SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 16, No. 5, pp. 1028–1033, 1997
Printed in the USA
0730-7268/97 $6.00 + .00

Keller Ruessler 1997

THE TOXICITY OF MALATHION TO UNIONID MUSSELS: RELATIONSHIP TO EXPECTED ENVIRONMENTAL CONCENTRATIONS

ANNE E. KELLER* and D. SHANE RUESSLER
National Biological Service, 7920 NW 71 St., Gainesville, Florida, 32653, USA

(*Received* 1 *May* 1996; *Accepted* 8 *October* 1996)

Abstract—The acute toxicity of malathion to glochidia, juvenile, and adult freshwater mussels was determined at pH 7.5 in soft water and at pH 7.9 in moderately hard reconstituted fresh water at 25°C and 32°C. Nine species were tested in one or more life stages. Glochidia tests were conducted for 4, 24, or 48 h, while juvenile and adult exposures lasted 96 h. Overall, *Utterbackia imbecillis* was the least sensitive species for all exposure conditions and life stages. The LC50 values for glochidia tested at 25°C ranged from 7 mg/L for *Lampsilis siliquoidea* (4 h) to 324 mg/L for *U. imbecillis* (48 h). At 32°C, glochidia LC50s were 119 mg/L for *Villosa lienosa* (48 h) and 374 mg/L for *U. imbecillis* (24 h). Tests with juvenile mussels produced 96-h LC50s ranging from 24 mg/L for *Lampsilis straminea claibornensis* at 25°C to 219 mg/L for *U. imbecillis* at 25°C. The 96-h LC50s for three species of adult mussels were greater than the highest malathion exposure concentration of 350 mg/L. These values are considerably higher than the reported 48-h LC50 of 1 μg/L for *Daphnia magna* and the 96-h LC50 of 0.76 μg/L for *Gamarus fasciatus* but are similar to 96-h LC50s for some fish. Expected environmental concentrations should not be lethal to unionids.

Keywords—Unionids Malathion Acute toxicity Mussels

INTRODUCTION

Native freshwater mussels (family, Unionidae) are among the most imperiled fauna in the United States [1,2]. Of the nearly 300 species present, 200 have been identified as threatened, endangered, or in decline [2,3]. While many possible causes have been identified, pesticides and other contaminants are believed to be contributors to the loss of mussel fauna [1,2,4,5]. However, little is known about how toxic even common pesticides are to unionid mussels.

Malathion is a commonly used organophosphorous insecticide because of its efficacy in mosquito and fruit fly control, low mammalian toxicity, and relatively short half-life [6]. A boll weevil control program (program) sponsored by the U.S. Department of Agriculture uses malathion in large areas of the cotton-growing states (e.g., Alabama, Texas, Mississippi). Buffer zone widths and application methods and rates were selected by program engineers to minimize nontarget exposures. Preliminary samples collected from streams adjacent to sprayed fields determined that malathion concentrations were generally below the 0.1-μg/L acute criterion for fresh water (U.S. Department of Agriculture, unpublished data) [7]. However, in exceptional cases residues were found to reach 10–30 μg/L immediately after malathion applications, dissipating within 4 h. The acute toxicity of malathion to native unionids had not been determined. Because endangered mussels inhabit the region, the U.S. Fish and Wildlife Service (USFWS), which has the major responsibility for protecting endangered species, imposed a "no exposure" limit for malathion in water bodies within the boll weevil program area, requiring wider buffer zones and restrictions on aerial spraying, until more data were available. This research was designed to provide data by which USFWS could better evaluate the risk malathion use posed to unionids.

For the control of boll weevils, malathion is applied most intensively during the middle of summer when glochidia, juvenile, and adult mussels are all present. Therefore, the acute toxicity of malathion was evaluated for all three life stages under temperature and pH conditions expected to be encountered in program areas. The nine mussels selected for the project were chosen because they are present in the unionid fauna of many program areas (Appendix), they represent different groups of mussels, they and may be good surrogates for the endangered mussels that could be exposed to malathion. One species tested, *Lampsilis subangulata,* is proposed for listing as an endangered species by the USFWS.

MATERIALS AND METHODS

Test conditions

Soft (pH 7.5) and moderately hard (pH 7.9) fresh water used as diluents were prepared by adding $MgCl_2$, NaCl, KCl, and H_2CO_3 to deionized water following Environmetal Protection Agency (EPA) methods [8] or by dilution of well water to comparable pH, hardness, and alkalinity (Table 1). The quality of dilution water was measured several times during its use. Water was filter-sterilized (0.2 μm) and aerated before use but not during the tests. Tests were performed at 25°C and 32°C under static conditions with a 12:12 photoperiod.

Pure malathion has a solubility limit of 145 mg/L in distilled water and is very slow to dissolve unless a carrier is used [6]. Therefore, test solutions were prepared by pipetting a concentrated stock of 96% pure malathion dissolved in analytical grade acetone into a volumetric flask that was then brought to volume with the appropriate test water. After being stirred for 30 to 45 min, 50% serial dilutions were made from the stock to produce final test solutions. Acetone controls were used in addition to the appropriate test water without malathion. Samples of test solutions were preserved with Na_2SO_4, acidified,

* To whom correspondence may be addressed. The current address of A.E. Keller is EPA-ESD, 980 College Station Rd., Athens, GA 30605-2700, USA.

Table 1. Summary of mean water quality characteristics for soft and moderately hard water used in mussel toxicity tests (standard deviations in parentheses)

Water	pH	Conductivity (µs)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)
Soft	7.53 (0.12)	131 (22)	47 (5)	40 (11)
Moderately hard	7.90 (0.23)	258 (56)	76 (19)	64 (12)

and frozen at −30°C until analyzed by gas chromatography [9]. Spike recoveries ranged from 100 to 126%. The method detection limit was 0.1 µg/L malathion.

The persistence of malathion depends on water temperature, pH, and whether or not bacteria are present [10–14]. Hydrolysis of malathion, the primary chemical degradation process, is more rapid in basic waters than in water with a pH below 7. Half-life estimates for malathion range from 12 h at pH 9 [13], to 5 months at pH 7 and below [11]. The presence of microbes enhances the speed of degradation [14] as does elevated temperature [6]. Toxicity tests were conducted in sterilized water, at 25°C and 32°C, for 48 and 96 h, at a pH of 7.5 or 7.9. Some loss or degradation due to volatilization and hydrolysis probably occurred at the higher temperature and pH. However, 25°C test water analyzed 24 h and 96 h after preparation showed little decrease in malathion concentration. Therefore, LC50 values were calculated by probit analysis based on initial malathion concentrations [15].

Glochidia tests

Mature glochidia (0.2–0.4 mm diameter) were collected from two or three adult female *Villosa lienosa, Villosa villosa, Utterbackia imbecillis, Megalonaias nervosa, Lampsilis teres,* or *Lampsilis siliquoidea* mussels. For each species, glochidia were pooled and placed in six-well polystyrene dishes containing test solutions. Two sets of three or four replicates, each containing 50 to 100 glochidia, were used for each of five test concentrations and two controls. Each species was tested separately.

Except in tests using *L. teres* and *M. nervosa,* one set of replicates was used to evaluate 24-h LC50s and the other set to measure toxicity at 48 h. Earlier experience indicated that *L. teres* glochidia were viable for only 4 to 6 h after removal from the female's marsupia (gills), and *M. nervosa* were viable for only 24 h. Therefore, tests with these species were concluded after 4- or 24-h exposures to malathion. When glochidia availability was limited, we performed tests only at pH 7.5 because malathion is more stable at lower pH [6]. Test results should then reflect worst-case conditions. The viability of glochidia (endpoint for LC50) was determined based on the ability of the glochidia to close when NaCl was added to the test chamber [16]. Once the numbers of live and dead glochidia were tallied for a time period (4, 24, or 48 h), those larvae were discarded.

Glochidia differ markedly from juveniles in that glochidia have no organs and absorb nutrients through pores in the shell rather than from the digestive tract. They also have inherently low survival rates [17,18]. Therefore, the 10% maximum control mortality used in many toxicity tests was increased to 20% for glochidia. The LC50s were calculated by probit analysis [15].

Juvenile tests

Most of the juvenile mussels (*V. lienosa, V. villosa, Lampsilis straminea claibornensis, L. subangulata,* and *Elliptio icterina*) used in toxicity tests were produced by infection of host fish in the laboratory. Glochidia were collected from two or three mussels of a species and mixed together. They were then pipetted onto the gills of fish held in aquaria containing well water until juvenile mussels were harvested by siphoning the aquarium bottom. *Utterbackia imbecillis* glochidia were cultured in the laboratory using in vitro techniques [19] R.G. Hudson and C. Shelbourne, unpublished manuscript. In vitro culture is preferable when successful because thousands of juveniles can be harvested from a single culture dish. However, such techniques have been developed for only a few species of mussels. Transformation to juveniles took from 7 to 19 d, depending on species. Once transformed, juvenile mussels were randomly distributed into 60- × 15-mm glass petri dishes containing the test solutions. Two to four replicates were used per concentration with each replicate containing 10 to 20 juveniles, depending on the availability of juveniles. Tests were performed under static conditions and mussels were not fed during testing, as described in earlier studies [20,21]. Cessation of both activity and heartbeat were used as measurement endpoints indicating death. The LC50s were calculated for each species and set of test conditions at 24 h, 48 h, 72 h, and 96 h using probit analysis [15].

Adult tests

Adult *V. lienosa* (2.5–5.0 cm), *E. icterina* (7–9.5 cm), and *U. imbecillis* (5–8 cm) mussels were exposed to five malathion concentrations and both acetone and dilution water controls in 5-gal aquaria. Five to ten mussels of one species were placed in each of two to four replicates per treatment. A smaller number of adult mussels was used compared to juveniles because of concerns about the impact of large harvests on local populations. Tests with *U. imbecillis* and *E. icterina* were conducted in moderately hard water (pH 7.9) at both 25°C and 32°C, while *V. lienosa* mussels were only tested at 32°C. Mussels were not fed during the 96-h tests. Death was determined based on cessation of siphoning activity and inability to react to stimulation (tapping on shell).

RESULTS

Glochidia tests

Successful glochidia toxicity tests were completed for six mussel species. For *L. teres,* tests ended at 4 h, while *M. nervosa* exposures were limited to 24 h. We were unable to calculate LC50s for most tests performed at 32°C because control deaths exceeded 20%. Therefore, we focussed our efforts on evaluating toxicity at 25°C.

Glochidia LC50s are summarized in Table 2. Two groups of mussels were identified based on test results—"tolerant" and "very tolerant"—compared to zooplankton and fish. In the first category are *L. siliquoidea, M. nervosa, L. teres,* and *V. lienosa,* which were 7,000 to 60,000 times more tolerant than the other organisms [22]. *Utterbackia imbecillis* and *V. villosa* fall into the very tolerant category because their LC50s were 100,000 to 400,000 times higher than fish or zooplankton [22]. The 4-h LC50 for *L. teres* glochidia was 28 mg/L (pH 7.5, 25°C). The LC50s after 24 h of exposure ranged from 8 mg/L for *L. siliquoidea* (pH 7.9, 25°C) to 406 mg/L for *U. imbecillis* (pH 7.5, 25°C). The LC50s after 48 h of exposure

Table 2. Malathion LC50s for glochidia of six species of mussels measured under various test conditions

Species	Water pH	Time (h)	Malathion concentration (mg/L) LC50	UCL[a]	LCL[a]
Temperature 25°C					
U. imbecillis	7.5	24	366	383	349
		48	324	343	310
V. lienosa	7.9	24	54	58	50
L. teres	7.5	4	28	32	25
L. siliquoidea	7.5	24	54	59	49
		48	59	69	50
	7.9	24	8	8.8	7.5
		48	7	7.4	6.2
M. nervosa	7.5	24	22	24	19
Temperature 32°C					
U. imbecillis	7.5	24	374	390	358
V. villosa	7.9	24	117	123	111
		48	119	128	110

[a] UCL and LCL are upper and lower 95% confidence limits.

ranged from 7 mg/L for *L. siliquoidea* (pH 7.5, 25°C) to 387 mg/L for *U. imbecillis* (pH 7.5, 25°C). *Utterbackia imbecillis* was the least sensitive species tested. Comparisons of temperature and pH effects on glochidia LC50s are not possible because fewer tests were completed successfully.

Juvenile tests

Twelve tests were completed using six species of juvenile mussels. These were performed on *V. lienosa, V. villosa, E. icterina, L. s. claibornensis,* and *L. subangulata* juveniles transformed on fish, and *U. imbecillis* juveniles transformed in vitro (Tables 3 and 4). Tolerant mussels, with LC50s that were 28,000 to 32,000 times the *Daphnia magna* LC50, included *E. icterina, L. s. claibornensis,* and *L. subangulata.* In the very tolerant group were *U. imbecillis, V. lienosa,* and *V. villosa,* with 96-h LC50s ranging from 100,000 to 200,000 times that of *D. magna* [22].

Table 3. Malathion LC50s for juvenile mussels in moderately hard water at 25 and 32°C

Species	Time (h)	Malathion concentration (mg/L) LC50	UCL[a]	LCL[a]
Temperature 25°C				
U. imbecillis	24	667	701	633
	48	363	375	352
	72	262	272	251
	96	219	229	210
V. villosa	24	431	454	407
	48	354	375	333
	72	255	270	240
	96	142	152	132
Temperature 32°C				
U. imbecillis	24	341	351	331
	48	196	204	188
	72	161	169	153
	96	74	83	65
V. lienosa	24	>231	—	—
	48	181	188	174
	72	154	161	148
	96	109	116	102

[a] UCL and LCL are upper and lower 95% confidence limits.

Table 4. Malathion LC50s for juvenile mussels in soft water for 24 to 96 h at 25°C and 32°C

Species	Time (h)	Malathion concentration (mg/L) LC50	UCL[a]	LCL[a]
Temperature 25°C				
U. imbecillis	24	568	587	549
	48	365	382	348
	72	295	311	279
	96	215	228	202
E. icterina	24	61	64	59
	48	54	56	52
	72	50	52	47
	96	32	34	30
L. s. claibornensis	24	62	66	59
	48	48	51	46
	72	40	42	37
	96	24	26	22
L. subangulata[b]	24	43	45	41
	48	32	33	30
	72	32	34	30
	96	28	29	26
V. lienosa	24	463	485	440
	48	192	200	183
	72	140	146	134
	96	111	116	105
Temperature 32°C				
U. imbecillis	24	391	408	374
	48	280	297	263
	72	165	177	154
	96	40	46	34
V. lienosa	24	263	273	262
	48	160	170	150
	72	96	105	87
	96	74	82	66
V. villosa	24	326	340	313
	48	220	229	211
	72	199	208	190
	96	180	189	171

[a] UCL and LCL are upper and lower 95% confidence limits.
[b] Test did not meet normality test.

Twenty-four-hour LC50s for juveniles ranged from 43 mg/L for *L. subangulata* at pH 7.5 and 25°C, to 667 mg/L for *U. imbecillis* in moderately hard water at 25°C. At 48 h, LC50s ranged from 32 mg/L for *L. subangulata* to 365 mg/L for *U. imbecillis* (pH 7.5, 25°C). After a 3-d exposure, the lowest was 32 mg/L for *L. subangulata,* and the highest juvenile LC50 was 295 mg/L for *U. imbecillis* (pH 7.5, 25°C). Finally, 96-h LC50s ranged from a low of 24 mg/L for *L. s. claibornensis,* to a high of 219 mg/L for *U. imbecillis* (pH 7.5 and 25°C).

The impact of higher temperature on toxicity was also noted. The 96-h LC50 for juvenile *U. imbecillis* at 25°C and pH 7.5 was 215 mg/L malathion, while at 32°C the LC50 was 40 mg/L. The 96-h LC50 for juvenile *U. imbecillis* at 25°C and pH 7.9 was 219 mg/L vs. 109 mg/L at 32°C. At 25°C, the 96-h LC50 for *V. lienosa* at pH 7.5 was 111 mg/L, while at 32°C it was 74 mg/L. In some cases, water pH was found to affect the toxicity of malathion to juvenile mussels. The 96-h LC50s for juvenile *U. imbecillis* were 7.4 mg/L malathion at pH 7.9 and 40 mg/L at pH 7.5 at 32°C. The *V. lienosa* mussels had LC50s of 74 mg/L at pH 7.5 and 109 mg/L at pH 7.9. However, pH did not appear to affect LC50s calculated for tests with juvenile *U. imbecillis* conducted at 25°C. At a pH of 7.9, the 96-h LC50 was 219 mg/L, while at pH 7.5 it was 215 mg/L.

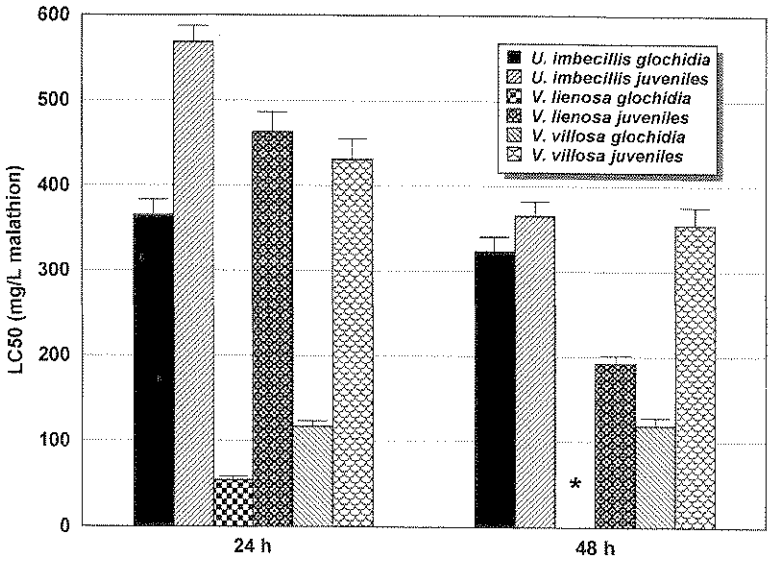

Fig. 1. Comparisons of malathion LC50s for glochidia and juvenile mussels. Bars indicate 95% confidence intervals. Asterisk indicates that no data were available for *Villosa lienosa* glochidia at 48 h.

Adult tests

Fewer toxicity tests were performed with adult mussels because of concerns about the impact of over harvesting and because they were found to be very tolerant of acute exposures to malathion. Fifty percent mortality was not observed for *V. lienosa, U. imbecillis,* or *E. icterina* at concentrations of up to 350 mg/L after 96 h of exposure. However, adults in malathion concentrations of 115 mg/L and greater were slow to close when disturbed, and at exposures of 150 mg/L or higher some animals never totally closed. By day 3, mussels in concentrations above 150 mg/L also produced large amounts of mucus.

DISCUSSION

In general, glochidia and juvenile mussels exhibited similar insensitivity to malathion. Glochidia LC50s had a greater range at 24 or 48 h (7–374 mg/L) than did juvenile mussel LC50s at 96 h (24–219 mg/L), and where both early life stages for a species were tested, glochidia usually had lower LC50s (Fig. 1). However, the overlapping confidence intervals (Tables 2–4) indicate that there are no real differences in the sensitivity of these early life stages. Adult mussels were less sensitive to malathion than the early life stages, a result that might have been expected based on similar findings with fish, insects, and other typical test organisms [22,23]. No adult mussel LC50 was calculable after 96-h exposures at concentrations up to 350 mg/L.

Mussel toxicity tests conducted at 32°C reflect the direct impact of temperature on survival in that many such tests failed due to high mortality in controls. In the three sets of juvenile tests conducted at the same pH, LC50s were lower at 32°C than at 25°C (Fig. 2). Because malathion degrades very quickly at 32°C (≅87% in 48 h) [11], it appears that high temperature was the major cause of mortality. Dimock and Wright [24] noted a similar decrease in the survival rate of juveniles of two mussel species exposed to elevated temperatures in the absence of contaminants, recording 96 h LT50s (50% thermal limit) at 31 to 33°C.

Two types of test water were used, soft at pH 7.5 and moderately hard at pH 7.9, primarily to evaluate toxicity at different pHs. Water pH can affect toxicity because malathion degrades more slowly in acidic conditions [6,12,14]. Some comparisons between LC50s calculated for the same species at different pHs followed this pattern. However, tests conducted on juvenile *U. imbecillis* and glochidia from *L. siliquoidea* did not. More research into pH effects on the toxicity of malathion is needed to better understand these inconsistencies.

The study's findings indicate that the toxicity of malathion to unionid mussels varies by species and test temperature but in general is much lower than for most other aquatic organisms. From the perspective of the boll weevil program, the most important result from this study was that all of the LC50s were found to be in the mg/L range, while the typical measured concentration of malathion in water bodies adjacent to sprayed areas was <0.1 μg/L and highest concentrations only reached 30 μg/L and dissipated within 4 h. Such a difference between environmental concentration and faunal sensitivity suggests that there would be no acute lethality to unionid mussels in the program area due to properly applied malathion.

The use of an EPA approach to measure risk further supports this conclusion. The EPA compares the expected envi-

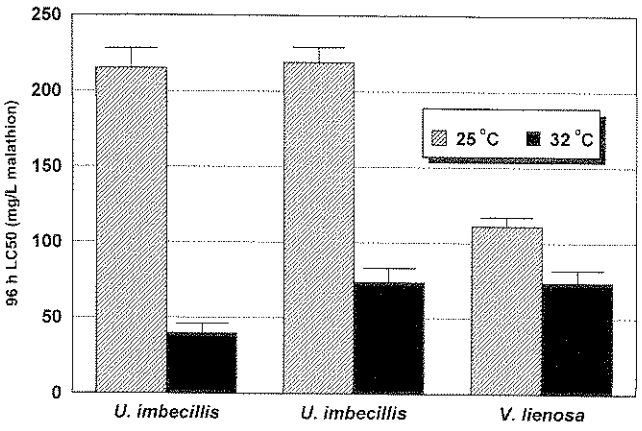

Fig. 2. Comparisons of malathion LC50s for juvenile toxicity tests performed at 25°C and 32°C. Bars indicate 95% confidence intervals.

ronmental concentration (EEC) to a value equal to one-tenth the LC50 for the most sensitive aquatic organism. The aquatic life criterion of 0.1 μg/L was calculated in this way based on the *Daphnia* LC50 of 1 μg/L. If endangered species are involved, the lowest aquatic LC50 is divided by 20 to determine the risk of pesticide exposure. When the EEC exceeds one-tenth or one-twentieth of the LC50, then a risk exists and restrictions on pesticide use may be imposed [25]. This more conservative approach is used because it is believed that species may have become endangered due to their heightened sensitivity to contaminants such as pesticides [25]. While no endangered species were tested, unionid surrogates were used to better represent endangered mussels. If glochidia and juvenile mussel LC50s are divided by 20, we get a range of 0.350 mg/L (*L. siliquoidea*) to 22 mg/L (*U. imbecillis*) malathion for glochidia and a range of 1.4 mg/L (*L. s. claibornensis*) to 11 mg/L (*U. imbecillis*) malathion for juveniles. These values are 10 to 730 times greater than the 30-μg/L maximum concentration measured in water bodies adjacent to sprayed fields and are much higher than the present aquatic acute criterion [7].

The literature on malathion toxicity to unionids is limited to a few mainly biochemical or physiological studies [26–29]. In an examination of sublethal impacts, Desi et al. [26] found that malathion diminished glochidial activity. Concentrations ranging from 1 μg/L to 100 μg/L significantly reduced the valve opening/closing activity of glochidia of *Anodonta cygnea* during exposure periods of 1 to 5 d. While reduced valve activity could decrease the likelihood that glochidia would attach to host fish, this endpoint is difficult to compare to LC50s calculated in the current study.

Current test results for adult mussels are consistent with studies that have found measurable changes in physiological responses only at malathion concentrations that are greater than would ordinarily be expected in the environment [6,27]. Adult *A. cygnea* were found to exhibit significantly reduced adductor muscle activity in 48-h tests with malathion at concentrations of 10 and 100 mg/L [26], whereas a 50% decrease in mantle cilia activity was recorded in adult *Lamellidens marginalis* exposed to 3.5 mg/L malathion [28]. These effects could reduce a mussel's ability to regulate respiration, protect itself from harsh conditions or avoid predation, and decrease both its ventilatory and filter-feeding capacity. Muley and Mane [29] recorded a lower respiration rate in three mussel species inhabiting Indian rivers following exposure to concentrations similar to LC50s determined in the present study. None of these impacts were acutely lethal, but it is likely that such physiological effects would reduce the fitness and survival of mussels over a long period of time.

CONCLUSIONS

Data produced from this and other studies [6,26–29] have shown that unionid mussels are less sensitive to acute exposures to malathion than are many other aquatic fauna. For comparison, the 48-h LC50 for the amphipod, *Gamarus fasciata,* is 0.76 μg/L, and for the zooplankter, *D. magna,* is 1 μg/L. The 96-h LC50 for the stonefly, *Pteronarcella badia,* is 6.2 μmg/L, and fish 96-h LC50s range from 20 μg/L for bluegill to 12.9 mg/L malathion for black bullhead [22]. Based on the results of this study, the EPA aquatic acute criterion of 0.1 μg/L [7] adequately protects endangered mussels in areas sprayed with malathion. As a result, the US FWS lifted its no exposure restriction on the boll weevil control program.

Acknowledgement—The authors received invaluable cooperation from Ron Berger and Susan Bright from the U.S. Department of Agriculture Animal Plant Inspection Service in completing this research and very helpful critiques of the manuscript from Jim Dwyer and Parley Winger of the National Biological Service.

REFERENCES

1. **Master, L.** 1990. The imperiled status of North American aquatic animals. *Biodiver. Network News* **3**:1–2, 7–8.
2. **Williams, J.D., M.L. Warren, Jr., K.S. Cummings, J.L. Harris** and **R.J. Neves.** 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* **18**:6–22.
3. **U.S. Fish and Wildlife Service.** 1994. Endangered and threatened wildlife and plants. *Fed. Reg.* **59**:58982–59028.
4. **U.S. Fish and Wildlife Service.** 1990. White cat's paw pearly mussel recovery plan. Twin Cities, MN.
5. **U.S. Fish and Wildlife Service.** 1994. Clubshell (*Pleurobema clava*) and northern riffleshell (*Epioblasma torulosa rangiana*) recovery plan. Hadley, MA.
6. **Mulla, M.S., L.S. Mian** and **J.A. Kawecki.** 1981. Distribution, transport, and fate of the insecticides malathion and parathion in the environment. *Residue Rev.* **81**:1–172.
7. **U.S. Environmental Protection Agency.** 1986. Quality criteria for water 1986. EPA 440/5-86-001. Office of Water Regulations and Standards, Washington, DC.
8. **Lewis, P.A., D.J. Klemm, J.M. Lazorchak, T.J. Norberg-King, W.H. Peltier** and **M. Heber.** 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-91/002. U.S. Environmental Protection Agency, Cincinnati, OH.
9. **Ford, J.H., C.A. McDaniel, F.C. White, R.E. Vest** and **R.E. Roberts.** 1975. Sampling and analysis of pesticides in the environment. *J. Chromatogr. Sci.* **13**:291–295.
10. **Mulla, M.S.** 1963. Persistence of mosquito larvicides in water. *Mosq. News* **23**:234–237.
11. **Konrad, J.G., G. Chesters** and **D.E. Armstrong.** 1969. Soil degradation of malathion, a phosphorodithioate insecticide. *Soil Sci. Soc. Am. Proc.* **33**:259–262.
12. **Weiss, C.M.** and **J. Gakstatter.** 1964. The decay of anticholinesterase activity of organic phosphorus insecticides on storage in waters of different pH. *Adv. Water Pollut. Res.* **1**:83.
13. **Eichelberger, J.W.** and **J.J. Lichtenberg.** 1971. Persistence of pesticides in river water. *Environ. Sci. Technol.* **5**:541–544.
14. **Paris, D.F.** and **D.L. Lewis.** 1973. Chemical and microbial degradation of ten selected pesticides in aquatic systems. *Residue Rev.* **45**:95–124.
15. **Gelber, R.D., P.T. Lavin, C.R. Mehta** and **D.A. Schoenfeld.** 1985. Statistical analysis. In G.M. Rand and S.R. Petrocelli, eds., *Fundamentals of Aquatic Toxicology.* Hemisphere, New York, NY, USA, pp. 110–123.
16. **Jones, R.O.** 1950. Propagation of fresh-water mussels. *Prog. Fish Cult.* **1**:13–25.
17. **Lasee, B.A.** 1991. Histological and ultrastructural studies of larval and juvenile *Lampsilis* (Bivalvia) from the upper Mississippi River. Ph.D. thesis. Iowa State University, Ames, IA, USA.
18. **McMahon, R.F.** 1991. Mollusca: Bivalvia. In J.H. Thorp and A.P. Covich, eds., *Ecology and Classification of North American Freshwater Invertebrates.* Academic, New York, NY, USA, pp. 315–399.
19. **Keller, A.E.** and **S.G. Zam.** 1990. Simplification of in vitro culture techniques for freshwater mussels. *Environ. Toxicol. Chem.* **9**:1291–1296.
20. **Keller, A.E.** and **S.G Zam.** 1991. The toxicity of selected metals to the freshwater mussel *Anodonta imbecillis. Environ. Toxicol. Chem.* **10**:539–546.
21. **Keller, A.E.** 1993. Acute toxicity of several pesticides, organic compounds and a wastewater effluent to the freshwater mussel, *Anodonta imbecillis, Ceriodaphnia dubia* and *Pimephales promelas. Bull. Environ. Contam. Toxicol.* **51**:696–702.
22. **Mayer, F.R.** and **M.R. Ellersieck.** 1986. Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. *U.S. Fish Wildl. Serv. Publ. 160.*
23. **Rand, G.M.** and **S.R. Petrocelli.** 1985. *Fundamentals of Aquatic Toxicology.* Hemisphere, New York, NY, USA.
24. **Dimock, R.V.** and **A.H. Wright.** 1993. Sensitivity of juvenile freshwater mussels to hypoxic, thermal and acid stress. *J. Elisha Mitchell Sci. Soc.* **109**:183–192.

25. **Urban, D.J.** and **N.J. Cook.** 1986. Hazard Evaluation Division, Standard Evaluation Procedure, Ecological Risk Assessment. EPA 540/9-85-001. U.S. Environmental Protection Agency, Washington, DC.
26. **Desi, I., G. Dura, L. Gönzi, Z. Kneffel, A. Strohmayer** and **Z. Szabó.** 1976. Toxicity of malathion to mammals, aquatic organisms and tissue culture cells. *Arch. Environ. Contam. Toxicol.* **3**: 410–425.
27. **Mulla, M.S.** and **L.S. Mian.** 1981. Biological and environmental impacts of the insecticides malathion and parathion on nontarget biota in aquatic systems. *Residue Rev.* **78**:101–135.
28. **Ahamad, I.K., M. Sethuraman, M.R. Begum** and **K.V. Ramana Rao.** 1979. Effect of malathion on ciliary activity of fresh water mussel, *Lamellidens marginalis* (Lamarck). *Comp. Physiol. Ecol.* **4**:71–73.
29. **Muley, D.V.** and **U.H. Mane.** 1987. Malathion induced changes in oxygen consumption in two species of freshwater lamellibranch molluscs from Godavari River, Maharashtra State, India. *J. Environ. Biol.* **8**:267–275.
30. **Burch, J.B.** 1975. *Freshwater Unionacean Clams (Mollusca: Pelecypoda) of North America.* Malacological Publications, Hamburg, MI, USA.

APPENDIX

Zoogeographic distribution of mussel species used in malathion toxicity tests [30]

Species	Distribution
Utterbackia imbecillis	United States east of the Rocky Mountains, generally
Megalonaias nervosa	Throughout the Mississippi River system; the Tombigbee and Alabama rivers, east to the Ochlockonee River in Florida
Villosa lienosa	Alabama-Coosa River system to Appalachicola River, Florida; from Texas to the lower Mississippi River, north to the lower Ohio and Wabash River, and east to southwestern Georgia and Florida
Villosa villosa	Appalachicola River system east to the St. Marys River system of Georgia and in peninsular Florida
Elliptio icterina	Escambia River drainage of Alabama and Florida, east to the Atlantic Coast and north in Atlantic coastal drainages to southern North Carolina
Lampsilis teres	Mississippi drainage, peninsular Florida west to Mexico
Lampsilis siliquoidea	Mississippi River, St. Lawrence interior drainage, and the Canadian interior basin (not in the Tennessee and Cumberland systems)
Lampsilis subangulata	Appalachicola, Chattahootchee, Flint, and Ochlockonee River drainages of Georgia and Florida
Lampsilis straminea claibornensis	Suwannee River system, west to eastern Louisiana