



An example of reduction and refining (Projects 114-08 and 123-10, Rufli)

Testing for acute toxicity in fish: Fewer fish and less suffering

In order to determine the ecotoxicity of the effects of a substance on the environment, the acute fish toxicity test must be carried out to determine the level of acute toxicity according to OECD guideline 203.

Statistical analyses by the Basle ecotoxicologist Hans Rufli involving thousands of data series from fish tests showed that results of equal quality can be obtained using six instead of seven fish, as prescribed by the guideline. Moreover, the fish could be removed from the test much earlier by applying a recognised criterion of “moribund” instead of “dead”, which reduced the suffering of the fish involved. In order to put these proposals into practice with regard to the 3R principles of reduction and refining, they would have to be included in the universally valid OECD guideline.

The level of toxicity of chemical substances is often expressed as an LC50 value. This corresponds to the concentration of the test substance in water, soil or air that kills 50% of the test organisms within a given period of observation. For the acute fish toxicity test, seven fish must be exposed at least five times to various concentrations of the test substance in order to determine at what concentration 50% of the fish die within 96 hours. Fish are also used for the preliminary tests in which the approximate level of critical concentration is determined. Tens of thousands of fish are used for such tests every year.

The aim of Hans Rufli from ecotoxsolutions in Basle and his research partners was to design a more efficient test that would provide results of equal quality using fewer fish. Through retrospective analysis of over 4,000 data series from previous fish tests they were able to produce a typical dose-effect curve. On the basis of these results they then carried out simulated tests and compared their results with those of tests carried out according to OECD guideline 203. It became apparent that for each test group of 7 fish one fish could be eliminated without compromising the quality of the results obtained. This corresponds to 14% of the fish used. This might appear to be a small difference, but in view of the total number of such toxicity tests carried out every year it is a significant number of fish.

Furthermore, the meeting of specialists which was organised as part of the project revealed that, for the preliminary tests where the critical concentration for the acute toxicity test was determined, i.e. where either no fish or all the fish died quickly, fish embryos instead of adult fish could just as well be used.

“Moribund” instead of “dead”

During the standard test, which normally lasts for 96 hours, the fish often waste away in the highest concentrations and die at a certain point. If a recognised “moribund” criterion were adopted the 50% limit could be established at a much earlier point. The suffering experienced by the fish could be reduced by up to 92 hours.

How can “moribund” be defined in this case? Rufli drew up a list of possible visible criteria such as unnatural swimming behaviour, loss of balance, hyperventilation, bulging eyes.

For this testing method, which reduces the number of fish required, international agreement first needs to be reached concerning the “moribund” criterion. Subsequently it needs to be included in OECD guideline 203. The ultimate aim is to instigate a demand from an OECD country, such as Switzerland, for the corresponding modification of the universally valid OECD guidelines.

http://www.forschung3r.ch/en/projects/pr_114_08.html

http://www.forschung3r.ch/en/projects/pr_123_10.html

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