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A global initiative to refine acute inhalation studies through the use of 'evident toxicity' as an endpoint: Towards adoption of the fixed concentration procedure

Fiona Sewell [a, *], Ian Ragan [b], Tim Marczylo [c], Brian Anderson [d], Anne Braun [e], Warren Casey [f], Ngaire Dennison [g], David Griffiths [h], Robert Guest [h], Tom Holmes [i], Ton van Huygevoort [j], Ian Indans [k], Terry Kenny [l], Hajime Kojima [m], Kyuhong Lee [n], Pilar Prieto [o], Paul Smith [p], Jason Smedley [q], William S. Stokes [r], Gary Wnorowski [s], Graham Horgan [t]

[a] NC3Rs, UK
[b] Board Member, NC3Rs, UK
[c] Public Health England, UK
[d] Harlan Laboratories, Switzerland
[e] INERIS, France
[f] NICEATM, USA
[g] Home Office, UK
[h] Harlan Laboratories, UK
[i] Exponent International Limited, UK
[j] WIL Research, The Netherlands
[k] Health and Safety Executive, UK
[l] Huntingdon Life Sciences, UK
[m] JaCVAM (Japanese Center for the Validation of Alternative Methods), NIHS (National Institute of Health Sciences), Japan
[n] Korea Institute of Toxicology, South Korea
[o] EURL ECVAM, Systems Toxicology Unit, Institute for Health and Consumer Protection, European Commission, Joint Research Centre, Ispra, Italy
[p] Charles River Laboratories, Edinburgh, UK
[q] Charles River Laboratories, OH, USA
[r] U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Animal Care, USA
[s] Product Safety Laboratories, USA
[t] BioSS, UK

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ABSTRACT

Acute inhalation studies are conducted in animals as part of chemical hazard identification and characterisation, including for classification and labelling purposes. Current accepted methods use death as an endpoint (OECD TG403 and TG436), whereas the fixed concentration procedure (FCP) (draft OECD TG433) uses fewer animals and replaces lethality as an endpoint with 'evident toxicity.' Evident toxicity is defined as clear signs of toxicity that predict exposure to the next highest concentration will cause severe toxicity or death in most animals. A global initiative including 20 organisations, led by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) has shared data on the clinical signs recorded during acute inhalation studies for 172 substances (primarily dusts or mists) with the aim of making evident toxicity more objective and transferable between laboratories. Pairs of studies (5 male or 5 female rats) with at least a two-fold change in concentration were analysed to determine if there are any signs at the lower dose that could have predicted severe toxicity or death at the higher concentration. The results show that signs such as body weight loss (>10% pre-dosing weight), irregular respiration, tremors and hypoactivity, seen at least once in at least one animal after the day of dosing are highly predictive (positive predictive value > 90%) of severe toxicity or death at the next

Abbreviations: fixed concentration procedure, FCP.

* Corresponding author.
E-mail address: fiona.sewell@nc3rs.org.uk (F. Sewell).

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highest concentration. The working group has used these data to propose changes to TG433 that incorporate a clear indication of the clinical signs that define evident toxicity.

1. Introduction

1.1. Background

Acute inhalation studies are conducted in animals as part of chemical hazard identification and characterisation. Current accepted methods, LC50 (OECD TG403 (OECD, 2009a)) and the acute toxic class (ATC) (TG436 (OECD, 2009b)) use death as an endpoint. These are described in more detail below. In an effort to reduce animal numbers and to improve welfare, an alternative fixed concentration procedure (FCP) was proposed in 2004 (draft OECD TG433 (OECD, 2004) which replaced lethality as an endpoint with 'evident toxicity.' This was defined as those signs of toxicity that predict severe toxicity or death in most animals at the next highest concentration of the chemical. The FCP was dropped from the OECD work plan in 2007 because of a lack of evidence for comparable performance with TG403 and TG436, suspected sex differences in the level of toxic effects (since the FCP was originally proposed to use females as the default sex) and the ill-defined and subjective nature of evident toxicity. The first two issues have been resolved (Price et al., 2011; Stallard et al., 2011) through work supported by the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) thereby leaving only the definition of evident toxicity to be determined. To this end, the NC3Rs launched a global initiative involving 20 organisations with the aim of making evident toxicity more objective and transferable between laboratories. The group shared data on the clinical signs recorded during acute inhalation studies for 172 substances, the majority of which fell under the category of dusts and mists (from completed studies held in the archives of participating laboratories), and determined which signs have high positive predictive value (PPV) for severe toxicity or death at the next highest concentration (as described below). The draft OECD TG433 is now back on the OECD work plan, pending the outcome of this work.

1.2. Acute inhalation studies

The two existing guidelines (TG403 and TG436) are described here in some detail because the data used for the analysis in this paper originated from studies run according to these protocols. The FCP (draft TG433) is the preferable method for investigation of acute inhalation toxicity for classification and labelling purposes based on animal welfare grounds (preventing unnecessary suffering by eliminating the need to test at higher actual lethal doses). This method has been shown to be comparable with both existing methods in estimating the toxic class to which a substance belongs (Stallard et al., 2011).

Table 1
GHS classifications for LC_{50} by inhalation.

GHS category	Vapours (mg/L)	Dusts and mists (mg/L)	Gases (ppm)
1 (most toxic)	≤0.5	≤0.05	≤100
2	>0.5 and ≤2	>0.05 and ≤0.5	>100 and ≤500
3	>2 and ≤10	>0.5 and ≤1	>500 and ≤2,500
4	>10 and ≤20	>1 and ≤5	>2,500 and ≤20,000
5 (least toxic)	>20	>5	>20,000

GHS, Globally Harmonised System; LC_{50}, median concentration; ppm, parts per million.

1.2.1. LC_{50} method (TG403)

The LC_{50} of a substance is the concentration that can be expected to cause death in 50% of the animal population, where 'death' is defined as compound-related mortality within 14 days. The LC_{50} is used to classify substances (dust and mists, vapours, and gases) under the Globally Harmonised System of Classification and labelling of chemicals (GHS) (OECD, 2001). The test specifies that 10 animals (5 males and 5 females) should be exposed at each of three concentration levels. The concentration levels should be sufficiently spaced to enable construction of a mortality curve and an estimate of the LC_{50} to be obtained. The LC_{50} is then used to classify the toxicity of the chemical, according to Table 1 and as illustrated in Fig. 1.

1.2.2. Acute toxic class method (TG436)

The acute toxic class (ATC) method (TG436) has been accepted as an alternative method to the LC_{50} test (OECD TG403). Whilst the test uses fewer animals, death is still used as an endpoint. The test specifies that 6 animals (3 males and 3 females) are tested at fixed concentrations that form the upper limit of the GHS categories (e.g. 0.05, 0.5, 1 and 5 mg/L for dusts and mists) (Table 1). The starting concentration is either the highest concentration, or that which is expected to lead to mortality in some of the exposed animals, based on prior information. At each concentration decisions are based on the number of observed deaths from the combined group of animals. Either a classification is made or testing continues at the next higher or lower concentration, depending on the starting concentration, as shown in Fig. 2.

1.2.3. The fixed concentration procedure (FCP) (TG433)

The FCP test method is similar to the ATC method above but decisions and classifications are instead based on evident toxicity – clear signs of toxicity such that it can be predicted that exposure to the next highest concentration would cause death in most animals. The draft FCP protocol starts with a sighting study in which single female animals are exposed sequentially to one or more concentrations. Information from the sighting study can be used to classify the substance (if there is death at the lowest concentration the substance is classified into the most toxic class) or to guide decisions for an appropriate starting concentration of the main study. Comparison of the FCP test with the existing methods showed that, in the absence of sex differences, the results are similar (Price et al., 2011). Since the original FCP design proposed testing in female rats, the NC3Rs working group suggested the inclusion of a modified sighting study to take into account any sex differences in sensitivity. This involves the testing of one male and one female, to choose the most sensitive gender to take forward to main study testing. The main study then uses females (unless males are indicated as the more sensitive sex), where groups of five animals are exposed at each concentration until a decision on classification can be made. As for the ATC, substances are tested at fixed concentrations that form the upper limit of the GHS categories (e.g. 0.05, 0.5, 1 and 5 mg/l for dusts and mists) (Table 1). At each concentration decisions are based on the number of deaths and/or the number of animals experiencing evident toxicity, and either a classification is made or testing continues at the next higher or lower concentration, depending on the starting concentration (Fig. 3).

An issue that needed to be addressed by the group is the