THE EFFECTS OF NAPHTHALENE ON THE HEMOGLOBIN CONCENTRATION AND OXYGEN CONSUMPTION OF *DAPHNIA MAGNA*

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PREFACE

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CHAPTER I

INTRODUCTION

Toxic substances entering the aquatic environment from industry, agriculture, and other activities produce detrimental effects on the biota. Animals take in chemicals through their respiratory surfaces, skin, and by ingestion (Leeuwangh 1978). Biological magnification throughout the food chain increases the concentration of some harmful substances in animal tissue to levels much higher than the surrounding water. Relatively little is known about the safe levels of these compounds. Standard tests on the effects of toxicants should be developed to preserve the aquatic environment.

The release of aromatic hydrocarbons such as naphthalene into the environment creates a need for testing their effects on the biota. These compounds are dispersed by the combustion of fossil fuels and by industrial processes (Herbes et al. 1976). Naphthalenes are found in all crude oil and are the predominant water soluble components of many refined oil products (Boylan and Tripp 1971, Anderson et al. 1974a). Naphthalene and alkyl naphthalenes are thought to be the major toxic substances found in water soluble fractions of petroleum (Anderson et al. 1974a, b).

Although practical tests are needed for assessing the effects of harmful substances, inherent problems arise. It is not possible to reproduce all the ambient conditions of an organism. The results of a test depend on factors such as oxygen saturation, temperature, and pH (Sprague 1970). The interaction of secondary effects with the toxicant may be more important than the effects of the toxicant alone. Immobilization and death can be determined, but safe concentrations of compounds cannot be easily inferred from these data (Leeuwangh 1978). Discretion must therefore be used when applying laboratory tests to environmental situations.

Daphnia are commonly used in aquatic toxicity bioassays (Buikema et al. 1976). Results using these crustaceans are often less variable than those using fish (Crosby and Tucker 1966, Macek and Sanders 1970, Gilderhus 1967). Daphnids are relatively easy to maintain and are highly sensitive to toxic chemicals (Leeuwangh 1978). For example, they are more sensitive to cadmium than either fish or phytoplankton and are therefore good indicators for toxicity of this element (Marshall 1978). Daphnid bioassay techniques have advantages over chemical methods of evaluation because they are less specific and can be used to detect, qualitatively or quantitatively, a wide range of toxicants. They are more sensitive than many conventional chemical methods (Frear and Boyd 1967).

Daphnia magna and Daphnia pulex are abundant in the temperate agricultural belt and are an important part of

the diet of fish (Pennak 1953). D. magna is the most widely used cladoceran in toxicity testing and is considered to be representative of predominant zooplankton in testing sensitivity to toxicants (Anderson et al. 1948). Daphnids have been used in tests with crude oil and emulsifiers (Dowden 1965), insecticides (Sanders and Cope 1966, Wollerman and Putman 1955), refinery wastes (Dorris et al. 1974), aquatic herbicides (Crosby and Tucker 1966), metals (Anderson 1948), and other substances from industrial wastes (Anderson et al. 1948).

Testing the response of certain organisms to naphthalene can aid in determining its potential harm to other organisms. D. pulex, for example, concentrates naphthalene 100 fold (Southworth et al. 1978) which could have a pronounced effect on predaceous fish. This compound is taken up rapidly by fish (Lee et al. 1972). Tissues of animals exposed to oil show that naphthalene is accumulated in greater quantities than other aromatic hydrocarbons and is also the last to reach undetectable levels after the organisms are placed in uncontaminated water (Anderson et al. 1974b). The presence of these compounds in fish tissue could cause effects in tertiary consumers such as man.

In addition to testing survival of organisms exposed to a toxicant, various physiological tests have been proposed. Certain toxicants alter oxygen consumption (Obreshkove and and Ketchum 1937, Viehoever and Cohen 1938b, Sigmon 1979). The concentration of hemoglobin has been shown to vary

according to different environmental stimuli (Green 1956). Hemoglobin concentrations and respiration rates may be correlated (Kobayashi 1974), and measuring both parameters should provide a better indication of respiratory activity. The objectives of this project were to determine the acute and chronic effects of naphthalene on the mortality, concentration of hemoglobin, and oxygen consumption of D. magna.

CHAPTER II

LITERATURE REVIEW

Daphnia

Taxonomy

Daphnia have leaflike appendages characteristic of the subclass Branchiopoda. The order Diplostraca to which these organisms belong contains animals which are enclosed to some extent in a bivalve carapace. In the suborder Cladocera, the water fleas, most representatives have a carapace that does not enclose the head. *Daphnia* are sometimes classified under the order Cladocera rather than Diplostraca and the suborder classification is not used (Pennak 1953).

Structure and Function

Daphnia are enclosed in a transparent carapace which excludes the head and antennae. The total length of a single organism is usually less than 5 mm (Hickman 1967), although one female *Daphnia magna* reared in the laboratory reached 6 mm (Green 1954). The first pair of antennae are small, while the second pair are long and branched and produce water movement for locomotion and feeding. Movement

occurs in short spurts; hence, the common name water flea (Hickman 1967).

The head of Daphnia contains a compound eye and an ocellus. The compound eye contains about twenty elements and exhibits continuous oscillatory or scanning movement (Gregory 1967). Daphnia are responsive to certain wavelengths of light. When stimulated by these wavelengths they perform what one author terms 'color dances' (Lincoln 1971).

The feeding mechanism of Daphnia is a complex one. The mouth is located at the junction of the head and trunk. It receives food which has been filtered by specialized structures in the thoracic region (Barrington 1967). The trunk appendages are bordered with fine setae for trapping food (Barnes 1980). After material is gathered by the filtering apparatus, it is transferred to a food groove which leads to the mouth. The movement of substances along this groove takes place as a result of water movement caused by the forward and backward motion of the limbs. Adhesive material is secreted by the walls of the food groove and nutritive particles are pushed into the mouth by the appendages surrounding it, especially the first maxillae (Dahl 1956).

The intestine of Daphnia, as in other arthropods, may be divided into three regions (Quaglia et al. 1976). The first is near the region of contact with the stomodeum. The midgut contains two small diverticula and is lined with microvilli that are often bifurcate. The surface area for

absorption is increased about 62 times which is one of the highest values found in the arthropods (Hecker et al. 1974). The midgut is the site for holocrine enzyme secretion in which apical plasma membranes are broken down and their cell contents freed (Schultz and Kennedy 1976). The posterior region absorbs materials after they have been digested (Lockwood 1967).

Gas exchange takes place through the epipodite located on the trunk appendages and the general integument (Barnes 1980). There is an 80% exchange between the hemolymph and the outside media every 2 min in D. magna (Green 1961). The respiratory pigment hemoglobin has been found in these animals and its concentration in the hemolymph varies because of environmental factors (Fox 1948). When these organisms are placed in water with low O_2 tension, they begin to synthesize additional hemoglobin. Daphnia can increase the concentration of this pigment by about 10 fold if necessary (Waterman 1960).

The heart of Daphnia lies anterior to the brood pouch. It is a saclike structure with only two ostia (Barnes 1980). Blood enters these ostia and leaves via an opening at the anterior end of the heart. No blood vessels are present, but mesenteries help to guide the circulation of hemolymph (Pennak 1953). The heart rate of D. magna averages approximately 240 beats per minute. Acetylcholine causes heartbeat to be more powerful and rhythmic in nature (Obreshkove 1942). Carbon dioxide causes a reversible

inhibition of heart contractions (Fox 1933).

The brain lies dorsal to the esophagus, hence the term supraesophageal mass is also used. Nerves run to the compound eye, the ocellus, the antennules, and to the subesophageal mass which consists of ganglia that connect to several body regions (Petrunkevitch 1929). Several sites in the nervous system indicate neurosecretory activity; the brain's ventral and anterior surfaces, the optic ganglion, two regions within the circumenteric connective, and certain cells at the junctions of the ventral nerve cords with the second ventral commissure (Halcrow 1969).

Life Cycle

The reproduction of *Daphnia* takes place primarily by means of parthenogenesis. The oviducts deposit eggs into a dorsal brood chamber. These eggs require varying lengths of time to develop. *D. magna* eggs, for instance, complete development in about 46 h at 25° C when cultured *in vitro*. These eggs are thought to be nutritionally self sufficient (Obreshkove and Fraser 1940a, b). The eggs have also been found to contain the respiratory pigment hemoglobin (Fox 1948). At the end of the developmental period, live young or neonates are evacuated from the brood pouch, molting takes place, and new eggs are deposited.

Borradaile and Potts (1961) have given a good description of the types of eggs that are produced by these

organisms. The first type, the summer eggs, contain a small amount of yolk and develop parthenogenetically. They develop into females that also reproduce parthenogenetically (Green 1961). The second type of eggs, the winter ones, contains considerable yolk. These eggs need to be fertilized in the brood pouch and they are shed with the next molt in a case termed an ephippium. The ephippium is formed by a thickening of the cuticle. Parthenogenesis is not totally abandoned even during the times when sexual reproduction is taking place.

The production of winter or ephippial eggs that develop into males takes place usually twice a year (Borradaile and Potts 1961). Overcrowding and inadequate food can cause some of the eggs to develop into males (Green 1961). Males are found in Daphnia populations in autumn and spring. They are smaller in size with larger antennules than the females and have a hook on the first legs for clasping (Pennak 1953). In D. magna, the metabolic rate of males is usually higher than that for females (MacArthur and Baillie 1929a,1929b).

Daphnia pass through many instars during their life-span. The termination of each instar is marked by molting of the carapace which allows a short period of growth (Green 1961). At 25° C the number of preadult instars for laboratory D. magna varies from four to six (Anderson and Jenkins 1942). The life span of this particular species was found to be about 702 h at 28° C and 1074 h at 18° C

under laboratory conditions (MacArthur and Baillie 1929a). Under better culture methods, the average longevity at 25° C is approximately 960 h or 17 instars (Anderson and Jenkins 1942).

Neonates become reproductively functional soon after birth. At room temperature (18°-23° C) D. magna females reach sexual maturity in 6 to 10 days. At this time the first brood is produced and the female is from 2.49 to 2.60 mm in size (Anderson 1932). The number of eggs in the first brood was found to proportional to the length of the organism (Green 1954). Parthenogenetic egg production during the life of a female D. magna increases in early broods but decreases in the latter ones (Anderson and Jenkins 1942).

The heads of some species of Daphnia undergo a phenomenon known as cyclomorphosis. A structure described as a helmet, which is an elongated form of the head, has been observed. Cyclomorphosis occurs seasonally, the well developed helmets being found in summer (Coker 1939). This event is thought to be caused by environmental factors such as turbulence, predation pressure (Brooks 1965), and temperature (Coker 1939). The individuals with helmets are thought to have increased survival potential (Brooks 1965).

Some zooplankton possess a periodic vertical migration pattern. This movement is predominantly upward during early evening and downward during early morning. In one study of Colorado lake fauna, the average extent of migration ranged

from 0.6 m for Daphnia longispina to 8.8 m for Diaptomus shoshone (Pennak 1944). The stimulus for this phenomenon is thought by some to be a change in light intensity (Ringelberg 1966) which is independent of the direction of the source (Harris and Mason 1956). Another suggestion is that the age of the organism, the temperature of the water, and the condition of the culture medium is the primary sign of phototropism (Clarke 1932). D. magna has been shown to display a circadian rhythm of about 28 h when exposed to continuous light (Ringelberg and Servaas 1971).

Culture Methods

Many culture techniques have been used for the rearing of Daphnia. Banta (Galtsoff et al. 1937) stated that the essential food for most cladocerans is bacteria and single celled algae. His medium which inherently contained these organisms consisted of garden soil, horse manure, and pond water. This formula has been used by many experimenters and is often modified. Cottonseed was found to be a good substitute for manure (Chipman 1934). Another culture medium consists of boiled lettuce leaf suspension (Hyman 1937). After 2 or 3 days, Daphnia are added directly to this mixture. This ration as well as Banta's must be renewed frequently or supplementary feeding is necessary.

Recently the use of artificial or reconstituted media has become common. One such example contains calcium

acetate, antibiotics, albumin, trace elements, water soluble vitamins, folic acid, B_{12}, calcium pantothenate, choline, pyridoxal, inositol, thiamine, nicotinamide, riboflavin, biotin, and putrescine. This diet has been found to be superior in quality for 14 species of the family Daphnidae and actually increases the life span of Moina macropa (Murphy 1970). At least 200 generations have been produced from a chemically defined mineral medium enriched with vitamin B_{12}, thiamine, and 1% of a dilute organic matter (D'Agostino and Provasoli 1970). A manufactured salt mixture has been used in distilled water with moderate success. After 32 generations in this medium, the population lost its viability (Wesson 1932). Although many advantages exist for using a chemically defined medium, the cost is expensive.

Numerous foods are suitable for culturing Daphnia. Yeast cells have been used as the sole food source (Bond 1934 and Anderson 1944). Soybean meal and urea (Viehoever 1935, 1938), raw liver (Hyman 1937), bran infusion and liver (Schluchter 1937), and a green algae with trout chow suspension (Westlake et al. 1978, Biesinger and Christensen 1972) are examples. The bacterial growth that is stimulated by the organic matter added to the medium also provides food for the Daphnia.

The use of cultured strains of algae can be practical in rearing cladocerans. Chlamydomonas reinhardii has been used as the sole food organism and has been shown to

be satisfactory for 14 species of daphnids (Murphy 1970). Scenedesmus obliquis and C. reinhardii have been successful as food organisms when the culture medium was enriched with dilute organic matter (D'Agostino and Provasoli 1970). When fed Scenedesmus, Daphnia were found to be more productive than when given yeast alone (Dewey and Parker 1964). Chlorella, which has been used in some studies, is suspected of causing toxic effects (Pratt et al. 1945). Ankistrodesmus was found to have an assimilation efficiency of 100% (Schindler 1971). It has been concluded that algae alone are nutritionally inadequate as food for Daphnia, even though they are a main constituent of the diet (Taub and Dollar 1968).

Physical and chemical conditions of the water are important factors in aquatic invertebrate cultures. The pH range at which D. magna can survive is about 6.0 to 9.5 (Anderson 1946). Optimum values have been shown to fall between 8.1 and 8.5 (MacArthur and Baillie 1929a) or 7.0 and 7.8 (Krishnamoorthi 1965). The temperature maxima are 44° C for D. pulex, 42° C for D. longispina, and 41° C for D. magna (Brown 1929). Egg bearing adults are more sensitive to increasing temperatures than immatures. Daphnia raised under constant temperatures show decreased upper limits of temperature tolerance over those that are subjected to natural fluctuations (Goss 1980).

Use as a Biological Indicator

Daphnia are useful organisms for biological testing and many authors have pointed out their advantages as laboratory animals. They are transparent and easily observed under a microscope (Viehoever 1937). *D. magna* has been an important experimental tool because it is (a) easily handled, (b) readily available, (c) intermediate in sensitivity to chemicals, and (d) valuable ecologically as fish food (Freeman and Fowler 1953). These invertebrates exhibit a normal swimming behavior that is so characteristic that it may be considered an important toxic symptom (Viehoever and Cohen 1938).

Anderson (1944) has pointed out many advantages in using *Daphnia* in experimentation. They are small organisms that require little space for rearing. Their life span is relatively short and they are easy to culture using bacteria or some equivalent as food. These crustaceans can be raised individually or in mass cultures. *Daphnia* mature early and reproduce within their first week of life. Twenty or more young may be produced per brood, thus a female can potentially produce 400 or more offspring. One of the main advantages in using these animals is their attribute of parthenogenetic reproduction which insures genetic uniformity within broods. *Daphnia* are important as fish food and if they are destroyed the fish may evacuate the area in search of new food.

Oxygen Consumption

Physiology

In branchiopods the epipodite, a saclike structure on the thoracic limbs, is thought to function as a gill. The entire integumentary surface probably is involved in gas exchange to some extent (Barnes 1980). The epipodites are inefficient as respiratory surfaces because of their low permeability. The external pO_2 is thus four to five times higher than that inside the organism (Fox 1945a). Water is moved over the epipodites by the rhythmic beating of the appendages. Ventilation is controlled by the rhythmic center within the nervous system. Low pO_2's increase ventilation and high pO_2's decreases it in D. pulex (Wolvekamp and Waterman 1960). Daphnia contain hemoglobin which is thought to function as a respiratory pigment at low pO_2's (Fox 1948).

Variation

A number of physical factors affect oxygen consumption rates of Daphnia. The metabolic rate slowly decreases with senescence (Obreshkove 1930). Oxygen uptake increases with size but on a unit weight basis, smaller organisms consume oxygen at a higher rate (Richman 1958). Light intensity affects the interaction between oxygen consumption rates and body size (Buikema 1972). As expected, rising temperatures increase respiration of these organisms (Obreshkove and

Abramowitz 1932). High population densities increase the amount of oxygen consumed per animal. D. magna, for example, consume about 1 µl/h/animal at 21° C at densities of 1 animal/0.25 ml, but they consume only 0.43 µl/h/animal when densities are 1 animal/12 ml (Zeiss 1963). Schindler (1968) found that food type, concentration, and energy content are important in affecting rates of oxygen uptake in D. magna. Variation exists between neonates originating from the same mother (Obreshkove and Banta 1930).

Oxygen content of the water and the reproductive state of the female affect the respiration rates in Daphnia. Females carrying six or more eggs in their brood pouch exhibit higher respiration rates than nonovigerous ones (Schindler 1968). Above critical oxygen tensions (30-40% air saturation), the oxygen consumption rates of D. magna are independent of O_2. Below this level oxygen uptake is proportional to hemoglobin concentrations in the animal (Kobayashi 1974). Filtering rates, which are often compared with respiration rates, decrease when these cladocerans are subjected to water containing less than 3 mg O_2/l. This rate increases after about 12 h and is a result of the ability of these animals to increase hemoglobin synthesis (Kring and O'Brien 1976).

Effects of Toxic Substances

Little research has been done in this area using Daphnia. On exposure to N/10,000 KCN, the rate is initially decreased by about 20% (Obreshkove and Ketchum 1937). Benzedrine and some of its derivatives slow movements of the appendages associated with the uptake of oxygen. The animals recover, however, when placed in water containing no benzedrine. Yohimbine, piperine, and capsaicin reduce respiratory movements in Daphnia (Viehoever and Cohen 1938 b). Respiration is also depressed by nicotine, epinephrine, strychnine, and metrazol (Sollmann and Webb 1941). A 3 mg/l concentration of 2,4-D and 2,3,5-T increases respiration at 30° C in D. pulex (Sigmon 1979).

Laboratory Determinations

The micro Winkler technique has been used to measure oxygen uptake in daphnids. This consists of placing a large number of the organisms into a stoppered container with a known volume of water. Initial oxygen concentrations are determined on duplicate containers (Zeiss 1963). After a period of several hours a portion of the water is titrated with sodium thiosulphate (Schindler 1968).

Respirometers are also used in measuring oxygen consumption rates. Obreshkove (1930) used a Thunberg microrespirometer consisting of two bottles connected to a horizontal capillary tube containing a drop of kerosene. A

reduction in pressure in the flask containing the Daphnia is shared equally between the two bottles. Oxygen consumption equals twice the volume of displacement of the kerosene drop. Gilson respirometers which function on a similar principle have also been used (Sigmon 1979).

Recently, the polarographic method has been used in respiration studies (Hoshi and Inada 1973). This system consists of a platinum electrode held at a constant voltage. Oxygen is reduced on the platinum surface causing a current to flow. The magnitude of the current produced depends upon the amount of oxygen that comes in contact with the platinum. The current varies linearly with oxygen tension (Kanwisher 1959). Metals which plate out on the electrode decrease its sensitivity. The electrode is therefore covered with a pure KOH solution and a teflon or polyethylene membrane. These membranes allow a known amount of oxygen diffusion. The electrode is placed in water containing organisms to determine the rate of oxygen consumption per unit time.

Hemoglobin

Background

A red color often seen in the blood of Daphnia is caused by hemoglobin (Lankester 1871). This pigment has been found in D. magna, D. pulex, D. obtusa, D havalina, D. curvostris (Fox 1948, Fox, et al. 1949,

1951), D. thomsoni, D. carinata, D. hodgsoni, and D. longispina (Green 1956). Hemoglobin is present in the muscles, brain, fat cells (Fox 1955), and parthenogenetic eggs (Teissier 1932), but has not been detected in ephippial eggs (Fox 1948).

The properties of this pigment are well studied in Daphnia. The hemoglobin molecules appear to be of two sizes, one having a weight of 400,000 and the other 34,500 Daltons (Svedberg and Erikson-Quensel 1934). The higher molecular weight form predominates under normal conditions and is stable from pH 4.5 to 10.5. The other form increases in percentage of the total hemoglobin with pH and reaches 100% of the total at a pH of about 11.0 (Svedberg and Hedenius 1933). It has been estimated that 24 subunits are present in the hemoglobin molecule of D. pulex (Ar and Schejter 1970). Daphnia hemoglobin is about 0.334% iron (Hoshi and Kobayashi 1971). The absorption peaks for D. magna are 576, 542, and 414 nm for oxyhemoglobin and 570, 539, and 419 nm for carboxyhemoglobin. The milli-molar extinction coefficients approximate those of human hemoglobin (Hoshi and Kobayashi 1971).

Physiology

Daphnid hemoglobin functions as a respiratory pigment at low partial pressures of oxygen. More hemoglobin is synthesized as the oxygen level of the surrounding water

decreases (Fox 1948). Daphnia can increase their hemoglobin concentrations by at least six times (Hildeman and Keighley 1955). Oxygen binding to Daphnia hemoglobin requires higher oxygen pressures than other invertebrate hemoglobins (Fox 1948). Values for half saturation of D. magna hemoglobin, for example, are 2.0 (10° C) and 3.1 (17° C) mm Hg, while at the other extreme, Chironomus riparius and Tubifex sp. exhibit values of 0.5 (10°C) and 0.6 (17° C) mm Hg (Fox 1945). The P_{50} (20° C) for D. magna hemoglobin is reached at 14% O_2 saturation (Hoshi and Yahagi 1975). Under increasing oxygen concentrations, the in vivo O_2 dissociation curve of pink Daphnia (raised under low oxygen levels) approximates the curve of relative respiration rates (Hoshi and Inada 1973).

Hemoglobin synthesis, which is thought to take place in the fat cells and ovaries (Smaridge 1956), is influenced by several factors. Organisms not fed for 5 days although kept in water of low dissolved oxygen content, have no detectable hemoglobin (Fox 1948). Vitamin B_{12} and iron increase hemoglobin production. Ferrous salts are more effective than ferric ones in increasing hemoglobin levels (Fox and Phear 1953). Hemoglobin is produced in large quantities in early life and later decreases probably because of a slower metabolic rate (Green 1956, MacArthur and Baillie 1929a). After molting or laying parthenogenetic eggs, hemoglobin production increases until it reaches a maximal level when well

developed embryos exist in the female's brood pouch (Fox et al. 1949). Males have a greater capacity for hemoglobin synthesis than females (Green 1956).

Hemoglobin is lost from Daphnia blood at certain stages in the life cycle. The pigment is deposited from the female's blood into the parthenogenetic eggs (Dressel 1948) which lowers her concentration of hemoglobin by as much as one-third (Smaridge 1956). When Daphnia are transferred from poorly aerated to well aerated water, they lose about 80% of their hemoglobin to the eggs (Green 1956). These crustaceans have maxillary or shell glands which are excretory in function and have observed to be red (Klotzche 1913). This red color is caused by oxyhemoglobin (Fox 1948). Iron found in high concentrations in the shell glands of Daphnia losing hemoglobin suggests that iron is being excreted after breakdown of the pigment (Smaridge 1956).

Variation

Daphnid hemoglobin concentrations vary with the organism's physiological state, changes in the external environment, and other factors. They vary inversely with the dissolved oxygen content of the water (Fox 1948, Fox et al. 1949). At high temperatures, female Daphnia contain more hemoglobin than at low temperatures which is due in part to a lower rate of egg production (Fox and Phear 1953, MacArthur and Baillie 1929a). This pigment is found in low

concentrations immediately prior to egg deposition and it is at its highest level just before the release of neonates at the end of the instar (Dresel 1948). The variation of hemoglobin in a population of parthenogenetically reproducing females is therefore due largely to stages of the instar. The size of the animal is a factor in the amount of this pigment present in the blood (Green 1956). Variation in hemoglobin content between species such as D. pulex and D. obtusa has been shown (Chandler 1954) as well as differences between races of the same species (Green 1956).

Testing

Early work on hemoglobin in cladocerans has been performed using a hemoglobin index developed by Fox (1948). The standard consists of a small amount of the experimentor's blood diluted with distilled water containing saponin for hemolysis and sodium bicarbonate to prevent breakdown of heamatin. Fifty ml of this solution are placed in a wedge-shaped optical glass trough. The trough is set up under a microscope with an accompanying paper scale. Daphnia are placed on a microscope slide and the tint of oxyhemoglobin at the base of the second antennae is matched with that of the standard by moving the trough left or right. The number from the scale is then recorded and averaged for 10 Daphnia to obtain a hemoglobin index for the population.

A modification of the cyanohemoglobin method (Drabkin and Austin 1932) has been used in Daphnia studies

(Hildemann and Keighly 1955). Daphnia are placed on a watch glass containing distilled water. They are then blotted dry and transferred to a centrifuge tube containing cold distilled water. The animals are macerated with a glass rod and centrifuged for about 5 min. Then 0.1% $K_3Fe(CN)_6$ is added and after 10 min 0.1% KCN is added to convert methemoglobin to cyanohemoglobin. The solution is then placed in O. D. cuvettes and monitored for transmittance at 415 nm.

Naphthalene

Background (Donaldson 1958)

This aromatic compound was found in coal tar by Garden in 1820 and the name naphthalene was later proposed (Kidd 1821). A structure of two fused benzene rings was theorized by Erlenmeyer in 1866 and the empirical formula was established in 1926 (Faraday). This structure was later confirmed and was written in the form used today.

Physical Properties

The physical properities of naphthalene have been well studied. In pure form, the compound is a white crystalline solid having a mol. wt. of 128.16, sp. gr. 1.145 at 20° C, m.p. 80.1° C, and b.p. 217.9° C. The vapor pressure of the crystalline form is 0.177 mm of mercury at 30° C and 0.0648 at 20° C. Naphthalene therefore readily sublimes at room temperature. The solubility of this compound in

water is 0.0344 g/l at 25^{o} (Donaldson 1958). A higher solubility occurs in organic solvents such as ethanol in which 5.29 g of naphthalene per 100 g of solvent can be dissolved at 15^{o} C (Bohon 1951). The main absorption bands of this compound are at wavelengths 2210, 2850, and 3100 Å (Badger 1954), while the fluorescent emission bands are 31,000, 30,000 and 28,900 cm^{-1} (Clar 1952).

Uses and Distribution

Naphthalene has a variety of uses in home and industry. It is an ingredient in making dyes, emulsifiers, preservatives, and tanning agents. Naphthalene has been used in insect control and is commonly the constituent of moth balls. It also has other chemical uses in industry (Donaldson 1958).

This aromatic compound is found predominantly in crude oil, refined oil (Laughlin 1979), and coal tar (Donaldson 1958). It is often a large percentage of the water soluble fraction (WSF) of oil. Naphthalene was found in one study to make up 27.6% of the WSF in crude oil derived from the North Sea (Corner et al. 1976).

Oil spills and oil leaks from internal combustion engines are typical routes for naphthalene to enter the environment. It has been estimated that more than 1 million metric tons of oil and its products are released into the ocean annually (Blumer and Thomas 1956b). Aromatic hydrocarbons then enter the food chain where they become more

stable (Blumer and Thomas 1965a,b).

Toxicity Studies on Naphthalene

The toxic effects of hydrocarbons such as naphthalene have been studied over the past few decades. Rabbits and rats used in early studies were found to excrete unconjugated 1- and 2-napthol as well as other related metabolites when injected with naphthalene (Corner and Young 1954, Booth and Boyland 1949). Rabbits develop cataracts when dosed with naphthalene over a period of several days (Bourne and Young 1934). This is thought to be a result of a reduction in the amounts of cysteine, which are used in detoxification of the naphthalene. The oxygenation of the lens which relies on cysteine is thus impared.

Fish have also been used in evaluation of naphthalene effects. This compound appears to be taken up predominantly through the gills and is accumulated by the gut, liver, and flesh. Some of the compounds found stored in the gall bladder, heart, and skin after naphthalene exposure are naphthalene, hydroxynaphthalene, and 1-2 dihydro-1,2 dihydroxynaphthalene (Lee et al. 1972, Roubal et al. 1977). Neff et al. (1976) found that maximal uptake was accomplished in 1 or 2 h in which the gall bladder attained a concentration of approximately 2300 mg/l total naphthalenes, while the brain reached 620 mg/l. Metabolities of naphthalene containing alkyl substitutions are accumulated to a higher degree than unsubstituted ones (Roubal et al. 1978).

Uptake and excretion of aromatic hydrocarbons in fish depends on their partitioning between the exposure water and the tissue lipids (Neely et al. 1974). The binding of hydrocarbons such as naphthalene is thought to be due to hydrophobic interactions (Stone 1975). Maximal incorporation of naphthalene into the tissues occurs within about 48 h of initial exposure, while release is not fully complete until about 200 h after removal from contaminated water (Neff et al. 1976). The levels of naphthalene were shown to reach 4232 µg/l in Salmo gairdneri, the rainbow trout, after 48 h of exposure to 9.2 µg/l naphthalene (Varanasi et al. 1978).

Aquatic invertebrates are often used in determining naphthalene toxicity. The concentration of this compound was found to be about five to nine times higher in the soft tissues of the clam, Rangia cuneata than that of the exposure water (Neff and Anderson 1975). In Calanus helgolandicus, a copepod crustacean, uptake of naphthalene by means of food ingestion is more important quantitatively than absorption from the water (Corner et al. 1976). Blue crabs (Callinectes sapidus) assimilate 2 to 10% of naphthalene from food. The hepatopancreas is the site for over half of this metabolism (Lee et al. 1977). Spot shrimp (Pandalus platyceros) convert naphthalene to compounds such as alcohols, dihydrodiols, sulphates and quinones (Sanborn and Malins 1980). Concentrations in excess of 3 mg/l are toxic to Elasmopus pectenicius (Lee and Nichol

1978).

Daphnia have recently been used in tests with naphthalene. Equilibrium between water and naphthalene uptake by D. pulex is reached within 24 h. The concentration of this compound in the lipids appears to be in equilibrium with the surrounding solution. Daphnia accumulate naphthalene about 100 fold (Southworth et al. 1978). The 24 and 48 h LC50 values for D. magna exposed to naphthalene are 17 and 8.6 mg/l, respectively, while 0.6 mg/l creates no observable effect (LeBlanc 1980).

CHAPTER III

MATERIALS AND METHODS

Cultures

The stock cultures of Daphnia were housed in 2l liter glass aquaria filled with tap water that had aged in a plastic storage bottle for at least 48 h. Water was added periodically to replace water that had evaporated. The temperature of the stock populations were maintained at room temperature (22-25^{o} C). The pH and conductivity were kept between 7.9-9.0 and 510-875 µmhos, respectively. High dissolved oxygen levels existed because of mechanical aeration. An automatic timer (Sears Model 32) attached to two 40 watt flourescent lights produced a 16 h photoperiod. Dark plastic on the exterior of the aquaria reduced light fluctuations. The temperature and pH of the culture water were measured periodically.

Food was prepared by mixing 5 g of Purina trout chow and 10 g of alfalfa in 250 ml of water (Biesinger 1975). This suspension was mixed for about 2 min in a Waring laboratory blender. It was strained through cloth and stored in a closed container under refrigeration. Fresh food was prepared every 2-3 wk. The stock cultures were fed every other

day; a feeding rate of about 1 ml/l/wk (Biesinger 1975).

Rearing organisms of known age was accomplished by a modification of a technique used by Anderson (1944). Two female Daphnia from the stock cultures were placed in a 125 ml Erlenmeyer flask containing 100 ml of aged tap water. These flasks were kept in a room temperature water bath and no aeration was provided. Two drops of food suspension were added every other day. The flasks were checked for dead organisms and neonate production. If dead mothers were found, they were removed and replaced by females from the stock culture. When young were observed, their mothers were temporarily transferred by pipette to a small beaker. The neonates were poured into a large beaker along with the water contained in their respective flasks. The adults were put back into flasks containing fresh water and fed one or two drops of food (depending on whether their reproduction was on a feeding day or not). All neonates from a 24 h period were housed in 1 liter beakers and placed in a 25^{o} C water bath consisting of a partially filled aquarium with a thermostatically controlled heater.

Exposure to Naphthalene

Acute naphthalene tests were conducted in 1 liter beakers containing 500 ml of aged tap water. Because of the limited solubility of naphthalene in water, the desired concentrations were obtained by adding a freshly made stock solution composed of the toxicant in 95% ethanol to the

beakers. Daphnids of known age were transferred with a pipette into the beakers. They were then put into a 25° C water bath with a glass cover. The test organisms were subjected to a 16 h light photoperiod and aeration was not applied.

The initial and final concentrations of naphthalene in the test solutions were measured spectrofluorometrically. Samples were analyzed on an Aminco-Bowman Spectrofluorometer using excitation and emission wavelengths of 290 and 330 nm, respectively. A 0.1 or 0.2 mm entrance slit was used, while the exit slit was set at 3.0 mm. Three 5 ml aliquots were pooled to make up each sample. A series of naphthalene standards were made daily containing concentrations of ethanol equal to the test solutions. Relative intensity values from these standards allowed the quantitation of naphthalene in the test solutions by way of regression analysis (Draper and Smith 1966).

Short term naphthalene studies were conducted to calculate LC50 values and physiological responses. Daphnia were fed initially and the pH (Corning Model 610A), dissolved oxygen, and temperature (YSI Model 54 A Oxygen Meter), conductivity (YSI Model 33 Conductivity Meter), swimming movements of the organisms, and the number of survivors were determined at 0, 24, and 48 h. LC-50 values for 24 and 48 h were determined using organisms up to 24 h old. These experiments were run at least three times and the dosage-mortality curves were determined by the use of probit (Finney

1952) and regression analysis (Draper and Smith 1966). Concentrations of approximately 1, 5, and 10 mg/l were chosen for physiological studies. The animals were conditioned to 25° C for approximately 1 wk before being used for experiments. A concentration of 0.95% ethanol was used in both the treatment and control solutions. Experiments were conducted within 24 h of initial exposure which prevented the concentration of naphthalene from reaching nondetectable levels. Separate controlled experiments on ethanol were run to provide adequate baseline data on the effects of this solvent.

Chronic studies on naphthalene were conducted similarly to the acute ones, but were 3 wk in duration. The exposure beakers were set up with 25 Daphnia, and the organisms were transferred to fresh test solutions containing 0.1% ETOH every 48 h. This separated the test animals from their young (Biesinger 1975), and replaced naphthalene lost by volatilization, photodecomposition, and biological degradation. The conditions of the water and the test organisms were checked at 48 h intervals just prior to feeding. One concentration (0.2 mg/l) was tested twice, using four replicates and four controls.

Oxygen Consumption

Oxygen consumption of Daphnia was determined by a polarographic method (Wickstrom and Krab 1978). The basic monitoring system consisted of a YSI Model 5331 oxygen electrode, a Gilson reaction chamber, a Cole-Parmer Micro V

magnetic stirrer, a Haake FK constant temperature water circulator, a Johnson Foundation Oxygen Electrode Amplifier and a chart recorder (Linear or Scientific Products). The water circulator was connected to the oxygen chamber by Tygon tubing; thus, 25°C water circulated constantly inside the jacket surrounding the chamber. A magnetic stirrer mixed the test solution in the chamber at a constant speed so that the oxygen consumed was determined from a linear tracing on the chart record. The glass chamber contained a steel mesh insert to prevent the Daphnia from being injured by the magnetic stir bar. Before oxygen consumption values were measured, organisms from the toxicity test were placed in about 200 ml of their test water which had been filtered using a 0.22 μm Millipore membrane filter. This minimized bacterial interference of the consumption readings. The beaker of Daphnia was then placed in 25° C water until the system was ready for measurements.

Setting up the polarographic system began with changing the membrane on the electrode. The system was calibrated by placing a 0.1 M KCl solution in the chamber. Calibration was accomplished by zeroing the chart recorder and adjusting the full scale on the amplifier. One ml of 0.1 M KCl holds 290 nmoles of oxygen at 25° C, (Smith 1928) so that a rate of oxygen consumption could be quantified.

After calibration, the system was ready for recording oxygen consumption. The chamber was rinsed three times with distilled water to remove all traces of the KCl solution.

Test water was then added to the chamber. After a 2-3 min equilibration, a linear rate for 6-8 min was established which represented the amount of oxygen uptake due to bacteria and other substances in the water. Five Daphnia were added directly to this chamber water and oxygen consumption was recorded for about 6-8 min. Oxygen consumption was determined from the difference between the linear rate of the organisms and the rate of the water alone.

Hemoglobin

The carboxyhemoglobin method of measuring total hemoglobin was used in this study (Frankel and Reitman 1963). Ten experimental animals were pipetted onto a clean surface and rinsed with distilled water three times. After carefully blotting the Daphnia with Kimwipes, they 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 and Keighley 1955). The homogenate was transferred to a 2 cc syringe attached to a Millipore apparatus and run through a 0.04 μm polycarbonate filter to remove debris. The filtrate was collected in a small test tube which was sealed immediately and put on ice.

Daphnia hemoglobin was converted to carboxyhemoglobin by bubbling carbon monoxide gas into the sample for about 1 min. The samples were measured for absorbance at 419 nm using a Beckman Model 24/25 spectrophotometer. The optical density in the Soret region of the sample was then inserted

into the Beer-Lambert equation to determine the concentration of hemoglobin in the sample. The millimolar extinction coefficient (EmM) determined by Hoshi and Kobayashi (1971) for carboxyhemoglobin (173.3) was used.

CHAPTER IV

RESULTS

Physical and Chemical Parameters

Physical and chemical conditions of the test water were maintained as constant as possible. The temperature range during all experiments was 22-26^{o} C. The low temperatures were due to an occasional malfunction of the submersible heaters. The temperature did not vary more than 2^{o} C during any 24 h period. The pH ranged from 8.0-8.6. Diel change was slight. Conductivity increased slightly due to evaporation and the accumulation of metabolic wastes. Diel variation never exceeded 30 µmhos and was usually less than 5 µmhos. The dissolved oxygen (DO) levels decreased after the test vessels were set up. This was probably due to respiration of Daphnia and bacteria since the treatment and controls showed comparable declines in DO. Dissolved oxygen ranged from 3.8 to 14.2 mg/l, but was usually between 6.0 and 8.0 mg/l after a 24 h test. DO was the only parameter that was tested for difference between control and treatment solutions. No significant difference was found between the means of the two containers (Snedecor and Cochran 1978).

Oxygen Depletion and Naphthalene Loss

Pilot tests were conducted to see if the test beakers could be covered to reduce the loss of naphthalene. When the vessels were covered with Saran Wrap (LeBlanc 1980), DO concentrations in the naphthalene treated containers dropped to as low as 0.6 mg/l. The control vessels contained 5.7 mg/l O_2. Daphnia mortality thus took place at low naphthalene concentrations, while survivors swam on the surface of the water. Unexposed animals were typically distributed almost randomly in the test beakers or swimming near the bottom. Subsequent tests were conducted 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 closed containers, the test vessels were left uncovered during the study.

Constant naphthalene concentrations were not maintained in uncovered containers. Naphthalene was lost probably because of photodecomposition, metabolic degradation, and volatilization. Naphthalene in high concentrations was lost at a higher rate than at low concentrations. This decrease could be considered analagous to a field situation such as an oil spill or periodic waste disposal in which the organisms would be exposed to a high initial concentration.

Behavioral Responses

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 of their containers. Survivors showed persistant sluggish behavior compared with the controls after 24 h. The organisms usually 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.

Controls

Ethanol (0.95%) caused slight decreases in Daphnia respiration and hemoglobin concentrations after 24 h. The hemoglobin concentration during one 24 h period showed statistically significant decreases (OSL < .01) from the control. However, no significant differences existed overall for either oxygen consumption or hemoglobin concentrations.

LC50

D. magna exposed to naphthalene for 24 and 48 h showed LC50 values of 13.2 and 3.4 mg/l, respectively (Figures 1 and 2), when analyzed by linear regression (Draper and Smith 1966). Probit analysis (Finney 1952) gave a 24 h LC50 of 6.6 mg/l and a 48 h of 4.1 mg/l.

Hemoglobin

Twenty four h exposure to naphthalene decreased

Figure 1. The 24 h Naphthalene LC50 for D. magna

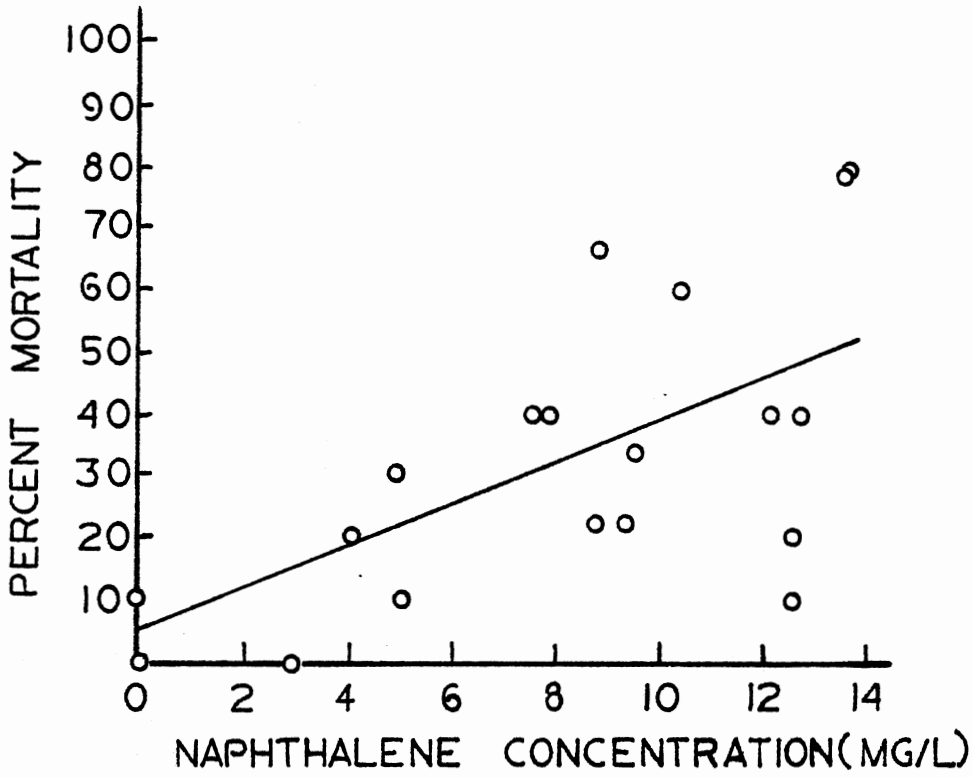
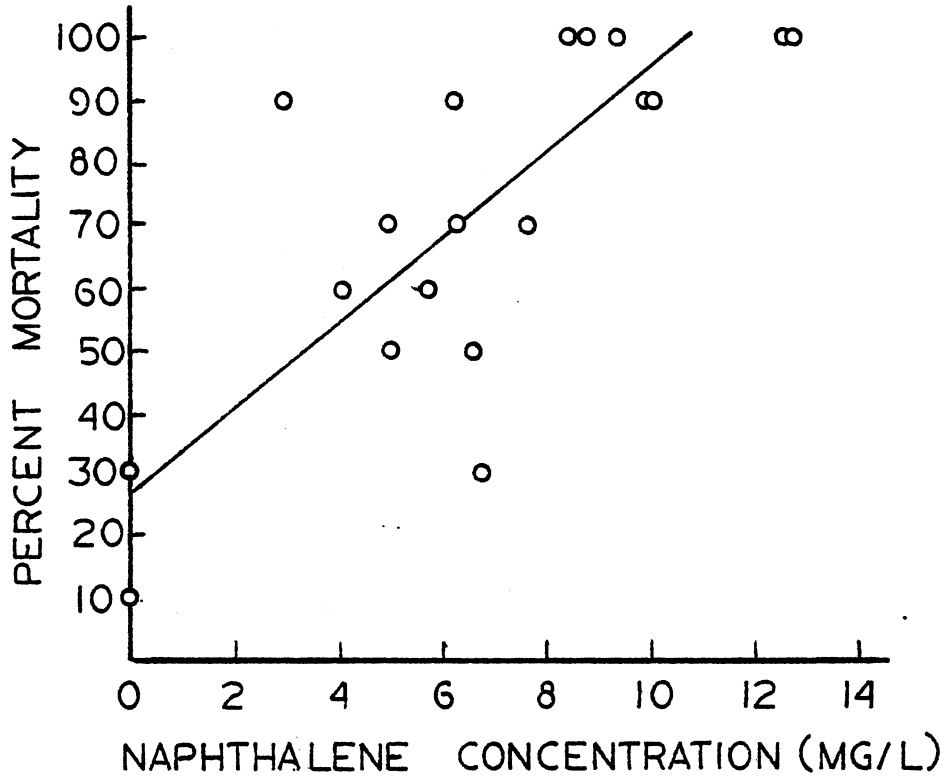

PERCENT MORTALITY
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
NAPHTHALENE CONCENTRATION(MG/L)

Figure 2. The 48 h Naphthalene LC50 for D. magna


PERCENT MORTALITY
100
90
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10
0
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12
14
NAPHTHALENE CONCENTRATION (MG/L)

hemoglobin in D. magna at concentration exceeding 1 mg/l (Table I). Values decreased from 102 nmoles/animal at 1 mg/l naphthalene to 67 nmoles/animal at 9 mg/l. Values were analysed as percent differences from their respective controls. A t-test (Snedecor and Cochran 1978) showed significant differences between the percent of hemoglobin lost at 9 mg/l compared to the control (OSL < 0.001), 1 mg/l (OSL 0.1 to 0.2), and 3 mg/l (OSL < 0.001) treated animals. The 23.6% loss at 5 mg/l was different from the control (OSL 0.1 to 0.2) and the 3 mg/l values (OSL 0.05 to 0.10).

Oxygen Consumption

Oxygen consumption of the organisms was decreased by a 24 h exposure to naphthalene (Table II). The nmoles/animal-/h of O_2 consumed was 44 in the controls and < 28 for animals exposed to 8 mg/l. Since temporal variation prevented testing significant differences in the actual values obtained, they were converted to percent difference from controls. Those receiving an input of 1 mg/l showed an average decrease of 10.2% from the controls, which was significant between the 0.1 and 0.2 levels. An input of 8 mg/l inhibited oxygen uptake by over 25% which was also significantly different from the control (OSL 0.005 to 0.010). The responses at 1 and 8 mg/l were also different from each other (OSL 0.025 to 0.050).

Chronic Experiments

Naphthalene decreased in the test beakers over the 48 h period between toxicant replacement in both experiments. The level of naphthalene decreased from 0.21 mg/l (s = 0.027) to an average of 0.03 mg/l (s = 0.01) after 48 h. Comparable decreases took place in both experiments.

Neonate production was affected by exposure to 0.2 mg/l naphthalene. On the third day of experiment one, numbers of offspring decreased significantly (OSL < 0.005) for an average of 2.5 in the controls to 0.4/adult in the naphthalene treatments. However, an increase in young (OSL < 0.005) took place at the end of the first experiment. A decrease (OSL < 0.005) occurred on day 13 of experiment two.

Oxygen consumption and hemoglobin concentrations were not affected significantly (OSL = 0.05) by a 3 wk exposure to 0.2 mg/l naphthalene. Measurements after 11 d of exposure showed similar results. At the end of the first chronic experiment, hemoglobin increased in the treatment animals (OSL between 0.1 and 0.2). No increase was observed in the second experiment.

TABLE I

THE EFFECT OF NAPHTHALENE ON THE HEMOGLOBIN CONCENTRATION OF *DAPHNIA MAGNA*

Naphthalene Concentration (mg/l)	Hemoglobin Concentration (nmoles/animal)	% Difference from Control
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)

TABLE II

THE EFFECT OF NAPHTHALENE ON THE OXYGEN CONSUMPTION OF *DAPHNIA MAGNA*

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

CHAPTER V

DISCUSSION

Physical and Chemical Parameters

Physical and chemical conditions of the test solutions must be considered when comparing studies. Guidelines have been established in an attempt to standardize the methods used in toxicity testing (Muller 1980). Substances in the test water may alter the toxicity of certain chemicals. For example, hardness of the water could affect the response of the organisms to naphthalene since the present experiments were conducted in an area known to contain hard water. Calcium has been shown to decrease toxicity of certain substances such as surfactants (Maki and Bishop 1979) and heavy metals (Dourdoroff and Katz 1953).

Oxidation of Naphthalene

Naphthalene decreased in the test solutions in several ways which lead to the production of other compounds, some of which were also toxic. Photooxidation of naphthalene takes place readily (Ludzack and Ettinger 1963). Bacteria are known to break down naphthalene to carbon dioxide and water by way of 1,2-dihydro-1,2-dihydroxy-naphthalene (Lee

and Anderson 1977). The respiration of these organisms could also influence oxygen depletion. Many studies have been performed on the metabolism of naphthalene by organisms. The spot shrimp (Pandalus platyceros) converts naphthalene to such compounds as naphthols and naphthoquinones (Sanborn and Mallins 1980). It is difficult to segregate the effect of these compounds on the toxicity of a test solution. The Daphnia used in the present study could metabolize naphthalene quickly to other compounds that may affect the test animals. Care must therefore be used when trying to evaluate the experimental data.

Behavioral Responses

The response of D. magna to naphthalene is similar to that found for other toxic compounds. Yohimbine, piperline, benzedrine, and capsaicin also reduce appendage movement (Viehoever and Cohen 1938b). As in the present study, the organisms recovered when the level of the toxicant decreased.

Ethanol

The use of ethanol as a solvent has an effect on the toxicity of naphthalene to Daphnia. However, organisms in later tests should have been affected in a similar manner since the concentrations of ethanol were equal in the control and treatment solutions. The threshold concentration of ethanol required for immobilization is 1.84% (Anderson

1944), approximately twice that used in this study.

LC50

LeBlanc (1980) reported higher values for D. magna (48 h = 8.6 and 24 h = 17.0 mg/l) than those in the present study. Physical and chemical conditions of the test water differed in the two studies. LeBlanc (1980) 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 and thus its concentration in the animal.

LC50 values determined in the present study are comparable to those found in other organisms. The fathead minnow (Pimephales promelas) exhibits comparable sensitivity to naphthalene (DeGraeve et al. 1980). Rainbow trout (Salmo gairdneri) (DeGraeve et al. 1980), the polychaete, Neanthes arenaceodentata (Rossi and Neff 1978), and grass shrimp, Palaomonetes pugla (Tatem 1976), are more sensitive to this toxicant than Daphnia. The pacific oyster (LeGore 1974) and the mosquito fish (Wallen et al. 1957) are relatively resistant to naphthalene toxicity. Their 96 h LC50 values are 199 and 150 mg/l, respectively.

Hemoglobin

It is difficult to relate the ranges of hemoglobin found in this study with those of others. Most of previous

works used hemoglobin indices (Hoshi and Kobayashi 1972, Fox 1948). These indices are only adequate for relative comparisons within an experiment. One study in which the hemoglobin was quantitated showed values ranging from 0.08 to 0.13 mg Hb/25 Daphnia (Hildemann and Keighley 1955).

As naphthalene concentrations increased, hemoglobin concentrations decreased after 24 h exposures (Table I). This could be due to hemoglobin excretion by the maxillary glands (Klotzche 1913, Fox 1948). Naphthalene may decrease the synthesis of this pigment, which would be analogous to daphnid response to low dissolved oxygen concentrations (Fox 1948, Fox et al. 1949) and low temperatures (Fox and Phear 1953, MacArthur and Baillie 1929a). The linear regression equation for the percent decrease in hemoglobin was not significantly different (OSL > 0.5) from the one for the percent decrease in oxygen consumption. The similarity in response of oxygen consumption and hemoglobin concentrations indicates that they may be related when the organisms are exposed to naphthalene. Inhibition of oxygen uptake would thus decrease the need for hemoglobin synthesis. Hemoglobin may have been lost when neonates were produced (Dresel 1948) which has been reported to lower the hemoglobin concentrations of the adults by 30% (Smaridge 1956).

Hemoglobin concentrations are decreased by naphthalene in other organisms. This toxicant causes hemolytic anemia in man (Haggerty 1956, Mackell et al. 1951). A decrease

occurs when naphthalene is ingested by dogs (Zuelzer and Apt 1949). These findings may not be directly compared with those of the present study since hemoglobin in those animals is contained within red blood cells, while daphnid hemoglobin is not (Fox 1948). Chironomus attenuatus, an insect with hemoglobin that is free in the hemolymph also exhibits decreases in hemoglobin concentrations when exposed to naphthalene (Darville, Personal Communication).

Oxygen Consumption

The values for oxygen consumption found in the present study are comparable to those of others in the literature. Kettle et al. (1980) showed values from 0.38 to 0.76 nmole/-animal/h for D. pulex. These values are smaller than those of the present study because the organisms were larger in this study with D. magna. Richman (1958) showed that body size is positively correlated with oxygen consumption. His values ranged from 0.95 to 0.124 nmole/Daphnia/h. Another study also showed values in this range (0.41 to 32.75µl/animal/h) (Buikema 1972). Hoshi and Inada (1973), using the same technique used in the present study had values for oxygen uptake ranging from 0.323 to 13.22 to 19.85 nmole/animal/h.

Oxygen consumption was negatively correlated with naphthalene concentrations. Decreases in the activity of the organisms with naphthalene exposure supported this relationship. Since the response was immediate, the effect

could have been on the nervous system. Naphthalene is a hydrophobic compound which may interact with lipids (Neely et al. 1974) of the nerve cell membranes decreasing their excitability and inhibiting impulse transmission (Chapman 1971).

Uptake of naphthalene by ingestion is greater than absorption from water (Corner et al. 1976) which may cause its effects to be related to energy use. Mitochondria are numerous in the microvilli of the midgut (Schultz and Kennedy 1976 and may be affected by toxicants. Mitochondria account for 98% of oxygen uptake in cultured Vero cells. 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 thus 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 attenuatus 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 and cultured Vero cells. The inhibition of electron transport is thus thought to be the cause of decreases in oxygen consumption.

Chronic Experiments

Naphthalene decreases in the experimental vessels could

have been due to several factors such as bacterial degradation of the compound (Lee and Anderson 1977) and photooxidation (Ludzack and Ettinger 1963). Naphthalene readily sublimes at room temperature (Donaldson 1958) and volatilization was probably the major cause of naphthalene decreases. In many previous static tests, the problem of toxicant loss was overlooked and the concentrations were assumed to remain constant for the duration of the experiments.

Neonate production decreased in both treatment and control groups. As the metabolism of the organisms decreases with age, so does the production of offspring (Anderson and Jenkins 1942). The increase of neonate production at the end of one experiment indicates that the organisms were under stress in the presence of naphthalene. Parkhurst et al. (1981) showed a similar increase in neonate production at 0.2 mg/l acridine after 28 d of exposure. This reproductive stimulation may have an ecological significance in regards to survival of the species (Kettle et al. 1980).

The increase of hemoglobin after 3 wk exposure to 0.2 mg/l of naphthalene in one experiment may be explained in several ways. Although little work has been done on the effects of toxic substances on hemoglobin, this pigment increases in concentration when the organisms are under oxygen stress (Fox 1948). As oxygen uptake is inhibited, the need for hemoglobin synthesis may also decline. It

appears that the Daphnia were experiencing a definite stress since the levels of hemoglobin would normally decrease as the animals aged (Green 1956, MacArthur and Baillie 1929a). The increase in neonate production may also account for the increase in hemoglobin concentrations since this pigment is present in maximal concentrations when embryos are present in the brood pouch (Fox et al. 1939).

CHAPTER VI

SUMMARY

1. Naphthalene solutions in closed containers became anoxic within 24 h. Test vessels were thus uncovered causing naphthalene concentrations to decrease after test initiation.

2. LC50 values were 13.2 and 3.4 mg/l at 24 and 48 h, respectively.

3. Naphthalene in concentrations greater than 5 mg/l caused decreases in swimming activity.

4. Neonate production was affected by 3 wk exposures to naphthalene. Initially a decline in numbers of offspring occurred. As the organisms aged, neonate production generally decreased. After 3 wk, the treatment groups in one experiment produced more young than the controls.

5. Hemoglobin levels decreased after 24 h exposures to 5 and 9 mg/l naphthalene. Decreases in synthesis of this pigment could account for lower hemoglobin levels.

6. Oxygen consumption was inhibited significantly by 24 h exposures to 1 and 8 mg/l naphthalene. This was thought to be caused by inhibition of electron transport enzymes.

7. Oxygen consumption and hemoglobin concentrations of

D. magna did not show significant (OSL < .05) effects from 3 wk exposure to 0.2 mg/l naphthalene.

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VITA 2

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