Variations by Sex and Body Size in the Avoidance Tests of Sodium Linear Laurylbenzene Sulfonate and Fenitrothion Using a Fish, Medaka *Oryzias latipes*

Hideo Hidaka* and Ryo Tatsukawa*

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This paper examined variations by sex and body size in the avoidance tests of LAS (sodium linear laurylbenzene sulfonate) and fenitrothion using a fish, adult medaka *Oryzias latipes*, in a small chamber system with a bisymmetric parallel-flow. Although these avoidance tests were repeated many times in different seasons, the reproducibility of the tests was excellent. No significant difference was found in between the male and female of same size. However, the avoidance concentrations of small medaka were apparently lower than those of large medaka, indicating that avoidance response to the aquatic contaminants may vary significantly with fish body size. Therefore, comparison of avoidance tests among different species of fish should take into consideration the fish size.

Although over the past two decades aquatic toxicology has concentrated primarily on lethality, growth, and reproductive success, recently behavioral test is becoming a promising field of study. However, behavioral toxicology has lagged in areas of environmental hazard and impact assessment because of difficulties in quantifying behavioral endpoints, which have a large variation associated with individual fishes and test conditions. So the standard testing methods have not been established yet.¹⁾

Many studies on fish avoidance response to aquatic contaminants have been reported since the work by Shelford and Alle (1913).^{2,3)} The test apparatus and procedures used so far varied so much and quite a few reports were questionable whether the testing methods, including the apparatus and procedures, were suitable for the purposes of respective studies. Therefore, it is difficult to compare quantitatively the past results of avoidance tests.⁴⁻⁶⁾

Thus, the authors have developed an apparatus and its working procedure, which is sensitive, rapid, reproducible and quantitative, for avoidance test by fish (medaka *Oryzias latipes*).⁷⁾ Using this method, anionic surfactants,⁸⁾ pesticides⁹⁾ and monochloramine⁹⁾ were tested. This test may be used as one of the standard methods for the ecotoxicological examination of chemicals to fish.

Avoidance behavior of fish varies very much

among individuals, test conditions and other factors. However, very few reports have dealt with these variations. Therefore, avoidance tests to LAS and fenitrothion were conducted several times in different seasons using adult medaka under the same test conditions which gave uniform results.^{7,8)} Furthermore, the variations by sex and body size on the avoidance responses of fish were studied in this paper.

Materials and Methods

The methods used here are the same as previously reported.⁸⁻⁹⁾ The outline is as follows: Adult medaka were used after acclimation for at least one month in a 50-l all-glass aquaria. The avoidance test was conducted in a small chamber system with a bisymmetric parallel-flow (Fig. 1). A fish is put into the small chamber and an overnight pretest acclimation was made under same conditions (without toxicants) as in the actual avoidance test, using a large container with 20 small chambers containing one medaka, respectively. After transferring the acclimated small chamber to the test trough, avoidance measurement was made for 10 min during the first 10-20 min of the experiment, adding a toxicant to one side of the water flow. The time required for one trial was 25 min. Fish were used only once. Usually, 10 trials were conducted for one concentration level of a chemical. The water tem-

* Department of Environment Conservation, Ehime University, Tarumi 3-5-7, Matsuyama 790, Japan (日高秀夫, 立川 涼: 愛媛大学農学部環境保全学科).

Fig. 1. Scheme of avoidance test trough.

This system consists of a test trough (made of 10 mm thick transparent acrylic resin plate) and exchangeable small chamber (made of 5 mm thick acrylic resin plate, downstream side transparent and other sides opaque blue). Measure in this figure represents outer side in mm. Rearing water of medaka flows through both sides into the test trough (2 l/min) and a toxicant solution or control water was added in each flow (10 ml/min). Rearing water and the toxicant were mixed thoroughly during their flow through two baffle plates, and these two water flows, the diluted toxicant solution and the control water, run parallel with a distinct boundary at the center of the small chamber. Water depth was adjusted to 5 cm and the average flow speed was 7 mm/sec in the small chamber. Putting a 1 mm thick opaque acrylic plate over the small chamber, both sides are illuminated at 500–520 lx by an overhead daylight lamp.

perature was maintained at $25.0 \pm 1.0^\circ\text{C}$ during the test.

The test results are expressed as avoidance ratio (AR), which is the percentage of time spent by a fish in the clean water of the test trough for 5 min during 15 to 20 min after the start of the experiment, using a mean of 10 trials for one concentration level of a chemical (mean AR). Furthermore, to compare more precisely with avoidance concentrations of chemicals, AR_{95} and its 95% confidence limit, which is the avoidance concentration of a chemical when the AR is 65% on the rising side of the avoidance curve, were calculated by regression analysis from the test results of at

least two concentrations at the higher and lower ranges of 65% AR on the linear increasing part of the avoidance curve.

The toxicants tested here were LAS and fenitrothion (dimethyl 4-nitro-*m*-toryl phosphorothionate).

Results and Discussion

From our previous studies,⁸⁻⁹⁾ it is found that the significant ($P=0.05$) mean AR compared to that of blank test is about 63%, and the mean AR above 60% can be regarded as the significant ($P=0.1$) avoidance. Also in this examination, the

Fig. 2. Avoidance responses of medaka to LAS.

Symbol	Test period	n	B.L. (S.D.)	B.W. (S.D.)
●	May-June 1980	80	25.1 (1.78)	0.27 (0.078)
○	July-Oct. 1983	60	25.0 (2.38)	0.30 (0.096)

B.L.: body length (mm)

B.W.: body weight (g)

Each plot represents mean of ten trials and bar shows its 95% confidence limit.

results confirmed by F-test for variance and t-test for differences of mean AR were similar to the previous findings.

Reproducibility of Test Results

The avoidance tests were conducted two times for LAS and three times for fenitrothion in different seasons of 1980-1983 (Figures 2 and 3). Notwithstanding the tests were performed in different seasons, no significant difference was found among the results of both chemicals respectively. Also, the values of AR_{95} showed good agreement (Table 1).

This study used reddish orange medaka (hime-

Fig. 3. Avoidance responses of medaka to fenitrothion.

Symbol	Test period	n	B.L. (S.D.)	B.W. (S.D.)
●	Aug. 1981	50	23.2 (1.86)	0.25 (0.074)
△	Apr.-May 1983	20	27.6 (2.25)	0.40 (0.109)
○	Oct.-Dec. 1983	30	26.6 (2.48)	0.34 (0.111)

B.L.: body length (mm)

B.W.: body weight (g)

Each plot represents mean of ten trials and bar shows its 95% confidence limit.

daka), which have been reared under the artificial environment for a long time as an aquarium fish in Japan. The medaka, rearing indoors at 25°C, spawns all the year around, and hatched fry grows as an adult within 3-4 months. Therefore, it is reasonable that the test results, performed by adult medaka under the same test conditions at $25.0 \pm 1.0^\circ\text{C}$, didn't show any seasonal variation.

The test results showed that the reproducibility of this method was excellent, which may be explained by the following reasons: healthy test fish was selected after acclimation for at least one

Table 1. Variation of the AR_{95} values of medaka

LAS*1		Fenitrothion*1	
Test period			
May-June 1980	13.5 (11.4-15.9)	Aug. 1981	74.3 (67.5-81.8)
July-Oct. 1983	13.0 (10.6-16.1)	Apr.-May 1983	73.1 (66.4-80.5)
		Oct.-Dec. 1983	72.8 (66.1-80.1)
Sex			
Male	13.1 (10.5-16.2)		73.4 (67.5-79.8)
Female	13.0 (11.0-15.3)		72.3 (64.4-81.3)
Body size			
Small*2	9.6 (8.2-11.4)		55.6 (53.4-57.9)
Large*2	18.7 (16.1-21.7)		80.6 (74.4-87.3)

*1 µg/l (95% confidence limit).

*2 See Fig. 5.

Fig. 4. Difference of avoidance response to LAS and fenitrothion between male and female of medaka.

Symbol	Sex	LAS			Fenitrothion		
		n	B.L. (S.D.)	B.W. (S.D.)	n	B.L. (S.D.)	B.W. (S.D.)
●	Male	68	24.8 (2.06)	0.27 (0.086)	39	26.1 (2.63)	0.33 (0.101)
○	Female	52	25.3 (2.11)	0.30 (0.092)	21	27.1 (2.29)	0.39 (0.118)

B.L.: body length (mm)

B.W.: body weight (g)

Each plot represents mean of 9–14 (male) or 6–11 (female) trials and bar shows its 95% confidence limit.

month, and, probably more importantly, the same rearing water of medaka was used both during pretest acclimation and test, that is, the water condition was kept constant throughout the experiments.^{6,7)}

Using these test results obtained by twenty trials for one test concentration of a chemical, we examined the variation of avoidance response.

Difference Between Male and Female

Though quite many papers on avoidance of fish have been published, the variation by sex in the avoidance responses of fish to aquatic contaminants have never been reported.

When divided the test results of Figures 2 and 3 into male and female, trial numbers of both sexes were different at test concentrations, but no significant differences were found in body size of both sexes (Fig. 4). As is generally known, the range of 95% confidence limit was broad at smaller number of the test trials; but here no variation of test results between male and female was found.

Although the AR of female was slightly higher than that of male at 30 µg/l of LAS, no significant difference was found at the other test concentrations. Moreover, no significant difference was recognized in AR₈₅ (Table 1).

As mentioned above, the medaka, rearing at

25°C, spawns all the year around. The spawning of medaka was observed in all the test periods of these experiments. Generally, the sexual difference appears most remarkably in the reproductive season. Therefore, it is safe to say that in the present experiments the medaka have not shown significantly different avoidance responses to chemicals between male and female.

Difference by Body Size

All the medaka used in the avoidance test were adult and the range of their body length was 20.3–32.0 mm. The test results were divided into two groups, by body length of 24.5 mm in LAS tests and of 26.5 mm in fenitrothion tests, the number of both size groups being nearly same. As shown in Fig. 5, the avoidance curve of small medaka shifted toward lower concentration and its peak concentration (exhibited the highest AR) shifted from 30 µg/l to 20 µg/l in the case of LAS. The AR₈₅ values of small medaka were apparently lower than those of large one in both LAS and fenitrothion respectively (Table 1). That is, the AR₈₅ values of large size fish were about 1.9 times in LAS and about 1.4 times in fenitrothion higher than those of small ones. The similar results were obtained when divided by body weight of fish.

Hose and Stoffel¹⁰⁾ tested the avoidance re-

Fig. 5. Difference of avoidance response to LAS and fenitrothion between large and small size in adult medaka.

Symbol	Size	LAS			Fenitrothion		
		n	B.L. (S.D.)	B.W. (S.D.)	n	B.L. (S.D.)	B.W. (S.D.)
●	Small	52	23.1 (0.96)	0.22 (0.050)	41	23.8 (1.83)	0.26 (0.064)
○	Large	68	26.5 (1.48)	0.34 (0.083)	29	28.7 (1.36)	0.44 (0.086)

B.L.: body length (mm)

B.W.: body weight (g)

Each plot represents mean of 8–11 (small) or 9–12 (large) trials and bar shows its 95% confidence limit.

sponse of juvenile blacksmith (*Chromis punctipinnis*, 4.0–10.0 cm in body length and 1–17 g in weight) to chlorinated seawater and found direct correlation between the weight of the fish and its avoidance concentration. Although their test methods are different from ours, the fact that the avoidance concentration of smaller size fish is lower, is similar with our results.

The Standard Methods of USA, in the section of selecting test organisms for bioassays,¹¹⁾ recommends organisms that are nearly uniform in size, with the largest individual not more than 50% longer than the shortest. Although this requirements was met in our tests, significant difference was found in the fish responses by body size. Therefore, it is necessary to consider carefully the variation of body size when comparing the avoidance tests among different species of fish and interpreting the results obtained in the laboratory as field situations.

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References

- 1) J. D. Giattina and R. R. Garton: in "Residue Reviews" (ed. by F. A. Gunter), Vol. 87, Springer-Verlag, New York, 1983, pp. 43–90.
- 2) S. R. Larrick, K. L. Dickson, D. S. Cherry, and J. Cairns, Jr.: *Hydrobiologia*, **61**, 257–265 (1978).
- 3) D. S. Cherry and J. Cairns, Jr.: *Water. Res.*, **16**, 263–301 (1982).
- 4) H. Hidaka and R. Tatsukawa: *Ecological Chemistry*, **7**(4), 17–26 (1985) (in Japanese).
- 5) H. Hidaka and R. Tatsukawa: *Ecological Chemistry* **8**(1), 17–27 (1985) (in Japanese).
- 6) H. Hidaka and R. Tatsukawa: *Arch. Environ. Contam. Toxicol.*, **14**, 565–571 (1985).
- 7) H. Hidaka, M. Suga, and R. Tatsukawa: *J. Agric. Chem. Soc. Jap.*, **57**, 571–579 (1983) (in Japanese).
- 8) H. Hidaka, M. Suga, and R. Tatsukawa: *J. Agric. Chem. Soc. Jap.*, **58**, 1–7 (1984) (in Japanese).
- 9) H. Hidaka, M. Hattanda, and R. Tatsukawa: *J. Agric. Chem. Soc. Jap.*, **58**, 145–151 (1984) (in Japanese).
- 10) J. E. Hose and R. J. Stoffel: *Bull. Environ. Contam. Toxicol.*, **25**, 929–935 (1980).
- 11) APHA-AWWA-WPCF: Standard Methods for the Examination of Water and Waste water, 15th ed., APHA, Washington D. C., 1980, pp. 615–743.