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Predictive value of laboratory tests with aquatic invertebrates: influence of experimental conditions

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Considering the difficulty of making meaningful extrapolations of laboratory bioassay data to real world situations, short-term tests have been carried out in a factorial pattern to determine the magnitude of effect variation resulting from changes in experimental abiotic conditions.

Three selected zooplankton species (the rotifer *Brachionus plicatilis*, the brine shrimp *Artemia salina* and the waterflea *Daphnia magna*) have been exposed to increasing concentrations of two chemicals (one inorganic and one organic) in different combinations of two major environmental variables.

For the brackish water rotifer *B. plicatilis* the acute toxicity of potassium dichromate and sodium laurylsulphate was determined in 16 different combinations of temperature and salinity (10–17–24–31°C and 5–20–35–50 ‰). For the marine crustacean *A. salina*, the acute toxicity of the same two chemicals was determined in 20 temperature–salinity combinations (10–15–20–25–30°C and 5–20–35–50‰) and for the freshwater crustacean *D. magna*, 16 combinations of temperature and water hardness (7–14–21–28°C and 80–320–560–800 mg/l $CaCO_3$) were assayed.

The entire study comprised nearly 300 complete toxicity tests. 24-h LC_{50} values (for *Artemia* and *Brachionus*) and 24-h EC_{50} values (for *Daphnia*) revealed that the variation in toxicity resulting from changing environmental conditions, is both species- and chemical-specific and (within the limits of this study) ranged from a minimum of a factor 2.5 to a maximum exceeding a factor of 100.

The necessity to take such variations into consideration in predictive hazard assessment studies is underlined.

Key words: *Brachionus plicatilis*; *Artemia salina*; *Daphnia magna*; Experimental abiotic condition

INTRODUCTION

For many years, short-term laboratory bioassays have been the major tool in evaluating the toxicity of chemicals. The ever returning problem is, however, the predictive value of such laboratory tests for extrapolation to real world situations. Most toxicity studies on aquatic biota are indeed performed in test conditions which

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are close to optimal. In nature, on the contrary, aquatic organisms must cope with environmental conditions which, during the year, may fluctuate considerably. Hansen (in White, 1984) underlined that even simple toxicity tests are rarely conducted under a sufficient variety of conditions to reflect the large range of 'potential' situations found in the field.

Many experimental studies have been made on the influence of environmental variables on the toxicity of xenobiotic compounds to organisms. Only a few publications, however, are dealing with the combined effect of environmental factors on pollutant toxicity. The influence of temperature-salinity combinations on toxicity, e.g. has been analyzed by Vernberg and Vernberg (1972), Vernberg et al. (1973), Vernberg et al. (1974), Jones (1975a,b) Gray (1976), Rosenberg and Costlow (1976), Hrs-Brenko et al. (1977), McKenney and Costlow (1977), Nelson et al. (1977), Laughlin and Neff (1979), MacInnes and Calabrese (1979), Theede et al. (1979), Cotter et al. (1982), Bryant et al. (1985a and b). Two studies were made on combined temperature-hardness influences (Cairns and Scheier, 1957; 1958). As emphasized by Nelson et al. (1977), a multivariable approach to pollutant studies provides a much more realistic idea of an animal's response in nature.

Considering the scarcity of information on the interaction of environmental factors and chemical toxicity, and the need to improve the predictive value of laboratory tests, a comparative study was undertaken to determine to what extent abiotic test conditions influence the sensitivity of a few selected freshwater and marine zooplankters in short-term bioassays. Series of factorial experiments have been designed to determine the combined effect of two abiotic variables on the toxicity of two chemicals: sodium laurylsulphate and potassium dichromate. The first series deals with the interacting effect of temperature and salinity on the toxicity of the two chemicals to two euryhaline invertebrates: the brackish water rotifer *Brachionus plicatilis* and the marine crustacean *Artemia salina*. The second addresses the combined effect of temperature and water hardness on the toxicity of the same two compounds to the freshwater waterflea *Daphnia magna*. The prerequisites in selecting the test range for the environmental parameters were that no increased mortality or other stress signs should occur in the controls during the 24-h test period and that the different experimental combinations should represent situations which can be encountered in the natural environment.

MATERIALS AND METHODS

The toxicity test

Artemia salina

All experiments with *A. salina* were carried out according to the standardized ARC-test (Artemia Reference Center-test) procedure developed at the Laboratory for Biological Research in Aquatic Pollution at the State University of Ghent in

Belgium. The ARC-test is a short-term routine bioassay that determines the 24-h LC_{50} of a mixed instar II–III population of a specific *A. salina* strain (Vanhaecke et al., 1980; Vanhaecke et al., 1981; Vanhaecke and Persoone, 1984).

Since *A. salina* strains from different geographic origins have different sensitivities to toxicants (Sorgeloos, 1981), all the experiments reported hereunder were carried out with reference cysts from the Artemia Reference Center.

Branchionus plicatilis

Since no standardized toxicity test method with rotifers was available at the time of the experiments, we developed a procedure to determine 24-h LC_{50} values with non-ovigerous females. All the bioassays were carried out in glass Petri dishes (diameter, 40 mm; height, 10 mm) filled with 5 ml of the respective toxicant concentration. After rinsing the rotifers in the corresponding toxicant dilution, five animals were transferred to individual petri dishes. Each dilution series of the chemical was tested in four duplicates. The petri dishes were incubated in darkness at the appropriate test temperatures and after 24 h, the number of dead rotifers was determined under a dissecting microscope. The rotifers were considered dead if no internal or external movement was observed within 10 s.

The *B. plicatilis* strain used was hatched from resting eggs originating from salinas near the Azov sea. The rotifers were reared in semi-continuous cultures in 1 liter glass serum bottles according to a technique described by Persoone and Sorgeloos (1975) for the culturing of algae. The rotifer cultures were kept under constant environmental conditions (20°C, 35‰, continuous illumination of 4000 lux and gentle aeration) in densities of approximately 50 animals/ml. Every second day one quarter of the culture was renewed and the rotifers in this fraction harvested. Algal food (*Dunaliella tertiolecta*) was added at the time of renewal to keep the concentration of algae at approximately 1×10^6 cells/ml.

Daphnia magna

All experiments were performed according to the EEC standard procedure (EEC, 1984) for determining the 24-h EC_{50} for *D. magna*.

The *D. magna* strain originates from a small pond near Antwerp (Belgium) and has been kept in (parthenogenetic) culturing in our laboratory since November 1982. The stocks were reared in 2 l aquaria in a synthetic medium, according to EEC-prescriptions (EEC, 1984); the medium was completely renewed three times weekly. Culture densities were kept below 50 animals/l, and the daphnids were fed daily 500×10^6 to 750×10^6 cells of the alga *Selenastrum capricornutum* and 100×10^6 to 125×10^6 cells of *Chlamydomonas reinhardti* for each liter of culture medium. The aquaria were placed in a temperature controlled cabinet with a 12:12 h light–dark cycle at 1000 lux light intensity at waterlevel. Offspring were separated at regular intervals. Test animals were 6–24 h juveniles, taken from cultures 3–5 wk old (broods 5–10).

Choice of test range for the environmental variables

The tolerance limits for temperature and salinity, or temperature and water hardness were determined for each of the three test species in preliminary experiments, first separately, then in combination. The prerequisite for selection was that the combination of the extreme values for the two environmental parameters should not induce mortality, nor cause any visible stress to the animals during the test period.

Artemia salina

Although *A. salina* is a euryhaline organism with a broad tolerance to temperature changes, important differences in tolerance for both environmental parameters are reported in scientific literature for brine shrimp strains from different geographical origin (Sorgeloos et al., 1976; Vanhaecke, 1983).

Our own preliminary experiments revealed that below 10°C, the nauplii of the reference strain became immobile and above 30°C mortality increased considerably; this confirms the findings of Vanhaecke (1983). Consequently, the experimental temperatures selected for our study ranged from 10–30°C. With regard to salinity the 3 to 300‰ tolerance range postulated by Bayly (1972) is definitely invalid for all *A. salina* strains. According to Von Hentig (1971) and Kristensen and Hulscher-Eneis (1972), mortality occurs at 5‰ for most strains. For our reference strain the lower salinity limit was about 5‰ and values higher than 50‰ induced mortality of the nauplii during the 24-h test period.

A 5×4 factorial experiment was thus conceived with temperatures of 10–15–20–25–30°C and salinities of 5–20–35–50‰, and to be replicated once. The combination 25°C and 35‰ salinity is identical to the environmental conditions of the standard ARC test.

Since brine shrimp nauplii can resist drastic important temperature and salinity changes (D'Agostino and Provasoli, 1968; Vanhaecke, 1983) nauplii were hatched and held under standard conditions of 25°C and 35‰ salinity. At the onset of the toxicity test the animals were transferred directly into the various temperature-salinity regimes, without intermediate acclimatisation.

Brachionus plicatilis

Ito et al. (1981) and Pascual and Yufera (1983) report that *B. plicatilis* populations can grow at temperatures of up to 40°C; however, the highest temperature in which our strain could survive 24 h was 35°C. Although Euteneuer et al. (1984) found that *B. plicatilis* females can survive at 4°C, the animals became virtually immobile below 10°C hence it was difficult to determine if they were alive.

B. plicatilis is a euryhaline species which tolerates salinities from 1 up to 96‰ (Worley, 1928; Ito, 1960; Walker, 1981). In preliminary experiments we found that rotifers survive a direct transfer from 35‰ to 5 or 70‰ for at least 24 h, even under extreme temperature conditions. Upon transfer to extreme salinities, the rotifers become very sluggish for a few hours and sink to the bottom; 24 h later they are, however, swimming actively.

In order to avoid stress from abrupt salinity changes, the test animals were exposed to each of the four salinities of the experimental design for two days prior to the experiment.

A 4×4 temperature-salinity factorial test was performed and replicated at 10–17–24–31°C for temperature and 5–25–45–65‰ for salinity. Experiments were performed in parallel at 25°C and 35‰ salinity to represent 'standard' test conditions.

Daphnia magna

Ivleva (1973) reported that *D. magna* no longer reproduces at 4°C although it can tolerate lower temperatures. This author furthermore indicated that *D. magna's* upper temperature resistance is approximately 36°C. Brown (1929), however, noted survival of the species at temperatures as high as 41°C whereas Goss and Bunting (1983) stated that at 30°C the animals are in a critical stress situation. In our own preliminary experiments, animals were transferred directly from 20°C to high or low temperatures. Since it appeared difficult to use swimming inhibition as test criterion in tests carried out at or below 5°C (the animals indeed become immobile at such low temperatures), 7°C was selected as the lower temperature limit. Since in preliminary tests, a 30% increase in mortality over the controls was noted after 48 h at 30°C, versus no difference at 28°C, the latter value was selected as the upper temperature limit.

Although *D. magna* is considered as a hard water species (Buikema et al., 1976), bioassays are often carried out in soft water (± 40 mg $CaCO_3$/l: Biesinger and Christensen, 1972; Baudouin and Scoppa, 1974; Cairns et al., 1978) or in medium hard water (± 100 mg $CaCO_3$/l: Parkhurst et al., 1981; Lal et al., 1983; Cowgill et al., 1985; Grothe and Kimerle, 1985; Lewis and Weber, 1985). In our experiments we used 80 mg $CaCO_3$/l as the low water hardness limit. Indeed, in preliminary tests using softer water (20 to 40 mg/l), mortality of the test animals after 48 h exceeded that of the controls (dilution water with 250 mg $CaCO_3$/l as described in the EEC standard). Although water hardness values greater than 800 mg/l did not affect survival (a finding corroborating the results of Leblanc and Surprenant, 1984), 800 mg/l was selected as the upper limit since the highest concentrations recorded for hard waters in nature seem to be approximately 1 000 mg $CaCO_3$/l (Cebedoc, 1959; US Geological Survey, 1979, in Pimentel and Bulkley, 1983).

A 4×4 factorial experiment was conducted as follows: 7–14–21–28°C and 80–320–560–800 mg/l $CaCO_3$. The series with potassium dichromate was repeated twice; that with sodium laurylsulphate once. Reference tests were also conducted according to the EEC standard test (20°C and 250 mg/l $CaCO_3$).

The test media

The artificial sea-water medium of Dietrich and Kalle (1957) was used for all bioassays with *Artemia* and *Brachionus*. Because of the small buffering capacity, the

$NaHCO_3$ concentration was increased twentyfold as recommended by Spotte (1979). Different salinity media were prepared by dilution with deionized water. The pH of the artificial seawater ranged from 7.9 to 8.1 with 90% oxygen saturation at the beginning of the tests.

For the *Daphnia* bioassays, the synthetic medium described in the EEC-standard (EEC, 1984) was used to prepare test waters with different water hardness. The various experimental media were obtained by proportionally increasing or decreasing the concentrations of the four salts of this synthetic medium to keep the Ca/Mg and Na/K ratios constant.

The test products

The compounds selected for this study are two reference chemicals frequently used for aquatic bioassays (Lee, 1980): sodium laurylsulphate (SLS), $CH_3(CH_2)_{10}$-CH_2OSO_3Na (Merck, grade 98–102%); hexavalent chromium as potassium dichromate $K_2Cr_2O_7$ (Fluka A.G., p.a.). All toxicant concentrations are expressed in mg/l and refer to the nominal concentrations at the onset of the experiments.

As a means of controlling the sensitivity of the test animals and the reliability of the experimental protocol, an internal control was performed with each series of tests in 'standard' test conditions; $K_2Cr_2O_7$ or SLS were used as reference toxicants. Whenever the EC_{50} or LC_{50} value of this internal control fell outside the allowed range, the data of the entire test series were considered unacceptable and the experiment was repeated.

Data analysis

The 24-h LC_{50} (or EC_{50})-values with the 95% confidence limits and the 'slope function *S*' were calculated according to Litchfield and Wilcoxon (1949). To evaluate the divergence between the LC_{50} (EC_{50}) values obtained in the selected sets of various environmental conditions ('factorial' values, *f*) and those under standard conditions ('standard' values, *s*), the ratios LC_{50} (EC_{50})-*f* to LC_{50} (EC_{50})-*s* were calculated and plotted in three-dimensional graphs. The LC_{50} (EC_{50}) values were subjected to a two-way analysis of variance (Model I) with replication within the subgroups, according to Sokal and Rohlf (1981). These calculations allowed us to quantify the significance of temperature and salinity effects, or temperature and hardness effects, as well as the interaction of both parameters, on the toxicity of the compounds.

RESULTS

The mean 24-h LC_{50} (EC_{50}) values of the different temperature–salinity or temperature–water hardness combinations and the range of the 95% confidence limits for the two chemicals and the three test species are summarised in Tables I–III.

TABLE I

Artemia salina – Mean 24 h LC_{50} values (mg/l) for potassium dichromate and sodium laurylsulphate, with 95% confidence limits.

Compound	Salinity (°C) ‰	5	20	35	50
$K_2Cr_2O_7$	10	182.5(167.0–199.5)	168.0(150.0–188.2)	263.5(229.8–298.7)	291.5(248.2–344.7)
	15	156.0(136.4–173.3)	160.0(144.9–178.2)	191.3(172.7–209.0)	206.5(169.6–248.5)
	20	128.0(100.0–158.8)	113.5(94.2–135.6)	48.5(39.8– 61.4)	52.3(42.9– 60.5)
	25	30.9(25.9– 36.4)	27.5(21.8– 35.6)	22.2(19.0– 26.2)	31.0(26.8– 35.8)
	30	14.1(10.9– 17.6)	8.8(6.1– 11.4)	14.2(11.5– 17.9)	20.6(16.2– 24.4)
SLS	10	154.0(140.2–167.5)	90.5(84.1– 98.3)	54.5(49.1– 58.8)	32.7(28.3– 36.7)
	15	120.0(106.6–139.9)	68.5(57.7– 81.9)	47.5(41.8– 54.3)	28.5(24.6– 32.5)
	20	88.5(77.3–104.8)	50.0(42.1– 56.7)	32.4(27.4– 38.8)	20.9(17.1– 25.3)
	25	53.1(44.3– 61.6)	40.1(36.6– 44.8)	21.5(18.8– 24.8)	16.7(14.5– 20.7)
	30	34.5(28.9– 40.9)	21.0(16.9– 25.5)	13.2(11.1– 16.0)	7.2(6.0– 8.9)

Artemia salina (*Table I*)

Potassium dichromate

The ratio between the highest and the lowest LC_{50} values (291.5 mg/l at 10°C and 50‰), and 8.8 mg/l at 30°C and 20‰ respectively) was as high as 33. The effect of salinity on the toxicity of potassium dichromate was less pronounced than the temperature effect. Indeed, at constant temperatures, the LC_{50} values fluctuated between a factor 1.3 and 2.6 for salinity changes but at constant salinity levels a 13- to 19-fold difference in LC_{50} values was noted with increasing temperature.

The ratios between the LC_{50} values at the different temperature-salinity combinations (LC_{50}-*f*) and the LC_{50} value under standard conditions (LC_{50}-*s* = 22.2 mg/l) were calculated, and are shown in Fig. 1. The effects at 25°C were similar at all salinity levels. The nauplii were more sensitive at 30°C than in standard conditions (25°C), with a maximum variation of 2.5 at 20‰ salinity; at lower temperatures a decrease in toxicity was noted with a maximum difference of a factor 13.

The ANOVA revealed that both the environmental variables and their interaction had a highly significant effect ($P < 0.001$) on the toxicity of potassium dichromate to brine shrimp nauplii.

Sodium laurylsulphate

LC_{50} values ranged from 7.2 mg/l (30°C–50‰) to 154.0 mg/l (10°C–5‰). At either constant temperature or constant salinity, toxicity increased approximately four times when either salinity or temperature were increased.

The LC_{50}-*f*/*s* ratios (LC_{50}-*s* = 21.5 mg/l) in the different temperature-salinity combinations are shown in Fig. 1. The highest LC_{50} value differed by a factor 7.2 from the standard value, the lowest LC_{50} by a factor 3.

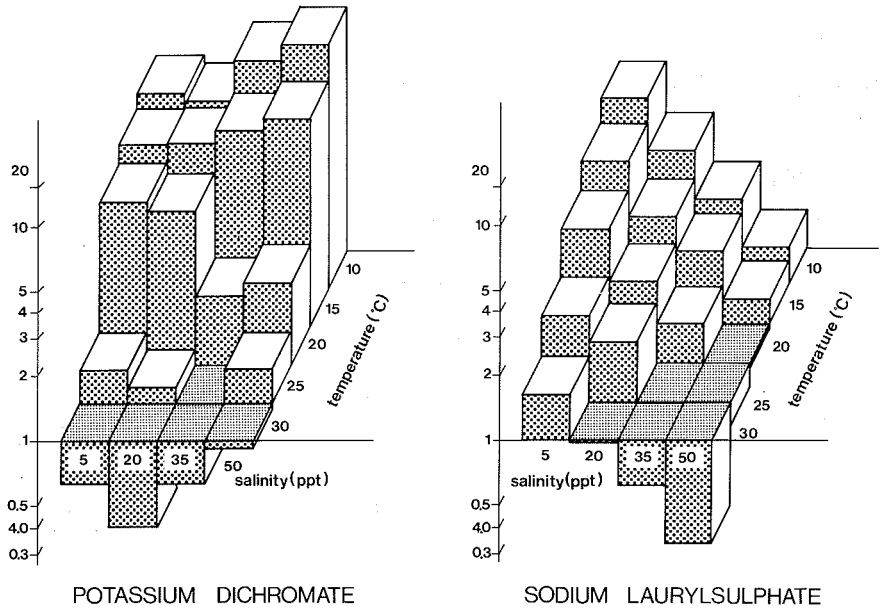

Fig. 1. *A. salina*: Ratios of 'factorial' to 'standard' 24 h LC_{50} values for potassium dichromate and sodium laurylsulphate. Ratios are expressed on a log scale.

Statistical analysis revealed that temperature and salinity, as well as their interaction, had a highly significant effect ($P<0.001$) on the toxicity of the detergent.

Brachionus plicatilis (*Table II*)

Potassium dichromate

The LC_{50} values varied by nearly five fold between the highest (690 mg/l at 17°C–45‰) and the lowest (130 mg/l at 31°C–5‰) values. The salinity effect was most pronounced; at constant temperatures a 2.3- to 4.6-fold increase in sensitivity with increasing salinity was noted, as compared to a maximum difference of a factor 1.7 resulting from temperature changes at constant salinity levels.

The LC_{50}-f/s ratios (LC_{50}-s = 347 mg/l) are represented (three-dimensionally) in Fig. 2. The highest LC_{50} differed by a factor 2 from the standard LC_{50}; the lowest by a factor 3.

The two-way analysis of variance of the data revealed a significant effect of both salinity ($P<0.001$) and temperature ($P<0.01$) as well as a significant effect of the interaction ($P<0.01$) of these two environmental parameters on the toxicity of potassium dichromate to the rotifers.

Sodium laurylsulphate

The range between the highest LC_{50} (29.4 mg/l at 10°C–5‰) and the lowest one

TABLE II

Brachionus plicatilis – Mean 24 h LC_{50} values (mg/l) for potassium dichromate and sodium laurylsulphate, with 95% confidence limits.

Compound	Salinity ‰ (°C)	5	25	45	65
$K_2Cr_2O_7$	10	227(208–259)	378(312–472)	500(437–577)	518(465–587)
	17	172(159–188)	344(262–407)	690(596–797)	457(412–504)
	24	146(124–170)	356(334–386)	645(571–736)	405(353–449)
	31	130(112–151)	238(208–271)	600(572–627)	345(302–396)
SLS	10	29.4(22.1–37.6)	18.0(15.3–20.4)	14.6(13.3–15.9)	16.1(13.1–20.3)
	17	27.9(22.3–34.1)	17.5(13.7–21.0)	12.7(11.5–13.6)	16.8(13.7–19.4)
	24	23.3(18.9–29.1)	18.8(17.5–19.8)	13.5(12.4–14.8)	10.9(7.8–14.1)
	31	17.7(15.0–20.8)	15.5(13.3–18.0)	13.2(12.4–14.5)	12.3(8.0–17.6)

(10.9 mg/l at 24°C–65‰) was a factor 2.7. The variation between LC_{50} values at constant salinities ranged from a factor 1.2 to 1.7. The increase in toxicity induced by salinity changes under constant temperature conditions was a factor 1.4 to 2.2.

As seen in Fig. 2, the highest and the lowest LC_{50} values differed respectively by a factor 2 and a factor 1.4 from the standard LC_{50} (15.4 mg/l).

Statistical analysis of the data indicated a significant effect of temperature ($P<0.05$) and salinity ($P<0.001$) on the toxicity of sodium laurylsulphate to *B. plicatilis*. However, the second order interaction term 'temperature × salinity' did not induce a significant effect on the toxicity of SLS.

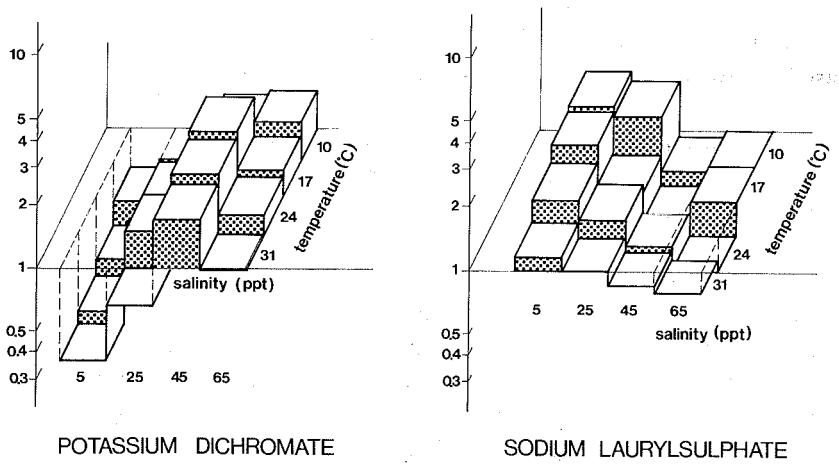

Fig. 2. *B. plicatilis*: Ratios of 'factorial' to 'standard' 24-h LC_{50} values for potassium dichromate and sodium laurylsulphate. Ratios are expressed on a log scale.

TABLE III

Daphnia magna - Mean 24 h EC_{50} values (mg/l) for potassium and sodium laurylsulphate, with 95% confidence limits.

Compound	Water hardness (mg/l) (°C)	80	320	560	800
$K_2Cr_2O_7$	7	0.46 (0.33 -0.71)	2.58(1.92-3.50)	4.7 (1.92-3.50)	7.9 (6.2 -9.4)
	14	0.30 (0.21 -0.43)	2.0 (1.2 -3.0)	4.1 (2.6 -6.5)	5.0 (3.4 -7.3)
	21	0.080(0.048-0.116)	1.04(0.68-1.73)	2.17(1.5 -2.7)	2.4 (1.4 -3.9)
	28	0.037(0.027-0.050)	0.77(0.57-1.18)	0.79(0.58-1.26)	0.79(0.57-1.03)
SLS	7	63 (49 -81)	79 (67-89)	84 (70-99)	75 (65-88)
	14	42 (30 -59)	57 (46-72)	74 (63-86)	78 (67-93)
	21	9.6(6.4-12.6)	32 (26-41)	24 (17-33)	39 (29-50)
	28	5.1(4.0- 6.5)	16 (14-17)	11 (8-13)	12 (10-13)

Daphnia magna (*Table III*)

Potassium dichromate

EC_{50} values ranged from 0.037 mg/l (at 28°C and 80 mg $CaCO_3$/l) to 7.9 mg/l (at 7°C and 800 mg $CaCO_3$/l). With constant water hardness and increasing temperature, toxicity increased 3- to 12-fold; at constant temperatures, however, a 16- to 30-fold decrease was noted with increasing water hardness.

From the EC_{50}-*f/s* ratios (EC_{50}-$s = 1.03$ mg/l) in Fig. 3, it appears that all combinations of water hardness lower than, and all temperatures higher than the standard conditions, resulted in a toxicity increase. For the most extreme conditions the ratio was as high as a factor 25. All other temperature-water hardness combinations decreased the toxicity of potassium dichromate for the test animals, up to a factor of 8.

A two-way variance analysis of the results revealed that both water hardness and temperature, as well as their interaction had a highly significant influence ($P < 0.001$) on the toxicity of $K_2Cr_2O_7$ to water fleas.

Sodium laurylsulphate

Average EC_{50} values varied from 5.1 mg/l (at 28°C and 80 mg $CaCO_3$/1) to 84 mg/l (at 7°C and 560 mg $CaCO_3$/l), as compared to 27.5 mg/l under standard conditions. At constant water hardness EC50-values varied up to a factor 12 with changing temperatures. At constant temperatures, a 1.3 to 4-fold increase in toxicity was noted between the high and low water hardness levels tested.

The EC50-f/s ratios calculated for the various temperature-water hardness combinations (Fig. 3) revealed that the detergent was always more toxic at 28°C than

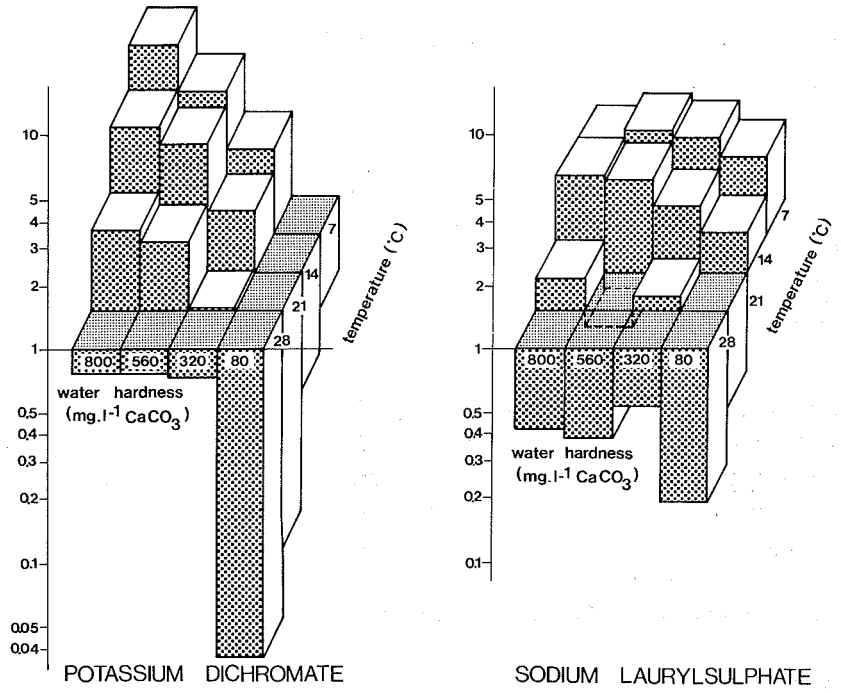

Fig. 3. *D. magna*: Ratios of 'factorial' to 'standard' 24 h EC_{50} values for potassium dichromate and sodium laurylsulphate. Ratios are expressed on a log scale.

under standard toxicity conditions; the maximum was a factor of 5. Most other temperature–water hardness combinations resulted in slightly higher EC50-values than that of standard conditions (maximum factor of 3).

The ANOVA test indicated that both water hardness and temperature had a highly significant effect ($P<0.001$) on the toxicity of sodium laurylsulphate. The interaction of the two abiotic factors was only significant at the $P_{0.01}$ level.

DISCUSSION

Chemical and biota dependent toxicity

From the nearly 300 toxicity tests presented above, one may conclude that LC50/EC50-values at the four or five temperatures and at the four salinities and water hardnesses tested, differed more for potassium dichromate than for sodium laurylsulphate for all three test species. This again clearly shows that the magnitude of variation in toxicity resulting from changes in the environmental conditions depends of the chemical compound.

TABLE IV

Magnitude of toxicity variation due to changes in experimental environmental conditions.

Test species	Highest LC_{50} (EC_{50}) ratio in 'standard' lab conditions to simulated field conditions		Ratio of highest to lowest LC_{50} in the various combinations tested out	
	Sodium lauryl sulphate	Potassium dichromate	Sodium lauryl sulphate	Potassium dichromate
A. salina	7	13	21	33
B. plicatilis	2	2	3	5
D. magna	5	27	16	213

The present study also demonstrates that toxicity patterns are also species specific. Under the conditions of the standard test, *B. plicatilis* e.g. was less sensitive to potassium dichromate than both *A. salina* and *D. magna*; the ratio between the LC50 of the rotifer and the EC50 of the waterflea was 300. Under extreme environmental conditions these differences become much smaller. For sodium laurylsulphate on the contrary, the LC50/EC50 values for the three test animals were quite similar in standard test conditions.

Influence of temperature

The influence of temperature on the acute toxicity of a chemical is a complex phenomenon and therefore difficult to predict. In addition to direct effects upon the metabolism of the test organisms there are also indirect effects such as increased solubility and diffusion rates of the substances. Detoxification and excretory mechanisms may offset the effect of this environmental variable (MacInnes and Calabrese, 1979; Graney et al., 1984; Niimi and Palazzo, 1985). Invertebrates usually show the classic response of increased sensitivity with increasing temperature (Vernberg and Vernberg, 1972; Schaefer and Pipes, 1973; Jones, 1975 a and b; Gray, 1976; Cairns et al., 1978; Capuzzo, 1979; MacInnes and Calabrese, 1979; Theede et al., 1979; Cotter et al., 1982; Bryant et al., 1985). Cairns et al., (1978) indicated that a considerable variation in sensitivity exists between the species used and that this variation depends on the chemical as well as on the test temperature. In the papers quoted above, the temperature ranges usually varied from 10 to 20°C. However, when determining variation of the effects of toxicants associated with small changes in temperature (17 and 23°C), Stephenson and Watts (1984) did not observe a consistent influence on the susceptibility of *D. magna* to potassium dichromate. This finding was confirmed by Cowgill et al. (1985) for the toxicity of phenol, diethanolamine and ethyleneglycol to the same test species. Contrary to the above statements, the latter authors found a significant decrease in sensitivity of both *D. magna* and *Ceriodaphnia dubia/affinis* for chlorobenzene at higher temperatures.

The results of our own bifactorial experiments with *A. salina, B. plicatilis* and *D. magna*, however, confirm the general notion that toxicity increases with increasing temperatures.

Influence of salinity

Scientific literature on the influence of salinity on the toxicity of pollutants generally indicates to an increasing tolerance for chemicals at increasing salinity levels (Vernberg and Vernberg, 1972; Vernberg et al., 1973; Jones, 1975b; Gray, 1976; McKenney and Costlow, 1977; Nelson et al., 1977; McInnes and Calabrese, 1979; Frank and Robertson, 1979; Theede et al., 1979; Bryant et al., 1985b; Laughlin and Neff, 1979). In the temperature–salinity study performed by Bryant et al., (1985a) practically no salinity–related toxicity variation was observed; Cotter et al. (1982) even detected an increased toxicity tolerance when the salinity was lowered. Several hypotheses have been formulated on the possible effect of salinity on the toxicity of xenobiotic compounds: changes in ionic strength (Lee, 1973), competitive inhibition with cations (Moore and Ramamoorthy, 1983) or anions (Riedel, 1985), and direct osmotic effects on the animals (Dalla Venezia et al., 1980). According to Rand and Petrocelli (1984) the effect of salinity depends in particular on the genetic nature of the test organisms used, which determines whether or not an organism can adapt to a given salinity and as a result express a different tolerance to toxicants. However, the influence of salinity on the toxicity of compounds is not only 'species', but also 'chemical' specific (Eisler, 1970). Forster and Tullis (1985) report that, at lower salinities, some organic chemicals become more toxic for *A. salina*–larvae, whereas others (such as hexane and 1-chloronaphtalene) do not. In our own experiments salinity significantly affected the toxicity of $K_2Cr_2O_7$ and SLS for *A. salina*–nauplii and *B. plicatilis*. Analogous to the effect of temperature, the salinity response was clearly both species and chemical dependent. Potassium dichromate became more toxic to *B. plicatilis* at low salinities, whereas the effect was temperature dependent for *A. salina*; at low temperatures a decrease in salinity indeed leads to increased toxicity. Inversely the organisms became less sensitive to the test compound at high temperatures for the same decrease in salinity. Contrary to the general rule reported for chemicals, we found that for the two marine test species used, sodium laurylsulphate was less toxic at lower salinities.

Influence of water hardness

Heavy metals are generally more toxic in soft water than in hard water (Abel, 1974; Buikeme *et al.*, 1974; Dave, 1978; Howarth and Sprague, 1978; Chakoumakos et al., 1979; Miller and Mackay, 1980; Müller, 1980). Competitive interaction with cations, particularly calcium, seems to be the cause of the decrease in toxicity. Gaus et al. (1985), however, reported that the toxicity of copper did not change within a

wide range of water hardness. Our own results with *D. magna* fully corroborate the finding of most authors that potassium dichromate becomes less toxic when water hardness increases. The acute toxicity changes of detergents under varying water hardness conditions are less uniform and clearly both chemical and species dependent. Alkylsulphates for example, are less toxic to fathead minnows in hard water (Henderson et al., 1959) and to rainbow trout and goldfish in soft water (Tovel et al., 1974). The sensitivity of the same fish species for non-ionic detergents is unaffected by water hardness (Tovell et al., 1975) but *D. magna* in turn is more sensitive to such detergents in soft water (Maki and Bishop, 1979). Our finding with sodium laurylsulphate using *D. magna* are in contradiction with those results of Tovel et al. (1974) and corroborate the hypothesis that the influence of water hardness on toxicity is not only chemical but also species dependent. According to Cairns and Scheier (1957, 1958), the hardness of the dilution water has a greater effect on the toxicity of zinc to snails and bluegills than do differences in temperature. Our own experiments dealing with the effect of temperature and water hardness on the toxicity of potassium dichromate to *D. magna* fully confirm the findings of the former authors. On the other hand, the sensitivity of the waterfleas to SLS was influence more by temperature than by water hardness.

Interaction of environmental variables on toxicity

Significant interaction on the toxicity was found with both chemicals for temperature–salinity and temperature–water hardness combinations for the three test animals. One exception was sodium laurylsulphate which showed no significant interactive effect between the two abiotic parameters for *B. plicatilis.*

Magnitude of toxicity variation

The necessity to take environmental factors into consideration when determining toxicity thresholds of chemicals is clearly demonstrated by our results with *A. salina* and *D. magna.* For both species significant differences were noted between LC50/EC50-values determined under 'extreme' conditions versus those found in 'standard' testing conditions. Standard procedures for *D. magna* toxicity tests issued by different governments and international organisations unfortunately do not clearly specify the characteristics of the waters to be used as dilution water, despite the warning by Muller (1980) that this is an important prerequisite. Our findings with the waterflea clearly demonstrate the need for more detailed standard procedures. Indeed, when comparing, for both chemicals used in the present study, the results obtained in the 21 °C series (which is close to the temperature outlined in the EEC standard) at different water hardness levels, to those obtained under 'standard' conditions (20 °C and 250 mg $CaCO_3 \cdot l^{-1}$), the largest difference in toxicity were noted for the soft water category (80 mg $\cdot l^{-1}$). Since, according to the standard protocol, bioassays with *D. magna* may be carried out in a water hardness range of 40

to 200 $mg \cdot l^{-1}$, the outcome can thus eventually vary by a factor of one order of magnitude! For *B. plicatilis* effect differences resulting from changing environmental conditions were much smaller and did not exceed the variability between replicates; according to literature, such variations can easily be a factor 2 (Canton and Adema, 1978; Buikema et al., 1980; Nebeker, 1982; Grothe and Kimerle, 1985; Lewis and Weber, 1985). In the present study the variability between replicates of the bioassays with *B. plicatilis* is in part due to the fact that the test procedure was new and not entirely mastered. In the meantime a standard protocol has since been developed (Snell and Persoone, 1987).

In Table IV the toxicity of the two chemicals to the three test species, in different sets of environmental conditions is presented in the form of two ratios. The highest ratio of LC50s (EC50s) for tests carried out in 'standard' laboratory conditions to those performed in other conditions (up to the limits of the tolerance range) gives an estimation of variation amplitude of toxic effects which may occur in nature. The ratio of the highest to the lowest LC50 noted in the various combinations performed on the other hand, gives an indication of how much environmental conditions may influence the toxicity of the compounds under investigation.

The data indicate that within the limits of the present study, toxicity variation is as low as a factor 3, whereas in others it may exceed two orders of magnitude.

With regard to the predictive value of standard laboratory bioassays as compared to 'real world' situations (as mimiced by the factorial combination) it appears that effects found in the laboratory can diverge from as little as a factor 2 to as much as a factor 27 from those that could eventually be encountered in the field. These variations, however, do not necessarily always imply an increase of toxicity. In fact, as clearly seen in Figs. 1, 2 and 3, changes in environmental conditions from standard lab conditions can result in an increase as well as a decrease of toxicity.

Consequently, laboratory data are in some cases underestimating and in others overestimating the toxic effects of chemical compounds in real world situations.

CONCLUSIONS

The major aim of this study was to determine the importance which variations in testing conditions can have on toxicity thresholds for aquatic invertebrates. The information forwarded is but a first step in this direction and only took into consideration a few of the many abiotic and biotic variables which may influence the sensitivity of aquatic biota. Nevertheless the outcome again confirms that the problem should be addressed from three sides: the environmental factor(s), the chemical compound(s) and the test species.

Although the experiments carried out thus far revealed interesting general information, many more data are needed to determine if it may ever be possible (e.g. through factorial experiments and Q.S.A.R.-analysis) to draw 'sensitivity variation patterns' for different classes of chemicals for different groups of organisms in dif-

ferent sets of natural conditions. Until such information has been generated, laboratory tests should for the benefit of hazard prediction be conducted in different sets of environmental conditions reflecting the range of 'potential situations' found in nature.

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