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ANNEX

PART A

CHEMICAL ACTIVE SUBSTANCES

SECTION 8

Ecotoxicological studies

Introduction

1. All available biological data and information which is relevant to the assessment of the ecotoxicological profile of the active substance shall be reported. This shall include all potentially adverse effects found during routine ecotoxicological investigations. Where required by the national competent authorities, additional studies, necessary to investigate the probable mechanisms involved and to assess the significance of these effects, shall be carried out and reported on.

2. The ecotoxicological assessment shall be based on the risk that the proposed active substance used in a plant protection product poses to non-target organisms. In carrying out a risk assessment, toxicity shall be compared with exposure. The general term for the output from such a comparison is 'risk quotient' or RQ. It shall be noted that RQ can be expressed in several ways, for example, toxicity:exposure ratio (TER) and as a hazard quotient (HQ). The applicant shall take into account the information from Sections 2, 5, 6, 7 and 8.

3. It may be necessary to conduct separate studies for metabolites, breakdown or reaction products derived from the active substance where non-target organisms may be exposed and where their effects cannot be evaluated by the available results relating to the active substance. Before such studies are performed, the applicant shall take into account the information from Sections 5, 6 and 7.

Studies undertaken shall permit characterisation of metabolites, breakdown or reaction products as being significant or not, and reflect the nature and extent of the effects judged likely to arise.

4. In the case of certain study types, the use of a representative plant protection product instead of the active substance as manufactured may be more appropriate, for example testing of non-target arthropods, bees, earthworm reproduction, soil micro-flora and non-target terrestrial plants. In the case of certain plant protection product types (for example encapsulated suspension) testing with the plant protection product is more appropriate to testing with active substance when these organisms will be exposed to the plant protection product itself. For plant protection products where the active substance is always intended to be used together with a safener and/or synergist and/or in conjunction with other active substances, plant protection products containing these additional substances shall be used.

5. The potential impact of the active substance on biodiversity and the ecosystem, including potential indirect effects via alteration of the food web, shall be considered.

6. For those guidelines which allow for the study to be designed to determine an effective concentration (EC_x), the study shall be conducted to determine an EC_{10}, EC_{20} and EC_{50}, when required, along with corresponding 95 % confidence intervals. If an EC_x approach is used, a no observed effect concentration (NOEC) shall still be determined.

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Existing acceptable studies that have been designed to generate a NOEC shall not be repeated. An assessment of the statistical power of the NOEC derived from those studies shall be carried out.

7. All of the aquatic toxicity data shall be used when developing a proposal for environmental quality standards (Annual Average EQS, AA-EQS; Maximum Acceptable Concentration EQS, MAC-EQS). The methodology for derivation of these endpoints is outlined in the 'Technical Guidance for Deriving Environmental Quality Standards[(1)]' for the Water Framework Directive 2000/60/EC of the European Parliament and of the Council[(2)].

8. 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.

9. Higher tier studies shall be designed and data analysed using suitable statistical methods. Full details of the statistical methods shall be reported. Where appropriate and necessary, higher tier studies shall be supported by chemical analysis to verify exposure has occurred at an appropriate level.

10. Pending the validation and adoption of new studies and of a new risk assessment scheme, existing protocols shall be used to address the acute and chronic risk to bees, including those on colony survival and development, and the identification and measurement of relevant sub-lethal effects in the risk assessment.

8.1. Effects on birds and other terrestrial vertebrates

For all avian and mammalian feeding studies, average achieved dose shall be reported, including where possible the dose in mg substance/kg body weight. Where dosing via the diet is utilised, the active substance shall be distributed uniformly in the diet.

8.1.1. *Effects on birds*

8.1.1.1. *Acute oral toxicity to birds*

The acute oral toxicity of the active substance to birds shall be determined.
Circumstances in which required

The effects of the active substance on birds shall be investigated except where the substance is included in plant protection products used, for example, in enclosed spaces and wound healing treatments where birds will experience neither direct nor secondary exposure.
Test conditions

A study shall be provided establishing the acute oral toxicity (LD_{50}) of the active substance. Where available, the study shall be performed with a quail species (Japanese quail (*Coturnix coturnix japonica*) or Bobwhite quail (*Colinus virginianus*)), since regurgitation is rare in these species. The study shall provide, where possible, LD_{50} values. The lethal threshold dose, time courses of response and recovery, the LD_{10} and LD_{20} shall be reported together with the no observed effect level (NOEL) and gross pathological findings. Where LD_{10} and LD_{20} cannot be estimated, an explanation shall be provided. Study design shall be optimised for the achievement of an accurate LD_{50}.

The highest dose used in tests shall not exceed 2 000 mg substance/kg body weight, however, depending on the expected exposure levels in the field following the intended use of the compound, higher doses may be required.

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8.1.1.2. *Short-term dietary toxicity to birds*

A study shall be provided establishing the short-term dietary toxicity. LC_{50} values, lowest lethal concentration (LLC), where possible, NOEC values, time courses of response and recovery and pathological findings shall be reported in such study. LC_{50} and NOEC values shall be converted to daily dietary dose (LD_{50}) expressed in mg substance/kg bw/day and NOEL expressed in mg substance/kg bw/day.
Circumstances in which required

A study on the dietary (five-day) toxicity of the active substance to birds shall only be required where the mode of action or results from mammalian studies indicate a potential for the dietary LD_{50} measured by the short-term dietary toxicity study to be lower than the LD_{50} based on an acute oral study. The short-term dietary toxicity test shall not be conducted for any other purpose than to determine intrinsic toxicity through dietary exposure, unless a justification of the need to do so is supplied.
Test conditions

The test species shall be the same as tested under point 8.1.1.1.

8.1.1.3. *Sub-chronic and reproductive toxicity to birds*

A study shall be provided establishing the sub-chronic and reproductive toxicity of the substance to birds. The EC_{10} and EC_{20} shall be reported. Where they cannot be estimated, an explanation shall be provided together with the NOEC expressed in mg substance/kg bw/day.
Circumstances in which required

The sub-chronic and reproductive toxicity of the active substance to birds shall be investigated, unless the applicant shows that exposure of adults, or exposure of nest sites during the breeding season is unlikely to occur. Such a justification shall be supported by information showing that no exposure or delayed effects will occur during the breeding season.
Test conditions

The study shall be conducted on the same species as tested under point 8.1.1.1.

8.1.2. *Effects on terrestrial vertebrates other than birds*

The following information shall be derived from the mammalian toxicological assessment based on the studies referred to in Section 5.

8.1.2.1. *Acute oral toxicity to mammals*

The acute oral toxicity of the active substance to mammals shall be determined and the LD_{50} expressed mg substance/kg bw/day.
Circumstances in which required

The effects of the active substance on mammals shall be investigated except when the substance is included in plant protection products used, for example, in enclosed spaces and wound healing treatments where mammals will experience neither direct nor secondary exposure.

8.1.2.2. *Long-term and reproductive toxicity to mammals*
Circumstances in which required

The reproductive toxicity of the active substance to mammals shall be investigated, unless a justification is provided by the applicant showing that exposure of adults, during the breeding season is unlikely to occur. Such a justification shall be supported by information showing that no exposure or delayed effects will occur during the breeding season.

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The most sensitive ecotoxicologically relevant mammalian long-term toxicological endpoint (NOAEL) expressed as mg substance/kg bw/day shall be reported. The EC_{10} and EC_{20} shall be reported together with the NOEC expressed in mg substance/kg bw/day. Where EC_{10} and EC_{20} cannot be estimated an explanation shall be provided.

8.1.3. *Active substance bioconcentration in prey of birds and mammals*

For active substances with a log Pow > 3, an assessment of the risk posed by bioconcentration of the substance in the prey of birds and mammals shall be provided.

8.1.4. *Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)*

Available and relevant data, including data from the open literature for the active substance of concern, regarding the potential effects to birds, mammals, reptiles and amphibians (see point 8.2.3) shall be presented and taken into account in the risk assessment.

8.1.5. *Endocrine disrupting properties*

Consideration shall be given to whether the active substance is a potential endocrine disruptor according to Union or internationally agreed guidelines. This may be done in consulting the mammalian toxicology section (see Section 5). In addition, other available information on toxicity profile and mode of action shall be taken into account. If as a result of this assessment, the active substance is identified as a potential endocrine disruptor, the type and conditions of the study to be performed shall be discussed with the national competent authorities.

8.2. **Effects on aquatic organisms**

Reports of the tests referred to in points 8.2.1, 8.2.4 and 8.2.6 shall be submitted for every active substance and supported with analytical data on concentrations of the substance in the test media.

When aquatic toxicity studies are conducted with a poorly soluble substance, limit concentrations lower than 100 mg substance/L may be acceptable, however precipitation of the substance in the test medium shall be avoided and a solubiliser, auxiliary solvent or dispersing agent shall be used when appropriate. Testing using the plant protection product may be required by the national competent authorities if no biological effects occur at the solubility limit of the active substance.

Toxicity endpoints (such as LC_{50}, EC_{10}, EC_{20}, EC_{50} and NOEC) shall be calculated on the basis of nominal or mean/initial measured concentrations.

8.2.1. *Acute toxicity to fish*

A study shall be provided on the acute toxicity to fish (LC_{50}) and details of observed effects.
Circumstances in which required

A test on rainbow trout (*Oncorhynchus mykiss*) shall be carried out.
Test conditions

The acute toxicity of the active substance to fish shall be determined. In order to minimise fish testing, a threshold approach to acute toxicity testing on fish shall be considered. An acute toxicity fish limit test shall be conducted at 100 mg substance/L or at an appropriate concentration selected from aquatic endpoints (points 8.2.4, 8.2.6 or 8.2.7) following consideration of the threshold exposure. When mortality is detected in the fish limit test an acute fish dose-response toxicity study shall be required to determine an LC_{50} for use in the risk assessment conducted in accordance with the relevant risk quotient analysis (see point 2 of the introduction of this Section).

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8.2.2. *Long-term and chronic toxicity to fish*
Circumstances in which required

A long-term or chronic toxicity study on fish shall be provided for all active substances where exposure of surface water is likely and the substance is deemed to be stable in water, that is to say there is less than 90 % loss of the original substance over 24 hours via hydrolysis (see point 7.2.1.1). A fish early life stage study shall be provided in these circumstances. However, if a fish full life cycle study is provided an early life stage study shall not be required.

8.2.2.1. *Fish early life stage toxicity test*

A fish early life stage toxicity test shall determine effects on development, growth and behaviour, and details of observed effects on fish early life stages. The EC_{10} and EC_{20} shall be reported together with the NOEC. Where EC_{10} and EC_{20} cannot be estimated, an explanation shall be provided.

8.2.2.2. *Fish full life cycle test*

A fish full life cycle test shall provide information on the effects on reproduction of the parental and the viability of the filial generation. The EC_{10} and EC_{20} shall be reported together with the NOEC.

For active substances that are not considered as potential endocrine disruptors, a fish full life cycle test may be required depending upon the persistence and bioaccumulative potential of the substance.

For active substances that fulfil the screening criteria on either of the fish screening assays, or for which there are other indications of endocrine disruption (see point 8.2.3), appropriate additional endpoints shall be included in the test and discussed with the national competent authorities.
Test conditions

Studies shall be designed to reflect concerns identified through lower tier testing, mammalian and bird toxicology studies and other information. The exposure regime shall be selected accordingly, taking account of the rates of application proposed.

8.2.2.3. *Bioconcentration in fish*

The test on bioconcentration in fish shall provide the steady-state bioconcentration factors, uptake rate constants and depuration rate constants, incomplete excretion, metabolites formed in fish and, if available, information on organ-specific accumulation.

All data shall be provided with confidence limits for each test substance. Bioconcentration factors shall be expressed as a function of both total wet weight and of the lipid content of the fish.

Data provided under point 6.2.5 shall be considered, where relevant, in addressing this point.
Circumstances in which required

The bioconcentration of the substance, shall be assessed where:

— the log Pow is greater than 3 (see point 2.7) or there are other indications of bioconcentration, and

— the substance is considered stable, that is to say there is less than 90 % loss of the original substance over 24 hours via hydrolysis (see point 7.2.1.1).

8.2.3. *Endocrine disrupting properties*

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Consideration shall be given to whether the active substance is a potential endocrine disruptor in aquatic non-target organisms according to Union or internationally agreed guidelines. In addition, other available information on toxicity profile and mode of action shall be taken into account. If as a result of this assessment, the active substance is identified as a potential endocrine disruptor, the type and conditions of the studies to be performed shall be discussed with the national competent authorities.

8.2.4. *Acute toxicity to aquatic invertebrates*
Circumstances in which required

The acute toxicity shall be determined for a *Daphnia* species (preferably *Daphnia magna*). For active substances with an insecticidal mode of action or which show insecticidal activity a second species shall be tested, for example Chironomid larvae or Mysid shrimps (*Americamysis bahia*).

8.2.4.1. *Acute toxicity to Daphnia magna*

A test shall be provided on the 24- and 48-hour acute toxicity of the active substance to *Daphnia magna*, expressed as the median effective concentration (EC_{50}) for immobilisation, and where possible, the highest concentration causing no immobilisation.
Test conditions

Concentrations up to 100 mg substance/L shall be tested. A limit test at 100 mg substance/L may be performed where the results of a range finding test indicate that no effects are to be expected.

8.2.4.2. *Acute toxicity to an additional aquatic invertebrate species*

A test shall be provided on the 24- and 48-hour acute toxicity of the active substance to an additional aquatic invertebrate species, expressed as the median effective concentration (EC_{50}) for immobilisation, and where possible, the highest concentration causing no immobilisation.
Test conditions

The conditions as set out in point 8.2.4.1 shall apply.

8.2.5. *Long-term and chronic toxicity to aquatic invertebrates*
Circumstances in which required

A long-term or chronic toxicity study on aquatic invertebrates shall be provided for all active substances where exposure of surface water is likely and the substance is deemed to be stable in water, that is to say there is less than 90 % loss of the original substance over 24 hours via hydrolysis (see point 7.2.1.1).

A chronic toxicity study shall be submitted on one aquatic invertebrate species. If acute toxicity tests have been conducted on two aquatic invertebrate species the acute endpoints shall be taken into account (see point 8.2.4) in order to determine the appropriate species to be tested in the chronic toxicity study.

If the active substance is an insect growth regulator, an additional study on chronic toxicity shall be carried out using relevant non-crustacean species such as *Chironomus* spp.

8.2.5.1. *Reproductive and development toxicity to Daphnia magna*

The aim of the test on reproductive and development toxicity to *Daphnia magna* shall be to measure adverse effects such as immobilisation and loss of reproductive capacity and to provide details of observed effects. The EC_{10}, and EC_{20} shall be reported together with the NOEC. Where EC_{10} and EC_{20} cannot be estimated, an explanation shall be provided.

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8.2.5.2. *Reproductive and development toxicity to an additional aquatic invertebrate species*

The test on reproductive and development toxicity to an additional aquatic invertebrate species shall measure adverse effects such as immobilisation and loss of reproductive capacity and provide details of observed effects. The EC_{10}, and EC_{20} shall be reported together with the NOEC. Where EC_{10} and EC_{20} cannot be estimated, an explanation shall be provided.

8.2.5.3. *Development and emergence in Chironomus riparius*

The active substance shall be applied to the water overlying sediment and effects on survival and development of *Chironomus riparius*, including effects on emergence of adults, shall be measured to provide endpoints for those substances considered to interfere with insect moulting hormones or that have other effects on insect growth and development. The EC_{10} and EC_{20} shall be reported together with the NOEC.
Test conditions

Concentrations of active substance in the overlying water and the sediment shall be measured to establish an EC_{10}, EC_{20} and a NOEC. The active substance shall be measured often enough to allow the calculation of test endpoints based on nominal as well as time-weighted average concentrations.

8.2.5.4. *Sediment dwelling organisms*

When accumulation of an active substance in aquatic sediment is indicated or predicted by environmental fate studies, the impact on a sediment-dwelling organism shall be assessed. The chronic risk to *Chironomus riparius* or *Lumbriculus* spp. shall be determined. An appropriate alternative test species may be used where a recognised guideline is available. The active substance shall be applied to either the water or the sediment phase of a water/sediment system and the test shall take account of the major route of exposure. The key endpoint from the study shall be presented in terms of mg substance/kg dry sediment and mg substance/L water and the EC_{10} and EC_{20} shall be reported together with the NOEC.
Test conditions

Concentrations of active substance in the overlying water and the sediment shall be measured to establish an EC_{10}, EC_{20} and a NOEC.

8.2.6. *Effects on algal growth*
Circumstances in which required

Testing shall be carried out on one green alga (such as *Pseudokirchneriella subcapitata*, synonym *Selenastrum capricornutum*).

For active substances that exhibit herbicidal activity a test on a second species from a different taxonomic group shall be performed such as a diatom, for example *Navicula pelliculosa*.

The EC_{10}, EC_{20}, EC_{50} and corresponding NOEC values shall be provided.

8.2.6.1. *Effects on growth of green algae*

A test shall be provided establishing EC_{10}, EC_{20}, EC_{50} for green algae and corresponding NOEC values for algal growth rate and yield, based on measurements of biomass or surrogate measurement variables.
Test conditions

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Concentrations up to 100 mg substance/L shall be tested. A limit test at 100 mg substance/L may be performed when results of a range-finding test indicate that no effects are to be expected at lower concentrations.

8.2.6.2. *Effects on growth of an additional algal species*

A test shall be provided establishing EC_{10}, EC_{20}, EC_{50} for an additional algal species and corresponding NOEC values for algal growth rate and yield, based on measurements of biomass (or surrogate measurement variables).
Test conditions

The test conditions as set out in point 8.2.6.1 shall apply.

8.2.7. *Effects on aquatic macrophytes*

A test shall be provided establishing EC_{10}, EC_{20}, EC_{50} and corresponding NOEC values for *Lemna* species growth rate and yield, based on measurements of number of fronds and at least one additional measurement variable (dry weight, fresh weight or frond area).

For other species of aquatic macrophytes, a test shall provide sufficient information to evaluate impact on aquatic plants and provide EC_{10}, EC_{20}, EC_{50} and corresponding NOEC values based on measurement of appropriate biomass parameters.
Circumstances in which required

A laboratory test with *Lemna* species shall be performed for herbicides and plant growth regulators and for substances where there is evidence from information submitted under point 8.6 of Part A of this Annex or point 10.6 of Part A of the Annex to Regulation (EU) No 284/2013 that the test substance has herbicidal activity. Additional testing may be required by the national competent authorities on other macrophyte species depending on the mode of action of the substance, or if clear indications of higher toxicity are apparent to dicotyledonous (for example auxin inhibitor, broad leaf herbicides) or other monocotyledonous (for example grass herbicides) plant species from efficacy or terrestrial non-target plants tests (see point 8.6 of Part A of this Annex and point 10.6 of Part A of the Annex to Regulation (EU) No 284/2013).

Additional aquatic macrophyte species tests may be undertaken on a dicotyledonous species, such as *Myriophyllum spicatum, Myriophyllum aquaticum* or a monocotyledonous species, such as aquatic grass *Glyceria maxima*, as appropriate. The need to perform such studies shall be discussed with the national competent authorities.
Test conditions

Concentrations up to 100 mg substance/L shall be tested. A limit test at 100 mg substance/L may be performed when results of a range-finding test indicate that no effects are to be expected.

8.2.8. *Further testing on aquatic organisms*

Further studies on aquatic organisms may be conducted to refine the identified risk and shall provide sufficient information and data to evaluate potential impact on aquatic organisms under field conditions.

Studies undertaken may take the form of additional species testing, modified exposure testing, microcosm or mesocosm studies.
Circumstances in which required

The need to perform such studies shall be discussed with the national competent authorities.
Test conditions

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The type and conditions of the study to be performed shall be discussed with the national competent authorities.

8.3. **Effect on arthropods**

8.3.1. *Effects on bees*

Effects on bees shall be assessed and the risk evaluated, including the risk deriving from residues of the active substance or its metabolites in nectar, pollen and water, including guttation. Reports of the tests referred to in points 8.3.1.1, 8.3.1.2 and 8.3.1.3 shall be submitted, except where plant protection products containing the active substance are for exclusive use in situations where bees are not likely to be exposed such as:

(a) food storage in enclosed spaces;

(b) non-systemic preparations for application to soil, except granules;

(c) non-systemic dipping treatments for transplanted crops and bulbs;

(d) wound sealing and healing treatments;

(e) non systemic rodenticidal baits;

(f) use in greenhouses without bees as pollinators.

For seed treatments the risk from drift of dust during drilling of the treated seed shall be taken into account. As regards granules and slug pellets the risk from drift of dust during application shall be taken into account. If an active substance is systemic and to be used on seeds, bulbs, roots, applied directly to soil, irrigation water, or applied directly to or into the plant, for example by spraying or stem injection, the risk to bees foraging those plants shall be assessed, including the risk deriving from residues of the plant protection product in nectar, pollen and water, including guttation.

Where bees are likely to be exposed, testing by both acute (oral and contact) and chronic toxicity, including sub-lethal effects, shall be conducted.

Where exposure of bees to residues in nectar, pollen or water resulting from systemic properties of the active substance may occur and where the acute oral toxicity is < 100 μg/bee or a considerable toxicity for larvae occurs, residues concentrations in these matrices shall be provided and the risk assessment shall be based on a comparison of the relevant endpoint with those residue concentrations. If this comparison indicates that an exposure to toxic levels cannot be excluded, effects shall be investigated with higher tier tests.

8.3.1.1. *Acute toxicity to bees*

Where bees are likely to be exposed, testing for acute oral and contact toxicity shall be performed.

8.3.1.1.1.*Acute oral toxicity*

A test for acute oral toxicity shall be provided establishing the acute LD_{50} values together with the NOEC. Sub-lethal effects, if observed, shall be reported.
Test conditions

The test shall be conducted with the active substance. Results shall be presented in terms of μg active substance/bee.

8.3.1.1.2.*Acute contact toxicity*

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A test for acute contact toxicity shall be provided establishing the acute LD_{50} values together with the NOEC. Sub-lethal effects, if observed, shall be reported.
Test conditions

The test shall be conducted with the active substance. Results shall be presented in terms of μg active substance/bee.

8.3.1.2. *Chronic toxicity to bees*

A test for chronic toxicity to bees shall be provided establishing the chronic oral EC_{10}, EC_{20}, EC_{50} together with the NOEC. Where the chronic oral EC_{10}, EC_{20}, EC_{50} cannot be estimated, an explanation shall be provided. Sub-lethal effects, if observed, shall be reported.
Circumstances in which required

The test shall be carried out where bees are likely to be exposed.
Test conditions

The test shall be conducted with the active substance. Results shall be presented in terms of μg active substance/bee.

8.3.1.3. *Effects on honeybee development and other honeybee life stages*

A bee brood study shall be conducted to determine effects on honeybee development and brood activity. The bee brood study shall provide sufficient information to evaluate possible risks from the active substance on honeybee larvae.

The test shall provide the EC_{10}, EC_{20} and EC_{50} for adult bees, where possible, and larvae together with the NOEC. Where EC_{10}, EC_{20}, EC_{50} cannot be estimated, an explanation shall be provided. Sub-lethal effects, if observed, shall be reported.
Circumstances in which required

The test shall be carried out for active substances for which sub-lethal effects on growth or development cannot be excluded, unless the applicant shows that it is not possible that honeybee brood will be exposed to the active substance.

8.3.1.4. *Sub-lethal effects*

Tests investigating sub-lethal effects, such as behavioural and reproductive effects, on bees and, where applicable, on colonies may be required.

8.3.2. *Effects on non-target arthropods other than bees*
Circumstances in which required

Effects on non-target terrestrial arthropods shall be investigated for all active substances except where plant protection products containing the active substance are for exclusive use in situations where non-target arthropods are not exposed such as:

— food storage in enclosed spaces that preclude exposure,
— wound sealing and healing treatments,
— enclosed spaces with rodenticidal baits.

Two indicator species, the cereal aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) and the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) shall always be tested. Initial testing shall be performed using glass plates and mortality (and reproduction effects if assessed) shall be reported. Testing shall determine a rate-response relationship and LR_{50}[(3)], ER_{50}[(4)] and NOEC endpoints shall be reported for assessment of the risk to these species in accordance with the relevant risk quotient analysis. If adverse effects can be clearly predicted

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from these studies then testing using higher tier studies may be required (see point 10.3 of Part A of the Annex to the Regulation (EU) No 284/2013 for further details).

With active substances suspected of having a special mode of action (such as insect growth regulators, insect feeding inhibitors) additional tests involving sensitive life stages, special routes of uptake or other modifications, may be required by the national competent authorities. The rationale for the choice of test species used shall be provided.

8.3.2.1. *Effects on Aphidius rhopalosiphi*

A test shall provide sufficient information to evaluate the toxicity in terms of LR_{50} and NOEC of the active substance to *Aphidius rhopalosiphi*.
Test conditions

Initial testing shall be performed using glass plates.

8.3.2.2. *Effects on Typhlodromus pyri*

A test shall provide sufficient information to evaluate the toxicity in terms of LR_{50} and NOEC of the active substance to *Typhlodromus pyri*.
Test conditions

Initial testing shall be performed using glass plates.

8.4. Effects on non-target soil meso- and macrofauna

8.4.1. *Earthworm — sub-lethal effects*

A test shall provide information on the effects on growth, reproduction and behaviour of the earthworm.
Circumstances in which required

Sub-lethal effects on earthworms shall be investigated where the active substance can contaminate soil.
Test conditions

Testing shall determine a dose-response relationship and the EC_{10}, EC_{20} and NOEC shall enable the risk assessment to be conducted in accordance with the appropriate risk quotient analysis, taking into account likely exposure, the organic carbon content (f_{oc}) of the test medium and the lipophilic properties (K_{ow}) of the test substance. The test substance shall be incorporated into the soil to obtain a homogenous soil concentration. Testing with soil metabolites may be avoided if there is analytical evidence to indicate that the metabolite is present at an adequate concentration and duration in the study conducted with the parent active substance.

8.4.2. *Effects on non-target soil meso- and macrofauna (other than earthworms)*
Circumstances in which required

Effects on soil organisms, other than earthworms, shall be investigated for all test substances, except in situations where soil organisms are not exposed such as:

(a) food storage in enclosed spaces that preclude exposure;

(b) wound sealing and healing treatments;

(c) enclosed spaces with rodenticidal baits.

For plant protection products applied as a foliar spray data on *Folsomia candida* and *Hypoaspis aculeifer* may be required by the national competent authorities. If data are available on both

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Aphidius rhopalosiphi and *Typhlodromus pyri* these may be used in an initial risk assessment. If concern is raised with either species tested under point 8.3.2, data on both *Folsomia candida* and *Hypoaspis aculeifer* shall be provided.

If data on *Aphidius rhopalosiphi* and *Typhlodromus pyri* are not available, then the data set out in point 8.4.2.1 shall be provided.

For plant protection products applied directly to soil as soil treatments either as a spray or as a solid formulation, testing shall be carried out on both on *Folsomia candida* and *Hypoaspis aculeifer* (see point 8.4.2.1).

8.4.2.1. *Species level testing*

A test shall provide sufficient information to perform an assessment of the toxicity of the active substance to the soil invertebrate indicator species *Folsomia candida* and *Hypoaspis aculeifer*.
Test conditions

Testing shall determine a dose-response relationship and the EC_{10}, EC_{20} and NOEC shall enable the risk assessment to be conducted in accordance with the appropriate risk quotient analysis, taking into account likely exposure, the organic carbon content (f_{oc}) of the test medium and the lipophilic properties (K_{ow}) of the test substance. The test substance shall be incorporated into the soil to obtain a homogenous soil concentration. Testing with soil metabolites may be avoided if there is analytical evidence to indicate that the metabolite is present at an adequate concentration and duration in the study conducted with the parent active substance.

8.5. Effects on soil nitrogen transformation

A test shall provide sufficient data to evaluate the impact of active substances on soil microbial activity, in terms of nitrogen transformation.
Circumstances in which required

The test shall be carried out where plant protection products 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 plant protection products for soil sterilisation, the studies shall be designed to measure rates of recovery following treatment.
Test conditions

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

8.6. Effects on terrestrial non-target higher plants

8.6.1. *Summary of screening data*

The information provided shall be sufficient to permit the evaluation of effects of the active substance on non-target plants.
Circumstances in which required

Screening data shall establish whether test substances exhibit herbicidal or plant growth regulatory activity. The data shall include testing from at least six plant species from six different families including both mono- and dicotyledons. The tested concentrations and rates shall be equal or higher than the maximum recommended application rate and at a rate either to simulate use pattern under field conditions, with testing conducted after the final treatment, or at a rate applied directly that takes in to account the accumulation of residues following multiple applications of the plant protection product. If screening studies do not cover the specified range

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of species or the necessary concentrations and rates, tests as set out in point 8.6.2 shall be carried out.

For assessment of active substances with herbicidal or plant growth regulatory activity screening data shall not be used. Point 8.6.2 shall apply.
Test conditions

A summary of available data from tests used to assess biological activity and dose range finding studies, whether positive or negative, which may provide information with respect to possible impact on other non-target flora, shall be provided, together with an assessment as to the potential impact on non-target plant species.

These data shall be supplemented by further information, in summary form, on the observed effects on plants during the course of field testing, namely efficacy, residues, environmental fate and ecotoxicological field studies.

8.6.2. *Testing on non-target plants*

A test shall provide the ER_{50} values of the active substance to non-target plants.
Circumstances in which required

For active substances that exhibit herbicidal or plant growth regulator activity, vegetative vigour and seedling emergence concentration/response tests shall be provided for at least six species representing families for which herbicidal/plant growth regulatory action has been found. Where, from the mode of action, it can be clearly established that either seedling emergence or vegetative vigour is effected, only the relevant study shall be conducted.

Data are not required, where exposure is negligible, for example in the case of rodenticides, active substances used for wound protection or seed treatment, or in the case of active substances used on stored products or in glasshouses where exposure is precluded.
Test conditions

Dose-response tests on a selection of 6 to 10 monocotyledon and dicotyledon plant species representing as many taxonomic groups as possible shall be provided.

8.7. **Effects on other terrestrial organisms (flora and fauna)**

Any available data on the effects of the product on other terrestrial organisms shall be submitted.

8.8. **Effects on biological methods for sewage treatment**

A test shall provide an indication as to the potential of the active substance on biological sewage treatment systems.
Circumstances in which required

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

8.9. **Monitoring data**

Available monitoring data concerning adverse effects of the active substance to non-target organisms shall be reported.

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(**1**) European Communities (2011) Publication ISBN: 978-92-79-16228-2.

(**2**) OJ L 327, 22.12.2000, p. 1.

(**3**) LR_{50}, abbreviation for 'Lethal Rate, 50 %', that is to say the application rate required to kill half the members of a tested population after a specified test duration.

(**4**) ER_{50}, abbreviation for 'Effect Rate, 50 %', that is to say the application rate required to cause an effect on half the members of a tested population after a specified test duration.

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