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INTRODUCTION OF MORIBUND CATEGORY TO OECD FISH ACUTE TEST AND ITS EFFECT ON SUFFERING AND LC50 VALUES

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Abstract—It has become common practice in many laboratories in Europe to introduce the criterion "moribund" to reduce the suffering in fish acute lethality tests. Fish with severe sublethal symptoms might be declared moribund and are removed from the test as soon as this occurs (premature discontinuation of experiment). Moribund fish affect main study outcomes as the median lethal concentration (LC50) derived on fish declared as moribund may be lower than the conventional LC50. This was evaluated by a retrospective analysis of 328 fish acute toxicity tests of an industry laboratory based on five different definitions of moribund, and of 111 tests from 10 other laboratories from Europe and the United States. Using the criterion of moribund 10 to 23% of the fish were being declared as moribund in 49 to 79% of the studies. In 36 to 52% of the studies, the $LC50_{moribund}$ was lower than the conventional LC50 depending on the definitions of moribund. An inclusion of the moribund criterion in an updated Organisation for Economic Cooperation and Development guideline for the acute fish toxicity test would reduce the period of suffering by up to 92 h, lowering the value of the main toxicity endpoint by a factor of approximately 2, and maximal by a factor of approximately 16. Environ. Toxicol. Chem. 2012;31:1107–1112. © 2012 SETAC

Keywords—Fish acute test   Moribund fish   OECD guideline 203   Reporting sublethal effects   Fish pain and suffering

INTRODUCTION

In the acute fish toxicity test according to the Organisation for Economic Cooperation and Development (OECD) guideline 203 [1], median lethal concentration (LC50) is assessed in terms of the concentration of substances at which 50% of the fish die within an exposure period of 96 h. The historical intent of the acute toxicity endpoints is to provide acute lethality endpoints such that effects can be extrapolated to the environment. Risk assessors rely on the LC50 to evaluate the potential impacts on the survival of wild fish, and chemicals are classified and labeled based on this endpoint. Several years ago, laboratories in the United Kingdom started to use the criterion of "moribund" in the acute fish test. In the meantime, it has become common practice in many laboratories in Europe as it reduces the terminal suffering of the fish.

Dictionary definitions of moribund include words and phrases such as "dying," "at the point of death," "in the state of dying," or "approaching death." However, these definitions are severely limited to laboratory animal research because they do not describe the moribund state in behavioral or physiological terms. Developing a sound approach to identifying the moribund state is crucial to its effective use as an experimental endpoint [2].

Fish with severe sublethal effects are declared moribund and are removed from the test as soon as this occurs (premature discontinuation of the experiment). However, the uncertain relationship between the sublethal endpoints, the moribund stage, and mortality render the use of moribund as subjective and highly variable as long as corresponding baseline data are lacking, and as long as the procedure of using the moribund stage in the test, and its evaluation and reporting, are not specified in the guideline. This greatly increases the likelihood of making type I errors, that is, incorrectly declaring a fish as moribund. The impact of using moribund on the study outcome as well as on reducing fish suffering has not been studied so far. Based on the fact that the moribund stage is already used in many laboratories when conducting fish acute tests, it is the aim of the present study to assess the magnitude of the potential decrease in the value of the main endpoint and its implications for risk assessment; to estimate the range of shortening fish suffering; and to provide information on how the test guideline should be adapted to reduce the subjectiveness introduced by using moribund to produce comparable results between laboratories.

MATERIALS AND METHODS

Results from a series of 512 LC50 studies (96 h) performed according to OECD guideline 203 from 1990 to 2001 in a Ciba-Geigy AG, Novartis, and Syngenta Crop Protection AG laboratory were compiled into a database called the industry laboratory database in the present study. This database was used for the retrospective analysis of data.

Additional information on this database can be found in Rufli and Springer [3], including the distribution of LC50-values and slopes, the number of partial mortalities (mortalities >0 and <100%), and differences between agrochemicals and other chemicals.

Before the data analysis could begin, the criteria for moribund state had to be defined. A first definition, referred to as definition A, arose from the reporting style of the studies of the industry laboratory on the observation of the sublethal symptoms of swimming behavior and loss of equilibrium over 48 h with severe symptoms (severity degree 3 in a range from 0 to 3). An example of the symptoms and degrees of severity as reported in a real-life study by the industry laboratory is given in Table 1.

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Table 1. Example of a real-life study with lethal and sublethal symptoms observed[a]

Concentration actual mean (mg/L)	Mortality (number of dead fish)				
	24 h	48 h	72 h	96 h	Moribund (%)
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

Concentration actual mean (mg/L)	Sublethal symptoms: Swimming behavior				Loss of equilibrium				Respiratory function				Exophthalmus				Pigmentation				Moribund (%)
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h	
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[b]	3[b]	3	3	3[b]	3[b]	3	3	0	0	1	1	0	0	0	0	0	0	0	0	100
2.5	3[b]	3[b]	3	3	3[b]	3[b]	3	3	2	2	2	2	0	0	0	0	0	0	0	0	100
5.0	3[b]	3[b]	3	3	3[b]	3[b]	3	3	3	3	3	3	0	0	0	0	0	0	0	0	100
8.1	3[b]	3[b]	3	3	3[b]	3[b]	3	3	3	3	3	3	0	0	0	0	0	1	1	1	100

[a] Explanation for sublethal symptoms: 0 = no symptoms; 1 = light; 2 = moderate; 3 = severe.
[b] Symptoms of swimming behavior and loss of equilibrium with a severity degree 3 over 48 h fulfill the requirements for the "moribund" stage according to definition A.

In the real-life study presented, fish showed severe sublethal symptoms expressed in terms of altered swimming behavior and loss of equilibrium from 24 to 96 h (i.e., over a period of 96 h). Based on the above definition, the fish would be declared moribund after 48 h. The fish would be removed immediately from the exposure, thereby reducing the severity of degree 3 suffering by 48 h in concentrations of 0.95, 2.5, 5.0, and 8.1 mg/L (28 fish in total). Moreover, applying the moribund criterion would result in all fish in these four concentrations being declared as moribund, whereas the death criterion led to an observation of 0, 0, 0, 14, and 57% mortality, respectively. Therefore, applying the moribund definition results in a lower LC50 of 0.49 (referred to as $LC50_{moribund}$ concentration) compared to an LC50 of 7.7 mg/L using the conventional method (factor 15.7 lower) and to a classification of "acute category 1 for hazardous to the aquatic environment (acute [short-term] aquatic hazard)" [4].

Based on definition A of moribund, a retrospective analysis of the industry laboratory studies was performed. Limit tests were not considered in the evaluation. This allowed determining the $LC50_{moribund}$ and the magnitude of the decrease of the $LC50_{moribund}$ compared to the LC50.

The $LC50_{moribund}$ was determined by the methods of Spearman-Kaerber [5], Berkson [6], and by probit analysis [7]. If considerable differences among the results of the different methods occurred, the concentration-effect curve was drawn on probability paper and the $LC50_{moribund}$ was determined graphically. Based on comparing the results of the various statistical methods to the concentration-effect curve, as well as the fact that the probit method should be used only with two or more partial mortalities [8], the following procedure to determine the $LC50_{moribund}$ evolved yielding the most scientifically reasonable results for the specific data sets (0, 1, >1 partial mortality): partial mortality 0, one concentration with 0% mortality and the next higher with all fish declared as moribund: method of Spearman-Kaerber; partial mortality 1, one concentration with 0%, the next higher with partial mortality (or between 0 and 100% of the fish declared as moribund), and the next concentration with all fish declared as moribund: method of Berkson; and partial mortality >1: probit analysis.

Finally, the distribution of the factors of the decrease from LC50 to $LC50_{moribund}$ was determined, as well as the frequency of a decrease and the number of fish affected by a shortened duration of suffering.

This procedure was repeated based on a second and third definition of moribund, definitions B and C, which were derived from the reporting style of the industry laboratory. Finally, definitions A to C were applied to studies from 10 different laboratories in Europe and the United States. In addition, definitions D and E of moribund, based on the reporting style of sublethal symptoms of these other laboratories, were evaluated for both the industry laboratory as well as for the other laboratories (Table 2).

RESULTS

Industry laboratory

The industry laboratory database consisted of 328 LC50 studies that were not limit tests, including 226 studies evaluating response to agrochemicals and 102 measuring response to other test materials.

Among the five sublethal symptoms reported, swimming behavior, equilibrium, and respiration (severity degree 3 during

Table 2. Definitions of moribund stage[a]

Definition A	Swimming behavior and loss of equilibrium over ≥48 h.
Definition B	Swimming behavior over ≥48 h, and at least a single observation of complete loss of equilibrium or strong ventilation.
Definition C	Swimming behavior over ≥48 h, and at least a single observation of complete loss of equilibrium, strong ventilation, or strong discoloration.
Definition D	At least a single observation of swimming behavior and complete loss of equilibrium or strong ventilation.
Definition E	At least a single observation of swimming behavior, and complete loss of equilibrium, strong ventilation or strong discoloration.

[a] All effects with severity degree 3.

two subsequent observations) were affected most frequently, namely in 53, 51, and 43% of the studies, respectively. Effects on pigmentation were less frequent with 21%, and exophthalmus was observed only in one study corresponding to 0.3%. Both swimming behavior and equilibrium were affected in 50% of the studies (164 of 328). In 96% of the studies, effects on swimming behavior and equilibrium were observed simultaneously. In only 3% of these studies swimming behavior was affected alone (equilibrium was not affected), and in 1% of these studies equilibrium was affected alone (swimming behavior was not affected). Effects on respiration were also observed to swimming behavior and loss of equilibrium in 82% (134 of 164 studies). In correspondence, effects on pigmentation were also observed in 5.5%. However, respiration was never affected when both altered swimming behavior and loss of equilibrium were observed. When three sublethal symptoms—swimming behavior, equilibrium, and respiration—were affected, effects on pigmentation were also observed in 33% of the studies (54 of 164). When neither swimming behavior nor equilibrium was affected strong ventilation did not occur, with the exception of 2.4% of the studies (8 of 328).

Based on definition A of the moribund stage, the fish in 45% of the tests were declared as moribund (49 and 38% for agrochemicals and nonagrochemicals, respectively), and 36% of all studies resulted in a decrease of the $LC50_{moribund}$ compared to the LC50 (41 and 28% for agrochemicals and nonagrochemicals, respectively). The distribution of the decrease factor was unimodal with a median of 1.8 (only studies resulting in a decrease were considered) (Fig. 1).

The maximum decrease was 15.7 observed for agrochemicals, whereas the corresponding value for nonagrochemicals was 3.6. The number of fish declared as moribund using definition A was 1,976 (fish exposed in concentrations, without blank and solvent controls). This corresponds to 13% of the total number of fish used. Therefore, for 1,976 fish, or 13%, the moribund stage would have reduced the severity of suffering degree 3 by 24 to 48 h when definition A was applied. Of these, 196 (1%) were declared as moribund after 24 h, 1,434 (10%) after 48 h, and 346 (2%) after 72 h of exposure.

Applying definition B for the moribund stage did not change the result much compared to definition A—three more studies were affected by the moribund stage (increase from 45–46%) and two more studies by a lower $LC50_{moribund}$ (increase from 36–37%). This had no effect on the median of the decrease factor. However, an additional 24 fish (+0.2%) were declared as moribund: seven fish more after 24 h, 28 fish more after 48 h, and 11 fish less after 72 h of exposure. Thus, the moribund stage was reached 24 h earlier for 35 fish using definition B.

Definition C, which also considered a strong change in pigmentation, did not further reduce the suffering or increase the number of studies affected compared to definition B.

However, with definitions D and E based on a single observation of altered swimming behavior, the number of studies declared as moribund increased by 15 to 60% compared to definition A (59.6 and 59.9% for definitions D and E, respectively). Similarly, the number of studies with a lower $LC50_{moribund}$ increased by 4 to 40% (by 8 and 13 studies to 39.8 and 40.2% for definitions D and E, respectively). At the same time, the number of fish declared as moribund rose by more than 50% to 3,115 and 3,157 fish for definitions D and E, respectively. Applying these definitions, 598 and 612 fish were already removed after a short exposure of 2 to 4 h (this additional observation time was introduced in 1993) and an additional 1,648 and 1,669 fish after 24 h, respectively. Contrary to this, the number of fish removed after 48 and 96 h of exposure decreased from 1,780 to 680. Thus, not only did the number of moribund fish increase considerably using definitions D and E, but also the duration of suffering was reduced. While 9.9% of moribund fish were removed after the initial 24 h of exposure using definition A, it was 79% with definitions D and E. This corresponds to 3.6 times the amount of fish removed early with definitions D and E (within 3–24 h), and 3.6 times more fish removed late for definition A to C (within 48–72 h). At the same time, a decrease of the $LC50_{moribund}$ was observed in 13 more studies with definition E as compared to definition A (121 studies with definition A, 134 with definition E).

Other laboratories

Apart from the industry laboratory, 111 nonlimit studies from 10 laboratories were available: six laboratories from Europe (Harlan, Ibacon, Safepharm, Springborn Smithers, Brixham, and Eurofins) and four from the United States (Springborn Smithers, Wilbury, ABC, Wildlife International). The various sublethal symptoms reported by these laboratories are shown in Table 3.

Increasing the number of sublethal symptoms to declare a fish as moribund, according to definitions A to E, did increase the number of studies with a moribund state and number of fish affected, step-by-step from 54 to 88 studies (49–79%), and from 676 to 1,555 fish (10–23%). A clear change was noticeable going from definitions A, B, and C with altered swimming behavior observed at two subsequent observations, to criteria D and E with a single observation. This change resulted in an increase of the number of moribund fish in the studies by a factor of 2.3 (676 with definition A, 1,555 with definition E), while the median of the toxicity increased from 1.8 to 1.9. The

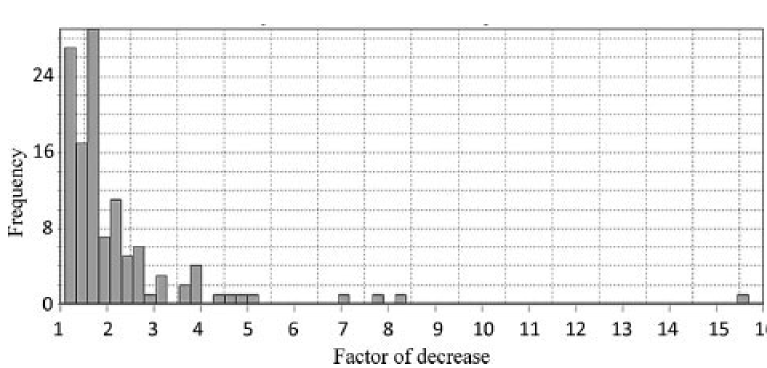

Fig. 1. Histogram of the decrease factor of $LC50_{moribund}$ compared to LC50.

Table 3. Sublethal symptoms reported by different laboratories

Swimming behavior[a]	Loss of equilibrium[a]	Respiratory function[a]	Pigmentation[a]	Other symptoms
Tumbling[b]	Tumbling[b]	Rapid respiration, strong ventilation, hyperventilation	Dark discoloured, darkended pigmentation, increased pigmentation	Strongly extended gills
Hyperexcitability	Partial loss of balance	Slow respiration	Discolored	Distended abdomen, abdominal distension, bloated, swollen abdomen
At surface, surfacing	Complete loss of equilibrium, keeling	Labored respiration	Changed color	Convulsions
Lying on the bottom,[c] sounding		Irregular respiration	Mottled	Mucus secretion
Erractic swimming		Gasping respiration		Hemorrhaging
Skittering		Gulping respiration		Exophthalmos
Diving		Coughing		Dilated pupils
Spiraling				Aggression
Twitching				Mouth open
Apathy, lethargic, weak				
Immobility, ceased swimming, quiescent				

[a] Abnormalities given in the Organisation for Economic Cooperation and Development guideline 203, fish acute toxicity test.
[b] Tumbling fish may be classified as showing both a change in swimming behavior and partial loss of equilibrium.
[c] Lying on the bottom may be allocated to altered swimming behavior, but may also show a loss of equilibrium.

factor for the maximum $LC50_{moribund}$ decrease compared to the LC50, however, changed from 5.6 to 8.2. Furthermore, fish were declared moribund earlier and were consequently removed earlier. For definitions A to C, 42% of the moribund fish were removed within 2 to 24 h of exposure and 75% for definitions D to E. Thus, 2.9 times the amount of fish was removed early based on definitions D to E (within 2–24 h), whereas for definitions A to C, 1.5 times more fish were removed late (only after 48–72 h).

DISCUSSION

The LC50 values are used in risk assessment and for classification and labeling of chemicals. The question arises, how suitable is the $LC50_{moribund}$ for the risk assessment compared to the LC50? Using the moribund stage instead of the death stage may result in a more accurate prediction in wild fish survival as moribund fish, for example, with altered swimming behavior and loss of equilibrium, are more susceptible to predation even if the sublethal symptoms might be reversible. In this respect, the $LC50_{moribund}$ might be a better endpoint to predict the impaired survival of a population and, therefore, a better endpoint for the risk assessment and classification than the LC50. However, based on the results of the present study, the assessment factors for $LC50_{moribund}$ may, on average, be lowered by a factor of 2 to set safe concentrations. Furthermore, fish with a moribund stage defined as showing effects for a longer period would be more likely to be caught by predators (48 h in definitions A to C, 24 h in definitions D and E). However, to relate the sublethal symptoms better to the moribund stage and actual death of a fish, to describe a moribund stage adequately, and to minimize the number of fish incorrectly declared as moribund, more research on this topic is required.

Within a test, the suffering of the fish gets reduced as more sublethal symptoms contribute to the moribund stage and with shorter required observation periods for these symptoms (definitions A to C with single observation vs definitions D and E with two subsequent observations). With more symptoms contributing to the moribund stage, criteria get "softer" and more fish will be declared as moribund. Thus, softer criteria reduce the suffering of the fish within a test (up to 7.9 more fish were removed after 3–24 h than after 48–72 h with the softer definition E than with definition A), and softer criteria increase the number of fish as well as the number of studies declared as moribund (up to 2.3 more fish were declared as moribund with definition E than with definition A and up to 1.6 more studies). At the same time, softer criteria may increase the number of fish incorrectly declared as moribund.

The introduction of the moribund stage may yield lower values of the $LC50_{moribund}$ compared to the LC50. If the $LC50_{moribund}$ is seen as a better prediction for the impaired survival of populations than the LC50, then the lowering of the endpoint value does not matter regarding its use in risk assessment and classification. However, to compare the values of $LC50_{moribund}$ with the huge database of historical LC50 values, it would be desirable to keep the decrease within limits. The magnitude of the decrease depends on two factors: (1) on the number of concentrations with fish declared as moribund (maximum factor observed of 15.7), which in turn depends on the definition of moribund and the sublethal symptoms observed (factor of 1.5 going from definitions A to E); and (2) on the spacing factor (interval) between concentrations. The potential decrease of the $LC50_{moribund}$ because of the spacing factor selected between concentrations corresponds to a factor of 3.5 (23.4/6.6 = 3.5), using the maximum spacing factor of 2.2 according to the guideline results in a ratio between the highest and lowest concentration of 23.4 and 6.6 when a small spacing factor of 1.6 is used. In the study yielding the highest decrease from the $LC50_{moribund}$ to LC50, the difference between the measured and nominal concentrations further contributed to the maximum factor of 15.7. Thus, using spacing factors of 1.6 to 1.8 rather than 1.9 to 2.2 would reduce the difference between the $LC50_{moribund}$ and LC50 (difference of up to a factor of 15.7) to the range of factors occurring between LC50 values derived from different laboratories, for example, a factor of 10 to 12 (for abamectin, LC50 values from different laboratories differed by a factor of 12—four species) [9]. For pyrethrum, extracted from the U.S. Environmental Protection Agency ECOTOX Database (http://epa.gov/ecotox), interspecies and interlaboratory differences averaged a factor of 10 using mean LC50 values per species (47 studies, five species).

Based on the discussion above, using moribund instead of death in the fish acute test may more accurately predict a situation where the survival of the fish population is impaired.

However, the moribund stage can be defined in various ways. The relationship between sublethal effects, the moribund stage, and death might be known for mammals, but not for fish. Research data need to be generated to adequately describe the moribund stage—approaching death—by sublethal symptoms in fish, and a unique definition of the moribund stage needs to be given in the guidelines to produce comparable results between laboratories.

For a view that moribund better predicts survival in the environment, definitions A to C are more adequate because the fish show symptoms for a longer period. However, the use of the moribund stage, at the same time, should reduce the suffering of the fish as much as possible. Reducing the suffering is better achieved with softer criteria such as in definitions D and E, while the number of fish incorrectly declared as moribund is increased. Consequently, a compromise needs to be found for defining the moribund stage, so that it serves the various purposes best.

Although the studies from different laboratories have shown that an evaluation of different ways of reporting the visible sublethal abnormalities in principle is possible, it is not only much easier, less subjective, and time consuming to interpret the sublethal symptoms when the reporting is done the same way in all laboratories, but also the declaration of moribund gets more consistent. Therefore, it is best if the way of reporting is specified in the guideline. It minimizes the differences between laboratories in the resulting declaration of the moribund stage and results will get more transparent and comparable between laboratories.

The impact of the way of reporting can also be seen when the two databases from the industry (database A) and the other laboratories (database B) are compared. In database A, the way of reporting is fixed; in database B, it is free and different in each laboratory. This is comparable to a situation in which the definition of the moribund stage and the way of reporting (reporting of sublethal effects and degree of effects) are specified in the guideline (database A), and a situation in which only the definition of moribund is specified (database B). Database A has a highest decrease from $LC50_{moribund}$ to LC50 of 15.7, and database B of 8.2. In database A, up to 17% of moribund fish were removed after 2 to 24 h, and in database B up to 75% depending on the definition of moribund. The reporting of sublethal effects (time of first observation and observation intervals) is in fact responsible for the difference in the reduction of the suffering between the two databases. In database B, the first observation time was earlier (2–24 h vs 6 h) and initial observation intervals were shorter (e.g., 2 h vs 18 h), both lead to fish being declared as moribund and removed from exposure at an earlier time. The way of reporting (type of sublethal effects reported, degree of effects reported individually or not and number of severity grades) also affects the magnitude of the decrease. However, the two databases are different in the distribution of the concentration–effect curve slopes. Database A is known to generally have steep slopes [3], which add to the decrease.

The industry database evaluation has shown that symptoms of sublethal effects proposed in OECD guideline 203, such as swimming behavior and loss of equilibrium, are ambiguous and cannot be separated clearly; for example, when observations like tumbling or lying on the bottom are made. Both swimming behavior and loss of equilibrium usually occur simultaneously and in only 3 and 1%, respectively, was one criterion observed alone. Therefore, the types of abnormalities specified in the guideline need to be nonambiguous.

Sublethal effects can be scored in various ways, for example, by the individual fish or by the whole batch of fish in a tank. Furthermore, different scoring scales can be used. Sometimes, it is difficult or impossible to score individual fish because of the coloration or turbidity of the test solution or the movement of the fish, for instance. Despite these difficulties, stating the number of organisms exhibiting the specified sublethal effects would probably be the best way, because this increases the ability to characterize the effects on fish from the exposure to the test substance.

CONCLUSIONS

Introducing the moribund state reduces the suffering of fish, though it may change a compound's 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 92 h. The median of the decrease of $LC50_{moribund}$ in relation to the LC50 was by a factor of approximately 2; the maximum factor observed was 15.7. Although reducing suffering is desirable, a decrease in the measured toxicity endpoint has consequences on classification and risk assessment.

To produce comparable results between laboratories when moribund is used requires the following specifications in an updated test guideline: (1) a unique definition of the moribund state in fish, (2) specification on the type of visible abnormalities to be reported, and (3) specifications on the degree of the effects.

Different definitions of the moribund stage lead to a different number of fish declared as moribund. Furthermore, a different definition may affect the difference between the $LC50_{moribund}$ and the LC50, because this depends on the number of concentrations with fish declared as moribund. Therefore, a unique definition of the moribund stage needs to be given in an updated guideline. For animal welfare reasons, the best choice of criteria for the definition of moribund reduces the suffering for as many fish as possible. To compare an $LC50_{moribund}$ to the huge database of historical LC50 values, the difference between the two toxic actions should be kept low. Because the magnitude of the $LC50_{moribund}$ decrease compared to the LC50 not only depends on the definition of moribund alone, but also on the spacing factor between concentrations, it is recommended that low spacing factors between 1.6 and 1.8 are used. Lower spacing factors result in a potentially lower decrease.

The type of visible abnormalities to be reported should be specified in the guideline. A similar way of reporting in different laboratories facilitates data interpretation and minimizes the differences between laboratories in declaring fish as moribund. To further reduce differences, the selected types of abnormalities need to be nonambiguous. Furthermore, there should not be a fixed link between two different sublethal effects (e.g., swimming behavior and pigmentation). Unusual behavior (reduced activity and or orientation to the bottom or surface of the test vessels, dark pigmentation), as reported in some laboratories, wrongly suggests that swimming behavior and pigmentation always occur simultaneously.

The degree of effects should be reported in a clearly defined way. In our view, reporting is best done by stating the specific number of organisms exhibiting the specified abnormalities, if possible, as this increases the ability to characterize the effects on fish from the exposure to the test substance.

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