The regulatory use of the LD_{50} test in the light of scientific and animal welfare considerations

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The LD_{50} dose of a test substance is the amount, which causes the death of 50 per cent of the dosed animals. This figure has a long tradition in the toxicity testing of drugs, cosmetics and pesticides. It is now achieving an important role in the regulation of new and old chemicals as exemplified with the 6. amendment of the 1967 directive of the 1967 directive of the EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. This directive went into force September 18th 1981.

In the 6th amendment chemicals having a LD_{50} value less than 25 mg per kg b. w. are classified as very toxic, less than 200 mg as toxic and less than 2000 mg as harmful. The test description for the LD_{50} test requires by EEC is in accordance with the OECD guidelines adopted May 1981.

The OECD guidelines for acute oral toxicity – designed mainly for rats – prescribes the use of 5 females and 5 males per dose level (OECD guideline no 401, 402 and 403). For dose levels less than 5000 mg/kg body weight at least 3 dose levels producing a dose response curve should be used. If a test with at least 5000 mg/kg body weight produces no compound related mortality, full study using three dose levels are not necessary (limit test) according to OECD.

The animals should be observed for a period of at least 14 days. A careful clinical examination should be made at least once a day. Cageside observations should include changes in the skin and fur, eyes and mocous membranes, as well as changes in the respiration, blood circulation, and nervous system, leading to tremors, convulsions, salivation, diarrhoea, lethargy, sleep or coma. Gross necropsy of all animals and microscopic examination should be performed.

In the evaluation of test results the OECD-guideline states that the LD_{50} value is a relatively coarse measurement, useful only as a reference value for classification and labelling purposes and for an expression of the lethal potential of the test substance by the route of exposure (oral, dermal, by inhalation).

The guideline interpretates the LD_{50} value as an estimate of relative toxicity of a substance in a test species, and underlines that extrapolation of the results of acute oral toxicity studies and oral LD_{50} values in animals to man is valid only to a very limited degree.

Scientific considerations

From a scientific point of view the test design made by OECD cannot be critizised because the design re-

commends us to obtain far more relevant data than just values causing death from the test. Such data may – as underlined by OECD – also form the basis for establishing the dosage regimen in subchronic and other repeated dose studies as well as providing initial information on the mode of toxic action of the test substance.
The expert group of OECD has met the regulatory demand by designing a technically optimal test for the LD_{50} determination which also provides the more exact information for planning and design of multiple exposure toxicological studies.

Animal welfare considerations
The use of at least 30 rats per test just to determine that dosage killing 50 % of the animals is from ethical and animal welfare points of view considered a too coarse and narrow minded use of mammals in obviously painful experimentation. The goal of the animal welfare associations – and most toxicologists as well – is therefore to avoid the simplistic LD_{50} determination for regularly purposes. Most people agree to the general need of using the necessary number of animals to explore and predict the order of magnitude of human health risks. Every use of animals should lead to a broadening of the total knowledge of the biological activity of the tested compound in order to justify the experimentation.

Ways to go
In order to apply data from both a scientific point of view and an animal welfare point of view in the regulatory decision it is therefore important to leave the simplistic LD_{50} measurement as the regulatory tool.
Several possibilities are obvious. The most optimal being replacing the use of intact animals with more simple test systems such as cell cultures. Such *in vitro* methods, however, still need time consuming and costly refinements and validation related to correlation to wellknown and recognised biological parameters in the intact animal. These developments need to be encouraged and followed closely.
The actual regulatory need of acute toxicity data enabling regulatory authorities to classify and label chemical compounds have to be accepted both by scientists and animal welfare people.
The way to go, is to optimize the OECD guideline for acute toxicity testing through the OECD-updating procedure. Most probably this ought to performed through a two step procedure.
The first and immediate step should be in a scientific guided approach to include instruction in the guidelines to perform the test as a sequence of tests each including just one dose level on a few animals. The procedure should be to begin the testing the first day with the lowest dosage, continuing after some hours with the following (double) dosage. This will allow time for observation of the symptoms induced in an elucidating cause of death. Testing of higher doses should be stopped when the dosage at which animals start to die within 24 hours is reached.
The regulatory need is only to classify a chemical within the broad

classes "very toxic", "toxic" and "harmful" (LD_{50} less than 25, 25–200 and 200–2000 mg/kg b.w./day). This ranking can in most cases, where LD_{50}-values are not borderline, be obtained by less precise estimates from few dead animals. For the same reasons also the number of animals per group might be lowered substantially for most chemicals, using more precise estimates for borderline chemicals.

The second step in the future development of the acute toxicity tests within OECD could be to apply a scoring system in the test. Scoring systems are already applied in the acute dermal irritation/corrosion test (OECD guideline 404) and the acute eye irritation/corrosion test (OECD guideline 405). So no formal difficulties exist. The difficulty will be to balance out the score values for the symptoms and between the different degrees of symptoms and – finally to correlate the total sum to present classification system based on LD_{50} values in such a way that the new system does not violate the present system, but creates a valuable alternative. Some ideas how to develop such a system are given in table 1.

Table 1.

Main symptoms in acute toxicity tests to creak the basis for score values.

Actual score values for the single symptoms still have to be established. The severity of the symptoms expressed as the sum of the individual scores together with the actual dosage leading to this summarized scores taken together from the basis for the classification of the chemical as very toxic, toxic or harmful.

Symptoms/observations
Skin and fur
Eyes
Mucous membranes
Respiration
Circulatory system
Diarrhoea
Salivation
Behaviour
Tremors
Convulsions
Weight loss
Lethargy longetivity
Coma longetivity
Time to death

Conclusion and summary

The present regulatory use of the LD_{50} data does not justify the unscientific thorough technical performance of acute toxicity tests according to the OECD-guidelines neither from a scientific point of view nor from a animal welfare point of view.

In the short run the present use of the OECD-guidelines ought to be simplified using fewer animals in a sequential dosing scheme.

In the long run the LD_{50} value should be replaced by a score value including for symptoms and severity of symptoms.