ANNEX

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

PART B

MICRO-ORGANISMS INCLUDING VIRUSES

5. Effects on human health

Introduction

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

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

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

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

— specify risk and safety phrases (once introduced) for the protection of man, animals and the environment to be included on packaging (containers),

— identify relevant first aid measures as well as appropriate diagnostic and therapeutic measures to be followed in the event of infection or another adverse effect in man.

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

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

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

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

Tier II studies must be conducted if tests under Tier I have shown adverse health effects. The type of study to be performed depends on the effects observed in the Tier I studies. Before

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

5.1. *Basic information*

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

5.1.1. *Medical data*

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

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

5.1.2. *Medical surveillance on manufacturing plant personnel*

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

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

5.1.3. *Sensitisation/allergenicity observations, if appropriate*

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

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

Available reports from the open literature on the micro-organism or closely related members of the taxonomic group (relating to clinical cases), where they are from reference journals or official reports, must be submitted together with reports of any follow-up studies undertaken. Such reports are of particular value and shall contain complete descriptions of the nature, level and duration of exposure, as well as the clinical symptoms observed, first aid and therapeutic measures applied and measurements and observations made. Summary and abstract information is of limited value.

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

5.2. *Basic studies*

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

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

5.2.1. *Sensitisation*[(1)]
Aim of the test

The test will provide sufficient information to assess the potential of the micro-organism to provoke sensitisation reactions by inhalation as well as with dermal exposure. A maximised test has to be performed.
Circumstances in which required[(2)]

Information on sensitisation must be reported.

5.2.2. *Acute toxicity, pathogenicity and infectiveness*

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

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

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

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

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

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

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

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

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

The inhalation toxicity[3], pathogenicity and infectiveness of the micro-organism must be reported.

5.2.2.3. *Intraperitoneal/subcutaneous single dose*

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

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

5.2.3. *Genotoxicity testing*
Circumstances in which required

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

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

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

It is important that a flexible approach is adopted, with selection of further tests being dependent upon interpretation of results at each stage.
Test conditions[4]

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

Genotoxicity of viruses shall be studied on infectious isolates.

5.2.3.1. *In vitro studies*
Circumstances in which required

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

5.2.4. *Cell culture study*

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

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

Short-term toxicity studies must be designed to provide information as to the amount of the micro-organism that can be tolerated without toxic effects under the conditions of the study. Such studies provide useful data on the risks for those handling and using preparations containing the micro-organism. In particular, short-term studies provide an essential insight into possible cumulative actions of the micro-organism, and the risks to workers who may be intensively exposed. In addition short-term studies provide information useful in the design of chronic toxicity studies.

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

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

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

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

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

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

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

5.2.5.1. *Health effects after repeated inhalatory exposure*

Information on the health effects after repeated inhalatory exposure is considered necessary, particularly for the risk assessment of the occupational setting. Repeated exposure might

influence the clearance capacity (e.g. resistance) of the host (human). Furthermore, for proper risk assessment the toxicity after repeated exposure to contaminants, growth medium, co-formulants and the micro-organism needs to be addressed. It should be kept in mind that the co-formulants in the plant protection product can influence the toxicity and infectiveness of a micro-organism.
Circumstances in which required

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

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

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

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

Information on resistance to antibiotics must be provided.

(END OF TIER I)
TIER II

5.3. *Specific toxicity, pathogenicity and infectiveness studies*

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

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

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

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

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

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

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

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

See point 5.4 of Part A.

Circumstances in which required

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

(END OF TIER II)

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

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

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

(**1**) The available methods for testing dermal sensitisation are not suitable for testing micro-organisms. Sensitisation by inhalation is most probably a greater problem compared with dermal exposure to micro-organisms but so far, there are no validated test methods. Development of these kinds of methods is therefore of great importance. Until then, all micro-organisms should be regarded as potential sensitisers. This approach also takes into consideration immuno-compromised or other sensitive individuals in the population (e.g. pregnant women, new-born children or elderly).

(**2**) 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.

(**3**) An inhalation study may be replaced by an intratracheal study.

(**4**) 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.