

n-ドデシルベンゼンスルホン酸ナトリウムによるヒメダカ,*Oryzias latipes*に対する急性毒性値の再現性

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Reproducibility of Acute Toxicity Data of Sodium *n*-Dodecylbenzenesulfonate to Red Killifish, *Oryzias latipes*

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Abstract

A series of acute toxicity tests of sodium *n*-dodecylbenzenesulfonate (C₁₂LAS) to red killifish *Oryzias latipes* were carried out in order to find out the sensitivity of test organisms to this compound. The LC₅₀ values obtained by Doudoroff's graphic interpolation method were 4.4 ± 0.4 mg/ℓ (n=9) at 48 h and 4.3 ± 0.3 mg/ℓ (n=4) at 96 h. The 48 h LC₅₀ values ranged from 4.0 to 6.0 mg/ℓ in 21 out of 24 tests and the 96 h LC₅₀ values were in the same range in all the 8 tests performed. The acute toxicity values thus obtained for C₁₂LAS to red killifish were highly reproducible under the controlled feeding and testing conditions, though the fish were supplied from different breeding district and at different seasons, and the sensitivity of red killifish to the compound was very uniform. In addition, the test substance, C₁₂LAS, was suggested to be a suitable reference substance for acute toxicity evaluation in fish.

Acute fish toxicity (LC₅₀ values) of chemical substances has been measured using various fish species¹⁾. Red killifish, *Oryzias latipes*, has been mainly used for the LC₅₀ measurement in Japan. This is a fish chosen in the Japanese Industrial Standard²⁾ (JIS method, hereafter) and the official method of OECD Guidelines for Testing of Chemicals³⁾. Tadokoro and Maeda⁴⁾ reported that the variance in LC₅₀ values of ring tests for 15 chemicals was within 3-fold in the red killifish.

Authors carried out a series of acute toxicity tests with red killifish under fixed test conditions, selecting sodium *n*-dodecylbenzenesulfonate (C₁₂LAS) in order to find out the sensitivity of test organisms to this compound.

Materials and Methods

Test Substance

Sodium *n*-dodecylbenzenesulfonate (C₁₂LAS) (Wako Pure Chemical Industries, Ltd., Japan) was used. The test substance used was isomers having sulfophenyl group (expressed ϕ) in different positions and the relative ratios determined by high-performance liquid chromatography were 2 ϕ : 22.9 %, 3 ϕ : 21.2 %, 4 ϕ : 21.8 %, 5 and 6 ϕ : 34.1 %, respectively.

Dilution Water

Tap water dechlorinated with activated carbon was used for acclimation of fish and toxicity tests. The wa-

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ter was analyzed every six months and met Suisanyo-suikijun (Water Quality Criteria for Fisheries)⁵⁾. Especially, analytical data of toxic substances such as pesticides and heavy metals showed less than the upper limits of these criteria. Water hardness was 67 ± 8 mg/l (mean \pm SD) as CaCO₃. Residual chloride determined was less than 0.01 mg/l all the time.

Fish and Their Feeding Conditions

Red killifish, *Oryzias latipes*, were supplied from a commercial source. The fish were disinfected with 1 % of sodium chloride solution for 2-4 days, then acclimated in a 70-l glass aquarium with a circulating filtration apparatus filled with dechlorinated tap water more than a week. The fish were delivered 11 times during February 1990 to March 1992. The number of fish delivered was 259 ± 48 (mean \pm SD) per aquarium. The fish were fed commercial food (TetraMin Staple Food, TetraWerke, Germany) equivalent to 1 % body weight per day, and not fed for 24 h before the start of the test. Water temperature was set at 25 °C for the entire period of acclimation.

Acute Toxicity Test Procedure

The JIS method for acute toxicity test was used for C₁₂LAS. The test were carried out at several concentrations including at least 4.0 and 6.0 mg/l under the test conditions shown in Table 1. The surviving number of fish at each concentration was recorded after 48 h in 24 tests and after 96 h in 8 tests with water renewal at 48 h. The single LC₅₀ values were estimated by a graphic interpolation method⁶⁾ in case that the mortalities were upper and under 50 % except 0 or 100 %. The number of fish in the acute toxicity test was 10 per 3 l for each group in a 5-l glass aquarium. The fish were not fed during the study.

Table 1. Test conditions*

Item	Mean \pm SD
Total length of fish (cm)	2.66 \pm 0.31
Body weight of fish (g)	0.21 \pm 0.08
Water temperature (°C)	24.2 \pm 0.8
Dissolved oxygen concentration (mg/l)	8.42 \pm 0.73
pH	7.30 \pm 0.45

* At the start of tests.

Results and Discussion

The results of 48 h and 96 h acute toxicity tests of C₁₂LAS are shown in Table 2 and Table 3, respectively. Fig. 1 illustrates an example of LC₅₀ values estimated by a graphic interpolation method. Mean values of single LC₅₀ estimated by this method were 4.4 ± 0.4 mg/l (n=9) at 48 h and 4.3 ± 0.3 mg/l (n=4) at 96 h. The 48 h LC₅₀ values were between 4.0 and 6.0 mg/l in 21 out of 24 tests except that test number 9, 14 and 15 were outside this range. The 96 h LC₅₀ values were in the same range in all the 8 tests, indicating small variation of the data. Breeding districts of red killifish were specified in the test number 14 through 24. Consequently, significant differences in the sensitivity by breeding districts of the fish could not be observed. Toxicity of sodium linear alkylbenzenesulfonate (LAS) was influenced by water hardness⁷⁻⁹⁾. Studies^{10,11)} carried out in the JIS method by which we have done showed the same results as ours in similar condition of water hardness.

These results indicates that LC₅₀ values of C₁₂LAS for red killifish were highly reproducible under the controlled feeding and testing conditions, though the fish were supplied from different breeding district and at different seasons, and the sensitivity of red killifish was very uniform.

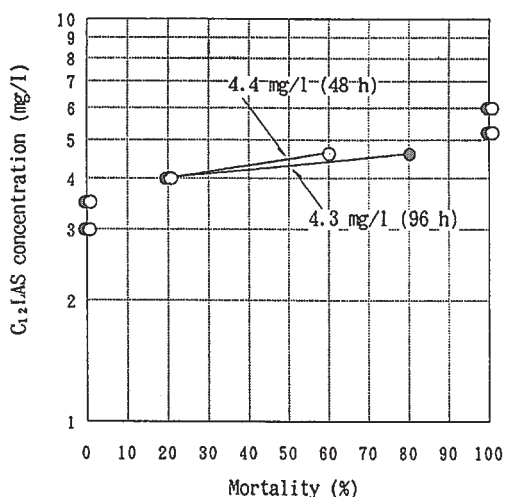


Fig. 1. An example of LC₅₀ values estimated by a graphic interpolation method (test No. 24). ○; 48 h, ●; 96 h.

Table 2. LC₅₀ values of C₁₂LAS after 48 h exposure to red killifish

Test No.	Test fish		Estimated LC ₅₀ (mg/ℓ)	Mortality (%)	
	Date of supply	Breeding district		4 mg/ℓ	6 mg/ℓ
1	Feb. 1990.	—	4.0–6.0	30	90
2	Sep. 1990.	—	4.0–6.0	10	100
3	Sep. 1990.	—	4.0–6.0	0	70
4	Oct. 1990.	—	4.0–6.0	0	70
5	Nov. 1990.	—	4.0–6.0	0	60
6	Nov. 1990.	—	4.0–6.0	0	100
7	Nov. 1990.	—	4.0–6.0	10	100
8	Nov. 1990.	—	4.0–6.0	40	100
9	Feb. 1991.	—	<4.0	60	100
10	Jun. 1991.	—	4.0–6.0	0	100
11	Jun. 1991.	—	4.0–6.0	40	100
12	Jul. 1991.	—	4.0–6.0	0	100
13	Jul. 1991.	—	4.0*	50	100
14	Jan. 1992.	Tochigi	<4.0	60	100
15	Jan. 1992.	Tochigi	3.5*	100	100
16	Jan. 1992.	Tochigi	4.0–6.0	20	100
17	Jan. 1992.	Tochigi	4.0–6.0	10	100
18	Mar. 1992.	Aichi	4.8*	10	100
19	Mar. 1992.	Aichi	4.6*	0	90
20	Mar. 1992.	Aichi	4.8*	20	100
21	Mar. 1992.	Aichi	4.3*	20	100
22	Mar. 1992.	Saitama	4.3*	20	100
23	Mar. 1992.	Saitama	4.6*	40	100
24	Mar. 1992.	Saitama	4.4*	20	100

* Nine single values could be obtained by Doudoroff's graphic interpolation method (mean±SD, 4.4±0.4).

Table 3. LC₅₀ values of C₁₂LAS after 96 h exposure to red killifish

Test No.	Test fish		Estimated LC ₅₀ (mg/ℓ)	Mortality (%)	
	Date of supply	Breeding district		4 mg/ℓ	6 mg/ℓ
17	Jan. 1992.	Tochigi	4.0–6.0	10	100
18	Mar. 1992.	Aichi	4.0–6.0	20	100
19	Mar. 1992.	Aichi	4.0–6.0	0	90
20	Mar. 1992.	Aichi	4.6*	20	100
21	Mar. 1992.	Aichi	4.0–6.0	30	100
22	Mar. 1992.	Saitama	4.2*	20	100
23	Mar. 1992.	Saitama	4.0*	50	100
24	Mar. 1992.	Saitama	4.3*	20	100

* Four single values could be obtained by Doudoroff's graphic interpolation method (mean±SD, 4.3±0.3).

In addition, HgCl₂ was proposed as a reference substance especially for validation of fish condition¹²⁾. Since there are advantages with respect to handling, use and waste disposal, and abundant data related to acute toxicity of LAS, C₁₂LAS is suggested to be suitable as a reference for acute toxicity evaluation in fish.

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n-ドデシルベンゼンスルホン酸ナトリウム (C₁₂LAS) を用い、ヒメダカ、*Oryzias latipes* の急性毒性試験を複数例実施した。その結果、供試魚の入手時期や生産地を変えても、一定の飼育管理と試験方法のもとでは、急性毒性値の再現性は高く、ヒメダカの感受性は均一であると考えられた。また、C₁₂LAS は、使用から廃棄までの取扱い上の利点からも、HgCl₂ に代わる魚類急性毒性試験の基準物質としての可能性が示唆される。