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Original Article

Effect of Potassium Dichromate on the Survival and Reproduction of *Daphnia magna*

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ABSTRACT

An attempt was made to evaluate chronic effect of Potassium dichromate on Daphnia magna, semi-statically. Based on the results of an acute immobilisation study (48 hour EC50), the test item concentrations of 0.03, 0.05, 0.1, 0.2 and 0.4 mg/L of Potassium dichromate was selected for the chronic investigation. During the study, physico-chemical parameters of M4 medium were recorded and maintained within the range as stated in the OECD guideline no.211 and the validity criteria were met during the study. Based on the results, the EC50 of Potassium dichromate (based on % inhibition of reproduction) was determined as 0.19 mg/L with 95% confidence limits of 0.16 - 0.22 mg/L. The NOEC and LOEC were determined as 0.05 and 0.1 mg/L. Based on number of offspring reproduced, the NOEC and LOEC were determined as 0.03 and 0.05 mg/L, respectively. Based on survival of parental animal, the EC50 of Potassium dichromate was determined as 0.20 mg/L with 95% confidence limits of 0.15 - 0.24 mg/L. The NOEC and LOEC were determined as 0.1 and 0.2 mg/L, respectively. There is no published data available in the public domain for chronic effect of Potassium dichromate to Daphnia magna. The data on Potassium dichromate is an important prerequisite for test laboratories because it is used as reference test item for acute and reproduction studies to validate the test. Hence, an attempt was made to generate data for the Potassium dichromate under laboratory conditions.

Keywords: *Daphnia magna, Potassium dichromate, Reproduction test.*

INTRODUCTION

Daphnia magna is commonly used in aquatic toxicity studies for the risk assessment of different chemicals because of its suitability for laboratory testing such as relatively small, short life cycle, parthenogenetic reproduction, high fecundity, ubiquitous occurrence and ease of laboratory handling [1, 2]. In acute tests, the measured parameter is mortality, while in chronic test, the inhibition of normal reproduction, survival of parent animal and growth are the most important end points for toxicity evaluation. The study methods are well defined in many international guidelines and being adopted by laboratories which perform these studies. *Daphnia magna* 21 days survival and reproduction test has been used as the standard method to evaluate chronic toxicity of pollutants and waste water to aquatic invertebrate [3, 4]. Many laboratories are performing acute toxicity study than the reproduction test using *Daphnia magna*. The data on Potassium dichromate is an important prerequisite for test laboratories because it is used as reference test item for acute and reproduction studies to validate the test.

MATERIALS AND METHODS

Daphnia magna primary culture was obtained from Marinco Bioassay Aqua Culture Lab, United States. The culture used in this study was maintained in the test facility by periodical culturing. *D. magna* was cultured individually in 100 mL glass beaker containing 50 mL of aerated M4 medium (**Table 1**) and fed with 3.4 – 3.9 × 10^7 cells/mL concentrated algal solution of *Pseudokirchneriella subcapitata* (Strain SAG 61.81) [5]. The medium was changed thrice in a week and the medium was also checked for its physico-

chemical parameters such as pH, temperature, dissolved oxygen and hardness [6]. Potassium dichromate ($Cr_2K_2O_7$) used in this study was procured from ACROS ORGANICS 2440 Geel, Belgium.
Separate stock solution of individual trace elements, macronutrients and vitamins were prepared in deionised water. The required volume of M4 medium was prepared prior to experiment by adding the trace elements and macro nutrient stock solutions to deionised water and kept in continuous aeration. Vitamins were added shortly before use of M4 medium.

Table 1: M4 Medium Nutrients

M4 Medium Nutrients			
Stock solutions	**Chemical Name**	**Formula**	**mg/l**
Trace elements			
1	Boric acid	H_3BO_3	57190
2	Manganese chloride	$MnCl_2.4H_2O$	7210
3	Lithium chloride	LiCl	6120
4	Rubidium chloride	RbCl	1420
5	Strontium chloride	$SrCl_2.6H_2O$	3040
6	Sodium bromide	NaBr	320
7	Sodium molybdate	$Na_2MoO_4.2H_2O$	1230
8	Cupric chloride	$CuCl_2.2H_2O$	335
9	Zinc chloride	$ZnCl_2$	260
10	Cobalt chloride	$CoCl_2.6H_2O$	200
11	Potassium iodide	KI	65
12	Sodium selinite	Na_2SeO_3	43.8
13	Ammonium vanadate	NH_4VO_3	11.5
14	EDTA*	$Na_2EDTA.2H_2O$	5000
15	Ferrous sulphate*	$FeSO_4.7H_2O$	1991
Macro nutrients			
16	Calcium chloride	$CaCl_2.H_2O$	293800
17	Magnesium sulphate	$MgSO_4.7H_2O$	246600
18	Potassium chloride	KCl	58000
19	Sodium hydrogen carbonate	$NaHCO_3$	64800
20	Sodium silicate	$Na_2SiO_3.9H_2O$	50000
21	Sodium nitrate	$NaNO_3$	2740
22	Potassium phosphate monobasic	KH_2PO_4	1430
23	Potassium phosphate dibasic	K_2HPO_4	1840
Vitamin stock solutions			
24	Thiamine hydrochloride	--------	750
25	Cyanocobalamine (B_{12})	--------	10
26	Biotine	--------	7.5

* Both EDTA and Ferrous sulphate solution were prepared separately, poured together and autoclaved.

An acute immobilisation test was conducted to determine 48 hours EC50 [7] of Potassium dichromate. Based on our earlier experience, the study was performed with nominal concentrations of 0.43, 0.52,

0.62, 0.74, 0.89 and 1.07 mg/L (Factor 1.2). In each test item concentration four replicates were maintained (100 mL capacity glass beakers) and each containing 5 neonates (<24 hours old) in 50 mL of M4 medium. The beakers were covered with glass plate to prevent the evaporation of test medium. The test was performed at 18-22^{o}C with a photoperiod of 16 hours light and 8 hours dark. During the experiment, neonates were not fed. In each test beaker, the immobilised neonates were recorded at 24 hours and 48 hours of exposure. The percent immbolisation was calculated as follows,

Percent immbolisation = (No. of daphnids immobilised/ No. of daphnids exposed) ×100

Based on the results of acute test, *D. magna* were exposed to Potassium dichromate at the concentrations of 0.03, 0.05, 0.1, 0.2 and 0.4 mg/L semi-statistically for a period of 21 days and the study was performed in accordance with OECD guidelines No.: 211. Neonates (offspring) used in this study were less than 24 hours old. The test item stock solution was prepared by dissolving 5 mg of Potassium dichromate in 50 mL of M4 medium and the required test item concentrations were prepared by diluting with the M4 medium. Ten offspring individually were exposed to each test item concentration and control. During the study period, 50 mL of test solution was exposed to each test beaker and the test medium was renewed three times in a week. Daphnids were fed with algal solution thrice in a week and the total organic carbon content of algal feed was in the range of 0.1 - 0.2 mg C/*Daphnia*/day. The number of live offspring produced by each parent *Daphnia* in all test item concentrations was removed and counted daily and mortality of the Daphnids was recorded throughout the study period.

During the study period, physico-chemical parameters such as pH, dissolved oxygen, temperature and hardness were determined once in a week in fresh and old media and all the parameters were found within the normal range as stated in the OECD guideline No.: 211.

A photoperiod of 16 hours light and 8 hours dark was maintained in the test room with an illuminance of 600 lux light intensity using fluorescent lamps and temperature in the range of 18-22°C was also maintained during the study period.

The percent inhibition of reproduction for each test item concentration was calculated as follows,

Percent inhibition = (C – T)/C×100

Where, C = Mean No. of offspring in the control group

T = Mean No. of offspring in the treatment group

Microsoft Excel 2003 was used to calculate % immobilisation data for acute toxicity study and % inhibition of the reproduced offspring for chronic toxicity study. All the statistical analysis was calculated using a software TOXSTAT 3.5 version [8]. The EC50 (Effective concentration) value indicates 50% reduction in the reproductive output of *D. magna*. Estimation of the EC50 values with 95% confidence limits, NOEC (No observed effect concentration) and LOEC (Lowest observed effect concentration) for acute toxicity study and % inhibition of reproduction, EC50 values with 95% confidence limits for survival of parent animal were calculated by probit analysis. NOEC and LOEC values for survival of parent animal were determined using Fisher's exact test. NOEC and LOEC values for mean number of offspring reproduced by parent animal were determined by Bonferroni t test. In all the statistical analysis the significant level was 0.05.

RESULTS AND DISCUSSION

In acute immobilisation test, 100% immobilisation was recorded in the concentrations of 0.89 and 1.07 mg/L. The concentration 0.43 mg/L did not affect the mobility of the *D. magna*. The EC50 (48 h) of Potassium dichromate was determined as 0.64 mg/L with 95% confidence limits of 0.61- 0.68 mg/L. The NOEC and LOEC were determined as 0.52 and 0.62 mg/L (**Table 4**).

Mean number of live offspring produced by *Daphnia* in each concentration and control were represented in **Table 2**. The data showed that there was a decline in the number of live offspring in the test item concentrations from 0.03 to 0.4 mg/L recorded 80.2 – 23.5 mean number of live offspring. In control, recorded mean number of live offspring was 97.4.

At the end of the study (21 days), all parent *Daphnia* were survived in the test item concentrations of 0.03 and 0.05 mg/L and control. The survival of parent *Daphnia* in 0.1, 0.2 and 0.4 mg/L of test item concentrations were 9, 5 and 2. First offspring were recorded on Day 9 in control, 0.03 and 0.05 mg/L, whereas first offspring were recorded on Day12 in the test item concentrations of 0.1, 0.2 and 0.4 mg/L (**Table 3)**. Highest percent inhibition of reproduction rate (75.87%) was recorded in the test item concentration of 0.4 mg/L while lowest percent inhibition of 17.66% was recorded in 0.03 mg/L (**Figure 1**). Significant effects in reproduction and mortality were found between the test item concentrations and the control.

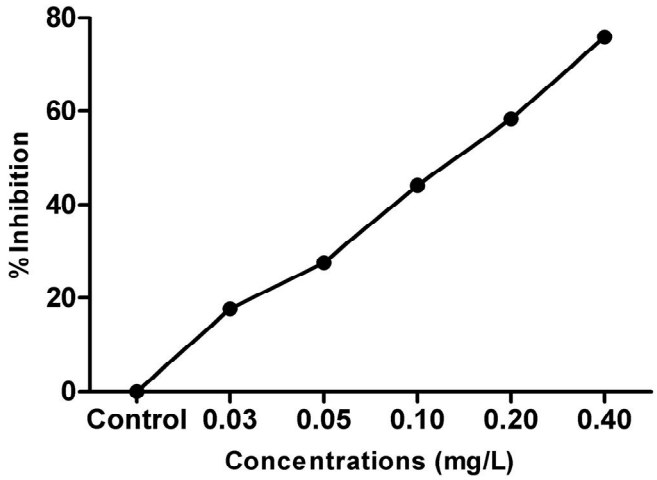

Figure 1 Concentrations vs Percentage Inhibition of *Daphnia magna* exposed to Potassium dichromate
Figure indicates the percent inhibition on the basis of number of offsprings reproduced against concentrations of Potassium dichromate

Based on the present investigation, the EC50 of Potassium dichromate (based on inhibition of reproduction) was determined as 0.19 mg/L with 95% confidence limits of 0.16 to 0.22 mg/L. The NOEC and LOEC were determined as 0.05 and 0.1 mg/L, respectively. Based on number of offspring, the NOEC and LOEC were determined as 0.03 and 0.05 mg/L, respectively. Based on survival of parental animal, the EC50 of Potassium dichromate was determined as 0.20 mg/L with 95% confidence limits of 0.15 to 0.24 mg/L. The NOEC and LOEC were determined as 0.1 and 0.2 mg/L, respectively (**Table 4**).

Table 2: Mean number of offspring reproduced by *Daphnia magna* at the end of 21 days

Conc. (mg/L)	Control	0.03	0.05	0.1	0.2	0.4
R_1	104	17	63	59	-	-
R_2	87	48	58	45	33	28
R_3	103	90	41	53	54	19
R_4	70	103	98	35	-	-
R_5	81	88	78	73	41	-
R_6	115	121	51	46	36	-
R_7	116	97	54	-	-	-
R_8	83	63	112	67	39	-
R_9	96	63	103	47	-	-

R_{10}	119	112	47	65	-	-
Mean ± SD	**97.4 ± 16.8**	**80.2 ± 32.1**	**70.5* ± 25.6**	**54.4* ± 12.4**	**40.6* ± 8.1**	**23.5* ± 6.4**

R – Replicate, '-'indicates mortality of parent daphnid

* Significantly different with control at P<0.05 –Bonferroni t - test

Table 3: Chronic Toxicity Results of Potassium dichromate on *Daphnia magna* at 21 days exposure Period

Concentrations (mg/L)	Survival of parent animal at the end of study	First offspring produced on
Control	10	Day 9
0.03	10	Day 9
0.05	10	Day 9
0.1	9	Day 12
0.2	5*	Day 12
0.4	2*	Day 12

* Significantly different with control at P<0.05 – Fisher's Exact test

Table 4: Data on EC50 (with 95% confidence limits), NOEC and LOEC

Result	Based on acute toxicity test (48 hours)	Based on inhibition of reproduction	Based on survival of parent animal	Based on number of offspring reproduced
EC50	0.64 mg/L	0.19 mg/L	0.20 mg/L	-
95% confidence limits	0.61 to 0.68 mg/L	0.16 to 0.22 mg/L	0.15 to 0.24 mg/L	-
NOEC	0.52 mg/L	0.05 mg/L	0.1 mg/L	0.03 mg/L
LOEC	0.62 mg/L	0.1 mg/L	0.2 mg/L	0.05 mg/L

The results of acute tests for chromium element obtained in this work are similar to corresponding 48-h EC50 reported in the literature by other authors (0.42 mg/L) (0.229 mg/L) (0.29 mg/L) [9 - 11].

The results obtained from chronic tests revealed that Potassium dichromate significantly inhibited reproduction and growth of *D. magna* (**Figure 1**). The effects of reproduction observed in chronic tests with chromium are in good agreement with some authors [11, 12]. According to them, Potassium dichromate concentrations higher than 0.1 mg/L and Sodium dichromate concentrations higher than 0.05 mg/L significantly decrease offspring production and swimming ability of *D. magna*. Diamantino et al. 2000 recorded different EC50 values for chromium on reproduction (0.047 mg/L), mortality (0.524 mg/L), total growth (0.233 mg/L) and NOEC, LOEC values in chronic test. These findings are well correlated with our present findings.

The results of this study indicate degrees in toxicity level of Potassium dichromate to *D. magna* evaluated in acute and chronic tests. Thus, Potassium dichromate seems to be highly toxic. There is no published data available for Potassium dichromate as reference test item of *Daphnia* reproduction study. Hence, an attempt was made to generate data for the Potassium dichromate under laboratory conditions adopting standard guidelines.

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REFERENCES

1. Adema, D.M. (1978). *Daphnia magna* as a test animal in acute and chronic toxicity tests. *Hydrobiologia*., 59:125-134.
2. Meer, C. VAN Der., Teunissen, C. & Boog, T.H F.M. (1988). Toxicity of Sodium, Chromate and 3,4-dichloroaniline to crustaceans. *Bull. Environ. Contam. Toxicol*., 40: 204 – 211.
3. ASTM. (1980). Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. American for the Testing and Materials, Philadelphia.Report. E-790-80.
4. OECD Guideline for Testing of Chemicals (No. 211, Adopted: 3rd October, 2008). *Daphnia magna* Reproduction Test.
5. John, P. CLARE. (2002). Daphnia: An Aquarist guide, Version 3.2.
6. APHA. (1998). Standard methods for the examination of water and waste water. APHA, AWWA, WPCF, Washington 20th Edition.
7. OECD Guideline for Testing of Chemicals (No. 202, Adopted: 13th April, 2004). Daphnia sp.,Acute Immobilisation Test.
8. TOXSTAT 3.5 version, 2003 - WEST, Inc. and Dave Gulley. (1996). Univ. of Wyoming; Central Avenue, Cheyenne, WY82001, USA.
9. Baird, D.J., Barber, I., Bradley, M., Soares, A.M.V.M. & Calow, P. A. (1991).Comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* Straus. Ecotoxicol. Envitron. Saf., 21: 257-265.
10. Guilhermino, L., Diamantino, T.C., Ribeiro, R., Goncalves, F. & Soares, A.M.V.M. (1997). Suitability of test media containing EDTA for the evaluation of acute metal toxicity to *Daphnia magna* Straus. *Ecotoxicol. Envitron. Saf*., 38: 292-295.
11. Diamantino, T.C., Guilhermino, L., Almeida, E. & Soares, A.M.V.M. (2000). Toxicity of sodium molybdate and sodium dichromate to *Daphnia magna* Straus evaluated in acute, chronic and acetylcholinesterase inhibition tests. *Ecotoxicol. Envitron. Saf*., 38: 292-295.
12. Anger, G., Halstenberg, J., Hochgeschwender, K., Uecker, G., Korallus, U., Knopf, H., Schmidt, P. & Ohlinger, M. (1986). Chromium compounds In Ullmann's Encyclopedia of Industrial Chemistry (F. Thomas Campbell, R. Pfefferkorn, and J.F. Rounsaville, Eds,), Editorial Advisory Board, Weisheim Vol.A7, pp.67-97.