Bull. Environ. Contam. Toxicol. (2001) 66:379–385

DOI: 10.1007/s00128-001-0016-z

Bulletin of Environmental Contamination and Toxicology

Acute and Chronic Toxicity of Potassium Chloride (KCl) and Potassium Acetate ($KC_2H_3O_2$) to *Daphnia similis* and *Ceriodaphnia dubia* (Crustacea; Cladocera)

L. R. P. Utz,* M. B. C. Bohrer

Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Brazil

Received: 28 March 2000/Accepted: 18 December 2000

Petroleum exploitation is one of the main human activities generating substances which can potentially contaminate surrounding terrestrial or aquatic environments. The majority of the extraction is performed on land, where, in addition to vegetation damage, the drilling fluid utilized during the perforation can contaminate the aquifer or freshwater bodies near the petroleum well (Soria and Chavarria, 1978). Drilling fluids are complex mixtures of various components which control several aspects of the drilling operation such as cooling the drill during the perforation of wells and preventing the disruption of the surrounding rock layers. During the work, a large amount of the fluid is discharged, and, after use, it is pumped into reservoirs where it is recycled (Price II et al., 1986). The contact with aquatic environments can take place during the perforation or disposing of the fluid and, in the case of freshwater environments, its salinity can damage the ecosystem by killing sensitive species (Gillenwater and Ray, 1989). The salinity of the drilling fluid is due to substances used to inhibit the swelling and dispersion of water-sensitive shale formations (Stansbury, 1986). The most common salt used in drilling fluid for that purpose is Potassium Chloride (KCl) because it is cheap, efficient, and easy to obtain. Despite these advantages, the toxicity of the ion chloride is the main reason for its restricted use (Gillenwater and Ray, 1989). Some studies have been performed in order to find a substitute for KCl with all its advantages, but lower toxicity (Gillenwater and Ray, 1989). Experiments showed that Potassium Acetate ($KC_2H_3O_2$) can efficiently replace Potassium Chloride, with the advantage of not having the restrictions imposed to the ion chloride. However, before the large-scale use of $KC_2H_3O_2$ is recommended, it is important to perform direct studies of its effect on living organisms, which has not been attempted so far.

The present work aims to evaluate the acute and chronic toxicity of Potassium Acetate and Potassium Chloride using *Daphnia similis* and

**Present address:* Department of Biology, University of Maryland, College Park, MD 20742, USA
Correspondence to: L. R. P. Utz

Ceriodaphnia dubia as test organisms. These two freshwater species were chosen because both are standardized for use in the evaluation of toxicity of substances as well as industrial wastewater.

MATERIALS and METHODS

Neonates of *Daphnia similis* (less than 16 h old) used in the acute tests were obtained from females cultured in natural spring water (adjusted to a total hardness of 44 mg/L as $CaCO_3$) at 20°C ±2°C and photoperiod of 16h light and 8h darkness (CETESB, 1992). The females were fed daily with the Chlorophycea algae *Monoraphidium dibowskii* cultured in Oligo medium (CETESB, 1992) at a concentration of 1 X 10^5 cells/mL. For the acute tests, concentrations were obtained from preliminary tests (EPA, 1993) ranging from 0.2 to 5.0 g/L for both potassium salts, with definitive tests concentrations ranging from 0.6 to 3.0 g/L. Tests were carried out in test tubes with 10 mL of final solution maintained for 48h in a test chamber at the same temperature used for the cultures and a photoperiod of 24h darkness. In all concentrations and in the control there were four replicates, each with five organisms. For all tests EC50;48H (Median Effective Concentration) was calculated by the Trimmed Spearman-Karber method (Hamilton et al., 1977).

For the chronic tests, neonates of *Ceriodaphnia dubia* (less than 16 h old) were obtained from females cultured individually. These females were kept in beakers with 30 mL of culture water with the same parameters used for *Daphnia similis*. The culture was maintained at 25°C ± 2°C and fed daily with 1X10^5 cells/mL of the Clorophyceae algae *Monoraphidium dibowiskii* cultured in Oligo medium, and approximately 1 mL of a mixture of ground dried trout and yeast (CETESB, 1992). Test concentrations were obtained from acute preliminary tests (EPA, 1994) with concentrations ranging from 0.1 to 2.5 g/L for both salts. The final concentrations used in the chronic tests ranged from 0.3 to 0.6 g/L for Potassium Acetate and from 0.6 to 1.25 g/L for Potassium Chloride. During the test, the organisms were kept in beakers with 30 mL of test solution. Each concentration and the control had ten replicates with only one organism per beaker. Chronic tests were maintained at the same temperature used for the cultures and a photoperiod of 16-h light and 8-h darkness. The test solution was renewed every day and the organisms were fed and observed daily. Aspects such as growth, fecundity and longevity were observed. Growth parameters were obtained daily by measuring three females of the control and of each concentration in a light microscope. Fecundity was recorded by counting neonates of each female in the control and in the test concentrations. Longevity parameters were assessed by keeping the tests until the death of all organisms had occurred. Growth and fecundity parameters were also observed until the death of all organisms in the control and test concentrations which happened on the 12^{th} day of experiment for both salts. To calculate significant differences in the fecundity, longevity and growth, the Computer Program TOXSTAT 3.3 (Gulley et al., 1991) was used (EPA, 1993).

RESULTS and DISCUSSION

Daphnia similis showed EC50;48H values that ranged from 0.69 g/L to 1.19 g/L in the acute tests with Potassium Chloride (Table 1). In the tests with Potassium Acetate the EC50;48H values ranged from 1.05 g/L to 1.21 g/L (Table 2). Although the acute toxicity range of both salts overlapped, the value observed at the beginning of the range is lower for KCl, suggesting a higher sensitivity of *Daphnia similis* to this salt.

Table 1. Evaluation of the Acute Toxicity (EC50;48H) of Potassium Chloride (KCl) to *Daphnia similis*. Values are expressed in g/L.

TEST	EC50;48H (95% confidence interval)
1	1.19 (1.11 – 1.28)
2	0.69 (0.66 – 0.73)
3	1.08 (1.00 – 1.16)

Table 2. Evaluation of the Acute Toxicity (EC50;48H) of Potassium Acetate ($KC_2H_3O_2$) to *Daphnia similis*. Values are expressed in g/L.

TEST	EC50;48H (95% confidence interval)
1	1.15 (1.06 – 1.25)
2	1.05 (0.96 – 1.15)
3	1.21 (1.09 – 1.34)

In the chronic tests, no statistically significant difference was observed in the growth of *Ceriodaphnia dubia* for $KC_2H_3O_2$, but a significant difference ($P<0.05$) was observed for KCl where all organisms at higher concentrations (0.8; 0.9; 1.0 and 1.25 g/L) of this salt died on the first day of experiment (Table 3). Also, a statistically significant difference in longevity ($P<0.05$) relative to the control was recorded in the experiment with KCl (Table 4). No difference in longevity between test concentrations and control was found for $KC_2H_3O_2$. With regard to fecundity, both salts showed statistically significant differences ($P<0.05$) at the lowest tested concentration when compared to the control (Tables 5 and 6). The single overlapping concentration in the experiments with KCl and $KC_2H_3O_2$ (0.6 g/L) showed a significant reduction in fecundity for both salts. In addition, a significant effect on longevity was observed for KCl. The higher concentrations obtained in the acute preliminary tests with Potassium Chloride in comparison with Potassium Acetate could be attributed to sensitivity differences between test populations used in both experiments.

Table 3. Results obtained from the Tukey statistical test (Gulley et al., 1991) for *Ceriodaphnia dubia* growth data in the chronic experiment with Potassium Chloride (KCl).

GROUP	IDENTIFICATION	MEAN (mm)	GROUP						
			1	2	3	4	5	6	7
1	CONTROL	0.560	-	•	•	+	+	+	+
2	0.6 g/L	0.550	•	-	•	+	+	+	+
3	0.69 g/L	0.532	•	•	-	+	+	+	+
4	0.8 g/L	0.323	+	+	+	-	•	•	•
5	0.9 g/L	0.329	+	+	+	•	-	•	•
6	1.0 g/L	0.316	+	+	+	•	•	-	•
7	1.25 g/L	0.316	+	+	+	•	•	•	-

+ significant ($p = 0.05$) • non-significant

Table 4. Results obtained from the Tukey statistical test (Gulley et al., 1991) for *Ceriodaphnia dubia* longevity data in the chronic experiment with Potassium Chloride (KCl).

GROUP	IDENTIFICATION	MEAN (Days)	GROUP						
			1	2	3	4	5	6	7
1	CONTROL	12.0	-	+	+	+	+	+	+
2	0.6 g/L	7.5	+	-	•	+	+	+	+
3	0.69 g/L	4.5	+	•	-	+	+	+	+
4	0.8 g/L	1.0	+	+	+	-	•	•	•
5	0.9 g/L	1.0	+	+	+	•	-	•	•
6	1.0 g/L	1.0	+	+	+	•	•	-	•
7	1.25 g/L	1.0	+	+	+	•	•	•	-

+ significant ($p = 0.05$) • non-significant

Table 5. Results obtained from the Dunnett statistical test (Gulley et al., 1991) for *Ceriodaphnia dubia* fecundity data in the chronic experiment with Potassium Chloride (KCl).

GROUP	IDENTIFICATION	MEAN (N° of neonates)	T Stat.
1	Control	17.2	-
2	0.6 g/L	4.9	5.67*
3	0.69 g/L	1.5	7.23*

*significant ($p = 0.05$)

Table 6. Results obtained from the Dunnett statistical test (Gulley et al., 1991) for *Ceriodaphnia dubia* fecundity data in the chronic experiment with Potassium Acetate ($KC_2H_3O_2$).

GROUP	IDENTIFICATION	MEAN (N° of neonates)	T Stat.
1	Control	16.9	-
2	0.3 g/L	4.2	7.98*
3	0.35 g/L	5.2	7.35*
4	0.42 g/L	3	8.35*
5	0.5 g/L	7	6.22*
6	0.6 g/L	3	8.23*

* significant ($p = 0.05$)

The major problem of the contact of the drilling fluids with fresh or brackish water environments is their high salt concentration, which can kill all species that are sensitive to salinity changes. Hynes (1971) reported that a considerable proportion of the salinization of lakes with non-toxic salts is a result of human activities, some of them related to petroleum exploitation. In some places salinity changes can result in a faunal alteration, as has been documented in the Aral Sea (Aladin, 1991). In the last 30 years the salinity in this lake has changed from 8-10‰ to 30-32‰ leading the whole Cladocera fauna to extinction. More sensitive species disappeared when the salinity was 12‰ while the more resistant died when the salinity reached 30‰ (Aladin, 1991). Salinity alterations sometimes cause only a sublethal effect, affecting mainly the organisms' reproduction. Leblanc and Surprenant (1984) demonstrated that *Daphnia magna* treated with high concentrations of KCl showed a significant reduction in egg production in comparison with animals that did not receive the same treatment. Schuytema et al. (1997), in an experiment with estuarine sediments, found that reproduction of *Daphnia magna* was affected by the salinity of the sediment. Those results are in agreement with the data presented in this paper, where a significant difference in fecundity between the treatments and the control was observed for both potassium salts, and a difference in longevity and growth was also observed for Potassium Chloride. In addition, KCl showed a higher acute effect for *Daphnia similis* when compared with $KC_2H_3O_2$. In this study, a toxic effect (lethal or sublethal) on the test organisms was observed for the two salts, suggesting that both can cause an environmental damage when used at high concentrations. The significant difference observed in the fecundity at the lowest test concentration of $KC_2H_3O_2$ demonstrates that this salt is not innocuous, and can cause sublethal effects at concentrations that are within the same order of magnitude as those tested for KCl. Since the results presented here are based on a single chronic test for each salt, performed in a similar but not fully overlapping concentration range, further studies, including also other components of the drilling fluid, are necessary to predict more precisely its potential effects on the organisms present in each affected ecosystem.

Acknowlegments: We thank CENPES (Centro de Pesquisas Leopoldo de Mello, PETROBRAS, Brazil) for financial and technical support, as well as Eduardo Eizirik, Carlos E. Güntzel, Nade J. C. Monteiro, Alexandre Arenzon and one anonymous reviewer for helpful suggestions and comments. Financial support for L.R.P. Utz was provided by CNPq, Brazil.

REFERENCES

Aladin NV (1991) Salinity tolerance and morphology of the osmoregulation organs in cladocera with special reference to Cladocera from the Aral Sea. Hydrobiologia 225: 291-299

CETESB (1992) Manual de Avaliação de poluentes a organismos aquáticos. Companhia de Tecnologia e Saneamento Ambiental. São Paulo, SP. 37pp.

Gillenwater KE, Ray CR, (1989) Potassium acetate adds flexibility to drilling muds. Technol. Oil Gas J 10: 99-102

Gulley DD, Boelter AM, Bergman H.L, (1991) TOXSTAT 3.3. Computer Program

Hamilton MA, Russo RC, Thurton RV, (1977) Trimmed Spearman-Karber Method for estimating Median Lethal Concentrations in Toxicity Bioassays. Environ Sci Technol 11: 714-719

Hynes HBN, (1971) The biology of polluted waters. University of Toronto Press. Toronto, Buffalo 202 pp.

Leblanc GA, Surprenant DC, (1984) The influence of mineral salts on fecundity of the water flea (*Daphnia magna*) and the implications on toxicity testing of industrial wastewater. Hydrobiologia 108: 25-31

Price II WA, Macauley JR, Clark JR, (1986) Effects of drilling fluids on

Thalassia testudinum and its epiphytic algae. Environ Exp Bot 26: 321-330
Soria FL, Chavarria JM, (1978) Tecnicas de defensa del medio ambiente. Editorial Labor S.A. España. Tomo II. p 721-1334
Schuytema GS, Nebeker AV, Stutzman TW, (1997) Salinity tolerance of *Daphnia magna* and potential use for estuarine sediment toxicity tests. Arch Environ Contam Toxicol 33: 194-198
Stansbury M, (1986) A new field procedures improve KCl/ polymer drilling mud results. Technol Oil Gas J 7: 49-52
U.S. Environmental Protection Agency (1993) Methods for estimating the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 4th. ed. EPA 600/4–90/027F Cincinnati, OH.
U.S. Environmental Protection Agency (1994) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 3rd. ed. EPA 600/4 – 91/002 Cincinnati, OH. p 293