A global initiative to refine acute inhalation studies through the use of 'evident toxicity' as an endpoint: towards adoption of the Fixed Concentration Procedure

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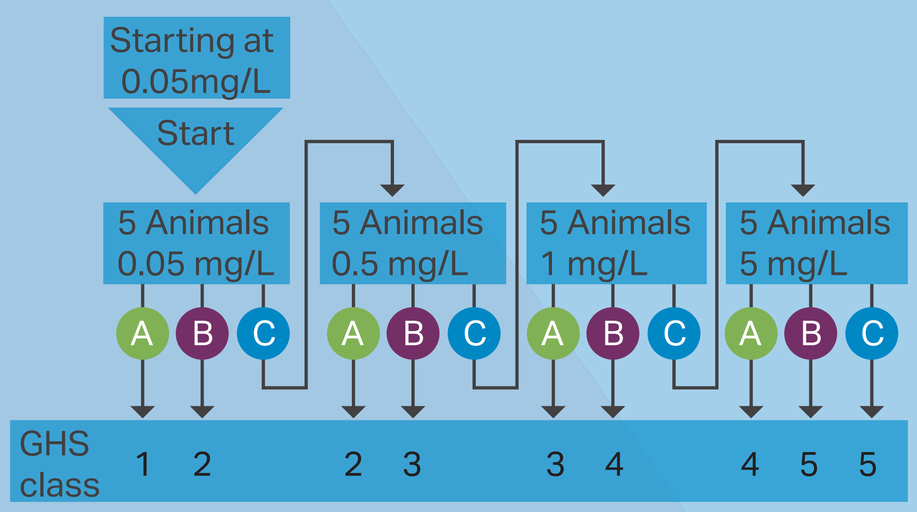
Summary

- Acute inhalation studies are conducted in animals for hazard identification and risk classification. Current accepted methods use death as an endpoint (OECD TG403 and TG436).
- The fixed concentration procedure (FCP) (draft OECD TG433) uses fewer animals and replaces lethality as an endpoint with 'evident toxicity' - signs that predict exposure to a higher concentration will cause severe toxicity or death in most animals (Figure 1).
- The FCP was dropped from the OECD workplan due to concerns regarding comparable performance with TG403 and TG436, suspected sex differences and the subjective nature of evident toxicity. The first two issues have been resolved[1,2].
- A global initiative including 19 organisations, led by the NC3Rs, has collected data on the clinical signs observed during inhalation studies in rats. This data has been used to develop a revised TG433 that clearly defines evident toxicity clinical signs.

Figure 1: FCP protocol example with starting concentration of 0.05mg/L



Outcome

- A: 2 or more deaths
- B: 1 or more with evident toxicity and/or 1 death
- C: Neither death nor evident toxicity

Data collection and analysis

- A clinical signs recording system was developed and used to collect data on over 40 clinical signs observed in individual animals during acute inhalation studies.
- Data for 188 substances, tested in rats at two or more concentrations, were shared by seven laboratories.
- Studies using five rats per group, with no death at the lower dose and at least a two-fold concentration change were selected for analysis. Male and female studies were also analysed separately. 268 pairs of studies were analysed.
- Data were analysed to determine whether clinical signs observed in animals at a lower concentration predict 'toxicity' at a higher concentration. 'Toxicity' was defined as death or euthanasia in two or more animals at the higher dose.
- Positive predictive values (PPV), specificity and sensitivity were determined for clinical signs occuring from day one onwards.

Results

- There was a range of clinical signs recorded, relating to behaviour, posture, appearance, secretions/excretions or respiratory related signs.
- In the absence of death or euthanasia at the same dose, some signs were highly predictive of toxicity at the next highest dose. Table 1 shows the most predictive signs for male and female studies combined. With the exception of ano-genital staining, which was more prevalent in females, there was no significant difference between males and females.

Table 1: Highly predictive signs

Clinical sign	Predictivity (%)*	Specificity (%)	Sensitivity (%)
Hypoactivity	100.0 (92.4-100.0)	100.0	18.4
Tremors	100.0 (68.8-100.0)	100.0	3.9
Body weight loss	94.0 (84.6-98.4)	95.1	22.7
Irregular respiration	89.0 (80.9-94.5)	85.2	35.3
Body staining	88.5 (71.8-97.0)	95.1	11.1
Ano-genital staining	86.4 (67.3-96.4)	95.1	9.2
Faeces reduced	85.3 (70.4-94.4)	91.8	14.0
Naso-ocular discharge	85.0 (71.4-93.7)	90.2	16.4
Noisy respiration	81.2 (71.9-88.4)	73.8	33.3
Hunched posture	78.8 (66.3-88.3)	66.3	19.8
Gasping	76.5 (52.5-92.0)	93.4	6.3

* with 95% confidence intervals.

Conclusions and recommendations

- The data have been used to form a draft recommendation for the recognition of evident toxicity, to provide more guidance on Outcome B in the FCP protocol (Table 2).
- These recommendations will be used to propose a revised TG433 that addresses all three areas of concern (i) comparability (ii) potential for sex differences and (iii) the recognition of evident toxicity.

Table 2: Guidance on the recognition of evident toxicity

Evident toxicity has been reached if one or more animals display any one of the listed signs (from day one onwards):

Increasing predictivity

- Hypoactivity
- Irregular respiration
- Body weight loss (>10%)
- Irregular respiration
- Body staining
- Ano-genital staining*
- Faeces reduced
- Naso-ocular discharge
- Noisy respiration
- Hunched posture
- Gasping**

Toxicity at the next highest dose is highly likely.
Substance can be classified according to Outcome B

*This sign is more prevalent in females than in males. **This sign is more often associated with studies where there is death, once evident toxicity has already been exceeded.

References

1. Price, C., N. Stallard, S. Creton, I. Indans, R. Guest, D. Griffiths, and P. Edwards. 2011. A statistical evaluation of the effects of gender differences in assessment of acute inhalation toxicity. *Human & experimental toxicology*. 30:217-238.
2. Stallard, N., C. Price, S. Creton, I. Indans, R. Guest, D. Griffiths, and P. Edwards. 2011. A new sighting study for the fixed concentration procedure to allow for gender differences. *Human & experimental toxicology*. 30:239-249.

3Rs impact

- Using this guidance, toxicity at the higher dose could have been predicted in 73% of studies. Use of animals at a higher dose would have been avoided.
- Adoption of TG433 over currently accepted methods has the potential to reduce animal use and refine acute inhalation studies, through the use of evident toxicity rather than death as an endpoint.

National Centre for the Replacement Refinement & Reduction of Animals in Research

International industry collaboration

The recommendations were developed by an international expert group which includes 19 organisations (chemical laboratories, contract research organisations, government and regulatory bodies) from Europe, the US, Korea and Japan led by the NC3Rs.

NC3Rs , BioSS, Public Health England, Charles River Laboratories, Exponent International Limited, Harlan Laboratories, Health and Safety Executive, Home Office, Huntingdon Life Sciences, INERIS, JaCVAM, Joint Research Centre, European Commission, Korean Institute of Toxicology, NICEATM, OECD, Product Safety Laboratories, US Department of Agriculture, WIL Research.