ANNEX

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

PART A

CHEMICAL SUBSTANCES

8. Ecotoxicological studies

Introduction

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

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

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

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

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

— classify the active substance as to hazard,

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

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

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

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

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

Test substance

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

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

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

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

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

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

Test organisms

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

8.1. *Effects on birds*

8.1.1. *Acute oral toxicity*

Aim of the test

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

Circumstances in which required

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

Test conditions

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

Test guideline

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

8.1.2. *Short-term dietary toxicity*

Aim of the test

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

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

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

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

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

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

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

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

8.2. *Effects on aquatic organisms*

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

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

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

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

The test must always be carried out.
Test conditions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

8.2.3. *Bioconcentration in fish*
Aim of the test

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Where environmental fate and behaviour data required in Section 7 report that an active substance is likely to partition to and persist in aquatic sediments, expert judgement shall be used to decide whether an acute or a chronic sediment toxicity test in required. Such expert judgement shall take into account whether effects on sediment dwelling invertebrates are likely by comparing the aquatic invertebrate toxicity EC_{50} data from points 8.2.4 and 8.2.5 with the predicted levels of the active substances in sediment from data in Section 9 of the Annex to Regulation (EU) No 545/2011.
Test conditions

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

8.2.8. *Aquatic plants*

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

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

8.3. *Effect on arthropods*

8.3.1. *Bees*

8.3.1.1. *Acute toxicity*
Aim of the test

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

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

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

8.3.1.2. *Bee brood feeding test*

Aim of the test

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

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

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

8.3.2. *Other arthropods*
Aim of the test

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

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

— food storage in enclosed spaces,
— wound sealing and healing treatments,
— rodenticidal baits.

Test conditions

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

Two sensitive standard species, a parasitoid and predatory mite (e.g. *Aphidius rhopalosiphi* and *Typhlodromus pyri*) shall be tested. In addition to these, two additional species must also be tested, which shall be relevant to the intended use of the substance. Where possible and if appropriate, they shall represent the other two major functional groups, ground dwelling predators and foliage dwelling predators. Where effects are observed with species relevant to the proposed use of the product, further testing may be carried out at the extended laboratory/ semi-field level. Selection of the relevant test species shall follow the proposals outlined in SETAC — Guidance document on regulatory testing procedures for pesticides with non-target arthropods[(1)]. Testing must be conducted at rates equivalent to the highest rate of field application to be recommended.
Test guideline

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

8.4. *Effects on earthworms*

8.4.1. *Acute toxicity*
Aim of the test

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

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

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

8.4.2. *Sublethal effects*
Aim of the test

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

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

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

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

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

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

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

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

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

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

8.7. *Effects on biological methods for sewage treatment*

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

(**1**) From the Workshop European Standard Characteristics of beneficials Regulatory Testing (Escort), 28 to 30 March 1994, ISBN 0-95-22535-2-6.