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Use of "moribund" stage in the OECD fish acute toxicity test and its effects on suffering and LC50-values

Introduction:
It has become common practice in many laboratories in Europe to use the criterion of "moribund" to replace the "death" criterion in the acute fish test as it reduces the terminal suffering of the fish. Fish with **severe sublethal effects** are declared **Moribund**, e.g. acc. to example definition: **Swimming behavior** and **Loss of equilibrium** with severity degree **3** over 48h (Table), and are removed from the test as soon as this occurs (premature discontinuation of experiment).

Aim:
- To assess the magnitude of the potential decrease of the value of the main endpoint
- To estimate the range of shortening the suffering of fish
- To provide information on how the OECD guideline 203 should be adapted to reduce the subjectiveness introduced by the use of moribund

Table: Example of a real-life study with lethal and sublethal symptoms observed (Explanation for sublethal symptoms: 0: no symptoms; 1: light; 2: moderate; 3: severe); with:

LC50 = 7.7 mg/L
$LC50_{moribund}$ = 0.49 mg/L

Using moribund, fish are removed in concentrations of 0.95, 2.5, 5.0 and 8.1 mg/L (28 fish in total) **reducing degree 3 severity of suffering by 48h.**

Conc. actual mg/L	Mortality Number of dead fish 24 h	48 h	72 h	96h	%
Blank	0	0	0	0	0
0.25	0	0	0	0	0
0.95	0	0	0	0	0
2.5	0	0	0	0	0
5.0	0	0	1	1	14
8.1	0	4	4	4	57

Conc. actual mg/L	Sublethal symptoms: Swimming behaviour 24	48	72	96	Loss of equilibrium 24	48	72	96	Respiratory function 24	48	72	96	Exophthalmus 24	48	72	96	Pigmentation 24	48	72	96h	Moribund %
Blank	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.25	0	0	1	1	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0
0.95	3	3	3	3	3	3	3	3	0	0	1	1	0	0	0	0	0	0	0	0	100
2.5	3	3	3	3	3	3	3	3	2	2	2	2	0	0	0	0	0	0	0	0	100
5.0	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	0	0	0	100
8.1	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	1	1	1	100

Method:
A retrospective analysis of 328 fish acute toxicity tests of an industry laboratory was perfomed based on five different definitions of moribund, and of 111 tests from ten other laboratories from Europe and the US.

Results:
- The introduction of the moribund state reduces the suffering of the fish, though it may change a compounds hazard classification if it results in a lower $LC50_{moribund}$ compared to the LC50.
- In the studies evaluated, up to 23% of the fish were declared as moribund **reducing the suffering of severity grade 3 (severe distress) by up to 92h.**
- **Decrease of the $LC50_{moribund}$ relative to the LC50 by a factor of about 2 (median); maximum 15.7.**
- To produce comparable results between laboratories, an updated OECD guideline 203 requires: A unique definition of the moribund state in fish, 2.) Specifications on the type of visible abnormalities to be reported, 3.) Specifications on the degree of the effects.