Bull. Environ. Contam. Toxicol. (2004) 73:392–397

DOI: 10.1007/s00128-004-0441-x

Bulletin of Environmental Contamination and Toxicology

Acute Aquatic Toxicity of N-Methyl-2-Pyrrolidinone to *Daphnia magna*

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Received: 8 December 2003/Accepted: 13 May 2004

Volatile organic compounds (VOCs) are widely employed in lubricating, coating, and cleaning processes by many manufacturers. These solvents are largely released into the working and ambient environments through vaporization and diffusion. A significant proportion of these solvents are health hazards and even possible carcinogens, for instance, trichloroethylene. In order to protect human health and diminish the environmental burden of VOCs, industries are forced to find suitable replacements. N-methyl-2-pyrrolidinone (NMP) is a successful substitute in this matter. For example, the Marine Corps Multi-Commodity Maintenance Centers found that more than 97% of NMP vapor would be transferred into the aqueous phase in a wet scrubber (Campbell and Striebig 1999).

NMP is mainly used in the electronics industry but also in the cleaning and stripping of polymers and paints (Jonsson and Akesson 2003). The health effects of NMP to workers are neglected in the U.S. because the permissible exposure level (PEL) of NMP has not regulated by US OHSA (NIOSH 2000). However, a case report of stillbirth suggested that NMP resulted in this tragedy (Solomon et al. 1996). Thus, the developmental, maternal, and reproductive toxicity of NMP to rats has recently been studied extensively (Saillenfait et al. 2003; Saillenfait et al. 2002; Solomon et al. 1995; Hass et al. 1995; Hass et al. 1994). In the US, an estimated 71,000 workers were exposed to NMP, and 2.7 million consumers might be exposed (Solomon et al. 1996). Furthermore, based on its Henry's law constant of 1.6×10^{-3} Pa m^3/mol, more than 99% of NMP released into the environment is expected to partition into water (IPCS 2001). In fact, the existence of NMP in waters was identified worldwide, even in drinking-water supplies (IPCS 2001).

In Taiwan, the electronics industry is huge and is confined to a small area. The aquatic environment in the Taiwan science industrial park area, which includes almost 90% of the electronics manufacturers in Taiwan, may be contaminated by NMP. There has been no investigation into this matter in Taiwan, however. Very few data on the aquatic toxicity of NMP are available. This study examined the acute aquatic toxicity of NMP to *Daphnia magna* to assess the potential impact of

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NMP on the aquatic environment.

MATERIALS AND METHODS

Daphnia magna were supplied by the LUZCOGI Inc., Germany. Mass and individual cultures were maintained following the USEPA specifications (USEPA 1994; 2002) and fed with green algae (*Chlorella spp.*). *Daphnia magna* were cultivated in the institute for several generations using soft water with minimum algae food. The photoperiod was 16 h L：8 h D and the temperature was 25 - 26 °C. Growth and life span of the organisms were stable in the institute before usage for acute tests. Reference toxicity tests using sodium chloride were routinely assayed to monitor the responses of the organisms. All the test data from inoculated daphnids met with the guideline of the USEPA (USEPA 1994; 2002).

Daphnids younger than 24 hours were used in the acute toxicity assay with various NMP solutions. Water fleas were exposed to a series of five NMP concentrations (0.6, 1.25, 2.5, 5, 10 mg/L) as well as the dilution water. The dilution water was prepared by dissolving salts into softened and distilled water following the USEPA guideline (2002). The HPLC-grade NMP (purity > 99.9%) was purchased form Sigma-Aldrich and used to prepare the above test solution. The NMP concentration in the stock solution was confirmed by a GC-MS (Agilent Technology 6890N Network GC System, Agilent 5973 Network Mass Selection Detector, Column: HP-5MS). The operation conditions were as followed: injection temperature: 300 °C; flow rate: 2.7 mL/min; oven temperature: initial 40 °C for 8 minutes, then 4 °C/min to 90°C followed 20°C/min to 200 °C and hold for one minute. The recovery was 87.1% ± 0.7% (n = 4). Five organisms were transferred into each vessel with quadruple groups for the assay. The temperature, pH, dissolved oxygen and conductivity of test solutions were measured and recorded just before and after the tests with appropriate direct-reading instruments. The temperature, pH, dissolved oxygen and conductivity of test solutions were stable at 23 °C, 7.0, 7.8 mg/L, and 286 μmhos/cm, respectively. After 24-h and 48-h static non-renewal exposure, the survival numbers for each group was counted. Water flea activities were observed by eye. Test groups were gently stirred and organisms were observed for 10 ~ 15 seconds. Death was then determined for organisms lacking any movement after stirring. Survival data were then analyzed using the Toxstat^{TM} package with the probit procedure (Gulley 1996).

RESULTS AND DISCUSSION

Control survival was always 100% and met the USEPA criteria. The 24-hour and 48-hour median lethal concentrations (LC50) of NMP to *Daphnia magna* were estimated to be 2.50 mg/L (95% C.I.: 1.8 - 3.5 ppm) and 1.23 mg/L (95% C.I.: 1.0 - 1.5 ppm), respectively (Table 1). The estimated mortality curve is shown in Figure 1.

Table 1. Acute toxicity of some organic compounds (mg/L) to *Daphnia magna* and *Vibrio fischeri*

Compound	*Daphnia magna*		*Vibrio fischeri,* (5-min)
	24-h LC50	48-h LC50	
2-propanol			33800[j]
Acetone			16900[i]
Acetonitrile			21200[j]
Amitriptyline	1.15[a]		
Carbon Tetreachloride	69.37[c]		
Chloroform	64.23[c]		670[j]
Diazepan	4.27[a]		
Dichloromethane	1914[a]		3600[j]
Digoxin	24.21[a]		
Diisopropyl methylphosphonate		267[g]	
Dimethyl sulfoxide		24.6[h]	
Disulfoton	0.055[g]		
Ethanol	9788[b]	5680[b]	41700[j]
Ethyl Alcohol	13715[a]		
Ethylene Glycol	48582[a]		10900[j]
Formaldehyde	57		
Hexachlorophene	1.99[a]		
Lindane	14.54[a]		
Malathion	0.35[a]		
Methanol	4816	3289[b]	85100[j]
Methyl Alcohol	20803[a]		
Paraoxon	0.00055[b]	0.00019[b]	
Paraquat	1.17[a]		
Parathion	0.00219[b]	0.00216[b]	
p-Cresol	14[f]		
Pentachlorophenol	0.86[a]		
Phenol	9.13[a]		
Toluene	8[e]		
NMP	2.50[l]	1.23[l]	1500[k]

[a.] Lilius et al. 1994; [b.] Guilhermino et al. 2000; [c.] Khangarot and Ray 1988; [d.]Fernandez-Casalderrey et al 1994; [e.] Janssen and Persoone 1993; [f.] Kuhn et al. 1989; [g.] Burton et al. 2002; [h]. Barbosa et al. 2003; [i.] Layton et al. 1999; [j.] Cassells et al. 2000; [k.]Campbell and Striebig 1999; [l.]this study

The potential environmental hazard owing to NMP pollution was also of some concern, especially in the region of high-tech industry. Ambient NMP is expected to undergo atmospheric wet deposition (HSDB 1997), and NMP is also very highly mobile in soil (Swann et al. 1983). In addition, NMP is very stable and is not degraded highly mobile in soil (Swann et al. 1983). In addition, NMP is very stable and is not degraded by chemical hydrolysis (Akesson 1994; IPCS 2001). Therefore, the potential impact of NMP on the aquatic environment becomes crucial in areas where industries use great amounts of NMP. Compared with some other organic compounds, the toxicity of NMP is more severe than those of acetone, acetonitrile, dichloromethane, ethanol, methanol, and 2-propanol for *Vibrio fischeri* (Table 1). The data clearly indicate that NMP may severely affect the aquatic ecosystem once it is released or transported into the aquatic environment. Our results show that NMP can be recognized as the most toxic organic solvent to *Daphnia magna*, and only some pesticides are more toxic than NMP (Table 1). This paper calls for more detailed studies with regard to the impact of NMP on the aquatic environment.

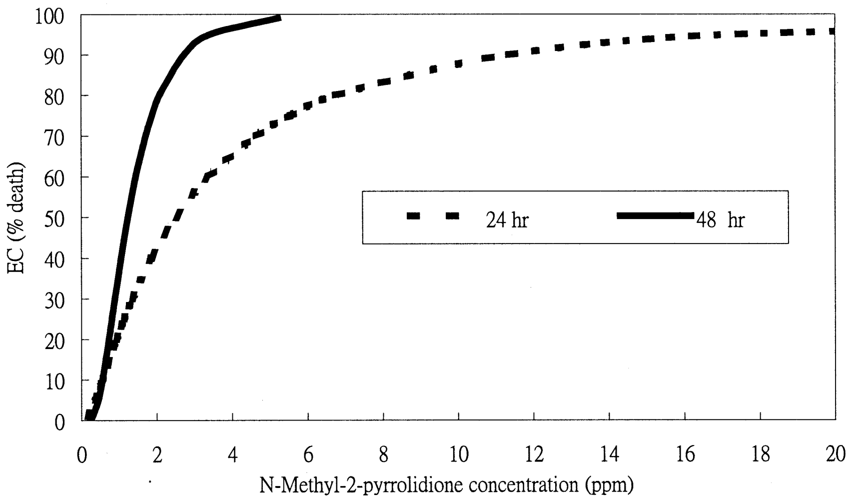

Figure 1. The estimated mortality of Daphnai magna vs. NMP concentration

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