SUBLETHAL EFFECTS OF PESTICIDES ON FISHES

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It is obvious that nearly any pesticide at a sufficient concentration in the water can kill fish and other aquatic animals. However, fish kills from pesticides are relatively rare, especially with the phasing out of the most toxic of the organochlorine insecticides. What is more insidious and has potentially longer-term impact are the effects of exposure of resident fish to concentrations well below lethal levels.

When a fish or other animal is exposed to a pesticide at a sublethal concentration, a wide variety of physiological and biochemical changes occur. These might represent an adaptation to the chemical stress that, if successful, will permit the fish to continue to function normally in its environment, or they might indicate partial failure of one or more physiological functions that can make the fish more vulnerable to predation or disease, less able to reproduce, slower to grow, etc.

Although the concept of target organ is useful in toxicology, especially when considering lethality, a given pesticide will usually affect multiple physiological/biochemical functions in a fish rather than just one, although the character of the response differs with the group of chemicals under investigation. The literature is extensive, so only a few examples will be included in this review. They were chosen to show the breadth of effects that may be seen and to illustrate some of the methodologies used to assess sublethal effects of pollutants on fishes. They are grouped as physiological, biochemical, behavioral, or those involving reproduction and growth, although such a classification is a bit arbitrary at times.

Physiological Changes

The respiratory system of a fish, with its extensive and very sensitive gill tissue, is generally the first point of impact from some harmful chemical. When exposed to a variety of pesticides, fish will begin to breath faster and cough because the chemical irritates the delicate gill tissue. These changes in breathing may occur at extremely low concentrations of the pesticide in the water. Because the respiratory movements can be monitored electronically, their measurement has been used recently in early-warning systems to detect chemicals (including pesticides) harmful to humans in municipal water supplies that are taken from rivers that have the potential for upstream spills or other contamination (Gruber and Diamond 1988). In this way, the fish acts as a sort of "canary in the mine," but death of the fish is not the end-point. Instead, a sublethal effect, which in this case is an aberrant breathing pattern, is the end-point of interest.

The swimming ability of fish can be tested by inducing them to swim in a water tunnel, which is similar in design to the wind tunnels used to test models of airplanes and cars, only it is filled with water. With such a device, the maximum swimming speed can be measured, as well as the ability to maintain position at a fixed speed (i.e., stamina). Chronic exposure to malathion has been shown to reduce the swimming speed and stamina of fish by as much as 70 percent (Post and Leasure 1974), which would be especially important for those species such as salmon that migrate up rapidly flowing rivers. The amount of swimming inhibition correlates approximately with the extent of acetylcholinesterase inhibition in the brain (discussed below), so the action of the pesticide is, at least in part, on the nervous system, although other systems (e.g., respiratory) that are important in swimming ability may also be affected.

A variety of blood chemistry and hematological changes have been observed in fish exposed to different pesticides (summarized by Heath 1987). These include increases or decreases in such parameters as hemoglobin concentration, hematocrit, serum enzymes, serum electrolytes, and blood glucose. Such clinical measurements are frequently used to assess health of humans and domestic animals, as they can indicate that a homeostatic mechanism (e.g., electrolyte balance) is compromised by a pesticide or that the animal is under some sort of nonspecific stress. Their use in fish health measurement is only beginning but is somewhat more difficult to use because of the extremely large number of fish species. Also, because various environmental factors such as temperature and photoperiod can have a considerable influence on them, "normal" levels for various blood values are not easily specified. And, there is a great need to determine the ecological relevance for the organism of changes in one or more of these substances in the blood (Merle and Mayer 1980). In this regard, Finlayson and Faggella (1986) exposed channel catfish and common carp to molinate, an herbicide, for 28 days and noted that concentrations that caused some mortality also caused decreases in hemoglobin and hematocrit. Thus, the hematological changes were no more sensitive than was mortality; however, detection of anemia such as this in a natural population during a routine monitoring program could be used to indicate a problem before large numbers of fish start turning belly up.

The collagen content of fish bones appears to be especially sensitive to toxaphene (Mayer et al. 1977). A lack of collagen can lead to abnormal growth and inability to swim correctly. Such changes were seen in fathead minnows with concentrations of toxaphene as low as 55 ng/L. Indeed, a variety of chemical pesticides (e.g., chlordecone, trifluralin, Dursban) have been found to cause abnormal bone growth in young fish and produce conditions such as scoliosis and longitudinal fusion of the vertebrae. These changes may be due, at least in part, to effects on the nervous system in addition to or rather than directly affecting bone growth.

Biochemical Changes

There are numerous potential biochemical changes that can be examined in fish exposed to pesticides, and quite a few have been. The one that has probably received the most attention is inhibition of acetylcholinesterase (AChE) in the brain. This enzyme is responsible for preventing the buildup of acetylcholine at synapses. The organophosphate and carbamate insecticides at very low concentrations are especially noteworthy for their ability to inhibit this enzyme. For this reason, the detection of inhibition has been used as a diagnostic tool for insecticide poisoning in birds, mammals, and to a lesser extent in fish. However, a variety of other substances have also been found to inhibit this enzyme when tested *in vitro*; whether this holds true *in vivo* remains to be determined (Heath 1987).

In general, the inhibition of AChE is a function of exposure concentration and duration, and there is a fairly good correlation between the active insecticide concentration in the brain and the extent of inhibition. A number of workers have noted that as much as a 70 to 80% loss of AChE activity may take place before death in a fish. There is a marked species difference in the sensitivity of this enzyme to OP and carbamate insecticides, so a dose that results in a rapid depletion of AChE in one species may result in no change in another (Gantverg and Perevoznikov 1984). Even though a large degree of inhibition is required before death, much lesser amounts of inhibition have been shown to result in reductions in swimming capacity (discussed above) and in ability to learn (Sun and Taylor 1983), two critical nervous system functions.

Another group of enzymes have received a great deal of attention because they are a target of organochlorine compounds. These are the ATPases associated with the plasma membranes and mitochondria of essentially all cells. A considerable amount of work (summarized at length by Murty 1986) has shown that the Na,K ATPases and Mg ATPases are clearly inhibited by organochlorine pesticides. The result of these inhibitions can be altered blood electrolytes and neurological functions, the latter being what most people associate with pesticide poisoning as it frequently causes changes in behavior of the fish that can be easily seen without sophisticated equipment.

Behavioral Effects

As a group, the organochlorine compounds usually cause the fish to become hypersensitive to external stimuli such as sounds or lights. In nature, such excessive bodily movements may make the fish more vulnerable to predation. The organophosphorus compounds may cause hypersensitivy or lethargy; the exact response varies with chemical and fish species.

Many species of fish school as a means to avoid predation and to reduce (by as much as 30%) the energy required for swimming. Weis and Weis (1974) noted that exposure of Atlantic silverside for

1 to 3 days to carbaryl (100ug/L) caused the school to occupy twice the area of controls and required a greater food supply to support it because of the larger energy demand for swimming.

Fish that are living in streams must be able to detect and orient into the current, a phenomenon referred to as rheotaxis. With the aid of a fairly sophisticated method, it has been found that exposure of trout to diquat (an herbicide) at doses similar to those used in the field but an order of magnitude below the lethal concentration causes a reduction in rheotaxis (Dodson and Mayfield 1979). This might cause exposed fish to drift downstream and possibly have problems feeding. It is interesting that in the same study, another herbicide, Simazine, caused no effect on rheotaxis, but, when combined with a wetting agent (Tween 80), there was an effect. Thus, the formulation can have an impact on how the pesticide affects fish.

Fish have the ability to detect and avoid, if given the opportunity, potentially harmful concentrations of some chemicals (summarized by Giattina and Garton 1983). This ability is measured in a chamber where one part has non-contaminated water while the other has water with the test chemical in it. The fish are observed to see whether they avoid (or perhaps are attracted) to the water with the chemical in it. There is considerable species and chemical variability in the response observed. In the context of this minisymposium, it is especially interesting to cite the study by Folmer (1976) in which herbicides were tested with rainbow trout fry at three different concentrations, one well below, one approximately at, and one well above the water concentration that would be expected when applied at recommended rates. The fry clearly avoided copper sulphate, Dalapon, and 2,4-D. They apparently were unable to detect glyphosate, endothal, or Diquat at any of the tested concentrations, although more recently it has been found that trout can avoid glyphosate at a concentration just below the lethal one, which is considerably above that found after field application (Hildebrand et al. 1982).

Avoidance can have interesting effects. For example, avoiding the chemical (assuming there is water that is non-contaminated that is available to the fish) helps them avoid possible harmful effects of the chemical. However, it also means that potentially large areas of habitat are at least temporarily rendered unusable by those fish. In any case, it is certainly not valid to assume that fish will merely avoid the chemical, as was shown with the three herbicides mentioned above.

Because many insecticides have strong effects on the nervous system, it is expected that they might reduce memory capability. This has been quantified using a shuttlebox avoidance-conditioning system with goldfish wherein the fish learns to avoid a shock when a light is turned on by moving to the other side of an aquarium. Sun and Taylor (1983) found that parathion at a dose commonly used for mosquito control in California caused a significant inhibition of the ability to learn this response.

Reproduction and Growth

It is a truism of aquatic toxicology that a chemical does not have to be lethal to eliminate a population; all that is required is for it to reduce reproduction, which involves not only production of fertilized eggs but also growth of the young fish. Free-swimming fry and juveniles are almost always more sensitive to both acute and sublethal doses of chemicals. Because of this sensitivity of young fish to chemicals, standardized toxicity tests have been developed for quantifying the toxicity of chemicals (USEPA 1985). The fathead minnow is the usual test organism and tests have been set up so they can be completed in seven days. Both survival and growth are measured so that acute as well as sublethal effects are determined. In this way a dose can be estimated that causes no effect, which goes by various names such as "Maximum Acceptable Toxicant Concentration" (MATC).

Eggs and yolk-sac fry are often rather resistant to acute doses of harmful chemicals, but teratogenic effects from chronic exposures of the eggs are common (Rosenthal and Alderdice 1976). And the chemicals may get into the eggs by way of the female fish rather than from fry being spawned in contaminated water. The overall result may be numerous malformed young fish that have little likelihood of survival.

The hormonal controls of reproduction in fish are easily as complex as those in other vertebrates. Pesticides may directly inhibit secretion of various hormones or may cause them to be metabolized excessively fast by the liver. The latter is the result of induction of the cytochrome P-450 system in liver cells that is used to detoxify harmful chemicals. This enzyme system also metabolizes sex hormones, so an excess of it causes reductions in these critical hormones and reduced reproduction (Lech et al. 1982).

Concluding Comments

The various ways that pesticides can cause physiological harm to fish are numerous and still poorly understood. Determinations of concentrations that are lethal are important but should not (and for the most part are not) the only information utilized in determining what are safe usage practices. In the future, sublethal effects will be considered much more by regulatory bodies.

Herbicides as a group tend to be less acutely toxic to fish than insecticides. However, their sublethal effects have been little investigated and may prove to be especially important. Furthermore, herbicides can have other effects on fish that are more indirect. For example, when masses of aquatic vegetation are killed by herbicides, the decomposition processes can easily deplete the dissolved oxygen in the water so the fish then suffer from hypoxia. Another more subtle effect is the elimination of aquatic plant life upon which some species of fish lay eggs or feed. Thus, we see that toxicity is not the only way which pesticides can affect resident fish.

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