

OECD GUIDELINE FOR TESTING OF CHEMICALS

Draft updated Test Guideline 402 on Acute Dermal Toxicity

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress and animal welfare considerations. The original acute Dermal Toxicity Guideline TG 402 was adopted in 1987. A number of recent publications have analyzed the results of acute oral and dermal toxicity studies for hundreds of pesticide active substances and thousands of industrial chemicals, finding that regulatory classifications based on oral acute toxicity data were equivalent to or more severe than those derived from dermal data in more than 98 percent of cases, calling into question the value of routine testing for this endpoint (1-5). OECD Guidance Document XXX on Waiving or Bridging of Mammalian Acute Toxicity Tests (6) identifies criteria for waiving of dermal acute toxicity studies, which should be reviewed prior to making a decision to conduct this study in the interests of limiting new animal testing for this endpoint to exceptional circumstances with compelling scientific justification.

2. Based on the recommendations of several expert meetings in 1999 (7), international agreement had been reached on harmonised LD₅₀ cut-off values for the classification of chemicals, ii) testing in one sex (usually females) is generally considered sufficient, and iii) in order for a point estimate to be meaningful, there is a need to estimate confidence intervals (CI) (8). Avoidance of using death of animals as either an exclusive or an intended end-point by incorporating evident clinical signs of toxicity as an end-point is encouraged as a basis for classification of the test chemical. This approach is also taken for this Guideline, in agreement with the OECD Guidance Document on Humane Endpoints (9).

3. An adaptation of the acute toxic class method set out in OECD Test Guideline 423 (10) is a stepwise procedure with the use of up to 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, further steps may be necessary to allow judgement on the acute toxicity of the test chemical. This procedure is reproducible, uses very few animals and is able to rank test chemicals in a similar manner to the other acute toxicity testing methods (e.g. Test Guidelines 420, 423 and 425). The acute toxic class method is based on biometric evaluations with fixed doses (11), adequately separated to enable a test chemical to be ranked for classification purposes and hazard assessment according to the Globally Harmonised System (GHS) for the classification of chemicals which cause acute toxicity (12).

4. Definitions used in the context of this Guideline are set out in Annex 1.

INITIAL CONSIDERATIONS

5. In the interest of both sound science and animal welfare, *in vivo* acute dermal toxicity testing should not be considered until all available data relevant to the potential dermal toxicity of the test chemical have been evaluated in a weight-of-the-evidence analysis. All available information on the test chemical should be considered prior to conducting the study. Such information will include the identity and chemical structure of the test chemical, its physico-chemical properties, the results of any other *in vitro* or *in vivo* toxicity tests on the test chemical, available (Q)SAR data and toxicological

1 data on structurally-related substances; the anticipated use(s) of the test chemical, the potential for
2 relevant human exposure and the expected use of the data generated. This information will assist in
3 the justification of conducting this study and, if so, the selection of an appropriate starting dose. It is a
4 principle of the method that only moderately toxic doses are used, and that administration of doses
5 that are expected to be lethal should be avoided.

6 6. Guidance on the waiver opportunities for this test method for a given purpose can be found
7 in the Guidance Document on Waiving or Bridging of Mammalian Acute Toxicity Tests (6).

8 7. While the emphasis is on estimating the toxicity ranges involved, the information generated
9 may be needed to permit the substance to be classified in accordance with Classification and Labelling
10 criteria (12). The information generated through acute toxicity testing is of particular value in
11 assessing hazards likely to arise in accident situations, yet, often, the actual data required in such
12 circumstances are beyond the scope of an acute toxicity study. If waiving is not justified scientifically
13 (6), the value or relevance for the data generated in this acute dermal toxicity study for the substance
14 should be reported.

15 ***Mixture or formulated end-use product:***

16 8. A comprehensive evaluation of mixture products (including pesticides, biocides, and other
17 formulated products, (12) has shown that the acute dermal toxicity is rarely greater than the acute oral
18 toxicity of that mixture. In fact, for the majority of cases (99%), the dermal LD₅₀ is ≥ 2000 mg/kg bw.
19 Therefore, it is recommended to use the waiver criteria for acute oral toxicity data (if oral LD₅₀ is
20 >2000 mg/kg bw) to avoid unnecessary testing. Further, *in vivo* data have been compared with the
21 GHS calculation methodology, see Chapter 3.1.3 Classification Criteria for Mixtures (12); in which
22 the classification category based on the Acute Toxicity Estimate (ATE) of each individual component
23 within the mixture is calculated. These data show $>98\%$ concordance (with no under-prediction, *i.e.*
24 false negatives), and thus would provide another means of waiving the dermal study. Whilst
25 synergistic effects among the components of a mixture are not expected, consideration shall be given
26 to the possible interactions that could contribute to the toxic potential of the total mixture.

27 9. If a waiver is not possible, a test for acute dermal toxicity may be carried out on a case by
28 case basis, providing a clear justification.

29 10. Test chemicals should not be administered at doses that are known to cause marked pain and
30 distress due to potential corrosive or severely irritant actions. The pH of the test chemical gives a
31 useful indication to this end. An *in vitro* study might be conducted to check the potential corrosivity of
32 the test chemical. Moribund animals or animals obviously in pain or showing signs of severe and
33 enduring distress shall be humanely killed, and are considered in the interpretation of the test results in
34 the same way as animals that died on test. Criteria for making the decision to kill moribund or
35 severely suffering animals, and guidance on the recognition of predictable or impending death, are the
36 subject of a separate Guidance Document (9).

37 11. If this study is conducted, both local and systemic effects can be investigated. Findings of
38 severe skin irritation (Grade 4 erythema or oedema) in the dermal study could be used instead of
39 performing a specific irritation study.

1 **PRINCIPLE OF THE *IN VIVO* TEST**

2 12. Groups of animals, of a single sex, are exposed to the test chemical in a stepwise procedure
3 using the appropriate fixed doses as set out in Annex 2. The initial dose level is selected on the basis
4 of a sighting study at the concentration expected to produce clear signs of toxicity without causing
5 severe toxic effects or mortality. Further groups of animals may be tested at higher or lower fixed
6 doses, depending on the presence or absence of signs of toxicity or mortality. This procedure
7 continues until the dose causing evident toxicity or no more than one death is identified, or when no
8 effects are seen at the highest dose or when deaths occur at the lowest dose.

9 13. The test chemical is applied to the skin in graduated doses to experimental animals, one dose
10 being used per group. Subsequently, observations of effects and deaths are made. Animals which die
11 during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and
12 necropsied.

13 14. Animals showing signs of severe distress and/or pain at any stage of the test or lesions
14 consistent with the humane endpoints should be humanely killed (9), and the test chemical assessed
15 accordingly.

16 **DESCRIPTION OF THE METHOD**

17 **Selection of animal species**

18 15. The adult rat is the preferred species to be used. In considering the most appropriate sex,
19 surveys of conventional acute oral (14) and acute inhalation toxicity tests (15) (16) show that usually
20 there is little difference in sensitivity between the sexes, but in those cases where differences are
21 observed, females are generally slightly more sensitive. Further evidence to this was obtained in a
22 recent review of dermal toxicity data generated across a breadth of products (13) which confirmed that
23 there is no sex difference in study outcome. Therefore, it is recommended that females should
24 normally be used. However, if knowledge of the toxicological or toxicokinetic properties of
25 structurally-related chemicals indicates that males are likely to be more sensitive then this sex should
26 be used. When the test is conducted in males, adequate justification should be provided.

27 16. Healthy young adult animals of commonly used laboratory strains should be employed.
28 Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing,
29 should be a young adult (at least 8-10 weeks old) with a size which facilitates the conduct of the test
30 (200-300 g) and its weight should fall within an interval of $\pm 20\%$ of the mean weight of any
31 previously dosed animals. Animals with healthy, intact skin are required.

32 **Housing and feeding conditions**

33 17. The temperature in the experimental animal room should be 22°C ($\pm 3^\circ\text{C}$). Although the
34 relative humidity should be at least 30% and preferably not exceed 70% other than during room
35 cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light,
36 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of
37 drinking water.

38 **Preparation of animals**

1 18. The animals are acclimatised to the laboratory conditions for at least five days prior to the
2 start of the study, using group-caging for welfare reasons. Animals are randomly selected for use in
3 the study and marked to provide individual identification.

4 19. Approximately 24 hours before the study, fur should be removed from the dorsal area of the
5 trunk of the test animals by clipping or shaving. Care must be taken to avoid abrading the skin, which
6 could alter its permeability. At least 10 % of the dorsal surface area should be clear for the application
7 of the test chemical. The weight of the animal should be taken into account when deciding on the area
8 to be cleared and on the dimensions of the covering.

9 **PROCEDURE**

10 **Administration of doses**

11 20. The test chemical should be applied uniformly over an area which is approximately 10 % of
12 the total body surface area. With highly toxic test chemicals the surface area covered may be less, but
13 as much of the area should be covered with as thin and uniform film as possible. Test chemicals
14 should be held in contact with the skin with a porous gauze dressing and non-irritating tape throughout
15 a 24-hour exposure period. The test site should be further covered in a suitable manner to retain the
16 gauze dressing and test chemical and ensure that the animals cannot ingest the test chemical. During
17 the 24-hour exposure period animals may be caged individually in order to avoid oral ingestion of the
18 test chemical by other animals in the cage.

19 21. When testing solids, which may be pulverised if appropriate, the test chemical should be
20 moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with
21 the skin. When a vehicle is used, the influence of the vehicle on penetration of skin by the test
22 chemical should be taken into account. The amount of vehicle used should be recorded (generally 0.5
23 to 1 mL are sufficient). Liquid test chemical are generally used undiluted.

24 22. At the end of the exposure period, residual test chemical should be removed, where
25 practicable using water or an appropriate solvent. Animals will be returned to group housing.

26 **Number of animals and dose levels**

27 23. The test chemical is administered to single animals in a sequential manner with no more than
28 2-3 animals used for each step. Generally, if an acute dermal toxicity study is required to be conducted
29 because the waiver criteria do not apply, the expected acute dermal toxicity will be unknown and or
30 high (*e.g.* LD₅₀ < 200 mg/kg body weight). Therefore, the starting dose is selected from one of three
31 fixed levels, 50, 200 or 1000 mg/kg body weight, based on the GHS Categories for acute dermal
32 toxicity (12).

33 24. When there is no information on a test chemical, for animal welfare reasons it is
34 recommended to use the starting dose of 2000 mg/kg body weight. The flow chart of Annex 2
35 describes the procedure that should be followed for each of the starting doses.

36 25. A period of at least 24 to 48 hours will be allowed between the testing of each animal,
37 though this will depend on the onset, duration, and severity of toxic signs. Treatment of animals at the
38 next dose level should be delayed until one is confident of survival of the previously dosed animal(s).
39 All animals should normally be observed for at least 14 days.

1 **Limit test**

2 26. In the rare case that a waiver justification cannot be made (6), or when data requirements do
3 not permit an alternative approach to be used, the option to conduct a limit test is available for
4 situations where the experimenter has information indicating that the test chemical is likely to be non-
5 toxic, *i.e.*, having toxicity only above regulatory limit doses. Information about the toxicity of the test
6 chemical can be gained from knowledge about similar tested chemicals, taking into consideration the
7 identity and percentage of components known to be of toxicological significance. In those situations
8 where there is little or no information about its toxicity, or in which the test chemical is expected to be
9 toxic, the main test should be performed.

10 27. A limit test at one dose level of 2000 mg/kg body weight may be carried out with three
11 animals. If test chemical-related mortality is produced, further testing at the next lower level may need
12 to be carried out.

13 **OBSERVATIONS**

14 28. Animals are observed immediately after dosing at least once during the first 30 minutes,
15 periodically during the first 24 hours, with special attention given during the first 2 to 6 hours after the
16 beginning of the exposure period, and daily thereafter, for a total of 14 days. However, the duration of
17 observation is not fixed but should be determined by the nature and time of onset of clinical signs and
18 length of recovery period. The times at which signs of toxicity appear and disappear are important,
19 especially if there is a tendency for signs of toxicity to be delayed (4). All observations are
20 systematically recorded, with individual records being maintained for each animal. Animals found in a
21 moribund condition and animals showing severe pain and/or enduring signs of severe distress should
22 be humanely killed without delay. When animals are killed for humane reasons or found dead, the
23 time of death should be recorded as precisely as possible.

24 29. Observations should include changes in skin and fur, eyes and mucous membranes, and also
25 respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and
26 behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation,
27 diarrhoea, lethargy, sleep and coma.

28 **Body weight**

29 30. Individual weights of animals should be determined on the day of, or immediately prior to
30 the administration of the test chemical and at least weekly thereafter. At the end of the test surviving
31 animals are weighed and then humanely killed.

32 **Pathology**

33 31. All test animals (including those that die during the test or are removed from the study for
34 animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should
35 be recorded for each animal. Microscopic examination of organs showing evidence of gross
36 pathology, or of the treatment site, in animals surviving 24 or more hours after the initial dosing may
37 also be considered because it may yield useful information.

1 **DATA AND REPORTING**

2 **Data**

3 32. Individual animal data should be provided. Additionally, all data should be summarised in
4 tabular form, showing for each test group the number of animals used, the number of animals
5 displaying signs of toxicity, the number of animals found dead during the test or killed for humane
6 reasons, time of death of individual animals, a description and the time course of toxic effects and
7 reversibility, and necropsy findings.

8 **Test Report**

9 33. The test report must include the following information, as appropriate:

10 *Rationale for in vivo testing: weight-of-evidence analysis of pre-existing test data:*

- 11 – description of relevant data available from prior testing;
- 12 – data derived in each step of testing strategy;
- 13 – weight-of-evidence analysis for performing *in vivo* study

14 *Test chemical*

- 15 - source, lot number, limit date for use, if available;
- 16 - stability of the test chemical itself, if known;
- 17 - solubility and stability of the test chemical in solvent, if known;
- 18 - measurement of pH, osmolality, and precipitate in the culture medium to which the test
19 chemical was added, as appropriate

20 *Mono-constituent substance:*

- 21 - physical appearance, water solubility, and additional relevant physicochemical properties;
- 22 - chemical identification, such as IUPAC or CAS name, CAS number, SMILES or InChI code,
23 structural formula, purity, chemical identity of impurities as appropriate and practically feasible, etc.

24 *Multi-constituent substance, UVCBs and mixtures:*

- 25 - characterized as far as possible by chemical identity (see above), quantitative occurrence and
26 relevant physicochemical properties of the constituents.

27 *Vehicle (if appropriate):*

- 28 – justification for use of vehicle and justification for choice of vehicle (if other than water).

1 *Test animals:*

- 2 – species/strain used;
- 3 – microbiological status of the animals, when known;
- 4 – number, age and sex of animals (including, where appropriate, a rationale for use of males
5 instead of females);
- 6 – source, housing conditions, diet, historical data *etc.*;
- 7 – details of food and water quality (including diet type/source, water source);
- 8 – method of randomisation in animal selection.

9 *Test conditions:*

- 10 – details of test chemical formulation, including details of the physical form of the material
11 administered;
- 12 – details of the administration of the test chemical including dosing volumes and time of dosing;
- 13 – the rationale for the selection of the starting dose;

14 *Results:*

- 15 – tabulation of response data and dose level for each animal (*i.e.*, animals showing signs of
16 toxicity including mortality, nature, severity and duration of effects);
- 17 – tabulation of body weight and body weight changes;
- 18 – individual weight of animals at the day of exposure, in weekly intervals thereafter, and at time
19 of death or euthanasia; – date and time of death if prior to scheduled sacrifice;
- 20 – time course of onset of signs of toxicity and whether these were reversible for each animal;
- 21 – necropsy findings and histopathological findings for each animal, if available.

22 *Discussion and interpretation of results.*

23 *Conclusions.*

24

25 **LITERATURE**

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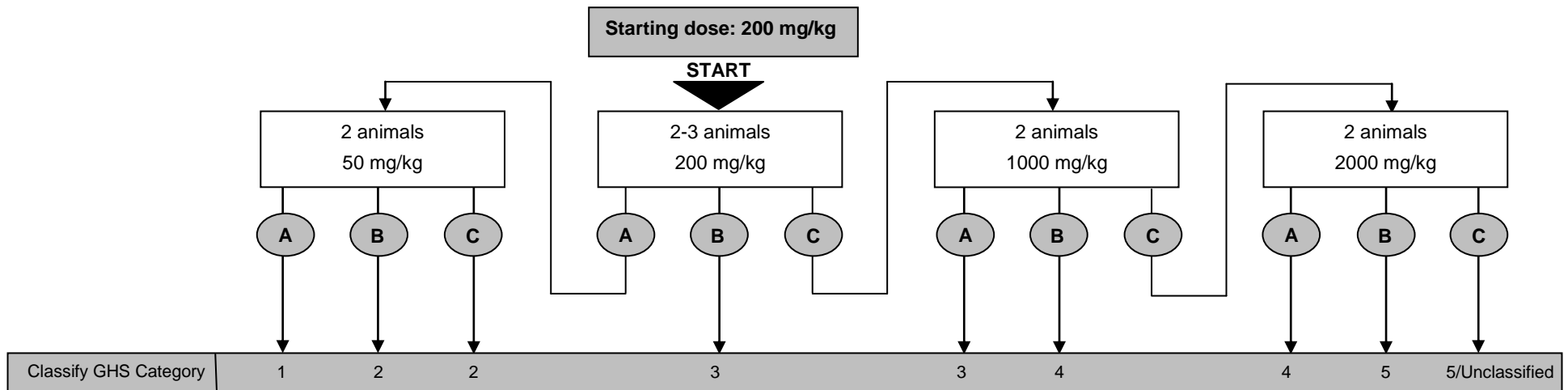
ANNEX 1

DEFINITIONS

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- 4 **Acute dermal toxicity** is the adverse effect caused by a substance following a single uninterrupted
5 exposure by dermal application over a short period of time (24 h or less).
- 6 **Evident toxicity** is a general term describing clear signs of toxicity following the administration of a
7 test substance, (see (16) for examples) such that at the next highest fixed concentration either severe
8 pain and enduring signs of severe distress, moribund condition (criteria are presented in the Humane
9 Endpoints Guidance Document No. 19 (4)) or probable mortality in most animals can be expected.
- 10 **Dose** is the amount of test substance administered. Dose is expressed as weight of test substance per
11 unit weight of test animal (*e.g.*, mg/kg).
- 12 **GHS:** Globally Harmonised Classification System for Chemical Substances and Mixtures. A joint
13 activity of OECD (human health and the environment), UN Committee of Experts on Transport of
14 Dangerous Goods (physical-chemical properties) and ILO (hazard communication) and co-ordinated
15 by the Interorganisation Programme for the Sound Management of Chemicals (IOMC).
- 16 **Impending death** is when moribund state or death is expected prior to the next planned time of
17 observation. Signs indicative of this state in rodents could include convulsions, lateral position,
18 recumbence, and tremor. (See the Humane Endpoint Guidance Document No. 19 (4) for more details).
- 19 **LD50** (median lethal dose) is a statistically derived single dose of a substance that can be expected to
20 cause death in 50 per cent of animals when administered by the given route of exposure. The LD50
21 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).
- 22 **Limit dose** refers to a dose at an upper limitation on testing (2000 or 5000 mg/kg).
- 23 **Moribund status** is being in a state of dying or inability to survive, even if treated. (See the OECD
24 Humane Endpoint Guidance Document No. 19 (4) for more details).
- 25 **Predictable death:** presence of clinical signs indicative of death at a known time in the future before
26 the planned end of the experiment, for example: inability to reach water or food. (See the Humane
27 Endpoint Guidance Document No. 19 (4) for more details).
- 28 **Test chemical:** designates what is tested.
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ANNEX 2

FLOWCHART FOR THE TESTING PROCEDURE



Outcome

- A** ► 2 Deaths (See paragraph 19 if 2-3 deaths)
- B** ► 1 with evident toxicity, and/or ≤1 death
- C** ► No evident toxicity and no deaths

