be determined and reported according to EEC method A 5.

### 2.7 *Technical characteristics of the biocidal product*

The technical characteristics of the biocidal product must be determined to permit a decision to be made as to its acceptability. If tests have to be performed, they must be done at temperatures compatible with survival of the micro-organism.

#### 2.7.1 *Wettability*



The wettability of solid biocidal products which are diluted for use (e.g. wettable powders and water dispersible granules), must be determined and reported according to CIPAC Method MT 53.3.

#### 2.7.2. *Persistent foaming*

The persistence of foaming of biocidal products to be diluted with water, must be determined and reported according to CIPAC Method MT 47.

#### 2.7.3. *Suspensibility and suspension stability*

The suspensibility of water dispersible products (e.g. wettable powders, water dispersible granules, suspension concentrates) must be determined and reported according to CIPAC Method MT 15, MT 161 or MT 168 as appropriate.

The spontaneity of dispersion of water dispersible products (e.g. suspension concentrates and water dispersible granules) must be determined and reported according to CIPAC Methods MT 160 or MT 174 as appropriate.

#### 2.7.4. *Dry sieve test and wet sieve test*

In order to ensure that dustable powders have a suitable particle size distribution for ease of application, a dry sieve test must be conducted and reported according to CIPAC Method MT 59.1. In the case of water dispersible products, a wet sieve test must be conducted and reported according to CIPAC Method MT 59.3 or MT 167 as appropriate.

#### 2.7.5. *Particle size distribution (dustable and wettable powders, granules), content of dust/fines (granules), attrition and friability (granules)*

The size distribution of particles in the case of powders, must be determined and reported according to OECD Method 110. The nominal size range of granules for direct application must be determined and reported in accordance with CIPAC MT 58.3, for water dispersible granules in accordance with CIPAC MT 170.

The dust content of granular biocidal products, must be determined and reported according to CIPAC Method MT 171. If relevant for operator exposure the particle size of dust must be determined and reported according to OECD Method 110.

The friability and attrition characteristics of granules, must be determined and reported once internationally agreed methods are available. Where already data are available they must be reported together with the method used.

#### 2.7.6. *Emulsifiability, re-emulsifiability, emulsion stability*

The emulsifiability, emulsion stability and re-emulsifiability of biocidal products which form emulsions, must be determined and reported according to CIPAC Method MT 36 or MT 173 as appropriate.

The stability of dilute emulsions and of preparations which are emulsions, must be determined and reported according to CIPAC Method MT 20 or MT 173.

#### 2.7.7. *Flowability, pourability (rinsability) and dustability*

The flowability of granular biocidal products must be determined and reported according to CIPAC Method MT 172.

The pourability (including rinsed residue) of suspensions (e.g. suspension concentrates, suspo-emulsions), must be determined and reported according to CIPAC Method MT 148.

The dustability of dustable powders must be determined and reported according to CIPAC Method MT 34 or another suitable method.

## 2.8. *Physical, chemical and biological compatibility with other products including biocidal products with which its use is to be authorised or registered*

### 2.8.1. *Physical compatibility*

The physical compatibility of recommended tank mixes must be determined and reported.

### 2.8.2. *Chemical compatibility*

The chemical compatibility of recommended tank mixes must be determined and reported except where examination of the individual properties of the biocidal products would establish beyond reasonable doubt that there is no possibility of reaction taking place. In such cases it is sufficient to provide that information as justification for not practically determining the chemical compatibility.

### 2.8.3. *Biological compatibility*

The biological compatibility of tank mixes must be determined and reported. Effects, such as antagonism, on the activity of the micro-organism after mixing with other micro-organisms or chemicals must be described. The possible interaction of the biocidal product with other chemical products to be applied under the expected condition of use of the biocidal product should be investigated, based on the efficacy data. Intervals between application of the biological biocidal product and chemical biocidal product should be specified, if appropriate, in order to avoid loss of efficacy.

## 2.9. *Summary and evaluation of physical, chemical and technical properties of the biocidal product*

A summary of all data and information provided under points 3.1 through 3.8, 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.

## **III. DATA ON APPLICATION**

The Information on intended uses of the product, together with its micro-organisms, must be sufficient to permit an evaluation of the biocidal product, including the nature and benefits that accrue following use of the product in comparison to suitable reference products or damage thresholds, and to define its conditions of use.

### 3.1. *Field of use envisaged*

The intended and potential uses should be indicated together with the fields of use. In addition, a detailed description of the overall use patterns of the product should be given. This information on the use envisaged should be sufficient to allow an approximate but realistic estimation of human and environmental exposure to the product.

In addition, give a detailed description of the overall use patterns. Information on the fields of use envisaged should be sufficient to allow for an approximate but realistic estimation of human and environmental exposure to the active substance.

### 3.2. *Mode of action*

The mode of action in terms, where relevant, of the biochemical and physiological mechanism(s) and biochemical pathways involved should be stated. Where available, the results of experimental studies must be reported.

Where it is known that in order to exert its intended effect the active substance must be converted into a metabolite or degradation product following application or use of a biocidal product containing it, justification should be submitted for why this metabolite or degradation product is not considered to be the active substance. In addition, available information relating to the formation of reactive metabolites or reaction products must be provided.

This information must include:

- the chemical name, empirical and structural formula, molecular mass, and CAS and EC (EINECS, ELINCS or No Longer Polymers list) numbers if available;
- the processes, mechanisms and reactions involved;
- kinetic and other data concerning the rate of conversion and if known the rate limiting step; and environmental and other factors effecting the rate and extent of conversion.

Indicate also if the actual active substance is the result of a combined action of different products (i.e. when such a combination is necessary to achieve the intended effect).

### 3.3. *Details of intended use*

This information on the use envisaged should be sufficient to allow an approximate but realistic estimation of human and environmental exposure to the product. Any relations which give case to exposure e.g. relevant product types should always involve further studies/estimation of human and environmental exposure.

For material preservatives of product types 6, 7, 9, and 10, the different use areas in which the material treated with the product is intended to be used should be indicated for these preservatives (e.g. indoors or outdoors, in cattle sheds, or in drinking water or food storage or processing or their facilities).

For product type 8, the hazard classes, as defined in the standard EN 335-1 (CEN 1992), in which wood treated with the product is intended to be used should be indicated for wood preservatives. For uses not described in this standard, such as curative or anti-sapstain products, see also guidance document by the European Wood Preservation Manufacturers' Group (EWPM 1996) describing also these other use sectors.

For product type 21, in addition to the fields of use, specify also if the product is intended to be used in marine environments, in brackish water and/or in fresh waters. The uses should also distinguish between for example, aqua-culture, buoys and other small static objects, sluice doors, harbour constructions, oil rigs, inlet pipes of cooling water systems, marine sensors, ships' hulls (e.g. deep sea, coastal, inland waterway vessels), etc.

Further information could be found in TNsG on data requirements for active substances and biocidal products.<sup>20</sup>

### 3.4. *Application rate*

Where applicable, the recommended dose of the product and the micro-organism per object (e.g. per surface area of the material to be protected or as a concentration in a water system) shall be submitted.

Further information could be found in TNsG on data requirements for active substances and biocidal products.<sup>21</sup>

### 3.5. *Contents of micro-organism in material used (e.g. in the application device or bait)*

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20 TNsG on data requirements for active substances and biocidal products, Lines 472-494.

21 TNsG on data requirements for active substances and biocidal products, Lines 1498-1505.

The content of the micro-organism(s) in the biocidal product and the content of the micro-organism in the material used for manufacturing of the biocidal product must be provided. These must include the maximum, minimum and nominal content of the viable and non-viable material.

The contents should be expressed in appropriate terms used for micro-organisms (number of active units per volume or weight or any other manner that is relevant to the micro-organism).

### 3.6. *Method of application*

The method of applying the biocidal product for the different intended uses must be provided. If the product is to be diluted, the substance used for dilution and concentration as a percentage of the active substance in the solution must be stated. A description of the application technique (e.g. dipping, spreading, spraying, automatic/manual dosing etc.) should be included.

The substances that may have to be added to the solution and their dosages must also be given. If certain technical device(s) will be used together with the biocidal product, a description of this device(s) should be provided.

Where applicable, the recommended dose of the product and the micro-organism per object (e.g. per surface area of the material to be protected or as a concentration in a water system) shall be submitted.

Further information could be found in TNsG on data requirements for active substances and biocidal products.<sup>22</sup>

### 3.7. *Number and timing of applications and duration of protection*

The maximum number of applications to be used and their timing must be reported. Where possible and necessary the interval between applications, in days, must be stated. The duration of protection afforded both by each application and by the maximum number of applications to be used, must be indicated.

Where relevant, it should be indicated how the applications differ in various areas of the Community due to the environmental differences. If such variations exist, then it should also be indicated the recommended duration of application and possible re-applications for every area of use.

Further information could be found in TNsG on data requirements for active substances and biocidal products.<sup>23</sup>

### 3.8. *Necessary waiting periods or other precautions to avoid adverse effects to human and animal health and the environment*

Where relevant, minimum waiting periods between the applications must be specified in order to avoid adverse effects to human and animal health and the environment.

Information on indications or assumptions that the use of the biocidal product in certain circumstances or environmental conditions might pose a risk to the humans, animals or the environment shall be submitted.

Where relevant, any conditions under which the biocidal product may be or not be used shall be stated and the reasons why such uses pose risks to human, animal health or the environment must be specified.

Further information could be found in TNsG on data requirements for active substances and biocidal products.<sup>24</sup>

### 3.9. *Proposed instructions for use*

The proposed instructions for use of the biocidal product, to be printed on labels and leaflets, must be provided.

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22 TNsG on data requirements for active substances and biocidal products, Lines 1489-1544.

23 TNsG on data requirements for active substances and biocidal products, Lines 1489-1544.

24 TNsG on data requirements for active substances and biocidal products, Lines 1489-1544.

### 3.10. *Category of users*

Category of users should be indicated such as: industrial, professional, general public (non-professional)

- industrial user, i.e. manufacturer of products (manufacture: all operations of purchase of starting materials and packaging materials, production, quality control release, storage, distribution of products and the related controls; where production can be defined as: all operations involved in the preparation of a product, from receipt of materials, through processing and packaging, to its completion as a finished product);
- professional user, including also other professional user than manufacturer; and
- non-professional user (the general public) at work-place and/or at home.
- Indicate user with the help of the categories given above, including also 'other professional user than industrial' where relevant.

The following are examples of the use(r) categories: vacuum impregnation of timber and addition of in-can preservatives are industrial use, preservatives for liquid-cooling and processing systems are used by professionals.

### 3.11. *Information on the possible occurrence of the development of resistance*

Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies should be submitted.

The information should also include the issue of cross-resistance. Where such information is not directly relevant to the uses for which authorisation is sought or to be renewed (e.g. different species of harmful organism), it must nevertheless be submitted as it may provide an indication of the likelihood of resistance development in the target population.

Where there is evidence or information to suggest that in commercial experimental use the development of resistance is likely, evidence must be generated and submitted as to the sensitivity to the substance on the part of the populations of the harmful organism concerned. In such cases a management strategy designed to minimise the likelihood of resistance or cross-resistance developing in target species must be provided.

### 3.12. *Effects on the materials or products treated with the biocidal product*

Where relevant, and based on the test data, any effects on the treated area should be reported. In particular, if the effect is an adverse effect.

Special attention should be paid towards the influence of formulation substances on the residue behaviour of the micro-organism and its metabolites.

## **IV. FURTHER INFORMATION ON THE BIOCIDAL PRODUCT**

### 4.1. *Packaging and compatibility of the biocidal product with proposed packaging materials*

Packaging to be used must be fully described and specified in terms of the materials used, manner of construction (e.g. extruded, welded etc.), size and capacity, size of opening, type of closure and seals. It must be designed in accordance with the criteria and guidelines specified in the FAO "Guidelines for the Packaging of Pesticides".

The suitability of the packaging, including closures, in terms of its strength, leak proofness and resistance to normal transport and handling, must be determined and reported according to ADR Methods 3552, 3553, 3560, 3554, 3555, 3556, 3558, or appropriate ADR Methods for intermediate bulk containers, and, where for the biocidal product child-resistant closures are required, according to ISO standard 8317.

The resistance of the packaging material to its contents must be reported according to GIFAP Monograph No 17.

#### 4.2. *Procedures for cleaning application equipment*

Cleaning procedures for both application equipment and protective clothing must be described in detail. The effectiveness of the cleaning procedure must be determined, using e.g. biotests, and reported.

#### 4.3. *Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment*

The information provided must follow from and be supported by the data provided for the active substance(s) and that provided under sections 7 and 8.

Where relevant intervals, re-entry periods or withholding periods necessary to minimize the presence of residues in or on treated areas or spaces, with a view to protecting man or animals, must be specified e.g. re-entry period (in hours or days) for man to buildings or spaces treated or waiting period (in days), between application and handling treated products.

Where necessary, in the light of the test results, information on any specific human health or environmental conditions under which the biocidal product may or may not be used must be provided.

#### 4.4. *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>25</sup> and Article 14 of Directive 1999/45/EC must be provided for the biocidal product.

#### 4.5. *Measures in the case of an accident*

Information must be submitted on procedures and methods to be used in case of accidents for rendering the effects of the biocidal product harmless in humans, animals or the environment.

Whether arising during transport, storage or use, detailed procedures to be followed in the event of an accident, must be provided and include:

- containment of spillages;
- decontamination of areas, vehicles and buildings;
- disposal of damaged packaging, adsorbents and other materials;
- protection of emergency workers and bystanders; and
- first aid measures.

#### 4.6. *Procedures for destruction or decontamination of the biocidal product and its packaging*

Methods to dispose safely of the biocidal product 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. The methods shall take into account the information submitted in Section 2.

Procedures for destruction and decontamination must be developed for both small quantities (user level) and large quantities (warehouse level). The procedures must be consistent with provisions in place relating to the disposal of waste and of toxic waste. The means of disposal proposed should be without unacceptable influence on the environment and be the most cost effective and practical means of disposal feasible.

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

The procedures must be consistent with provisions in place relating to the disposal of waste and of toxic waste (Directive 75/442/EEC<sup>26</sup>, as last amended by Regulation (EC) No 1882/2003<sup>27</sup>). The means of disposal proposed should be without unacceptable influence on the environment and be the most cost effective and practical means of disposal feasible. Information has to be submitted in regard to whether the presence of the micro-organisms in the waste makes it hazardous pursuant to Annex III to Directive 91/689/EEC<sup>28</sup>, and in particular if it displays one of the hazard-properties listed in that Annex, in particular infectiousness (H9).

#### 4.6.1. *Controlled incineration*

In many cases the preferred or sole means to safely dispose of biocidal products and in particular the formulants contained in it, contaminated materials, or contaminated packaging, is through controlled incineration in a licensed incinerator.

The applicant must provide detailed instructions for safe disposal.

#### 4.6.2. *Others*

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

#### 4.7. *Monitoring plan to be used for the active micro-organism and other micro-organism(s) contained in the biocidal product including handling, storage, transport and use*

The objective of a monitoring plan is to confirm and identify any assumptions regarding the occurrence and impact of potential adverse effects of the micro-organism or its use in the risk assessment is correct. Monitoring should take place after the placing on the market of a biocidal product containing micro-organisms as active substances.

The interpretation of the data collected by monitoring should be considered in the light of other existing environmental conditions and activities.

Where necessary in addition to point 3.11 Annex IVA the monitoring plans shall take into account the outcome of the risk assessment of the biocidal product containing the micro-organism and also take into account the characteristics of the formulants, the characteristics and scale of its intended use and the range of relevant environmental conditions where the biocidal product is expected to be used.

## V. ANALYTICAL METHODS

The provisions of this section only cover analytical methods required for post-registration control and monitoring purposes of the biocidal product. It is desirable to have a biocidal product without contaminants, if possible. The level of acceptable contaminants should be judged from a risk assessment point of view, by the competent authority and on a case-by-case basis. Information on analytical methods is required for assessing compliance with conditions for issuing authorisation for a biocidal product according to Article 5(1)(c). For products which are difficult to analyse a description of the problems should be given.

Both production and product must be subject to a continuous quality control by the applicant. The quality criteria for the product should be submitted. 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.

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26 OJ L 194, 25.7.1975, p. 39-41.  
27 OJ L 284, 31.10.2003, p. 1.  
28 OJ L 37, 31.12.1991, p. 20-27.

Descriptions of methods must be provided and include details of equipment, materials and conditions used. The applicability of existing CIPAC methods must be reported. As far as practicable these methods must employ the simplest approach, involve the minimum cost, and require commonly available equipment.

For this section the same definitions as set in Section VII, Annex IVA shall apply.

On request the following samples must be provided:

- samples of the biocidal product;
- samples of the micro-organism as manufactured for every source of production covered by the application;
- analytical standards of the pure micro-organism;
- analytical standards of relevant metabolites and all other components included in the residue definition;
- if available, samples of reference substances for the relevant impurities.

For this section the following applies:

Impurities: Any component (including contaminating micro-organisms and/or chemical substances) other than the specified micro-organism, originating from the manufacturing process or from degradation of the biocidal product 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 the degradation 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.

### 5.1. *Methods for the analysis of the biocidal product*

Methods, which must be described in full, must be provided for the identification and the determination of the content of the micro-organism in the biocidal product.

In the case of a biocidal product containing more than one micro-organism, methods capable of identifying and determining the content of each one should be provided.

Methods to establish regular control of the final product (biocidal product) in order to show that it does not contain other organisms than the indicated ones and to establish its uniformity, methods to identify any contaminating micro-organisms of the biocidal product, and methods used to determine the storage stability and shelf life of the biocidal product must be provided.

### 5.2. *Methods to determine and quantify residues*

Analytical methods for the determination of residues, as defined in Annex IIB, section 4 and Annex IVA, section 7 must be submitted unless it is justified that the information already submitted according to the requirements of Annex IIB, section 4 and Annex IVA, section 7 is sufficient.

## **VI. EFFICACY DATA**

The efficacy of the micro-organism is either determined by the level of infectivity and pathogenicity or due to its properties. A high infectivity rate and a high level of mortality of the target organism, will increase the efficacy of the micro-organism. The micro-organism could also exert a controlling effect by



compete out the other target organisms or by other biochemical means control the activity of the target organism.

The applicant must demonstrate that the biocidal product is effective and suitable for its intended use when applied according to its instructions for use. This can be confirmed by provision of data that may include laboratory studies, pilot plants or field test data or other relevant study data, the test conditions of which are comparable with the purpose applied for and which are comparable with the environmental characteristics relevant for the intended use. Further product-type-specific guidance is given in the guidance document on product evaluation in support of Annex VI of the Directive.

For field studies conducted outside the territory of the Member State in which the authorisation is being sought, a justification of the relevance of such data must be made. The extent of the information required will vary depending on the product type and proposed use pattern and upon the similarity of the conditions in the two countries. Justification may include, as relevant and appropriate, information on the target organism (*e.g.* comparison of genera/species and its relevance to the Member State in which authorisation is sought), meteorological parameters (*e.g.* mean temperatures and rainfall) and location details.

The test method should measure a response and, as appropriate, an end-point relevant to the label claims. The method should employ a reference product for comparison, if possible, and an untreated control. The efficacy test reports should contain dose response data for dose rates lower than the recommended rate. However, this may not be always possible for field studies.

Where earlier formulations of the product or other products containing the same active substance(s) are cited as supporting evidence, all relevant formulation details must be given and the relevance of this evidence to the current formulation must be fully justified.

Further information could be found in TNsG on data requirements for active substances and biocidal products.<sup>29</sup>

## VII. EFFECTS ON HUMAN HEALTH

For proper evaluation of the toxicity including potential for pathogenicity and infectivity of biocidal products sufficient information should be available on acute toxicity, irritation and sensitization of the micro-organism, as laid down in Annex IVA. If possible, additional information on mode of toxic action, toxicological profile and all other known toxicological aspects of the micro-organism should be submitted. Special attention should be given to co-formulants.

While performing toxicology studies, all signs of infection or pathogenicity should be noted. Toxicology studies should include clearance studies.

In the context of the influence that impurities and other components can have on toxicological behaviour, it is essential that for each study submitted, a detailed description (specification) of the material used, be provided. Tests must be conducted using the biocidal product to be authorized. In particular, it must be clear that the micro-organism used in the biocidal product, and the conditions of culturing it, are the same for which information and data are submitted in the context of Annex IVA.

A tiered testing system will be applied to the study of the biocidal product.

### 7.1. *Basic acute toxicity studies*

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 biocidal product, and in particular to establish, or indicate:

- the toxicity of the biocidal product;
- toxicity of the biocidal product relative to the micro-organism;

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<sup>29</sup> TNsG on data requirements for active substances and biocidal products, Lines 1564-1646.

- the time course and characteristics of the effect with full details of behavioural changes and possible gross pathological findings at post-mortem;
- where possible the 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 biocidal product to be classified in accordance with Directive 1999/45/EC. The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

### 7.1.1. *Acute oral toxicity*

#### Circumstances in which required

An acute oral test should always be carried out unless the applicant can justify in accordance with Article 8(5) that it would be scientifically unnecessary to conduct the test or technically not feasible.

#### Test guideline

The test must be carried out in accordance with Directive 92/69/EEC Method B1 or B1 bis.

### 7.1.2. *Acute inhalation toxicity*

#### Aim of the test

The test will provide the inhalation toxicity of the biocidal product.

#### Circumstances in which required

The test must be carried out where the biocidal product:

- is used with fogging equipment,
- is an aerosol,
- is a powder containing a significant proportion of particles of diameter <50 micrometer (> 1% on a weight basis),
- is to be applied from aircraft in cases where inhalation exposure is relevant,
- is to be applied in a manner which generates a significant proportion of particles or droplets of diameter <50 micrometer (> 1% on a weight basis).
- contains a volatile component at greater than 10%

#### Test guideline

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

### 7.1.3. *Acute percutaneous toxicity*

#### Circumstances in which required

An acute percutaneous test should always be carried out unless the applicant can justify in accordance with Article 8(5) that it would be scientifically unnecessary to conduct the test or technically not feasible.

#### Test guideline

The test must be carried out in accordance with Directive 92/69/EEC Method B3.

## 7.2. *Additional acute toxicity studies*

### 7.2.1. *Skin irritation*

#### Aim of the test

The test will provide the potential of skin irritancy of the biocidal product including the potential reversibility of the effects observed.

#### Circumstances in which required

The skin irritancy of the biocidal product must always be determined, except where the formulants are not expected to be skin irritant or the micro-organism is shown not to be skin irritant or where it is likely, as indicated in the test guideline, that severe skin effects can be excluded.

#### Test guideline

The test must be carried out in accordance with Directive 92/69/EEC Method B4.

### 7.2.2. *Eye irritation*

#### Aim of the test

The test will provide the potential for eye irritation of the biocidal product, including the potential reversibility of the effects observed.

#### Circumstances in which required

The eye irritancy of the biocidal product must be determined, where the formulants are suspected to be eye irritant, except where the micro-organism is eye irritant or where it is likely, as indicated in the test guideline, that severe effects on the eyes may be produced.

#### Test guideline

The eye irritation must be determined in accordance with Directive 92/69/EEC Method B5.

### 7.2.3. *Skin sensitisation*

#### Aim of the test

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

#### Circumstances in which required

The test must be carried out where the formulants are suspected to have skin sensitizing properties, except where the micro-organism(s) or the formulants are known to have skin sensitizing properties.

#### Test guideline

The tests have to be carried out in accordance with Directive 92/69/EEC Method B6.

### 7.3. *Data on exposure*

The risks for those in contact with biocidal products (operators, bystanders, workers), depend on the physical, chemical and toxicological properties of the biocidal product as well as the type of the product (undiluted/diluted), formulation type, and on the route, the degree and duration of exposure. Sufficient information and data must be generated and reported to permit an assessment of the extent of exposure to the biocidal product likely to occur under the proposed conditions of use.

In the cases where there is particular concern on the possibility of dermal absorption based on the information for the micro-organism available in Annex IVA, section 5 and section 8 or from the information provided for the biocidal product in the present section of Annex IVB, further dermal absorption data can be necessary.

Results from exposure monitoring during production or use of the product must be submitted.

The above mentioned information and data must provide the basis for the selection of appropriate protective measures including personal protective equipment to be used by operators and workers and to be specified on the label.

#### 7.4. *Available toxicological data relating to non-active substances*

A copy of the notification and the safety data sheet submitted in the context of Directive 1999/45/EC and Commission Directive 91/155/EEC<sup>30</sup> of 5 March 1991 defining and laying down the detailed arrangements for the system of specific information relating to dangerous preparations in implementation of Article 10 of Directive 88/379/EEC must be submitted for each formulant. All other available information should be submitted.

Where a substance of concern has been identified, the test(s) described in Annex II A section 6 and section 7 shall be required for these toxicologically relevant non-active substances contained in the biocidal.

#### 7.5. *Supplementary studies for combinations of biocidal products*

##### Aim of the test

In certain cases it may be necessary to carry out the studies as referred to under points 7.1 to 7.2.3 for a combination of biocidal products where the product label includes requirements for use of the biocidal product with other biocidal products and/or with adjuvants as a tank mix.

Decisions as to the need for supplementary studies must be made on a case-by-case basis, taking into account the results of the acute toxicity studies of the individual biocidal products, the possibility for exposure to the combination of the products concerned and available information or practical experience with the products concerned or similar products.

#### 7.6. *Summary and evaluation of effects on human health*

A summary of all data and information provided under section 7, 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.

### **VIII. RESIDUES IN OR ON TREATED MATERIALS, FOOD AND FEED**

The same provisions as detailed in Annex IVA, section 6, apply. The information required according to this section has to be provided unless it is possible to extrapolate the residue behaviour of the biocidal product on the basis of the data available for the micro-organism.

Special attention should be paid towards the influence of formulation substances on the residue behaviour of the micro-organism and its metabolites.

### **IX. FATE AND BEHAVIOUR IN THE ENVIRONMENT**

The same provisions as detailed in Annex IVA, section 7 apply. The information required according to this section has to be provided unless it is possible to extrapolate the fate and behaviour of the biocidal product in the environment on the basis of the data available in Annex IVA, section 7.

### **X. EFFECTS ON NON-TARGET ORGANISMS**

The information provided, taken together with that for the micro-organism(s), must be sufficient to permit an assessment of the impact on non-target species (flora and fauna), of the biocidal product, when used as

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OJ L 76, 22.3.91, p35.

proposed. Impact can result from single, prolonged or repeated exposure, and can be reversible, or irreversible.

The choice of the appropriate non-target organisms for testing of environmental effects should be based on the information on the micro-organism, as required in Annex IVA, and on the information on the formulants and other components, as required by the present Annex. From such knowledge it would be possible to choose the appropriate test organisms, such as organisms closely related to the target organism.

In particular, the information provided for the biocidal product, together with other relevant information, and that provided for the micro-organism, should be sufficient to:

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).

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

permit an evaluation whether special precautions are necessary for the protection of non-target species.

In general, much of the data relating to impact on non-target species, required for authorization of biocidal products, will have been submitted and evaluated for the inclusion of the micro-organism(s) in Annex I, IA or IB.

Where exposure data are necessary to decide whether a study has to be performed, the data obtained in accordance with the provisions of Annex IVA should be used.

For the estimation of exposure of organisms all relevant information on the biocidal product and on the micro-organism must be taken into account. Where relevant the parameters provided for in this section should be used. Where it appears from available data that the biocidal product has a stronger effect than the micro-organism, the data on effects on non target organisms of the biocidal product have to be used for the calculation of relevant effect/exposure ratios.

In order to facilitate the assessment of the significance of test results obtained, the same strain of each relevant species should where possible be used in the various specified tests for effects on non target organisms.

#### *10.1. Effects on birds*

The same information as provided in Annex IVA, point 8.1 has to be reported where it is not possible to predict the effects of biocidal product on the basis of the data available for the micro-organism, unless it can be justified that exposure of birds is unlikely to occur.

#### *10.2. Effects on aquatic organisms*

The same information as provided in Annex IVA, point 8.2 has to be reported where it is not possible to predict the effects of the biocidal product on the basis of the data available for the micro-organism, unless it can be justified that exposure of aquatic organisms is unlikely to occur.

#### *10.3. Effects on bees*

The same information as provided in Annex IVA, point 8.3 has to be reported where it is not possible to predict the effects of the biocidal product on the basis of the data available for the micro-organism, unless it can be justified that exposure of bees is unlikely to occur.

#### *10.4. Effects on arthropods other than bees*

The same information as provided in Annex IVA, point 8.4 has to be reported where it is not possible to predict the effects of the biocidal product on the basis of the data available for the micro-organism, unless it can be justified that exposure of arthropods other than bees is unlikely to occur.

#### *10.5. Effects on earthworms*

The same information as provided in Annex IVA, point 8.5 has to be reported where it is not possible to predict the effects of the biocidal product on the basis of the data available for the micro-organism, unless it can be justified that exposure of earthworms is unlikely to occur.

#### *10.6. Effects on soil micro-organisms*

The same information as provided in Annex IVA, point 8.6 has to be reported where it is not possible to predict the effects of the biocidal product on the basis of the data available for the micro-organism, unless it can be justified that exposure of non target soil micro-organisms is unlikely to occur.

#### *10.7. Additional studies on additional species or higher tier studies such as studies on selected non-target organisms*

Expert judgment 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.

Additional studies might include further studies on additional species or higher tier studies such as studies on selected non-target organisms.

##### *10.7.1 Terrestrial plants*

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

##### *10.7.2 Mammals*

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

##### *10.7.3 Other relevant species and processes*

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

#### *10.8. Summary and evaluation of effects on non-target organisms*

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.

## **XI. CLASSIFICATION, PACKAGING AND LABELLING OF THE BIOCIDAL PRODUCT**

As established in Article 20, proposals including justification for the classification and labelling of the biocidal product in accordance with the provisions set in Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances and Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and

administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous biocidal products must be submitted. The classification comprises of the description of the category/categories of danger and qualifying risk phrases for all dangerous properties. On the basis of the classification, a proposal for labelling including the hazard symbol(s) and indications of danger, risk phrases and safety phrases should be given. The classification and labelling shall be in regard to the chemical substances contained in the biocidal product. If necessary, specimens of proposed packaging should be submitted to the competent authority of a Member State.

Proposals for allocation to one of the risk groups outlined in Article 2 of Directive 2000/54/EC<sup>31</sup> of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work with justifications for the proposal together with indications on the need for products to carry the biohazard sign specified in Annex II to Directive 2000/54/EC.

## **XII. SUMMARY AND EVALUATION OF SECTIONS II to XI INCLUDING CONCLUSIONS OF THE RISK ASSESSMENT AND RECOMMENDATIONS**

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:

- prediction of distribution and fate in the environment, and the time courses involved;
- identification of non-target species and populations at risk, and prediction of the extent of potential exposure; and
- identification of precautions necessary to avoid or minimise contamination of the environment, and for the protection of non-target species.

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31 OJ L 262, 17.10.2000, p. 21.