ANNEX

PART A

CHEMICAL ACTIVE SUBSTANCES

SECTION 8

Ecotoxicological studies

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).

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*

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.

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

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

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

Changes to legislation:
There are outstanding changes not yet made to Commission Regulation (EU) No 283/2013. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.
View outstanding changes

Changes and effects yet to be applied to the whole legislation item and associated provisions

- Signature words omitted by S.I. 2019/556 reg. 21(4)
- Annex Pt. A s. 8 word omitted by S.I. 2019/556 reg. 21(5)(b)(xiv)
- Annex Pt. A s. 1 point 1.4 word substituted in earlier amending provision S.I. 2019/720, Sch. 2 para. 176(2)(a)(i) by S.I. 2020/1567 Sch. 2 para. 61
- Annex Pt. A s. 1 point 1.4.1 word substituted in earlier amending provision S.I. 2019/720, Sch. 2 para. 176(2)(b) by S.I. 2020/1567 Sch. 2 para. 61
- Annex Pt. B s. 9 words omitted by S.I. 2019/556 reg. 21(5)(c)(vi)
- Art. 1(1) Art. 1 renumbered as Art. 1(1) by S.I. 2019/556 reg. 21(2)(a)
- Art. 1(2) inserted by S.I. 2019/556 reg. 21(2)(b)