Guidance to support the use of evident toxicity in acute oral toxicity studies (OECD TG 420)

Fiona Sewell, National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs; ✉ fiona.sewell@nc3rs.org.uk); David Andrew, ERM; Marco Corvaro, Corteva Agriscience Italia S.r.l.; Thomas Holmes, Adama; Irene Manou, European Partnership for Alternative Approaches to Animal Testing (EPAA); Boris P. Müller, Symrise; Tim Rowan, EPAA; Graham Horgan, Biomathematics & Statistics Scotland (BioSS)

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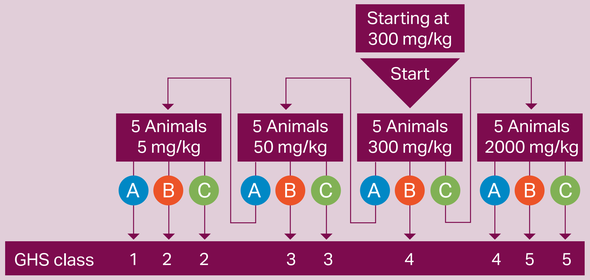
Introduction

Acute oral toxicity studies are conducted in animals as part of chemical hazard classification, for purposes such as classification and labelling. There is currently a choice of methods, OECD test guidelines (TG) 423 and 425, which use death as an endpoint, and the fixed dose procedure (FDP) (TG 420) which uses fewer animals and replaces lethality with 'evident toxicity' as an endpoint (Figure 1). Evident toxicity is defined as *'clear signs of toxicity that predict the next highest dose will cause severe toxicity or death in most animals'*.

Previous work has provided guidance to support the use of evident toxicity within acute inhalation studies [1,2] (TG 433), but the subjectivity and lack of guidance for acute oral toxicity studies may be preventing wider uptake of TG 420. A collaboration between the EPAA and the NC3Rs aimed to make the judgment of evident toxicity more objective and transferable between laboratories.



GHS Class	Dose (kg/mg)	Example
1 (most toxic)	≤ 5	≥50% death at 5 mg/kg
2	> 5 and ≤ 50	≥ 2 deaths at 5 mg/kg *and* up to 1 death or evident toxicity at 50 mg/kg
3	> 50 and ≤ 300	≥ 2 deaths at 50 mg/kg *and* up to 1 death or evident toxicity at 300 mg/kg
4	> 300 and ≤ 3000	Evident toxicity or 1 death at 300 mg/kg *or* no death or evident toxicity at 300 mg/kg *and* ≥ 2 deaths at 3000 mg/kg
5 (least toxic)	> 3000	Up to 1 death or evident toxicity at 3000 mg/kg

Figure 1. *The Fixed Dose Procedure (TG420) protocol, starting at 300 mg/kg.*

Data collection

Historical data on individual animal clinical signs recorded for acute oral toxicity studies were collected from 11 organisations (250 substances) (Figure 2). All studies were carried out in rat (typically 3M+3F or 5M+5F); animals were dosed orally and monitored daily for 14 days. Inclusion and exclusion criteria were applied (e.g., at least two doses per substance, at least 6-fold difference between doses, no death at lower dose, death at higher dose). The final dataset included 120 pairs of studies (68 substances), involving a total of 802 animals.

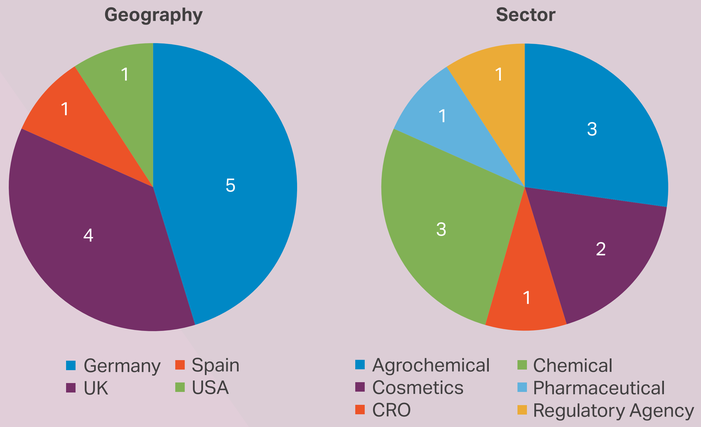


Figure 2. *Data collected (geography and sector).*

Data analysis

Pairs of studies were analysed to determine whether there were any clinical signs (or combination of signs) observed in animals at a lower dose that could predict toxicity at the next higher dose. For each pair of studies (lower dose, higher dose) toxicity was defined as death or euthanasia in more than two animals at the higher dose.

Based on at least one animal per group presenting with the clinical sign at least once, positive predictive values (PPV), specificity and sensitivity were determined:

- **PPV** = the proportion of time the presence of the clinical sign is predictive of toxicity at the next highest dose.
- **Sensitivity** = the proportion of toxicities associated with the clinical sign.
- **Specificity** = the proportion of non-toxicity associated with the absence of the clinical sign.

Results and discussion

There were a range of clinical signs recorded, relating to behaviour, posture, appearance, secretions or respiratory related signs. A large proportion of animals were found dead during the study or required euthanasia (240 animals; 29.9%). Some individual signs were highly predictive of toxicity with PPVs greater than 84% and high specificities (Table 1). High specificity values indicate that there is a low chance of a false-positive result.

Predictivity and specificity increased with increasing numbers of animals experiencing the sign per group, and/or with observations seen on increasing numbers of days, but with decreased sensitivity. Combinations of signs were also examined and some found to be highly predictive, e.g. nasal-ocular discharge and/or laboured respiration and/or ataxia and/or tremors (PPV = 100%).

Clinical signs	PPV (with 95% confidence intervals)		Specificity	Sensitivity
Ataxia	100%	(71.7 - 100)	100%	9.1%
Respiration laboured	100%	(47.3 – 100)	100%	4.0%
Nasal-ocular discharge	100%	(5.0 – 100)	100%	1.0%
Eyes partially closed	100%	(22.4 – 100)	100%	2.0%
Increased salivation	100%	(5.0 - 100)	100%	1.0%
Unkempt coat	100%	(22.4 – 100)	100%	1.0%
Lethargy	86.7%	(62.5 – 97.7)	94.1%	13.%
Respiration decreased	85.7%	(47.0 – 99.3)	97.1%	6.1%
Loose faeces	85.7%	(47.0 – 99.3)	97.1%	6.1%

Table 1. *Highly predictive and specific signs.*

Conclusions and recommendations

The data have been used to form draft recommendations on the recognition of evident toxicity, to provide more guidance on Outcome B in the FDP protocol (Figure 3). If the signs are observed in more than one animal during an acute oral toxicity study there is no need to conduct a further study at a higher dose, since this demonstrates that evident toxicity has been reached – testing at a higher dose will likely result in death or severe suffering.

Guidance on the recognition of evident toxicity

Evident toxicity has been reached if one or more animals display any one of the listed signs at any point within the study*:

Increasing predictivity

- Ataxia
- Respiration laboured
- Naso-ocular discharge
- Eyes partially closed**
- Increased salivation
- Unkempt coat
- Lethargy
- Respiration decreased
- Loose faeces
- Pilo-erection
- Hunched posture

Toxicity is highly likely to occur at the next highest dose

Substance can be classified according to outcome B in the FDP protocol

** Confidence increases with increasing numbers of animals, duration and/or severity*

*** Sign also associated with death – evident toxicity may have been exceeded*

Figure 3. *Draft guidance on the recognition of evident toxicity for acute oral toxicity studies.*

References

[1] Sewell *et al.* (2018) *Regulatory Toxicology and Pharmacology* 94: 22–32.

[2] Sewell *et al.* (2015) *Regulatory Toxicology and Pharmacology* 73(3): 770–9.

OECD Test Guidelines

- OECD (2009) Test Guideline 420: Acute Oral Toxicity – Fixed Dose Procedure.
- OECD (2009) Test Guideline 423: Acute Oral Toxicity – Acute Toxic Class Method.
- OECD (2009) Test Guideline 425: Acute Oral Toxicity – Up-and-Down Procedure.
- OECD (2017) Test Guideline 433: Acute Inhalation Toxicity – Fixed Concentration Procedure.

NC 3Rs

3Rs impact: the development of guidance to aid the recognition of 'evident toxicity' will support wider use of the FDP over currently accepted methods and has the potential to reduce the suffering and numbers of animals used when *in vivo* acute oral toxicity studies are required.

epaa The European Partnership for Alternative Approaches to Animal Testing