Bull. Environ. Contam. Toxicol. (1991) 46:461–465

Bulletin of Environmental Contamination and Toxicology

Influence of Sulfate, Ca, and Mg on the Acute Toxicity of Potassium Dichromate to *Daphnia similis*

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In the Daphnia test which is commonly employed for assessing effects of chemicals to aquatic environments, it is well known that the toxicity of chemicals is variable with test animals (Cowgill 1987; Girling and Garforth 1989) and also with the chemistry of test waters (Nebeker 1982; Berglind and Dave 1984). However, it is not clear what constituents in water affect toxicity of chemicals. We report here experimental results which show that sulfate, calcium and magnesium, commonly contained in aquatic environments, decrease the toxicity of $K_2Cr_2O_7$ which is recommended as a reference toxicant in the acute toxicity test.

MATERIALS AND METHODS

Daphnia similis were collected from a natural pond in Osaka prefecture and were cultured for three years in our laboratory. They were kept in a reconstituted water ($NaHCO_3$ 1.0mM, $CaCl_2.2H_2O$ 1.0mM, $MgSO_4.7H_2O$ 0.3mM, KCl 0.05mM). Stock cultures of daphnids were acclimated to the test temperature ($22 \pm 1^\circ C$) and to the photoperiod (16L : 8D), and were fed with suspensions of a mixed culture of green algae . The alga culture was maintained by A-MS medium (Keating 1985) supplemented with Cr and Ni at 5 ppb. Test waters were prepared from deionized and distilled water to which $NaHCO_3$ (1.0mM), buffering the test waters at pH about 8, and variable amounts of $CaCl_2.2H_2O$, $MgCl_2.6H_2O$ and Na_2SO_4 were added. The test waters were aerated for about 10 min to ensure air saturation and left for another 48 hr to be stabilized in the chamber. All reagents used were analytical grade.

Static acute toxicity tests without aeration, feeding or renewal were done in the chamber above. Neonate Daphnia, 24 ± 4hr old, were used for all toxicity tests. The static tests were conducted in 20-ml glass tubes containing 20 ml of test water and five Daphnia. A 1.25-fold dilution series of six nominal concentrations and a control were used for all tests. A total of 25 Daphnia was exposed to each concentration. Test results were checked with immobility after 24 and 48 hr exposure period. Immobility was defined according to Standard Methods(APHA 1989).

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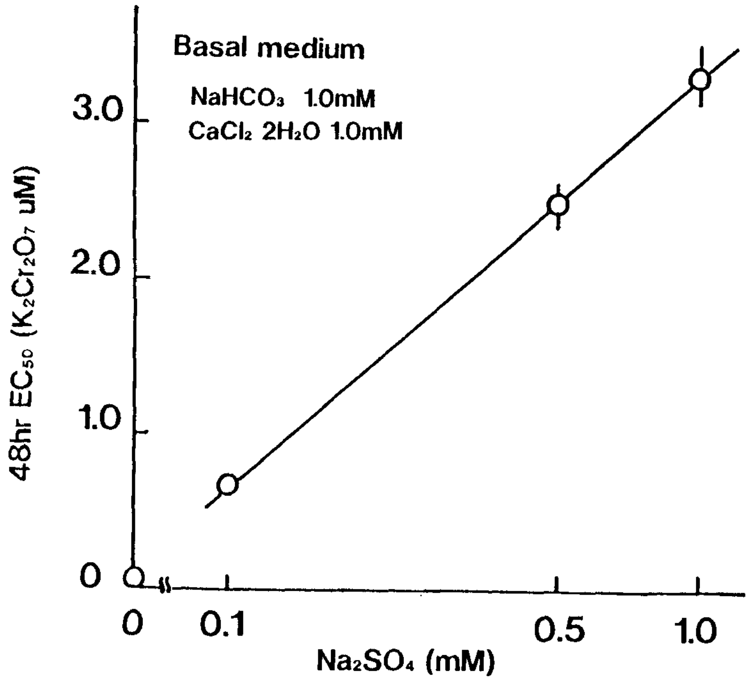

Figure 1. The influence of sulfate on the toxicity of potassium dichromate to _D. similis_.

The number of immobile organisms was recorded after 48 hr. The 48hr EC_{50} values and associated 95% confidence intervals were calculated by the probit procedure.

RESULTS AND DISCUSSION

The influence of sulfate on the toxicity of $K_2Cr_2O_7$ to _D. similis_ was examined using a test water containing 1.0mM $NaHCO_3$ and 1.0mM $CaCl_2.2H_2O$ equivalent to 100mg/L as hardness. Figure 1 shows that the toxicity of $K_2Cr_2O_7$ decreases strongly by the addition of sulfate and their EC_{50} values apparently are logarithmically proportional to the sulfate concentrations in the test waters.

It is widely accepted that the toxicity of chemicals is affected by pH, hardness and alkalinity of test water, but the effects of sulfate on the toxicity of $K_2Cr_2O_7$ to _Daphnia_ have not been reported. Muller(1980) reported that an increase of the total hardness of the test water having a ratio between Ca and Mg of 4 : 1 decreases the toxicity of $K_2Cr_2O_7$ significantly. However, he prepared the test water using $MgSO_4.7H_2O$ as Mg salt. The decrease of toxicity of $K_2Cr_2O_7$ is dependent on hardness, and he did not mention the effects of sulfate. Riedel(1985) found that the uptake of sulfate by an estuarine diatom was inhibited in competition with toxic concentrations of hexavalent chromium or vice versa and proposed the mechanism in which chromate is taken up as a sulfate analogue, but acts irreversibly and non-specifically on

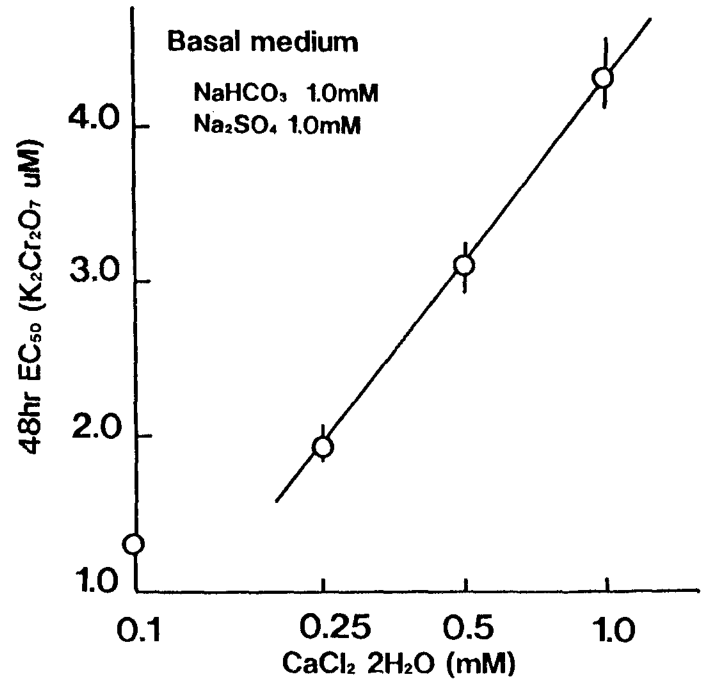

Figure 2. The influence of calcium on the toxicity of potassium dichromate to D. similis.

sulfate uptake site. According to his model, Daphnia may have an active site-to-sulfate transport which is attacked by chromate. Since sulfate occur in aquatic environments as a common constituent and sulfate salts are used as covenient reagents in preparing media, these effects of sulfate have been overlooked. It has been accepted that the biological action of a reagent is competitive with the other chemicals of similar chemical properties(Wood and Wang 1983).

The degree of toxicity of $K_2Cr_2O_7$ in a test water containing 1.0mM $NaHCO_3$ and 1.0mM Na_2SO_4 is dependent on the Ca concentration in a similar manner as described above(Figure 2). Recently, van der Meer et al.(1988) described in their research of toxic effects of sodium chromate and 3,4-dichloroaniline to crustaceans at different salinities that, in contrast to the results obtained with chromate, Palaemonetes varians larvae are equally sensitive to 3,4-dichloroaniline at 33 0/00 and 3.3 0/00. These situations in the toxicity of chromate may have been due to the decrease of sulfate and/or Ca in the low salinity test water. Murphy(1970) suggested that Ca was the only inorganic element essential to the survival of Daphnia pulex in culture media. In our experiments without feeding, D. similis neonates also died within 24 hr without Ca but survived more than 2 days in the medium containing only $CaCl_2.2H_2O$. These results suggest that the toxicity of $K_2Cr_2O_7$ depends on its competitive attack to the Ca binding site

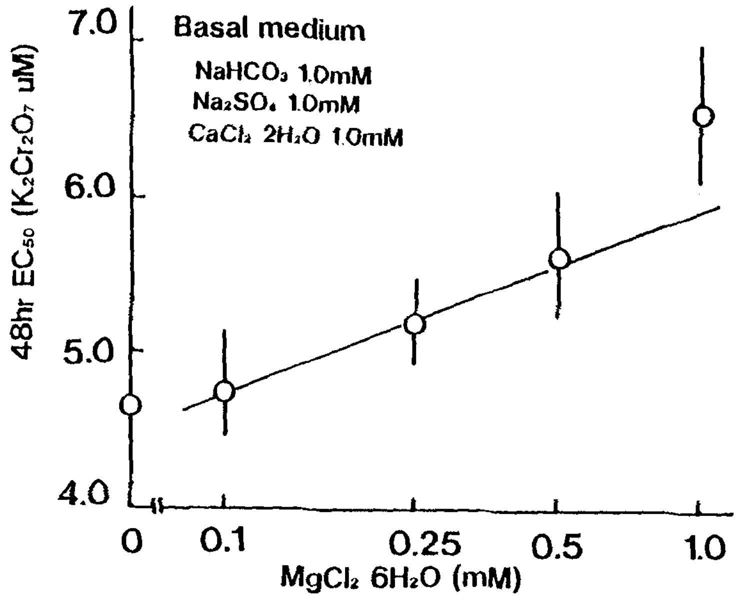

Figure 3. The influence of magnesium on the toxicity of potassium dichromate to D. similis.

in Daphnia, and/or on its combination with Ca such as $CaCrO_4$ or $CaCr_2O_7$. In spite of the need to use Ca in culture media of Daphnia, the effects of Ca on the toxicity of chemicals have not been widely studied. The toxicity of a variety of chemicals is interesting in connection with Ca metabolism in Daphnia.

In Figure 3, the influence of Mg on the toxicity of $K_2Cr_2O_7$ to D. similis is shown, and it is seen that its effect is weak. The test water used contained 1.0mM $NaHCO_3$, 1.0mM Na_2SO_4 and 1.0mM $CaCl_2.2H_2O$.

Although Daphnia do not definitely require Mg in culture media, Mg is an essential element to all organisms, and Ca and Mg appear to influence differently the toxicity of $K_2Cr_2O_7$. We are presently studying these phenomena.

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Received August 9,1990; accepted August 24,1990