ANNEX

PART A

CHEMICAL ACTIVE SUBSTANCES

SECTION 5

Toxicological and metabolism studies

5.2. Acute toxicity

The studies, data and information to be provided and evaluated shall be sufficient to permit the identification of effects following a single exposure to the active substance, and in particular to establish, or indicate:

(a) the toxicity of the active substance;

(b) the time course and characteristics of the effects with full details of behavioural changes, clinical signs, where evident, and possible gross pathological findings at post-mortem;

(c) the possible need to consider establishing acute reference doses (such as ARfD, aAOEL[(1)]);

(d) where possible mode of toxic action;

(e) the relative hazard associated with the different routes of exposure.

While the emphasis shall be on estimating the toxicity ranges involved, the information generated shall also permit the active substance to be classified in accordance with Regulation (EC) No 1272/2008. The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

5.2.1. *Oral*
Circumstances in which required

The acute oral toxicity of the active substance shall always be reported.

5.2.2. *Dermal*
Circumstances in which required

The acute dermal toxicity of the active substance shall be reported unless waiving is scientifically justified (for example where oral LD_{50}[(2)] is greater than 2 000 mg/kg). Both local and systemic effects shall be investigated.

Findings of severe skin irritation (Grade 4 erythema or oedema) in the dermal study shall be used instead of performing a specific irritation study.

5.2.3. *Inhalation*
Circumstances in which required

The acute inhalation toxicity of the active substance shall be reported where any of the following apply:

— the active substance has a vapour pressure > 1×10^{-2} Pa at 20 °C;

— the active substance is a powder containing a significant proportion of particles of a diameter < 50 μm (> 1 % on weight basis);
— the active substance is included in products that are powders or are applied by spraying.

The head/nose only exposure shall be used, unless whole body exposure can be justified.

5.2.4. *Skin irritation*

The results of the study shall provide information on the potential for skin irritancy of the active substance including, where relevant, the potential reversibility of the effects observed.

Before undertaking *in vivo* studies for corrosion/irritation of the active substance, a weight-of-evidence analysis shall be performed on the existing relevant data. Where insufficient data are available, they can be developed through application of sequential testing.

The testing strategy shall follow a tiered approach:

(1) the assessment of dermal corrosivity using a validated *in vitro* test method;

(2) the assessment of dermal irritation using a validated *in vitro* test method (such as human reconstituted skin models);

(3) an initial *in vivo* dermal irritation study using one animal, and where no adverse effects are noted;

(4) confirmatory testing using one or two additional animals.

Circumstances in which required

The skin irritancy study of the active substance shall always be provided. Where available, a dermal toxicity study shown not to produce irritation of the skin at the limit test dose level of 2 000 mg/kg body weight shall be used to waive the need for any dermal irritation studies.

5.2.5. *Eye irritation*

The results of the study shall provide the potential of eye irritancy of the active substance including, where relevant, the potential reversibility of the effects observed.

Before undertaking *in vivo* studies for eye corrosion/irritation of the active substance, a weight-of-evidence analysis shall be performed on the existing relevant data. Where available data are considered insufficient, further data may be developed through application of sequential testing.

The testing strategy shall follow a tiered approach:

(1) the use of an *in vitro* dermal irritation/corrosion test to predict eye irritation/corrosion;

(2) the performance of a validated or accepted *in vitro* eye irritation study to identify severe eye irritants/corrosives (such as Bovine Corneal Opacity and Permeability (BCOP) assay, Isolated Chicken Eye (ICE) assay, Isolated Rabbit Eye (IRE) assay, Hen's Egg Test - Chorio-Allantoic Membrane assay (HET-CAM)), and where negative results are obtained, the assessment of eye irritation using an *in vitro* test method for identification of non-irritants or irritants, and where not available;

(3) an initial *in vivo* eye irritation study using one animal, and where no adverse effects are noted;

(4) confirmatory testing using one or two additional animals.

Circumstances in which required

The eye irritancy of the active substance shall always be tested, except where it is likely that severe effects on the eyes may be produced based on criteria listed in the test methods.

5.2.6. *Skin sensitisation*

The study shall provide sufficient information to assess the potential of the active substance to provoke skin sensitisation reactions.
Circumstances in which required

The study shall always be carried out, except where the active substance is a known sensitiser. The local lymph node assay (LLNA) shall be used, including where appropriate the reduced variant of the assay. In case the LLNA cannot be conducted, a justification shall be provided and the Guinea Pig Maximisation Test shall be performed. Where a guinea pig assay (Maximisation or Buehler), meeting OECD guidelines and providing a clear result, is available, further testing shall not be carried out for animal welfare reasons.

Since an active substance identified as a skin sensitiser can potentially induce hypersensitivity reaction, potential respiratory sensitisation should be taken into account when appropriate tests are available or when there are indications of respiratory sensitisation effects.

5.2.7. *Phototoxicity*

The study shall provide information on the potential of certain active substances to induce cytotoxicity in combination with light, for example active substances that are phototoxic *in vivo* after systemic exposure and distribution to the skin, as well as active substances that act as photoirritants after dermal application. A positive result shall be taken into account when considering potential human exposure.
Circumstances in which required

The *in vitro* study shall be required where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution.

If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 $L \times mol^{-1} \times cm^{-1}$, no toxicity testing is required.

(**1**) aAOEL, abbreviation for 'Acute AOEL'.

(**2**) LD_{50}, abbreviation for 'Lethal Dose, 50 %', that is to say the dose required to kill half the members of a tested population after a specified test duration.

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)