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10. Aided by NIH grant AI05226 and ONR contract Nonr-1842(07) (I.W.S.); and by PHS (Div. of Air Pollution, Bureau of State Services) grant AP71-04 to J.B.M. We thank Judith Ruble for technical assistance. Analyses of *P. lophurae*–infected duck erythrocytes and plasma by Siddiqui (personal communication) indicate higher values for certain amino acids in normal plasma (proline, serine, glutamine, and asparagine), with similar increases in these amino acids during infection. He also found larger increments in infected erythrocytes, especially of methionine, leucine, tyrosine, phenylalanine, proline, valine, lysine, and histidine. These discrepancies may partially reflect differences in host age, diet, and genetic constitution, as well as modification in methodology.

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Toxicity of Aquatic Herbicides to Daphnia magna

Abstract. *Accurate determination of the acute toxicities of a series of 16 aquatic herbicides to* Daphnia magna *reveals that several of them may present a hazard to food-chain microfauna whether the compounds are environmentally destroyed or not.*

Microcrustaceans such as those of the genus *Daphnia* are of worldwide distribution and represent an extremely important elementary link in the food chain. *Daphnia magna* and *Daphnia pulex* are particularly abundant and form a significant part of the diet of both young and adult fish in ponds and lakes of temperate-zone agricultural areas (*1*).

Toxic effects of a wide variety of insecticides on species of *Daphnia* have been reported (*2, 3*). The low levels of these compounds unintentionally present in bodies of fresh water as a result of drift and run-off from agricultural applications may present a potential threat to organisms of the aquatic food chain through chronic exposure (*4*), but the amounts generally are well below those required for acute intoxication. The median dose for immobilization of *Daphnia magna* by toxaphene is 260 μg/liter (*3*), while the amount of this insecticide typically present in treated lake water, for instance, ranges from 40 to 1 μg/liter (*4, 5*). However, there is an increasing intentional use of herbicides for the direct control of aquatic weeds and algae in lakes, ponds, and waterways, and few published data describe their real or potential effects on aquatic microfauna.

A parthenogenic stock strain was reared for over 50 generations under constant conditions. One hundred female *D. magna* were maintained in each of six 1-gallon (3.8 liters) wide-mouth bottles containing 3.6 liters of deep-well tap water having the following constituents, in milligrams per liter: Ca (as $CaCO_3$), 40; Mg (as $MgCO_3$), 80; Cl, 24; SO_4, 37; B, 0.64; SiO_2, 28; Na, 81; K, 2.2; and Fe 0.0; *p*H was 8.12 and conductivity (μmhos at 25°C), 493. This medium was superior to any of a wide variety of synthetic hard water. Bottles were covered loosely, almost submerged in a glass aquarium at constant temperature, and continuously aerated. Rearing jars were held under constant illumination of approximately 1100 lu/m² from daylight-type fluorescent lamps above; temperature was maintained at 21.1± 0.05°C to produce optimum growth and reproduction. Animals were fed twice daily, by pipette, with 1.5 ml of freshly prepared 3.3 percent suspension (weight to volume) of crushed baker's yeast cake.

In order to obtain test organisms, nylon net was cemented across a flexible circle of plastic tubing so that the resulting disc fitted tightly into each culture jar at a distance of approximately 8 cm above the bottom. Within 15 minutes the normally photopositive first-instar daphnids ascended through the 30-mesh net which excluded almost all larger individuals, and culture water and animals above each net were siphoned into a single reservoir and randomized by agitation. Most of the water was then siphoned back into the original jar through a glass-tipped, 25-mm-diameter tube closed by the 40- to 50-mesh net which excluded all *Daphnia.* Young daphnids were washed several times with boiled deep-well water, held without food for about 2½ hours, and, about 1 hour prior to tests, were passed again through 30-mesh net to remove any that had moulted during the preparation. This convenient technique provided a regular supply of highly uniform animals apparently still similar in characteristics to those collected from nature.

The herbicides were technical standards from the respective manufacturers and were dissolved in nontoxic, reagent-grade acetone when the solubility in water was low. Tests were performed in boiled deep-well tap water to which commercial nonionic surfactant (Tween 20) was added to provide a concentration of 1 part per million (ppm). Twenty-five starved daphnids were transferred by dropper to 100-ml test and control beakers that contained 50 ml of the surfactant solution. Appropriate concentration of toxicant in either acetone or aqueous solution was applied by means of a microsyringe; generally, 50 μl of solution was introduced below the surface, and contents of the beaker were swirled for thorough mixing. After 26 hours, mobility was observed under strong illumination from above and then by illumination from below. Unaffected *Daphnia* swam vigorously toward the light; those that were unable to swim vertically 1 cm or more were considered immobile. Measurement at each concentration was replicated at least three times, data from a log series of dosages were plotted as percentage of immobility versus log concentration on log-probit paper to provide a median immobilization concentration (IC_{50}), and statistical significance was determined by the method of Litchfield and

Table 1. Toxicity, in terms of median immobilization concentrations (IC_{50}), of aquatic herbicides to *Daphnia magna.* Paraquat, diuron, and fenac (Na salt) are not currently registered for aquatic use. Parenthetic values represent 95 percent confidence limits.

Common name	IC_{50} (ppm)	Field use (ppm) (*7*)
Dichlone (Phygon)[a]	0.014 (.011–.017)	0.15
Molinate (Ordram)[b]	.70 (.46–1.05)	3.5
Propanil (Stam, Rogue)[c]	4.8 (3.8–6.6)	7
Sodium arsenite	6.5 (5.7–7.3)	10
Diquat[d]	7.1 (6.3–8.0)	3
Dichlobenil (Casoron)[e]	9.8 (8.8–10.7)	15
Paraquat[f]	11.0 (9.1–12.2)	2
Amitrole[g]	23 (15.3–44.4)	10
Amitrole T[h]	40 (14.3–112.0)	10
Endothall[i]	46 (36–57)	3
Diuron[j]	47 (41.6–53.1)	2
Silvex (K salt)[k]	100	2
Fenac (Na salt)[l]	>100	
Monuron[m]	106	2
MCPA[n]	>100	2
2,4-D[o]	>100	2

[a] 2,3-Dichloro-1,4-naphthoquinone. [b] *S*-Ethyl hexahydro-1*H*-azepine-1-carbothioate. [c] 3′,4′-Dichloropropionanilide. [d] 1,1′-Ethylene-2,2′-dipyridylium dibromide. [e] 2,6-Dichlorobenzonitrile. [f] 1,1′-Dimethyl-4,4′-dipyridylium dichloride. [g] 3-Amino-1,2,4-triazole. [h] Amitrole + ammonium thiocyanate. [i] 3,6-Endoxohexahydrophthalic acid. [j] 3-(3′,4′-Dichlorophenyl)-1,1-dimethylurea. [k] Potassium 2-(2′,4′,5′-trichlorophenoxy)propionate. [l] Sodium 2,3,6-trichlorophenylacetate. [m] 3-(4′-Chlorophenyl)-1,1-dimethylurea. [n] 2-Methyl-4-chlorophenoxyacetic acid. [o] 2,4-Dichlorophenoxyacetic acid.

Table 2. Comparative toxicity of aquatic herbicides to *Daphnia*, fish, and the rat (*9*). *Daphnia* were exposed for 26 hours at 21.1°C, rainbow trout for 48 hours at 12.8°C, and bluegills for 48 hours at 23.9°C. Rats received oral doses. IC_{50} is median immobility concentration; LC_{50}, median lethal concentration; and LD_{50}, median lethal dose.

Herbicide	IC_{50} (mg/liter)	LC_{50} (mg/liter)		LD_{50} (mg/kg)
	Daphnia	Rainbow trout	Bluegill	Rat
Dichlone	0.014		0.04*	1300
Molinate	.70	0.29	.48	720
Sodium arsenite	6.5	60	44	10
Dichlobenil	9.8	22	20	3160
Diuron	47		7.4	3400
Silvex (K salt)	100	21.9†	14.5†	650†
Fenac (Na salt)	>100	7.5	19	1780†

* 24 hours. † Free acid.

Wilcoxon (*6*). Under these conditions, toxicity measurements were highly reproducible.

Table 1 indicates the toxicity to *Daphnia* of some common aquatic herbicides in terms of median immobilization concentrations; controls were unaffected. Actual death in this organism proved very difficult to determine by such measures as heart beat, respiration, and eye movements, so that immobility represents only an index of toxicity rather than true lethality. While it is apparent that most of these pesticides are far less toxic than the majority of insecticides that have been reported, the relatively high concentrations usually required for effective herbicidal action suggest that several of them could present a very real danger to *Daphnia* under field conditions (*7*). Dichlone (a quinone), Molinate (a thiolcarbamate), Propanil (an anilide), sodium arsenite, and Dichlobenil (a nitrile) all may be employed at field rates in excess of the median toxic dose for *Daphnia*. Other common herbicides, such as 2,4-D, seemed to be completely innocuous.

Table 3. Eventual mortality of mobile *Daphnia* after 26-hour exposure to aquatic herbicides.

Compound	Dose (mg/liter)	Immobile *Daphnia* (%)	Death time* (days)
Paraquat	6	24	2
Paraquat	8	56	2
Paraquat	10	64	2
Paraquat	12	72	1
Paraquat	14	70	1
Amitrole	20	32	>60
Amitrole	50	56	>60
Amitrole	215	88	>30
Endothall	25	72	>30
Endothall	50	56	>30
Endothall	84	84	6

* Time required for death of all mobile organisms transferred to fresh medium. Periods greater than 30 days indicate the length of observation (no toxic effects).

Table 2 compares the effect of several herbicides, representing a range of toxicities for *Daphnia*, on two species of fish and the rat. Although the route and level of exposure probably are different, relative toxicities are apparent. Toxicity of these compounds varies considerably among several species of fish examined (*4, 8*), and the responses of *Daphnia* provide only a very rough predictability of potential hazard. It also appears that none of these aquatic species may be used to predict toxicity to rats that received oral doses of the herbicides. It is probable that very little toxicant enters either fish or *Daphnia* by the usual oral route, and their respiratory contact is undoubtedly far greater. Unfortunately, no comparable data on respiratory (inhalation) or even intravenous or intraperitoneal administration are available for mammals. As such information is developed in the future, it will be of interest to determine whether readily cultured and treated aquatic species could provide advance warning on respiratory hazards in higher animals and man.

In the field, most aquatic herbicides disappear quite rapidly; pools treated with Diquat (3 ppm), for example, showed negligible residues after 21 days (*4*), and a significant proportion was lost within 3 days. This disappearance may be due in part to uptake at the surfaces of plants such as algae, metabolism by microorganisms, or adsorption on soil. *Daphnia* is not a discriminating eater and may be exposed to the pesticides or their decomposition products on the plants, bacteria, and soil they continually ingest. In addition, most of the aquatic herbicides listed here have been found to decompose readily in aqueous solution under the influence of ultraviolet light or natural sunlight (*10*). Initial experiments on the effect of ultraviolet irradiation upon the toxicity of herbicide solutions produced erratic results, but there appears to be no doubt that the original physiological effects were altered.

Because of the disappearance of the parent herbicide under practical conditions, measurements of chronic toxicity were not attempted. However, in several instances *Daphnia* that survived 26-hour treatment were removed to fresh water and held. As shown in Table 3, lethal action may be delayed, and consequently standard IC_{50} values may not represent the total effect of exposure to environmental toxicants regardless of the degree to which the parent compounds or their decomposition products persist. In the presence of nontoxic herbicides, no effect on reproduction was observed.

Accurate field experiments on the effects of aquatic herbicides and their decomposition products on aquatic microfauna are needed. Although the present laboratory data can only provide a standard benchmark for outdoor studies, they indicate that toxicity of a pesticide to mammals cannot be used as an indication of environmental hazard to *Daphnia*, and vice versa; that the high degree of sensitivity of some aquatic weeds to herbicides may be surpassed by that of ecologically related food-chain animals; and that even brief exposure of *Daphnia* to certain compounds intentionally released in their environment may lead to more far-reaching effects on populations of these important animals than might be anticipated from data on acute toxicity.

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Stylet-Borne Virus: Active Probing by Aphids Not Required for Acquisition

Abstract. *Anesthetized aphids, whose stylets had been dipped into capillaries containing purified concentrated cucumber mosaic virus, acquired the virus and, after recovery from the anesthetic, were able to transmit it in a low percentage of cases. Although this study does not eliminate active probing as a means of virus acquisition, experimentally, it clearly establishes passive contamination of aphid mouthparts as a method of virus acquisition.*

Most plant viruses that are transmitted by aphids can be acquired and transmitted within seconds. Aphids lose the ability to transmit these viruses very rapidly unless they have access to another virus source (*1*). This type of transmission, originally called nonpersistent (*2*), has since been termed stylet-borne (*3*).

The very short times required for acquisition and transmission (*1*), and the inhibition of transmission by treating stylet tips of viruliferous aphids with ultraviolet light or formaldehyde (*4*), have been interpreted as evidence that these viruses are carried as contaminants of the stylets. On the other hand, a recent attempt to transmit henbane mosaic virus, which is stylet-borne (*3*), with anesthetized aphids whose stylets had been inserted into cells of infected leaves was unsuccessful (*5*). This was regarded as evidence that virus is not carried as a contaminant of the stylets, but that it must be acquired by active probing.

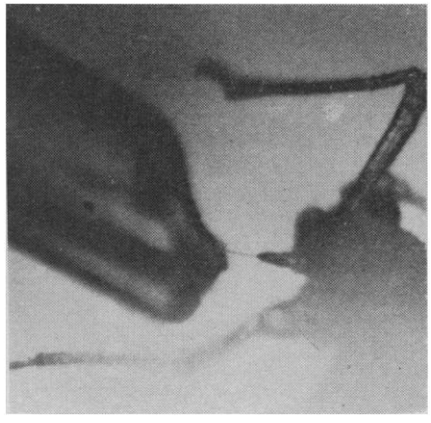

Fig. 1. Photomicrograph showing exposed stylets of an aphid (*Myzus persicae* Sulz.) being dipped into a capillary containing purified cucumber mosaic virus.

We have recently shown (*6*) that purified preparations of certain stylet-borne viruses can be acquired and transmitted by aphids that are allowed to make 20- to 40-second acquisition probes through a Parafilm membrane. Experiments described in this report were made to determine whether anesthetized aphids could be made viruliferous by dipping their stylets into a suspension of a purified stylet-borne virus.

The C-1, Imperial 78, and Wisconsin 102 strains (*7*) of cucumber mosaic virus were used. Viruses were propagated in tobacco *Nicotiana tabacum*, L., variety Havana 425) and purified by the method of Scott (*8*). Aphids (*Myzus persicae* Sulz.) were propagated on Tendergreen mustard (*Brassica perviridis* Bailey) as previously described (*6*); they were anesthetized while feeding on these plants by being exposed to a stream of CO_2 for 1 minute and then removed from the plants by suction with a fire-polished capillary pipette attached to a vacuum pump. Suction was adjusted so that the insect could be held securely by the dorsal surface of the abdomen without injury. Exposed stylets of aphids were dipped in a concentrated suspension of purified virus in a fine capillary (Fig. 1); the insects were then released and placed in a glass dish for recovery from the anesthetic, which occurred in 4 to 6 minutes after removal from CO_2. After recovery, aphids were placed singly on healthy tobacco seedlings, covered with a cellulose tube to prevent movement to another plant, and allowed a test feeding of 4 to 12 hours. Plants were sprayed with the insecticide mevinphos (2-methoxycarbonyl-1-methyl or vinyl dimethyl phosphate) and removed to the greenhouse where they were observed for development of symptoms.

Table 1. Transmission of strains of cucumber mosaic virus acquired by anesthetized aphids whose stylets had been dipped in purified virus suspensions or by aphids making probes through a Parafilm membrane. A single aphid was placed on each test plant.

Virus strain	Transmission* of viruses acquired by	
	Dipping stylets	Probes through membranes
Wis. 102	3/100	5/24
Imp. 78	2/138	10/128
C-1	13/100	6/24

* Transmission is expressed as the number of plants infected (numerator) out of the number tested (denominator).

To determine the transmissibility of virus preparations acquired by natural probes, aphids were allowed to acquire the virus through a Parafilm membrane, with the use of techniques previously described (*6*). Test feedings were the same as those described for the anesthetized insects. As controls, an equal number of aphids were removed from healthy mustard plants and placed directly on tobacco seedlings. These seedlings were then treated and maintained in the same way as test plants.

Results (Table 1) show that the three strains of cucumber mosaic virus were acquired by the anesthetized insects, although the rate of transmission by aphids that acquired virus in this manner was somewhat lower than the rate of those that acquired it after probing through the Parafilm membrane. In both cases, rate of transmission was considerably less than that which would be expected if the virus were acquired by aphids from infected leaves (*9*). None of the control plants became infected.

These results do not necessarily conflict with those obtained when exposed stylets were inserted into cells of infected leaves (*5*), since, in addition to the fact that a different virus was used, it is possible that the cells into which stylets were inserted did not contain virus. It has also been suggested (*10*) that aphids acquire stylet-borne viruses intercellularly. Although our study does not eliminate active probing as a means of virus acquisition, experimentally, it clearly establishes passive contamination of aphid mouthparts as a method of virus acquisition.

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