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Chronic Toxicity of Acrylonitrile and Acetonitrile to *Daphnia magna* in 14-d and 21-d Toxicity Tests

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Daphnia magna 21-d survival and reproduction test has been used as the standard method to evaluate chronic toxicity of pollutants and waste water to aquatic invertebrate (OECD 1986 ; U.S.EPA 1973 ; ASTM 1981). The requirement of a 21-d exposure period makes this a costly test. In addition, numerous laboratories have reported problems with the lack of consistent and sustained reproduction as well as inadequate survival over the 21-d period. These realities oftendetract from the overall credibility of daphnia test data and the utility of the test species (Adams and Heidolph 1985).

Adams and Heidolph (1985) compared the *Daphnia magna* 21-d and 14-d survival and reproduction test methods with 30 tests of 21-d chronic toxicity and 18 tests of 48-hr acute toxicity. The results showed that the MATC (Maximum Allowable Toxicant Concentration) after 21-d exposure might be exactly estimated with the reproduction and survival data of 14-d tests. So they suggested that the standard protocols for *D.magna* chronic test be changed to a requirement of only a 14-d period of exposure instead of 21-d. This proposal was supported by the test results of other researchers (Gersich 1990). This study performed *Daphnia magna* 21-d and 14-d survival and reproduction tests of two pollutants, acrylonitrile and acetonitrile, which were the important pollutants in the wastewater from the petro-chemical industry. Acrylonitrile is the priority pollutant in many countries including the United States, China and Germany. The sensitivity of the two methods was compared according to the results. This paper also discussed Adams and Heidolph's suggestion about using 14-d test method instead of 21-d test method. The purpose of this study is to evaluate the feasibility of *D.magna* 14-d test in the chronic toxicity assay for invertebrate animals.

MATERIALS AND METHODS

Daphnia magna used in this paper was provided by the Aquatic Organism Institute, Chinese National Academy of Science. Daphnia less than 24 hr of age were used as test organisms. All test organisms were cultured in our laboratory with *Selenastrum*

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capricornutum as food. Acrylonitrile (>99% purity) was bought from No.3 Shanghai Chemical Reagent Plant, while acetonitrile (>99% purity) was obtained from Shanghai Chemical Reagent Trade Center.

The dilution water was tap water dechlorinated by active carbon.

Table.1 The quality of dilution water

Hardness	1.86±0.08 mg ($CaCO_3$)/L
COD	1.423±0.321 mg/L
DO	7.5-8.0 mg/L
pH	6.5-7.5

Two static acute toxicity tests were conducted according to the requirements of OECD Guidelines for Testing Chemicals (OECD 1987). The tests were conducted without food in the dilution water and the chemical concentrations were not measured. The test solution was changed once every day. Test temperature was 24±l°C. Mortality was measured at 48 hr and 48-hrLC_{50}s were calculated. The parameters of water quality were measured at the beginning and the end of each test by the standard methods (APHA-AWWA-WPCP 1985).

Two chronic renewal tests were performed following OECD Guidelines for Testing Chemicals (OECD 1987). All tests were one generation, 21 days long, and were started with daphnia less than 24 hr of age. There were four treatment groups and one control group in each test, four breakers per group and three daphnia per 150 mL breaker. Each breaker had 100 mL test solution. The test solution was renewed once per day. The photoperiod was 16 hr light and 8 hr dark, and the light density was 2000 Lux. The test temperature was the same as that in acute tests. The tested concentrations of the two chemicals were showed in Table 2. The daphnia were feed with *Selenastrum capricornutum* at the concentration of 5.0-6.0 x 10^5 cell/mL. Survival and reproduction were measured every day.

The 14-d chronic renewal toxicity tests were the same as 21-d chronic tests except for the test period.

For the acute tests, the probit method was used to calculate LC_{50}(Hamiton et al. 1977). For the chronic tests, the reproduction data did not satisfy the normal distribution hypothesis, and every group had four breakers. So Steel's Many-One Rank test (U.S.EPA 1985a) was used to calculate the NOEC (No Observable Effect Concentration), LOEC (Lowest Observable Effect Concentration), and ACR (Acute Chronic Ratio) for reproduction. Fisher's Exact (U.S.&PA 1985a) method was used to arrive at NOEC, LOEC and ACR for survival. ACR is defined as the ratio of 48-hrLC_{50} and ChV (Chronic Value), while ChV is the geometric mean value of NOEC and LOEC.

RESULTS AND DISCUSSION

The acute and chronic test data of the two chemicals are presented in Tables 2 and 3. In this study, the deviation of pH was less 0.3, and the concentrations of dissolved oxygen in the test solution were higher than 5 mg/L. So pH and DC satisfied the requirerents of OECD about pH and DO (1987). The survival percentages of control groups in acrylonitrile and acetonitrile tests were 100% and 92% respectively at the end of tests. At the test temperature, female daphnia should have 5 broods during 21 days, and the mean numbers of young per female daphnia in control groups were 231 and 244 respectively in acrylonitrile and acetonitrile tests. So the mean numbers of young per female daphnia during one brood were 46.2 and 48.6. Thus the survival and reproduction results were both in accordance with the requirements of OECD (1987).

Renewal method might be used in daphnia chronic toxicity tests according to the literature of U.S.EPA (1985b). The results of Paul's study also indicated that the endpoints of flow-through tests were not more sensitive than these of renewal tests (Paul 1987). In the acetonitrile and acrylonitrile tests of this study, the survival percentages of control groups were 92% and 100% respectively, and the mean numbers of young per female daphnia during one brood were 48.8 and 46.2. These data showed that survival and reproduction in renewal tests with enough feed and under other proper conditions would not be worse than these of flow-through tests. As long as the contaminant concentrations were stable in renewal tests, the satisfying and reliable results might be gained through renewal tests.

Adams and Heidolph (1985) conducted 30 21-d daphnia chronic toxicity tests and 18 acute toxicity tests to compare 13 endpoints according to the MATCs calculated on the basis of the test data. The results indicated that there was only minor difference between 14-d and 21-d MATCs. Therefore they recommended that standard method for daphnia chronic test be changed to a 14-d period of exposure instead of 21-d without significant loss of accuracy. Gersich (1990) conducted daphnia chronic toxicity tests with three pollutants, including boric acid, aniline and 2,4-dichlorophenol, for the same purpose as that of Adams and Heidolph (1985). Their results showed that the ChVs of 14-d and 21-d tests were very similar. ChVs Ratios of 14-d and 21-d tests were less than 2 for all the three pollutants. The chronic values of 2,4-dichlorophenol gained from the two test methods were virtually identical. So they supported the use of the 14-d exposure period instead of 21 days to evaluate the chronic toxicity of pollutants to aquatic invertebrate animals.

For the two pollutants tested in this study, the sensitivity of reproduction and survival in 14-d test was the same as that in 21-day tests. So the authors concluded that the 14-d test might be used instead of 21-d test except that 21-d chronic toxicity test is specially demanded.

Table 2. Survival and reproduction of *D. magna* during 14-d and 21-d chronic tests .

chemicals	conc.	surv. (%)		reproduction*	
	mg/L	21-d	14-d	21-d	14-d
Acetonitrile	control	92	92	244	148
	80	92	92	234	151
	160	100	100	218	140
	320	92	100	208	129
	640	67	83	199	119
Acrylonitrile	control	100	100	231	140
	0.25	83	92	230	131
	0.50	100	100	210	133
	1.0	92	100	209	130
	2.0	75	92	205	126

* The number of neonates per female.

Table 3. NOEC,LOEC,ChV and ACR of four pollutants in the 21-d tests and 14-d tests.

pollutants		Acrylonitrile#		Acetonitrile#	
		14-d	21-d	14-d	21-d
48-hrLC_{50}		10		$3.6x10^3$	
Repro-duction	NOEC*	0.5		$1.6x10^2$	
	LOEC	1.0		$3.2x10^2$	
	ChV	0.71		$2.3x10^2$	
	ACR	16		16	
Survival	NOEC	2.0		$6.4x10^2$	
	LOEC	> 4.0		> $13x10^2$	
	ChV	> 2.8		> $9x10^2$	
	ACR	< 3.6		< 4.0	

*.The unit of NOEC,LOEC,ChV is mg/L. ACR has no unit.
#.The results of 14-d and 21-d tests were identical.

Compared with 21-d test, 14-d test has significant advantages in the following respects: 1)the test period is shortened to 14 days without compromising its scientific integrity and reducing its sensitivity; 2) there is adequate survival at the end of 14-d test while some reports have shown that there was not enough survival

at the end of 21-d texts (Adams and Heidolph 1985). In this study, however, the survival percentages of control groups at the end of 14-d tests were the same as those in 21-d tests.

The relative sensitivity of growth and survival in *D. magna* 21-d test depends on different pollutants, but reproduction is the most sensitive among the three parameters in most tests (Gersich 1985). The results of 14-d tests (Adams and Heidolph 1985) were similar with those of 21-d tests mentioned above. In this study the sensitivity of survival and reproduction was emphasised while growth was not measured. The results indicted that reproduction was more sensitive than survival in both 14-d and 21-d tests for two pollutants. The ChVs of reproduction in acrylonitrile and acetonitrile tests were a quarter of those of survival.

REFERENCES

Adams WJ, Heidolph BB (1985) Short-cut chronic toxicity estimates using *Daphnia magna.* In: Cardwell RD, Purdy R,and Bahner Rc (ed) Aquatic Toxicology and Hazard Assessment. Seventh symposium, ASTM STP 854, ASTM, Philadelphia, pp 87-103

APHA-AWWA-WPCF (1985) Standardmethods for the examination of water and wastewater. 16th ed., American Public Health Association, Washington, DC, U.S.A.

ASTM Task Group on Daphnias (1981) Draft proposed standard practice for conducting *Daphnia magna* chronic toxicity tests in a flow-through system. Draft No.3, ASTM Subcommittee E47.01 on Aquatic Toxicology, American Society for Testing &Materials, Philadephia.

Gersich FM (1990) Evaluation of 14-day static renewal toxicity test *with Daphnia* magna strauss. Arch Environ Contam Toxicol 19:72-76

Gersich FM (1985) The sensitivity of chronic endpoints used in *Daphnia magna* life cycle tests. In: Bahner RC and Hansen DJ (ed) Aquatic Toxicology and Hazard Assessment. 8th symposium, ASTM STP 891, ASTM, Philadephia, pp 245-252

OECD (1986) OECD Guidelines for Testing Chemicals.

Paul CF (1987) Chronic toxicity of 4-Nitrophenol to *Daphnia magna* strauss under static-renewal and flow-through conditions. Bull Environ Contam Toxicol 36:730-737.

Hamiton MA, Russo RC, Thurston RV (1977) Trimmed Sepearman-Karver method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11(7):714-719

U.S.EPA (1973) Water quality criteria 1972. National Academy of Sciences, U.S.EPA, Research series, Washington, DC

U.S.EPA (1985a) Short-term method for estimating the chronic toxicity of effluent and receiving waters to freshwater organisms. 2nd ed., EPA600/4-85/014, U.S.EPA, Washington, DC

U.S.EPA (1985b) Guidelines for deriving numerical national water quality criteria for the protection of aquatic organism and their uses. National Technical Information Service, Accession Number PB85-227040. U.S.EPA, Washington, DC