III.6 Grasshopper Treatment Effects on Aquatic Communities

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Concern about potential for adverse effects on endangered species from inadvertent exposure to insecticides was partially responsible for initiation of the Grasshopper Integrated Pest Management (GHIPM) Project. Investigation of effects of grasshopper control operations on aquatic communities was one aspect of the Project and had two major emphases.

The first emphasis was evaluation of the toxicity of carbaryl and malathion to two federally endangered fishes that inhabit rivers of the Colorado River Basin (the Colorado River and tributaries in Wyoming, Colorado, Utah, New Mexico, and Arizona). The second area of research involved environmental monitoring of the effects of operational grasshopper insecticide applications on aquatic invertebrates and fish in ponds and streams. Results of these studies provide information on potential effects of pesticide application practices and allow evaluation of adequacy of no-spray buffer zones around aquatic habitats.

Toxicity Testing With Endangered Fishes

The Colorado pikeminnow *(Ptychocheilus lucius)* and bonytail *(Gila elegans)* are large minnows historically found throughout the Colorado River Basin. Populations of both species have declined as a result of interactions with introduced fishes, construction of dams, and habitat modification. Young Colorado pikeminnow and bonytail occupy shallow, low-velocity, near-shore nursery habitats. These habitats have low rates of water exchange, and pesticides deposited in them may persist in sufficient concentration and duration for toxic effects to occur.

The timing of grasshopper control programs coincides with the presence of potentially sensitive early life stages of Colorado pikeminnow and bonytail in nursery habitats. But the infrequency and low application rate of pesticide use in Federal grasshopper control programs present a minor risk to these endangered fishes in comparison to other hazards, such as cropland chemicals, instream flow changes, and introduced (exotic) species. Nevertheless, data are needed on the IPM chemical effects.

Because of uncertainty in predicting the sensitivity of Colorado pikeminnow and bonytail to carbaryl and malathion, Beyers et al. (1994) estimated toxicity of these chemicals using methods recommended by U.S. Environmental Protection Agency and the American Society for Testing and Materials. The toxicity of technical carbaryl, Sevin® 4-Oil, and technical malathion was estimated by determining (1) 96-hour median lethal concentrations, and (2) concentrations that affected survival and growth in 32-day early life-stage tests (Beyers 1993, Beyers et al. 1991 and 1994).

One concern responsible for initiation of toxicological studies was that Colorado pikeminnow or bonytail might be supersensitive to carbaryl or malathion. To evaluate this possibility, we compared the sensitivity of Colorado pikeminnow and bonytail to other commonly studied fishes. We concluded that Colorado pikeminnow and bonytail were 2 to 10 times more sensitive to carbaryl than fathead minnow *(Pimephales promelas)* but were about as sensitive to malathion as fathead minnow (Beyers et al. 1994, Mayer and Ellersieck 1986). Some pesticide formulations are more toxic than their technical compounds; however, toxicity of Sevin 4-Oil (49 percent carbaryl) is approximately one-half that of technical carbaryl. No synergistic or antagonistic toxic effects due to formulation of carbaryl as Sevin 4-Oil were observed.

Results of standardized toxicity tests provided quantitative description of toxicant effects, but the tests did not simulate chemical exposure conditions likely to occur in the field. Therefore, we conducted studies of brain acetylcholinesterase (AChE) inhibition in order to estimate toxicant effects at a scale consistent with the duration of exposure and concentration range typically observed in the field. AChE activity was measured in Colorado pikeminnow after 24-hour *in vivo* exposure to technical carbaryl or malathion (Beyers and Sikoski 1994).

A comparison of the potency of the 2 toxicants showed that technical carbaryl was about 13 times more toxic than malathion to Colorado pikeminnow. Toxicant concentrations that significantly affected AChE activity were 15 times lower for carbaryl and 4 times lower for malathion than concentrations that affected growth or survival in 32-day early life-stage tests. These differences were attributed to development of physiological tolerance over the 32-day period used for early life-stage tests, and greater sensitivity of biochemical processes (AChE inhibition) compared to whole-organism responses (growth or survival).

Environmental Monitoring

Insecticides used to control grasshopper infestations pose a potential hazard to fish and invertebrates because, although no-spray buffer zones are observed around aquatic habitats, pesticide may be deposited by drift or mobilized from upland areas by runoff. We investigated effects of several aerial grasshopper control pesticide applications within the Little Missouri National Grasslands in western North Dakota (Beyers et al. 1995, Beyers and Myers 1996).

Environmental monitoring in aquatic habitats involved collection of water samples for pesticide analysis and study of sublethal and lethal effects on invertebrates and fish. In pond studies, we used enclosures called mesocosms to divide a portion of a pond into independent experimental units. Each mesocosm contained sediment, plants, and invertebrates that occurred naturally in the pond. We monitored survival of invertebrates within mesocosms for up to 4 days after pesticide application. *In situ* toxicity tests using naturally occurring invertebrates were also conducted with mesocosms.

The effects of pesticide application on river-dwelling organisms in the Little Missouri River were investigated on two separate occasions. Potential effects on aquatic invertebrates were investigated by quantifying daytime invertebrate drift. Normally, aquatic invertebrate drift in rivers is low. However, when pesticides are introduced, catastrophic drift may occur as invertebrates attempt to avoid toxicant exposure or suffer toxic effects (Wiederholm 1984). Sublethal effects on fish in the Little Missouri River were evaluated by studying fish-brain AChE inhibition. AChE activity of flathead chub *(Platygobio gracilis)* collected from control and treatment sites before and after pesticide application was measured.

Results of monitoring showed that when the standard 500-ft (152-m) no-spray buffer was employed, trace amounts of pesticide were always detected in aquatic habitats. The amount of deposition was dependent on the size of the aquatic habitat; smaller ponds had higher pesticide concentrations. Detection of trace amounts of pesticides does not necessarily result in biological effects on aquatic organisms.

We intensively studied six ponds but found evidence of direct mortality of pond-dwelling organisms in only one. On this occasion, a 0.6-acre (0.23-ha) pond containing abundant amphipods was monitored during an application of Sevin 4-Oil. All amphipods in treatment enclosures died within 24 hours of pesticide application. Subsequent collections confirmed that the amphipod population in the pond had declined. Amphipods are known to be extremely sensitive to carbaryl and malathion (Mayer and Ellersieck 1986). Other taxa in the pond appeared to be unaffected by the application.

Studies in the Little Missouri River during a drought year (1991), when discharge and the dilution potential of the river was low, detected an increase in invertebrate drift during the first 3 hours after pesticide application (Beyers et al. 1995). This increase was primarily composed of Ephemeroptera, especially Heptageniidae. There was no change in drift at the reference site. Subsequent sampling during the day of pesticide application showed that the increase in invertebrate drift was transient and undetectable after 3 hours.

The biological significance of increased invertebrate drift due to pesticide application is uncertain but probably of minimal consequence. The increase in invertebrate drift was mostly due to Ephemeroptera; other taxa were unaffected. Because a relatively small portion of the Little Missouri River was within the spray block (3.2 river-miles or 5.2 river-km), mortality was probably compensated by recolonization from unaffected organisms living in the substrate or upstream. Thus only a portion of the invertebrate community may have been affected, and the likelihood of rapid recovery of affected populations was high. Analyses of brain AChE activity in flathead chub showed that fish were not affected by the pesticide application. Similar monitoring studies conducted during a year when precipitation was above average (1993) did not detect any increase in aquatic invertebrate drift or effects on fish (Beyers et al. 1995). The overall conclusion was that these grasshopper control operations had no biologically significant affect on aquatic resources.

A factor that may reduce the potential for toxic effects to aquatic organisms is the natural degradation of carbaryl and malathion. Both pesticides hydrolyze (decompose chemically) rapidly in waters with pH >7 (Beyers and

Myers 1996). All aquatic habitats monitored in North Dakota had pH's greater than 7. Although the amount of pesticide deposited in aquatic habitats may be potentially toxic to some aquatic life, the short duration of the exposure can reduce or eliminate toxic effects.

Our investigations were designed to detect AChE inhibition or invertebrate mortality within 96 hours of pesticide application. If toxic effects were manifested over a longer time scale it is unlikely that effects would have been detected by our investigations. Toxicity endpoints other than death of aquatic organisms (such as swimming ability, avoidance of predators, feeding behavior, and reproductive effects) also are receiving attention by others in the field of aquatic ecotoxicology (Nimmo and McEwen 1994).

A Note on Quality Assurance for Pesticide Monitoring

One of the reasons why carbaryl and malathion are used to control grasshopper infestations is that they degrade relatively rapidly in the environment. Short persistence assures less potential for nontarget effects; however, these qualities complicate sampling for pesticide analysis because, if precautions are not taken, degradation may continue to occur after a sample has been collected and pesticide concentration estimates will be in error.

An important aspect of quality assurance (QA) that can be used to guard against this eventuality is fortification (spiking with measured pesticide amounts) of similar environmental samples. Prior to pesticide application, samples for fortification should be collected at the same localities where pesticide monitoring samples will be collected. A known amount (for example, 1 mL) of a fortification standard should be added to each QA sample. To prevent investigator bias, QA samples should not be identified any differently than posttreatment monitoring samples. QA samples should be handled and submitted for chemical analysis along with other monitoring samples. In general, QA samples should be fortified to approximately 10 times the detection limit reported by the analytical laboratory and the number of QA samples should be about 10 percent of total number of samples submitted for analysis. If only a few monitoring samples are being collected (fewer than 10), then at least 2 QA samples should be submitted. Fortification standards should be obtained from the laboratory that will be conducting the analytical work (see Chapter III.9). When reporting results of pesticide monitoring, percent recovery from fortified samples also should be reported. The importance of including QA samples cannot be overstated: they provide the only method for judging accuracy of reported results.

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