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Effects of Naphthalene on the Hemoglobin Concentration and Oxygen Uptake of *Daphnia magna*

Julie Y. Crider, Jerry Wilhm, and H. James Harmon

Department of Zoology, Oklahoma State University, Stillwater, OK 74078

Naphthalenes are found in all crude oil and are the predominant water soluble components of many refined oil products. Naphthalene and alkyl naphthalenes are probably the major toxic substances found in water soluble fractions of petroleum (ANDERSON et al. 1974a). Thus, a need exists for testing the effects of naphthalene on the biota.

Daphnia magna is a commonly used crustacean in aquatic toxicity bioassays. Results using these crustaceans are often less variable than those using fish (CROSBY & TUCKER 1966). Daphnids are relatively easy to maintain and are highly sensitive to toxic chemicals. They have been used in tests with crude oil and emulsifiers (DOWDEN 1965), insecticides (SANDERS & COPE 1966, SIGMON 1979), refinery wastes (DORRIS et al. 1974), and aquatic herbicides (CROSBY & TUCKER 1966).

In addition to testing survival of organisms exposed to a toxicant, various physiological tests have been proposed. Certain toxicants alter oxygen consumption (VIEHOEVER & COHEN 1938b, SIGMON 1979). The concentration of hemoglobin has been shown to vary with different environmental stimuli (HOSHI & KOBAYASHI 1972). Hemoglobin concentrations and respiration rates may be correlated (KOBAYASHI 1974), and measuring both parameters should provide a better indication of acute effects of naphthalene on the mortality, concentration of hemoglobin, and oxygen consumption of D. magna.

MATERIALS AND METHODS

The stock cultures of Daphnia were housed in 21 liter glass aquaria filled with aged tap water with the following environmental conditions: temperature 22-25 C, 7.9-9.0 pH, and conductivity 510-875 µmhos. High dissolved oxygen levels resulted from mechanical aeration. An automatic timer attached to two 40 watt fluorescent lights produced a 16 h light photoperiod.

Food was prepared every 2-3 wk by mixing 5 g of Purina trout chow and 10 g of alfalfa in 250 ml of water for about 2 min in a Waring laboratory blender, straining through cloth, and refrigerating in a closed container. The stock cultures were fed every

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other day; a rate of about 1 ml/wk (BIESINGER 1975).

Organisms of known age were obtained by placing two female Daphnia from the stock cultures in 100 ml of aged tap water in a 125 ml Erlenmeyer flask (ANDERSON 1944). These flasks were kept in a room temperature water bath (aeration was not provided). Two drops of food suspension were added every other day. The vessels were checked daily for dead organisms and neonate production. All neonates from a 24 h period were housed in 1 liter beakers and placed in a 25 C water bath.

Acute naphthalene tests conducted in 1 liter beakers containing 500 ml of aged tap water. Because of the limited solubility of naphthalene in water, 95% ethanol was used to make a stock solution. Daphnids of known age were pipetted into the beakers which were placed in a covered 25 C water bath. The test organisms received a 16 h light photoperiod and no aeration.

The initial and final concentrations of naphthalene in the test solutions were measured on an Aminco-Bowman Spectrofluorometer using excitation and emission wavelengths of 290 and 330 nm, respectively. Relative emission intensity of standard solutions allowed the quantitation of naphthalene in the test solutions by regression analysis.

Short term naphthalene studies were conducted to calculate LC50 values and physiological responses. Daphnia of 24 h were fed initially 0.25 ml food/l and the pH, dissolved oxygen and temperature, conductivity, swimming movements, and the number of survivors were determined at 0, 24, and 48 h. These experiments were run at least three times and the dosage-mortality curves were determined by the use of probit and regression analyses. Physiological studies were made for concentrations of 1, 5, and 10 mg/l. The animals were conditioned to 25 C for approximately 1 wk. A concentration of 0.95% ethanol was used in both the treatment and control solutions. Separate control experiments on ethanol were run to study the effects of this solvent.

Oxygen consumption of Daphnia was measured polarographically. The basic monitoring system consisted of oxygen electrode, a glass reaction chamber, a magnetic stirrer, a constant temperature water circulator, a Johnson Research Foundation (Univ. of Pennsylvania, Philadelphia) oxygen electrode amplifier and a strip chart recorder. The water jacketed glass chamber (25 C) contained a steel mesh insert to prevent the Daphnia from injury by the magnetic stir bar. Before oxygen consumption values were measured, organisms were placed in 200 ml filtered (0.22 µm pore size) test water to minimize bacterial interference. The beaker of Daphnia was then placed in 25 C water until the system was ready for measurements. Five adults were added to the chamber and oxygen consumption was recorded for 6-8 min. Oxygen uptake was determined from the difference between the linear rate of the organisms and the rate of the water alone.

A carboxyhemoglobin method was used to measure total hemoglobin. Ten experimental animals were pipetted onto a clean surface and rinsed with distilled water three times. After blotting, the Daphnia were transferred to a homogenizer vessel containing 1 ml of 0.4% ammonium hydroxide. The organisms were macerated by hand for about 1 min (HILDEMANN & KEIGHLEY 1955). The homogenate was transferred to a 2 ml syringe attached to a Millipore apparatus and run through a polycarbonate filter (0.04 µm pore size) to remove debris. The filtrate was collected in a small test tube which was sealed immediately and put on ice. Daphnid hemoglobin was converted to carboxyhemoglobin by bubbling carbon monoxide gas into the sample for 1 min. The samples were measured for absorbance at 419 nm using a Beckman Model 24 spectrophotometer. The millimolar extinction coefficient determined by Hoshi and Kobayashi (1971) for carboxyhemoglobin (173.3) was used.

RESULTS

The temperature range during all experiments was 22-26 C with a range less than 2 C during any 24 h period. The pH ranged from 8.0-8.6 with little diel change. Conductivity ranged from 335-435 µmhos; diel variation was usually less than 5 µmhos. The dissolved oxygen (DO) was usually between 6.0 and 8.0 mg/l after a 24 h test.

Pilot tests were conducted to see if the test beakers could be covered with Saran Wrap (Le Blanc 1980) to reduce the loss of naphthalene. DO concentrations in the naphthalene treated containers dropped to as low as 0.6 mg/l, while uncovered vessels contained 5.7 mg/l O_2. Daphnia mortality was high in the covered beakers and survivors swam on the water surface. Subsequent tests on the oxygen consumption of solutions of naphthalene showed that anoxic conditions existed after 22 h in a closed chamber of 3 ml. Because of the loss of oxygen in covered containers, the test vessels were left uncovered during the study.

Exposure of D. magna to naphthalene produced immediate behavioral changes. In animals exposed to concentrations greater than 5 mg/l, the movement of the second antennae ceased resulting in the organisms coming to rest on the bottom. Survivors showed persistent sluggish behavior compared with the controls after 24 h. The organisms often recovered, however, after the toxicant concentration fell to nondetectable levels. Below 1 mg/l, the behavior of the treatment animals was indistinguishable from the controls.

D. magna exposed to naphthalene for 24 and 48 h showed LC50 values of 13.2 and 3.4 mg/l, respectively, when analyzed by linear regression. Because of the different weights given the data points, probit analysis gave a 24 h LC50 of 6.6 mg/l and a 48 h of 4.1 mg/l.

The hemoglobin concentration of the control and the treated organisms were significantly different ($P < 0.05$). Values

decreased from 102 nmoles/animal at 1 mg/l naphthalene to 67 nmoles/animal at 9 mg/l (Table 1).

TABLE 1. The effect of naphthalene on the hemoglobin concentration of Daphnia magna.

Naphthalene Concentration (mg/l)	Hemoglobin concentration (nmoles/animal)a	% Difference from control [a]
0	97 (±37)	---
1	102 (±53)	10.2 (±9.6)
3	75 (±18)	6.6 (±10.5)
5	72 (±13)	28.4 (±6.9)
9	67 (±27)	26.0 (±9.0)

[a]Values are means (±SD)

An f-test showed a significant ($p < 0.05$) decrease in hemoglobin existed after 24 h at 5 and 9 mg/l, but not at 1 and 3 mg/l.

Oxygen consumption of D. magna decreased during a 24 h exposure to naphthalene (Table 2). Inhibition of oxygen uptake was significant ($p < .025$) when the organisms were exposed to 10 mg/l. Ethanol (0.95%) caused no significant difference in either hemoglobin concentration or oxygen consumption.

TABLE 2. The effect of naphthalene on the oxygen uptake of Daphnia magna.

Naphthalene Concentration (mg/l)	Oxygen uptake (nmoles/animal/h)[a]	% Difference from control [a]
0	44 (±16)	---
1	37 (±13)	10.2 (±6.7)
8	28 (±9)	25.1 (±5.1)

[a]Values are means (±SD)

DISCUSSION

Naphthalene decreases in the experimental vessels could be due to several factors such as bacterial degradation of the compound (LEE & ANDERSON 1977) or photoxidation (LUDZACK & ETTINGER 1963). Since naphthalene readily sublimes at room temperature, volatilization was probably the major cause of naphthalene losses.

The behavioral response of D. magna to naphthalene is similar to that found for other toxic compounds. Yohimbine, benzedrine, and capsaicin reduce appendage movements (VIEHOEVER & COHEN 1938b). Organophosphate and chlorinated hydrocarbon pesticides also cause immobilization (SANDERS & COPE 1966).

LE BLANC (1980) reported higher values for D. magna (48 h = 8.6 and 24 h = 17.0 mg/l) than those found in the present study.

Physical and chemical conditions of the test water differed in the two studies; LE BLANC conducted his experiments at 22 C and pH ranged from 6.7 to 9.4. The organisms were fed in the present experiment which could increase the uptake of naphthalene (CORNER et al. 1976), its concentration in the animal, and thus the extent of effects.

LC50 values determined in the present study are comparable to those found in other organisms. The polychaete, Neanthese arenaceodentata (ROSSI & NEFF 1978), and grass shrimp, Palaomonetes pugla (TATEM 1976), are more sensitive to this toxicant than Daphnia (3.8 and 2.4 mg/l, respectively). The pacific oyster (LEGORE 1974) and the mosquito fish (WALLEN et al. 1957) are relatively resistant to naphthalene toxicity indicating large species differences in toxicity to naphthalene.

It is difficult to relate the ranges of hemoglobin found in this study with those of others since most of previous works used hemoglobin indices in which the color of the hemolymph was compared with a standard solution (HOSHI & KOBAYASHI 1972, FOX 1948). One study in which the hemoglobin was quantitated showed values ranging from 0.08 to 0.13 mg Hb/25 Daphnia (HILDEMANN & KEIGHLEY 1955). As naphthalene concentrations increased, hemoglobin concentrations decreased after 24 h exposures in the present study. This could be due to hemoglobin excretion by the maxillary glands (FOX 1948). Naphthalene may also decrease the synthesis of this pigment, which would be analogous to daphnid response to high dissolved oxygen concentrations (FOX 1948, FOX et al. 1949) and low temperatures (FOX & PHEAR 1953). The linear regression equation for the percent decrease in hemoglobin was not significantly different ($P > 0.5$) from that of the percent decrease in oxygen consumption. Inhibition of oxygen uptake may thus decrease the need for hemoglobin synthesis.

Mitochondria are numerous in the microvilli of the midgut (SCHULTZ & KENNEDY 1976) and may be affected by toxicants. Mitochondria account for 98% of oxygen uptake in cultured Vero cells (HARMON et al. 1981). At 15 mg/l of naphthalene, inhibition of 50% to NADH oxidase and 30% to NADH-cyt c reductase occurs. The oxygen uptake of intact cells is also inhibited 50% by 15 mg/l naphthalene (HARMON et al. 1981). This inhibition of oxygen consumption is comparable to that found in the present study in which a decrease of approximately 25% occurred at 8 mg/l.

Chironomus attenatus oxygen uptake is also inhibited by exposure to naphthalene (DARVILLE, Personal Communication). On exposure to 5 mg/l naphthalene, the oxygen uptake is decreased about 20% which is similar to that of Daphnia, cultured Vero cells, and isolated mitochondria. The inhibition of electron transport is thus thought to be a primary cause of decreases in oxygen consumption.

Acute tests that are more sensitive than mortality experiments are needed. Significant physiological responses have been shown in this study at toxicant levels which were lower than the 24 h LC50 value. Hemoglobin concentrations and oxygen uptake may therefore be useful tools in assessing water quality and its effects on the biota.

REFERENCES

ANDERSON, B. G.: Sew. Works J. 16, 1156 (1944).
ANDERSON, J. W. et al.: Mar. Biol. 27, 75 (1974a).
BIESINGER, K. E.: Fed. Regist. 40, 26902 (1975).
CORNER, E. D. S. et al.: J. Mar. Biol. Ass. UK 56, 121 (1976).
CROSBY, D. G., & R. K. TUCKER.: Science 154, 289 (1966).
DORRIS, T. C. et al.: OWR RB-025-Okla (1974).
DOWDEN, B. F.: J. Water Pollut. Control Fed. 34, 1010 (1965).
FOX, H. M.: Proc. Royal Soc. Ser. B. Biol. Sci. 135, 195 (1948).
FOX, H. M. et al.: Proc. Royal Soc. Ser. B. Biol. 136, 388 (1949).
FOX, H. M. & E. A. PHEAR: Proc. Royal Soc. Ser. B. Biol. 141, 179 (1953).
HARMON, H. J., et al.: ASM National Meeting, Dallas, Texas. March 1-6 (1981).
HILDEMAN, W. H. & G. KEIGHLEY: Amer. Nat. 89, 169 (1955).
HOSHI, T. & K. KOBAYASHI: Sci. Rep. Niigata Univ. Ser. D. Biol. 8, 65 (1971).
HOSHI, T. & K. KOBAYASHI: Sci. Rep. Niigata Univ. Ser. D. Biol. 9, 55 (1972).
KOBAYASHI, M.: Sci. Rep. Niigata Univ. Ser. D. Biol. 11, 1 (1974).
LE BLANC, G. A.: Bull. Environ. Contam. Toxicol. 24, 684 (1980).
LEE, R. F. & J. W. ANDERSON: Bull. Mar. Sci. 27, 124 (1977).
LEGORE, R. S.: PhD Thesis, Univ. of Wash. (1974).
LUDZACK, F. J. & M. B. ETTINGER: Purdue Univ. Eng. Bull Ser. 115 278 (1963).
ROSSI, S. S. & J. M. NEFF: Mar. Pollut. Bull. 9, 200 (1978).
SANDERS, H. O. & O. B. COPE: Trans. Amer. Fish. Soc. 95 165 (1966).
SCHULTZ, T. W. & J. R. KENNEDY: Tissue and Cell 8, 479 (1976).
SIGMON, C: Bull. Environ. Contam. Toxicol. 21, 822 (1979).
TATEM, H. E.: PhD Thesis, Texas A & M Univ. (1976).
VIEHOEVER, A. & I. COHEN: Amer. J. Pharmacol. 110, 526 (1938b).
WALLEN, I. E. et al.: Sew. Ind. Wastes 29, 695 (1957).