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Acute toxicity of DMAH to *Oryzias latipes* as modified by some influential factors*

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Abstract——As a new insecticide, less information about the aquatic biological toxicity of DMAH [N-(2,4-dimethylphenyl)-N'- methylamidine hydrochloride] is known. Acute 96h static bioassays were conducted with *Oryzias latipes* to assess the toxicity of DMAH and the influence of environmental factors. The results indicated that, at 23℃, the 24h, 48h and 96h LC_{50} are 14.6 mg/L, 13.06 mg/L and 12.39 mg/L, respectively, the safe concentration (SC) of DMAH to *Oryzias latipes* is 1.16—1.32 mg/L. The changes of temperature, pH value and hardness of water will significantly influence the toxicity of DMAH to fish.

Keywords: DMAH [N-(2,4-dimethylphenyl)-N'-methylamidine hydrochloride]; *Oryzias latipes*; acute toxicity.

1 Introduction

DMAH [N-(2,4-dimethylphenyl)—N'—methylamidine hydrochloride] is a new type of organic nitrogen insecticide, which was invented by Chinese scientist in the middle of 1980s. It can prevent pest insect in the orange, apple and cotton field effectively (Deng, 1991; Zhao, 1991). Some papers about its toxicity to mammal have been reported (Wang, 1992a; 1982; 1992b; Huang, 1989). Up to the present, less research work related to its aquatic biological toxicity has been carried out (Gong, 1994).

The wastewater containing insecticide drainaged into the natural waters constitutes a great threat to aquatic organisms and even the whole aquatic ecosystem (Zhuang, 1982). For elucidating the influence of the insecticide in wastewater on natural waters, more detailed information about its aquatic toxicity should be gotten. The toxicity research also provides the scientific base for the safe discharging of wastewater.

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Oryzias latipes is a small fish with the wide distribution in China. It is an general material for aquatic toxicity test with the advantage of short mature period, high breeding rate as well as domesticating easily (Huang, 1988).

In this article, we pay attention to the acute toxicity of DMAH to *Oryzias latipes* and the influence of environmental factors, in order to provide more information for drawing up the discharge standard of the DMAH contained in wastewater.

2 Materials and methods

The healthy, about the same size, 1-year fishes used for the toxicity test were offered by Aquatic Ecological Lab., Institute of Zoology, CAS. During the indoor 7d domesticating with the 48h aerated tap water, fishes were feed with the fresh bait (Chironomids larvae).

The 250 mg/L mother solution of DMAH for test were made up from the pure N'-(2,4-dimethylphenyl)-N-methylformamidine (DMA) by dissolving it into hydrochloric acid.

Tetradic 96—h static bioassays (control and five concentrations) with *Oryzias latipes* were conducted under acute toxicity test standard (Zhou, 1989). Each 17.5 cm-diameter glass container with 5 fishes was made up to 1.5 liter by blending concentrated solution with distilled water to gain the desired test concentration.

The pH of the test dilution water was adjusted in a large mixing vat and maintained at the desired level in the test vessels by the addition of 50% H_2SO_4 or 10 mol/L NaOH. Adding $CaCO_3$ was the method for changing the hardness in the test solution. Each of the test vessels was aerated by miniature air pump, dissolved oxygen levels were kept to be more than 80% of the saturation throughout the entire test period.

The pH was measured by using a Cole-Parmer pH meter. Dissolved oxygen and temperature were measured by using an O_2/temperature YSI Model 58 meter. Hardness determinations were conducted by EDTA titration method.

The whole bioassay was carried out under the natural light condition. Test solution was exchanged daily. Test fishes were randomly chosen from the domesticated ones. Fishes were not fed 24 hours prior to the start of the test, nor were they fed during the test.

3 Results and conclusions

3.1 The acute toxicity of DMAH to *Oryzias latipes*

All experimental conditions were kept in constant, the water temperature was 23℃. The test concentrations were 0, 4.5, 9, 18 and 36 mg/L. Calculations of the 24h, 48h and 96h LC_{50} and their 95% confidence intervals were determined using the probit regression of Finney (Finney, 1971). The results are shown in Table 1 and Fig. 1.

Table 1 LC_{50} of *Oryzias latipes* exposed to DMAH

Fish		Water			LC_{50}, mg/L		
Length, cm	Weight, g	Temperature, ℃	Hardness, $mgCaCO_3/L$	pH	24h	48h	96h
3.02±0.20	0.22±0.06	23	266—268	7.66—7.78	14.61±0.29 (15.70—3.61)	13.06±0.17 (13.91—12.28)	12.39±0.62 (13.22—0.61)

* The data in the parenthesis is the 95% confidence limit.

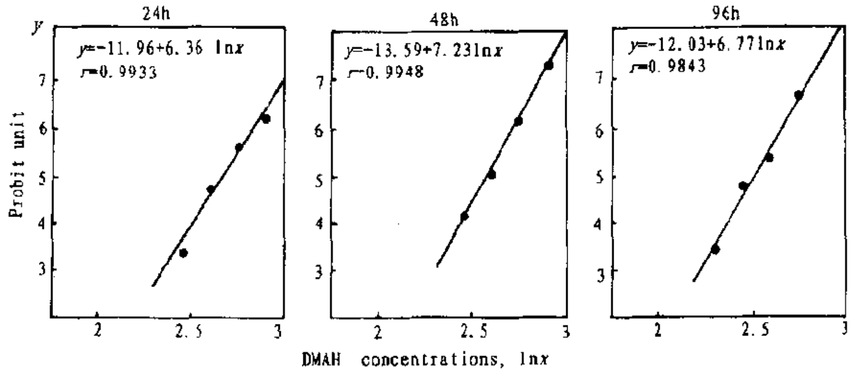

Fig. 1 Acute mortality of *Oryzias latipes* exposed to DMAH

The chi square test was carried out to check regression results. The X^2 value were 0.29 (24h), 0.36 (48h) and 0.94 (96h) respectively, all less than $X^2_{0.05}$(5.99). Regressions met the statistical requirement.

U.S. Environmental Protection Agency (EPA) proposed the 0.0001—0.1 range of application factor of insecticide in 1972. As an unstable insecticide, the 1.16—1.32 mg/L safe concentration (SC) of DMAH could be gotten by the 0.1 application factor.

3.2 Influence of environmental conditions on the toxicity of DMAH

3.2.1 Temperature

Based on the results of preparatory test, the test concentrations were designed as 0, 9, 18, 36, 54 mg/L (Table 2, Fig. 2).

Table 2 Influence on the acute toxicity of DMAH by reducing water temperature

Fish		Water			LC_{50}, mg/L		
Length, cm	Weight, g	Temperature, ℃	Hardness, $mgCaCO_3/L$	pH	24h	48h	96h
3.04±0.28	0.21±0.08	15	263—266	7.65—7.70	26.37±0.14 (30.10—23.11)	24.54±2.00 (28.68—21.13)	18.60±0.71 (22.50—15.57)

The results of chi-square test for 4 sets data were less than $X^2_{0.05}$, regressions met the statistical requirement.

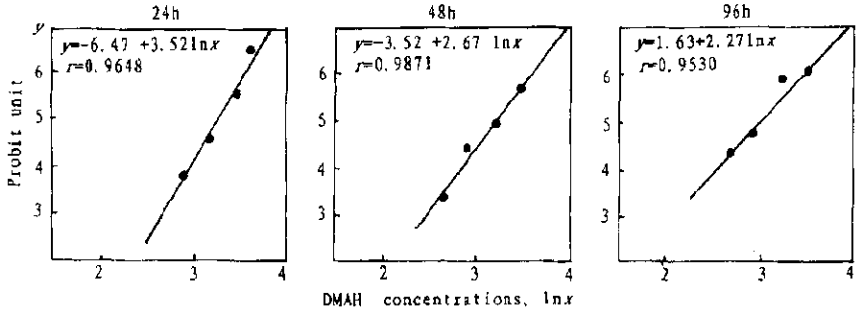

Fig. 2 Mortality of *Oryzias latipes* exposed to DMAH while reducing water temperature

3.2.2 pH

3.2.2.1 Increase of pH value

The test concentrations were 0, 4, 5, 9, 18, 36 mg/L (Table 3).

Table 3 Influence on the acute toxicity of DMAH by increasing pH value

Fish		Water			LC_{50}, mg/L		
Length, cm	Weight, g	Temperature, ℃	Hardness, $mgCaCO_3/L$	pH	24h	48h	96h
3.10±0.23	0.24±0.09	15	266−267	9.10 9.20	14.42±0.65 (16.23±12.81)	13.54±0.41 (14.90±12.30)	11.75±0.31 (13.60±10.18)

* The data in the parenthesis is 95% confidence limit

The chi-square test was carried out to check the experimental results, the X^2 value of 4 sets data were less than $X^2_{0.05}$. Regressions met statistical requirement.

3.2.2.2 Decrease of pH value

The pH value was reduced to be about 6.10 . In the first 24 hour, no dead fish was found in the maximum concentration 36 mg/L. The average 48h mortality in 36 mg/L was 10% as well as 25±7.1% for the average 96h mortality.

3.3 Hardness

The test concentrations were 0, 9, 18, 36, 54 mg/L.

3.3.1 Increase of water hardness

The X^2 values were less than $X^2_{0.05}$, regressions met the statistical requirement (Table 4).

Table 4 Influence on the acute toxicity of DMAH by increasing water hardness

Fish		Water			LC_{50}, mg/L		
Length, cm	Weight, g	Temperature, ℃	Hardness, $mgCaCO_3/L$	pH	24h	48h	96h
3.00±0.28	0.22±0.07	19	271−273	7.76−7.88	21.05±0.89 (24.11−18.43)	20.23±0.28 (22.56−18.15)	16.32±0.81 (18.82−14.20)

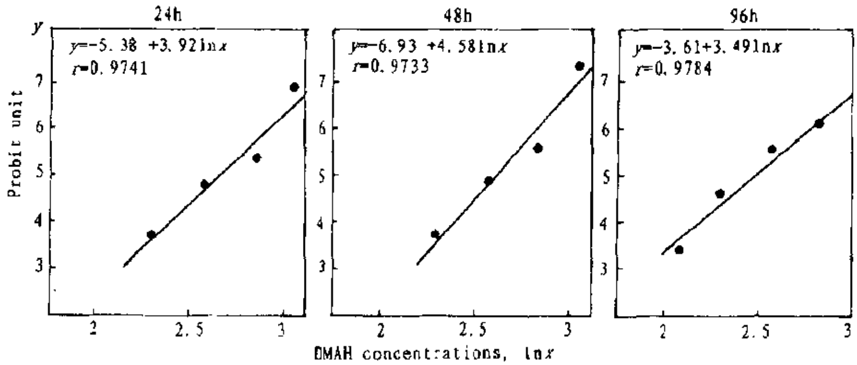

Fig. 3 Mortality of *Oryzias latipes* exposed to DMAH while increasing pH value

3.3.2 Decrease of water hardness

All X^2 values were less than $X^2_{0.05}$, regressions met the statistical requirement (Table 5; Fig. 4; Fig. 5).

Table 5 Influence on the acute toxicity of DMAH by reducing water hardness

Fish		Water			LC_{50}, mg/L		
Length, cm	Weight, g	Temperature, ℃	Hardness, $mgCaCO_3$/L	pH	24h	48h	96h
3.10±0.15	0.23±0.06	19	133—136	7.60—7.68	30.63±0.94 (37.37—25.26)	29.76±0.37 (37.93—23.69)	27.77±1.65 (38.94—20.66)

* The data in the parenthesis is 95% confidence limit.

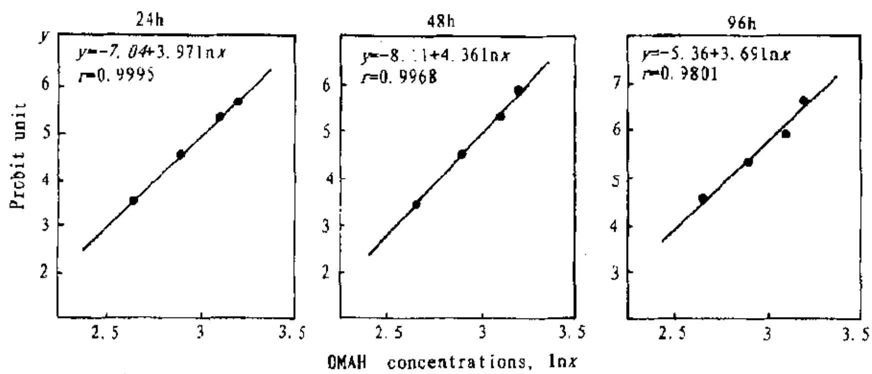

Fig. 4 Mortality of *Oryzias latipes* exposed to DMAH while increasing water hardness

Based on the results obtained, we can find that the temperature, pH value and harness of water can produce the great influence on the acute toxicity of DMAH, the increase of these parameters can promote their toxicity to *Oryzias latipes*.

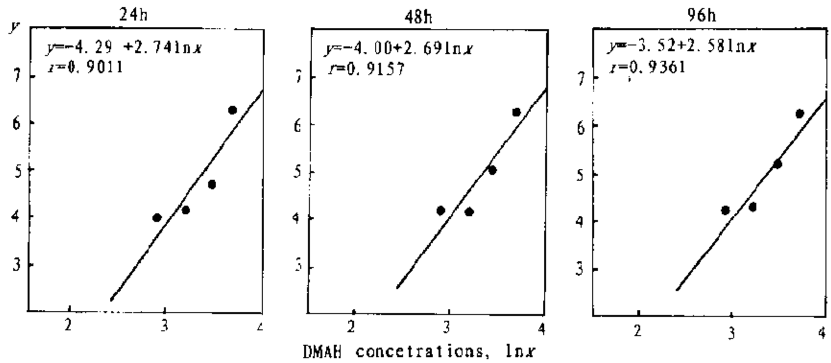

Fig. 5 Mortality of *Oryzias latipes* exposed to DMAH while reducing water hardness

4 Discussion

DMAH belongs to the amidine compounds, its toxicity is much higher than other similar chemicals for inhibiting the activity of monoamine oxide enzyme in animal body. Its LC_{50}s for large- and small-sized rats are 215 and 145 mg/kg (Wang, 1992). Comparing with this bioassay, we can find that the toxicity of DMAH to aquatic organisms is more serious than those to mammals, it is reasonable that using the toxicity reaction of aquatic organisms to DMAH as the bio-monitoring indicator in the production and application process.

Temperature is an important influencing factor to the aquatic toxicity of insecticide, it not only influence the behavior and metabolism of aquatic organisms, but also change the physical and chemical properties of insecticide. For the most insecticide, along with the increase of temperature, their toxicity becomes more serious (Mason, 1981). Iyatomi *et al.* (Iyatomi, 1958) found the endrin was several hundred times as toxic to carp (*Cyprinus carpio*) at 27℃ than that at 7℃ (Iyatomi, 1958). Cope substantiated the positive effects of increased temperature on the toxicity of pesticides (e. g., endrin, lindane, dieldrin, and aldrin) to bluegills (Cope, 1965). In this bioassay, while temperature was increased from 15℃ to 23℃, the 24h LC_{50} of DMAH to *Oryzias latipes* reduced from 26. 7 mg/L to 14. 61 mg/L accompanied by the decrease of 96h LC_{50}, from 18. 60 mg/L to 12. 39 mg/L, the toxicity of DMAH increased evidently. The increase of water temperature can promote the oxygen demand of the fish. Respiration and pesticide intake could be greater at higher than at lower temperature. Meanwhile, the enhance of fish metabolism activity would lead to the decrease of dissolved oxygen concentration and the increase of excreta discharged, all these would aggravate the toxicity of DMAH.

Hardness and pH value can directly influence the toxic effect of chemicals to aquatic organisms. In this test, we found that the increase of pH and hardness in water could enhance the acute toxicity of DMAH, on the contrary, the decrease of pH and hardness could reduce the toxicity of DMAH. Henderson *et al.* (Henderson, 1960) confirmed that the 96h LC_{50} of trichlorphon to fathead minnows in hard water (400 mg/L as calcium carbonate) was less toxicity than one-third of that in soft water (20 mg/L as calcium carbonate), he thought it was due to a rapid breakdown of the pesticide to more toxic degradation products at the higher pH. An activity half-life for antimycin was determined by changing the toxicity of antimycin solutions of pH ranging 6 to 10 (Marking, 1972). The half-life decreased from 310 at pH 6 to 1.5 at pH 10. It indicated that pH can influence the rate of degradation and the toxicity of a chemical. The decomposition product of DMAH is 4-amino-methyl benzoic acid (Wang, 1992). While pH increased from 7.5 to 9.0, the color of test solution became milky white, fishes were immobiled within short time, which was probably related to the decomposition product of DMAH under the alkalinity condition.

In the whole experiment, the increase of hardness would lead to the increase of pH value, and the reverse was also true. The relationship among pH, alkalinity, and hardness was well documented for the piscicide antimycin. Based on many basic studies conducted in the laboratory, followed by field studies, it appeared that the influence of hardness on the toxicity of pesticides to aquatic organisms followed the changes of pH value (Rand, 1985). The results of this toxicity test hold same views with Amend, who thought calcium and magnesium ions do not themselves affect the toxicity of pesticides unless accompanied by pH and alkalinity changes (Amend, 1974).

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