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Short communication

Effects of temperature and chemical formulation on the acute toxicity of pentachlorophenol to *Simocephalus vetulus* (Schoedler, 1858) (Crustacea: Cladocera)

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Abstract The influence of temperature on the acute toxicity of a technical formulation (86%) and pure formulation (99%) of pentachlorophenol (PCP) to less than 24-h-old *Simocephalus vetulus* neonates was determined with 48-h static toxicity tests. The technical grade PCP was significantly more toxic to *S. vetulus* than the pure PCP ($P < 0.05$). Sensitivity of *S. vetulus* to technical PCP also significantly increased with temperature ($P < 0.05$), but a significant temperature effect was not found with the pure PCP. The mean 48-h LC_{50} values for neonates exposed to technical PCP were 140 and 199 μg l^{-1} at 22°C and 16°C, respectively, and for those exposed to pure PCP were 262 and 304 μg l^{-1}, respectively.

Keywords cladoceran; *Simocephalus vetulus*; pentachlorophenol; acute toxicity; temperature

INTRODUCTION

The organochlorine compound pentachlorophenol (PCP) was extensively used in New Zealand for the preservation of sawn timber for almost 40 years until 1988. Recent investigations have shown that several sites used for timber treatment are heavily polluted with PCP, leading to extensive contamination of adjacent aquatic environments (Shaw 1990; National Task Group 1992; Gifford et al. 1993). PCP used for timber treatment in New Zealand was invariably industrial grade. Such formulations are known to be contaminated with other organochlorine compounds (WHO 1987) including other chlorophenols, chlorinated ethers, polychlorodibenzofurans (PCDFs) and polychlorodibenzodioxins (PCDDs). New Zealand's contaminated sites are likely to contain a range of such substances. The toxicity of PCP, in its pure form, has been extensively investigated and it has even been proposed as an organic reference toxicant (Davis & Hoos 1975; Adelman & Smith 1976; Lee 1980). However, little attention has been paid to the potentially toxic influence of formulation contaminants.

Aquatic toxicity testing is in its infancy in New Zealand and few studies have examined the suitability of New Zealand aquatic biota for laboratory toxicity tests. Freeman (1986) evaluated the potential of four acute toxicity tests, of which three were recommended: the Beckman Microtox test, the *Selenastrum capricornutum* 96-h growth inhibition test, and the *Daphnia magna* 24-h immobilisation test. These, and especially the last involving parthenogenetic neonates of the cladoceran *Daphnia magna*, have become established internationally as standard test procedures in the field of ecotoxicology.

Cladocerans play an important role in the freshwater environment. Often they predominate in the zooplankton and littoral microfauna and are therefore of major importance in the food chain, converting phytoplankton and bacteria into animal protein and making it available to higher animals such as fish. They are used extensively in toxicity tests because of their relative ease of culture in the laboratory, short generation time, and their sensitivity to potential environmental pollutants. Females reproduce parthenogenetically, thereby minimising genetic variability of individuals within

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and between tests (Leeuwangh 1978). Rapid population growth is possible with asexual reproduction, and under suitable conditions clutches may be produced every 2–3 days, providing a continual supply of animals for use in toxicity tests. *Daphnia magna* has been the most commonly used cladoceran in aquatic toxicity tests worldwide, but as this species does not occur in New Zealand it has only limited potential as a toxicity test species in this country. Information on the sensitivity of cladocerans found in New Zealand is limited to data from studies on these species overseas and a single study in New Zealand by Hickey (1989). *Simocephalus vetulus* is a littoral or shallow-water cladoceran which is found throughout New Zealand (Chapman & Lewis 1984) and is relatively large and easy to handle compared to many other cladocerans. These factors and the previous use of this species in toxicity tests (Mount & Norberg 1984; Hedtke et al. 1986; Hickey 1989) suggest that *S. vetulus* has potential as a test species in New Zealand.

Temperature significantly affects cladoceran life histories (Brown 1927; Korpelainen 1986) and in some instances may also affect a chemical's toxicity, although Mayer & Ellersieck (1986) report that 40% of the time temperature does not affect chemical toxicity. Because PCP increases metabolic oxygen demand (Weinbach 1957), its toxicity might be expected to increase with temperature, though tests using *Daphnia magna* and *Daphnia pulex* have shown no influence of temperature on PCP toxicity (Lewis & Horning 1991). Hedtke et al. (1986) found PCP toxicity to *S. vetulus* to increase with temperature although this study employed seasonal collection of test organisms requiring temporal separation of comparative tests. The test temperatures chosen for the current study lie within the range of summer temperatures experienced in New Zealand freshwaters. The higher test temperature of 22°C is near the summer maximum and close to the optimum temperature of 21°C for maintaining a healthy culture of *S. vetulus* (Sharma & Pant 1982, 1984). The lower temperature of 16°C was chosen to observe a temperature effect of toxicity without imposing significant thermal stress on animals cultured at the higher temperature.

As part of a more extensive study to evaluate the suitability of *S. vetulus* as a standard toxicity test organism (Willis 1994), we examined the toxicity of two formulations of PCP at 16° and 22°C to < 24-h-old *S. vetulus* neonates under controlled laboratory conditions.

METHODS

Culture conditions

A laboratory culture of *Simocephalus vetulus* was established from parthenogenetic females collected from Lake Rotokauri (NZMS260 S14 040800), Hamilton. The culture was maintained in the laboratory for 2 months before toxicity testing.

Five or more static cultures, each containing about 20 adult *S. vetulus*, were maintained in uncovered 500 ml glass beakers at 22 ± 1°C under ambient laboratory lighting (10 μE $m^{-2}s^{-1}$) and natural photoperiod. Cultures were maintained in soft synthetic freshwater (USEPA 1991) prepared using deionised water and reagent grade chemicals. Final pH was 7.8 ± 0.2 and hardness 40–48 mg $CaCO_3$ l^{-1}. Cultures were fed daily with a modified yeast, Cerophyll, and trout chow mixture (YCT - USEPA 1991), and the green alga *Chlamydomonas*. About 8–10 ml YCT and 2 ml of algae (4–6 × 10^6 cells ml^{-1}) were fed to the cultures each day which allowed a clearing of turbidity within 24 h.

Toxicity test protocol

About 24 h before beginning a test, gravid females with fully developed embryos in their brood chamber were sorted into 500 ml beakers to ensure that a sufficient number of neonates < 24 h old would be available for the test the following day. At the start of the test, neonates were randomly selected and placed individually into the test vessels using a Pasteur pipette. To ensure that the test solutions were diluted as little as possible, neonates were transferred in a minimum of culture medium.

The neonates were exposed for 48 h under static conditions to selected concentrations of technical grade PCP and pure PCP at two temperatures (16 and 22°C). Each test consisted of 4 PCP concentrations and a control. There were five replicates per concentration with 10 neonates in each beaker, giving a total of 50 animals per concentration. The test vessels (50 ml borosilicate glass beakers), each containing 50 ml of test solution, were placed in a temperature controlled water bath. Temperature was continuously monitored for the duration of the test and each test was replicated five times.

For tests at 22°C (culture temperature), the test chambers were equilibrated to the test temperature before the neonates were introduced. For tests at 16°C, neonates were placed in the test vessels at 22°C and the temperature was then progressively lowered to 16°C over a period of 2 h.

Toxicity tests were conducted under the same lighting and photoperiod regimen used for the adult cultures. The tests vessels were not aerated or covered and the animals were not fed during the test. The pH of all test solutions was measured at the beginning and end of each test. At test completion the animals were checked for mortality using failure to move after gentle prodding as the criterion. Tests were considered valid if control survival was greater than 80% (Weber & Peltier 1981).

The 48-h LC_{50} values and their confidence limits for each replicated toxicity test were calculated using Probit analysis (Finney 1971). Differences between test variances were compared using the *F*-test. Differences between mean LC_{50} values at 16°C and 22°C, and pure and technical grade PCP were compared using Student's *t*-test. Test precision was determined by calculating the relative standard deviation of the replicate LC_{50} values and expressed as percentage coefficient of variation (%CV) = (standard deviation × 100) / mean.

PCP formulations

A pure preparation (nominally 99%) and a technical formulation (nominally 86%) of pentachlorophenol supplied by Aldrich Chemicals were used in toxicity tests. The composition of these preparations were analysed by gas chromatograph mass spectrometry (Hewlett Packard 5890 GC with 5970 Mass Selective Detector) in the Department of Chemistry at the University of Waikato. The technical formulation was found to contain c. 20% tetrachlorophenol and small amounts (c. 5%) of other contaminants stated by the manufacturers to be dibenzodioxins and dibenzofurans. The pure PCP formulation was found to be contaminated with c. 5% of the minor impurities stated above.

Stock solutions were prepared by dissolving PCP in 95% ethanol (1 g l^{-1}) and were stored in the dark at 4°C. Test solutions were prepared by dilution of the PCP stock solution in soft synthetic water (pH 7.6–8.0, hardness 40–48 mg l^{-1} $CaCO_3$). Appropriate amounts of 95% ethanol were added to the controls.

RESULTS

The acute toxicity of pentachlorophenol was influenced by PCP formulation and temperature (Table 1). The technical grade PCP formulation was significantly more toxic to *S. vetulus* than the pure PCP ($P < 0.05$) at both 16 and 22°C. Sensitivity of *S. vetulus* to technical grade PCP was increased at the higher test temperature ($P < 0.05$). There was no significant temperature effect with pure PCP, although LC_{50} values were higher (indicating decreased toxicity) at 16°C with pure PCP. Test precision, or amount of variation between replicated tests as indicated by %CV, was slightly better at 22°C (16 and 17%) than at 16°C (18 and 24%) for both PCP formulations (Table 1).

Temperature did not significantly affect control survival (Table 2). Mean control survival was 90% at 16°C and 92% at 22°C. The range in control survival was greater at 16°C (48–100%), though very few tests were invalidated at either temperature by poor survival. Both the test standard deviations and the standard deviation for control survival were greater at the lower test temperature; however, this difference was not significant (*F*-test).

DISCUSSION

For temperatures which fall well within the lethal limits for a species, it is tempting to believe that sensitivity to toxicants will increase with

Table 1 48-h LC_{50} values (µg PCP l^{-1}) for < 24-h-old *Simocephalus vetulus* neonates. Means differ significantly at $P < 0.05$ (^{a,b}) and $P < 0.001$ (^{c}).

	Technical PCP		Pure PCP	
Test No.	22°C	16°C	22°C	16°C
1	121	178	330	349
2	155	231	218	253
3	162	169	257	348
4	152	171	277	201
5	111	244	230	368
Mean	$140^{a,c}$	$199^{a,b}$	262^{c}	304^{b}
SD	23	36	45	73
%CV	16	18	17	24

Table 2 Percentage control survival at 16 and 22°C for *Simocephalus vetulus*. Tests were invalidated when control survival was <80%.

	16°C	22°C
No. of tests	11	12
Range	48–100	71–100
Mean	90	92
SD	14.85	9.54
%CV	16.5	10.4
No. of invalid tests	1	2

temperature owing to an elevation of the organism's metabolic rate. However, this is not always so. The toxicity of DDT to *Simocephalus serrulatus* is greatest at lower temperatures (Sanders & Cope 1966). Lewis & Horning (1991) found no significant difference in the toxicity of sodium pentachlorophenate (NaPCP) to either *Daphnia pulex* or *Daphnia magna* neonates following an increase in temperature from 20 to 26°C. Although Hedtke et al. (1986) found *S. vetulus* adults to be significantly more sensitive to NaPCP at 24°C than at 18°C, this was a seasonal alteration of temperature and other factors such as nutritional status may have contributed to the result. In the present study the toxicity of PCP to *S. vetulus* was increased at the higher temperature of 22°C.

Differences in response may be attributed to both species and chemical differences, or to the period of temperature acclimation before the tests. Lewis & Horning (1991) acclimated *D. pulex* to each test temperature for 3 months before testing, whereas in the present study, *S. vetulus* was only acclimated to the higher of the two test temperatures. Cowgill et al. (1985) suggest that when test animals have been adjusted for at least 1 year to the test temperature, habitat, and diet, variability between tests will be less than when the animals have been acclimated for a shorter period of time. The high control survival (c. 90%) observed during this study at both test temperatures, and an overall test precision of 16–24% CV, suggest that test validity was not jeopardised by the lack of acclimation at the lower test temperature. Although an increase in the variability of test results was observed at the lower test temperature, possibly as a result of the abrupt temperature change at test initiation, this was not statistically significant. Such abrupt changes in temperature on exposure to a toxicant may add some environmental realism to a test regime as toxicants are often introduced to the environment in effluent discharges which may be different in temperature from that of the environment, although it is acknowledged that this introduces a compounding variable. However, any possible thermal shock induced by acute temperature change would be expected to increase mortality at the lower test temperature rather than the opposite effect observed.

Comparisons between the results of different toxicity tests are important to validate test procedures performed in different laboratories, but are complicated because test conditions such as water quality, temperature, and pH may vary between different studies or are not reported. In tests using compounds such as pentachlorophenol, the purity of the chemical used varies with the formulation and the manufacturer. Despite these difficulties comparisons between different studies still provide some indication as to the validity of the results from a particular series of tests. The acute toxicity of pure PCP (c. 95%) to *S. vetulus* at 22°C observed during this study (262 μg l^{-1}, pH 7.8) compares well with other reported values. At a temperature of 24°C (pH 7.8–8.0) Hedtke et al. (1986) reported a 48-h LC_{50} value of 204 μg PCP l^{-1} (94%). Hickey (1989) obtained a 24-h LC_{50} of 206 μg PCP l^{-1} (analytical) at 20°C (pH 7.9), and Mount & Norberg (1984) determined a 48-h LC_{50} of 217 μg PCP l^{-1} (temperature and PCP grade not reported).

Some of the toxicity of technical formulations of PCP may be attributed to contaminants, the nature and amount of which vary from one company to another and from batch to batch. The technical grade PCP (86%) used in this study was found to contain c. 20% tetrachlorophenol (TCP) which has been shown to be more toxic than PCP to *S. vetulus* by Mount & Norberg (1984), with LC_{50} values of 145 μg TCP l^{-1} and 217 μg PCP l^{-1}. Technical formulations of PCP also contain varying amounts of dibenzodioxins and dibenzofurans which are possibly more hazardous than the PCP, although their toxicity to cladoceran species is unknown. The technical grade PCP used in this study was significantly ($P < 0.05$) more toxic to *S. vetulus* than the pure PCP. In contrast, Stephenson et al. (1991) found technical PCP (86%, Stanchem) to be either as toxic or less toxic than pure PCP (99%, Aldrich) to three age classes of *D. magna*, suggesting that the contaminants in the technical formulation imparted no additional acute toxicity. The technical formulation used was not supplied from the same company as the PCP used in the present study and it was not chemically analysed by Stephenson et al. (1991), hence the contaminants present may have differed or been present in lower quantities in their formulation. The species difference may also have contributed to the different response observed. Unlike *S. vetulus*, *D. magna* is more sensitive to PCP than it is to TCP with LC_{50} values of 143 μg PCP l^{-1} and 406 μg TCP l^{-1} (Mount & Norberg 1984). Further work examining the specific toxicity of minor formulation contaminants is required to fully evaluate the nature of the enhanced toxicity of industrial grade PCP to *Simocephalus vetulus*.

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REFERENCES

Adelman, I. R.; Smith, L. L. 1976: Fathead minnows (*Primephales promelas*) and goldfish (*Carassius auratus*) as standard fish in bioassays and their reaction to potential reference toxicants. *Journal of the Fisheries Research Board of Canada 33*: 209–214.

Brown, L. A. 1927: Temperature coefficients for development in cladocerans. *Proceedings of the Society for Experimental Biology and Medicine 25*: 164–165.

Chapman, M. A.; Lewis, M. H. 1984: *An introduction to the freshwater Crustacea of New Zealand.* Auckland, Collins. 261 p.

Cowgill, U. M.; Takahashi, I. T.; Applegath, S. L. 1985: A comparison of the effect of four benchmark chemicals on *Daphnia magna* and *Ceriodaphnia dubia-affinis* tested at two different temperatures. *Environmental toxicology and chemistry 4*: 415–422.

Davis, J. C.; Hoos, R. A. W. 1975: Use of sodium pentachlorophenate and dehydroabietic acid as reference toxicants for salmonid bioassays. *Journal of the Fisheries Research Board of Canada 32*: 411–416.

Finney, D. J. 1971: *Probit analysis.* Cambridge University Press. 333 p.

Freeman, M. C. 1986: Aquatic toxicity tests: Comparative assessment of four acute tests and their application in New Zealand. *Water and Soil technical publication. 28.* 36 p.

Gifford, J. S.; Hannus, I. M.; Judd, M. C.; McFarlane, P. N.; Anderson, S. M.; Amoamo, D. H. 1993: Assessment of chemical contaminants in the Lake Rotorua catchment. Unpublished report for the Bay of Plenty Regional Council. New Zealand Forest Research Institute Limited. 93 p.

Hedtke, S. F.; West, C. W.; Allen, K. N.; Norberg-King, T. J.; Mount, D. I. 1986: Toxicity of pentachlorophenol to aquatic organisms under naturally varying and controlled environmental conditions. *Environmental toxicology and chemistry 5*: 531–542.

Hickey, C. W. 1989: Sensitivity of four New Zealand cladoceran species and *Daphnia magna* to aquatic toxicants. *New Zealand journal of marine and freshwater research 23*: 131–137.

Korpelainen, H. 1986: The effects of temperature and photoperiod on life history parameters of *Daphnia magna* (Crustacea : Cladocera). *Freshwater biology 16*: 615–620.

Lee, D. R. 1980: Reference toxicants in quality control of aquatic bioassays. *In*: Buikema, A. L.; Cairns, J. *ed.* Aquatic invertebrate bioassays, pp. 188–199. American Society for Testing and Materials. ASTM STP 715.

Leeuwangh, P. 1978: Toxicity tests with daphnids: Its application in the management of water quality. *Hydrobiologia 59*: 145–148.

Lewis, P. A.; Horning, W. B. 1991: Differences in acute toxicity tests results of three reference toxicants on *Daphnia* at two temperatures. *Environmental toxicology and chemistry 10*: 1351–1357.

Mayer, F. L.; Ellersieck, M. R. 1986: Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. U.S. Department of Interior Fish and Wildlife Service, Washington, DC. *Resource publication 160.*

Mount, D. I.; Norberg, T. J. 1984: A seven-day life-cycle cladoceran toxicity test. *Environmental toxicology and chemistry 3*: 425–434.

National Task Group Study Team Report 1992: New Zealand national task group on site contamination from the use of timber treatment chemicals: Pentachlorophenol risk assessment pilot study. Camp Scott Furphy.

Sanders, H. O.; Cope, O. B. 1966: Toxicities of several pesticides to two species of cladocerans. *Transactions of the American Fisheries Society 95*: 165–169.

Sharma, P. C.; Pant, M. C. 1982: Population dynamics of *Simocephalus vetulus* (O. F. Muller). *Journal of plankton research 4*: 601–618.

Sharma, P. C.; Pant, M. C. 1984: An energy budget for *Simocephalus vetulus* (O. F. Müller) (Crustacea: Cladocera). *Hydrobiologia 117*: 37–42.

Shaw, C. P. 1990: Timber preservation chemicals in the New Zealand environment: pentachlorophenol and chlordane. Unpublished report for the Ministry of the Environment. Cawthron Institute, Nelson. 52 p.

Stephenson, G. L.; Kaushik, N. K.; Solomon, K. R. 1991: Acute toxicity of pure pentachlorophenol and a technical formulation to three species of *Daphnia*. *Archives of environmental contamination and toxicology 20*: 73–80.

USEPA 1991: Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 4th ed. Weber, C. I. *ed.* Environmental Monitoring Systems Laboratory. U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA- 600/4–90–027.

Weber, C. I.; Peltier, W. 1981: Effluent toxicity screening test using *Daphnia* and mysid shrimp. Environmental Monitoring and Support Laboratory. U.S. Environmental Protection Agency, Cincinnati, Ohio, pp.1–49.

Weinbach, E. C. 1957: Biochemical basis for the toxicity of pentachlorophenol. *Proceedings of the National Academy of Science 43*: 393–397.

WHO 1987: Pentachlorophenol. *Environmental health criteria 71*. World Health Organisation, Geneva.

Willis, K. J. 1994: The effect of temperature on the acute toxicity of pentachlorophenol to early life stages of *Simocephalus vetulus* and *Oncorhynchus mykiss*. Unpublished MSc thesis, University of Waikato.