REVIEW:

ASSESSING EFFECTS OF PESTICIDES ON AMPHIBIANS AND REPTILES: STATUS AND NEEDS

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ABSTRACT

Growing concern about the decline of certain amphibian populations and for conservation of amphibians and reptiles has led to renewed awareness of problems from pesticides. Testing amphibians and reptiles as a requirement for chemical registration has been proposed but is difficult because of the phylogenetic diversity of these groups. Information from the literature and research may determine whether amphibians and reptiles are adequately protected by current tests for mammals, birds, and fish. Existing information indicates that amphibians are unpredictably more resistant to certain cholinesterase inhibitors, and more sensitive to two chemicals used in fishery applications than could have been predicted. A single study on one species of lizard suggests that reptiles may be close in sensitivity to mammals and birds. Research on effects of pesticides on amphibians and reptiles should compare responses to currently tested groups and should seek to delineate those taxa and chemicals for which cross-group prediction is not possible. New tests for amphibians and reptiles should rely to the greatest extent possible on existing data bases, and should be designed for maximum economy and minimum harm to test animals. A strategy for developing the needed information is proposed. Good field testing and surveillance of chemicals in use may compensate for failures of predictive evaluations and may ultimately lead to improved tests.

INTRODUCTION

The U.S. Fish and Wildlife Service (1990) recently released a list of endangered and threatened wildlife species that included 19 taxa of amphibians and 101 taxa of reptiles. More than a decade ago, a committee of scientists (Anon., 1977) reported to the European Committee for the Conservation of Nature and Natural Resources that at least 30% of Europe's amphibian species and 45% of its reptile species were in danger of extinction. Similarly, an earlier assessment in the United States (Bury, Dodd & Fellers, 1980) estimated that continued survival of at least 16% of native salamanders and 7% of frogs and toads was in jeopardy. More recent studies by the U.S. Fish and Wildlife Service (Corn, Stolzenburg & Bury, 1989) of the effects of acid precipitation on amphibian populations of the Rocky Mountain region noted declines of several species, but current evidence does not implicate acidification as a primary cause of these declines. Worldwide concern over declining amphibians prompted the U.S. National Research Council to sponsor a conference in February 1990 to summarize evidence and to seek explanations for the declines (Borchelt, 1990). Measures to conserve amphibians and reptiles have been slow, and threats from toxic chemicals in the environment are among the threats that have received insufficient attention. The success of governments in ameliorating the effects of toxic chemicals on other biota leads to the conclusion that it may also be possible to protect amphibians and reptiles.

Governments, particularly in the developed countries, use scientific evidence that certain chemicals are harmful to biota to restrict or even eliminate their use. The organochlorine pesticides, generally broad-spectrum, persistent pesticides, some of which accumulate in animal tissues, are a well-known example. The harm caused in the decades when information on effects was accumulating resulted in the requirement of batteries of tests to register new chemicals for sale and use. The process involved research that revealed mechanisms and extent of the harm, legislation, and regulations for enforcement of restricted applications to prevent a recurrence of past problems. The extent of continued losses of fish and wildlife determine the effectiveness of regulated applications of chemicals.

Losses of fish and birds attracted the most attention and initiated legislation and regulations for the application of chemicals. Concern was especially high for mammals because of the obvious implications for human health. Regulations specified fish, birds, and mammals as test subjects and required tests to reveal the kinds of toxicity that had been observed in natural populations of these organisms. If amphibians and reptiles were ever considered, it was presumed that tests conducted on fish, birds, and mammals would yield a range of toxicity data that, when evaluated and implemented with appropriate safety factors, would also protect these other groups.

The U.S. Environmental Protection Agency (EPA) recently (draft Revised FIFRA Guidelines Document - Subdivision E, March 1988) began consideration of pre-registration testing of chemicals for their acute lethal toxicity to amphibians and reptiles, using frogs (*Rana* spp. tadpoles) and adult lizards (*Anolis carolinensis*). In the proposal, works by Hall & Swineford (1980) and Hall & Clark (1982) were cited as examples of acceptable protocols for both classes. Several questions are raised by this proposal. Are existing safeguards adequate to protect amphibians and reptiles? Would requirement of the kinds of pre-registration testing proposed confer additional safeguards? Are more effective and more efficient tests available? And, lacking adequate information on which to answer the foregoing questions, how can the information needed be obtained?

We have reviewed the scientific evidence of the toxicity of chemicals, evaluated the adequacy of existing knowledge for protection of herpetofauna, and identified a strategy for research to provide missing information.

PROBLEMS IN EVALUATING RISK

Diversity of Amphibians and Reptiles

Taxonomic diversity. Reptiles are more diverse than any of the other land vertebrates. Crocodilians, for example, are more closely related to birds than to turtles. If relatedness is a good predictor of hazard of chemicals, effective predictors for all reptiles do not exist. Pough, Heiser & McFarland (1989), relying on the work of Smithson (1985) and others, stated that precise relationships among the major groups of vertebrates cannot be determined. Nevertheless, all indications are that the major groups of amniotes are both distinct and diverse. An *Anolis* lizard is probably not a good predictor of responses of crocodilians and turtles, for example, even though all three are in the same vertebrate class. Likewise amphibians and reptiles are likely to respond differently to chemicals.

Ecological diversity. Amphibians have complex life cycles, and more opportunities for exposure to chemicals and more potential routes of exposure than other vertebrates. Likewise, diversity in life history and survival strategies of amphibians and reptiles is extreme, as, for example, the differences in adaptations between iguanas and amphisbaenids illustrate.

Physiological diversity. As amniotes, reptiles can be expected to share many physiological and biochemical characteristics with birds and mammals, but as poikilotherms, can be expected to differ in their response to various environmental conditions. Amphibians likewise are physiologically diverse with different adaptations at different stages to accommodate morphological and ecological changes through the life cycle.

Geographic diversity. Amphibians and reptiles occupy a great variety of climatic and ecological zones, but achieve their greatest biological importance in the tropics. Most evaluations of effects of toxic chemicals on them have been done with temperate species.

Selectivity of Chemicals

Chemicals intended for use in the environment are screened on the basis of selective toxicity. An ideal pesticide is highly toxic to target pest organisms and non-toxic to other organisms. Of greatest concern to producers of pesticides is mammalian toxicity as it predicts hazard to humans. Most successful pesticides are highly toxic to invertebrate animals or to target kinds of plants and generally less toxic to vertebrates. The degree of toxicity to vertebrates varies, however, both among chemicals and among different groups of vertebrates. The following examples use toxicity values obtained from a summary of reports on the comparative toxicity of many pesticides to a variety of animal species (Kenaga, 1979) to illustrate some differences among selectively toxic chemicals. *Carbaryl* is a widely used and effective insecticide of very low toxicity to most vertebrates. *Parathion*, a broad-spectrum non-systemic insecticide, is highly toxic to birds and mammals, but of low hazard to fish. *Azinphos-methyl*, a non-systemic insecticide and acaricide, is much more toxic to birds than to fish. *Trifluralin*, a herbicide of low toxicity to birds and mammals, is among the most toxic chemicals to fish. The potential interaction of the great natural diversity among amphibians and reptiles and the intentional selectivity engineered into pesticides can result in diverse responses and in unpredicted hazards.

The Nature of Data on Chemical Hazards

Most available information on the hazard of chemicals to vertebrates is in data bases that were generated on groups of animals other than amphibians and reptiles. Probably the greatest volume is on mammals, primarily laboratory rodents. Another very large volume of information exists on fish. Less information is available on birds, but much of the knowledge from avian studies is relatively useful because of its focus on hazards to wild animals in the natural environment. For the great majority of chemicals, there is no information on hazards to amphibians or reptiles.

RESEARCH ON AMPHIBIANS AND REPTILES

Past research on hazards of environmental chemicals to amphibians and reptiles has not revealed complete answers to the questions posed, but may indicate how answers may be found.

Power, Clark, Harfenist & Peakall (1989) recently summarized research on the susceptibility of amphibians to toxic chemicals. The wide variety of studies included acute toxicity tests with 211 different pollutants, a variety of effects in the laboratory with 154 different substances or conditions, and field studies of the effects of 54 different pollutants. Test species or protocols have not been standardized. Use of 45 different species of amphibians in acute toxicity tests was reported under widely varied test conditions. The authors discussed the problems of selection of test species, life stages, and endpoints, and the common problems of test media, holding, rearing, and testing conditions, and length of the observation period. Multiple test species were suggested until more is known about interspecific differences in sensitivities. Available test guidelines were identified as inadequate.

An earlier summary of the literature on the effects of environmental contaminants on reptiles (Hall, 1980) was reasonably complete, but almost entirely concerned with organochlorines. Much of it was based on observations following field applications or on reports of chemical residues in tissues.

Until recently, it was commonly stated that research does not support the argument that any species of amphibian or reptile is more susceptible to any chemical contaminant than other kinds of vertebrates. The literature on environmental contamination is devoid of compelling evidence that adult and larval amphibians are more sensitive to chemicals than other land and aquatic vertebrates (Table 1). In fish culture, however, amphibians are often regarded as pests and the literature of fish culture and fish control reveals that at least two chemical toxicants are selectively toxic to amphibians. TFM (3-trifluoromethyl-4-nitrophenol) is a toxicant developed in the 1950s as a selective control for the sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes region of North America. Howell (1966) and Gilderhus & Johnson (1980) observed that mudpuppies (*Necturus maculosus*) and frog larvae are routinely killed by field applications of TFM, and suggested that they are perhaps

as sensitive as the target lamprey. Bioassays by Chandler & Marking (1975) indicated that larvae of gray treefrogs (*Hyla versicolor*), leopard frogs (*Rana pipiens*), and bullfrogs (*R. catesbeiana*) are 1.2 to 8.2 times as sensitive to TFM as several fish species for which comparable data (Marking & Olson, 1975) are available. Kane, Stockdale & Johnson (1985) and Kane & Johnson (1989) found that TFM is four times more toxic to bullfrog larvae than to fathead minnows (*Pimiphales promelas*) and that young larvae are selectively killed by TFM in ponds inhabited by fish.

Helms (1967) reported on experiments with another toxicant, formalin, to control tadpoles in fish production ponds, and Carmichael (1983) found that treatment with formalin selectively removes tadpoles in raceways containing fingerling largemouth bass (*Micropterus salmoides*). In fact, the tadpoles were more sensitive to formalin than nine species of fish tested by Bills, Marking & Chandler (1977) and far more sensitive than four of five species representing major invertebrate groups (Table 2). Only ostracods (*Cypridopsis* sp.) were more sensitive than tadpoles. The other arthropod species, the backswimmer (*Notonecta* sp.) and the freshwater prawn (*Palaemonetes kadiakensis*), were highly resistant. Because formaldehyde is a major air pollutant that readily dissolves in water to produce formalin, its increasing concentrations could conceivably have significant effects on amphibians in the environment.

Some research has questioned the validity of traditional toxicological methods and endpoints in evaluations involving amphibians and reptiles. Standard toxicological methods were developed over many years for homeothermic vertebrates, and a separate set was developed for use with fish. These methods may not be useful for amphibians and reptiles. Temperature regimes for toxicity tests on poikilotherms have been questioned. The review by Power *et al.* (1989) revealed that tests on amphibians have been conducted over a wide range of temperatures. Tests with death as an endpoint in relatively inactive species have been questioned because behavioural effects were sometimes noticeable at far below lethal exposure. For example, Hall & Swineford (1981) found that larvae of *Ambystoma opacum* were debilitated at levels of toxaphene far below lethal concentrations and probably would not have survived in the wild, although many ultimately recovered in the laboratory. Preliminary results from our laboratory indicate behavioural anomalies in *A. maculatum* exposed to cholinesterase inhibitors at one order of magnitude below lethal levels. Behavioural changes affecting feeding or escape could have lethal effects in the field. Studies of teratogenic effects (Cooke, 1981) indicated that abnormalities in amphibian larvae are produced at sublethal concentrations.

A moderate amount of work has been done on amphibian larvae, principally anuran tadpoles. Anurans are remarkably resistant to some cholinesterase inhibitors, the class of pesticides currently in greatest use, apparently many times more so than other vertebrates (Hawkins & Mendel, 1946; Edery & Schatzberg-Porath, 1960; Andersen, Aaraas, Gaare & Fonnum, 1977). Tailed amphibians may share this resistance; preliminary evaluations with *Ambystoma maculatum* larvae performed in our laboratory indicate similar resistance. Resistance permits the accumulation of tremendous body burdens that may be harmful to predators (Hall & Kolbe, 1980; Hall, 1990). Resistance appears to result from an inability of the chemicals to structurally bind with and inhibit amphibian cholinesterases (Potter & O'Brien, 1964; Wang & Murphy, 1982). Resistance may be fortuitous or may be an adaptation to naturally-occurring cholinesterase inhibitors in the environment; recent studies of *Anabaena flos-aquae*, a freshwater alga, revealed that it produces a sufficiently powerful cholinesterase inhibitor to kill livestock drinking from waters that support blooms (Cook, Beasley, Lovell, Dahlem, Hooker, Mahmood & Carmichael, 1989). Some results indicate that the apparent resistance to cholinesterase inhibitors may not apply to all amphibian species and all chemicals. For example, Sanders (1970) found the organophosphate carbophenothion to be the most toxic of 16 chemicals to tadpoles of the chorus frog (*Pseudacris triseriata*). Cooke (1981) implicated the carbamate oxymyl in the production of deformities in larvae of *Rana temporaria*, the European common frog. Although some or most synthetic cholinesterase-inhibiting chemicals may not unduly harm amphibians, the responses of amphibians could not have been predicted from results of testing other vertebrates, and another class of compounds could produce equally atypical results. Responses might be skewed toward lethality (as with TFM).

Almost no experimental evaluations of the sensitivity of reptiles to environmental chemicals have been made, although field studies were common in the era of organochlorines. In a study of the sensitivity of the green anole, *Anolis carolinensis*, to four organophosphates (Hall & Clark, 1982), responses seen were close to those of mallards and rats (Table 3). The study was intended to test the widely-held notion of "cold-blooded" and "warm-blooded" patterns of response to cholinesterase inhibitors.

A series of investigations summarized by Cooke (1981) related exposure to environmental pollutants and the occurrence of deformities in tadpoles, and indicated that the production of such deformities can be a sensitive indicator of pollution by certain chemicals. Cooke (1981) reported development and testing of a protocol in which caged tadpoles in waters receiving runoff or spray drift from agricultural fields could indicate chemical treatments hazardous to amphibians. Dumont, Shultz, Buchanan & Kao (1983) developed what they called the "FETAX" test, a protocol using embryos of the clawed frog (*Xenopus laevis*) as an assay for teratogenicity of chemicals and mixtures of contaminants. The primary purpose of the proposed test was neither screening agricultural chemicals nor protection of amphibian populations, but it might be adaptable for these purposes. Despite indications that examining production of abnormalities in embryos and larvae may be an economical and powerful tool for identifying chemical hazards, it has not been extensively used for testing new chemicals.

In summary, far too little is known to conclude that safety standards for other kinds of vertebrates are adequate for the protection of amphibians and reptiles. Existing evidence indicates that amphibians are more sensitive to a selective piscicide and to a prophylactic fishery chemical than most fish commonly tested, and strikingly more resistant to some cholinesterase-inhibiting compounds (Table 1) than other classes of vertebrates. Neither response could have been predicted from tests conducted on other vertebrate classes. Susceptibility of reptiles to selective pesticides is virtually unknown. Preliminary information (Hall and Clark, 1982) suggests similarity in responses to other amniote vertebrates, but this conclusion is based on the exposure of only one lizard species to four chemically similar compounds.

	BULLFROG	MALLARD	B/M
CARBAMATES			
BAYGON	595	9.4	63
CARBARYL	>4000	>2564	-
MEXACARBATE	566	3.0	190
NABAM	420	>2560	<0.2
ORGANOPHOSPHATES			
CHLORPYRIFOS	>400	76	>5
DEMETON	562	7.2	78
DIAZINON	>2000	3.5	>570
DICROTOPHOS	2000	4.2	476
PHORATE	85	0.6	140
TEMEPHOS	>2000	79	>25
TEPP	112	3.6	31
OTHER			
DDT	>2000	>2240	-
SODIUM MONO-FLUOROACETATE	54	5.9	9
STRYCHNINE	2.2	2.0	1.1

TABLE 1. Acute oral toxicity (LD_{50} in mg/Kg) of pesticides to bullfrogs (*Rana catesbeiana*) and mallards (*Anas platyrynchos*), and the relative sensitivity of bullfrogs compared to mallards. Mallards are, for example, 63 times as sensitive to Baygon as are bullfrogs. Data from Tucker & Crabtree (1970).

ORGANISM	LC_{50} (mg/l)			
	24 h	48 h	72 h	96 h
Ostracods[2] (*Cypridopsis* sp.)	1.15			1.05
Tadpoles of Three Species of Amphibians[3]	22 - 70	21 - 59	21 - 59	
Leopard Frog Larvae[4] (*Rana berlandieri*)	~40			
Four Species of Fish[3]	>70 - 87	49 - >100	45 - >100	
Six Species of Fish[2]	141 - 389			62.1 - 173
Largemouth Bass[4] (*Micropterus salmoides*)	~150			
Snail[2] (*Heliosoma* sp.)	710			93
Bivalves[2] (*Corbicula* sp.)	800			126
Freshwater prawn[2] (*Palaemonetes kadiakensis*)	1105			465
Backswimmer[2] (*Notonecta* sp.)	4500			835

1- In all tests, commercial formalin stated to be approximately 37% formaldehyde was used. 2- data from Bills *et al.* (1977). 3- data from Helms (1967) 4- data from Carmichael (1983)

TABLE 2. Toxicity of formalin[1] to aquatic organisms

	PARATHION	METHYL PARATHION	MALATHION	AZINPHOS-METHYL
ANOLIS	8.9	82.7	2324	98
MALLARD	2.1	10	1485	136
RAT	16	26	1840	15

TABLE 3. Acute oral toxicities (LD_{50} in mg/Kg) of four organophosphorus pesticides to three species of vertebrates. Data on *Anolis* from Hall & Clark (1982). Data on mallard and rat from Kenaga (1979)

PREREGISTRATION TESTING

Routine preregistration testing would be costly in financial terms and in animal subjects. Before making a commitment, the predictive value of generated data should be known. Amphibians may be expected to be exposed to chemicals in the water or by food and appropriate tests must expose larvae and transformed individuals by different media. One limited study (Hall & Swineford, 1979) compared routes of uptake in adult toads. Conclusions were that exposure to the organochlorine methoxychlor through water could be significant even in highly terrestrial amphibians. A battery of tests is therefore required, and some or all tests might have to be used for each chemical, depending on expected distribution of chemicals after their release into the environment. More than one species would be necessary to account for interspecies variability. For reptiles, route of exposure and choice of life stages present fewer problems, but predictability from one group to another is largely unknown. Until variability among and within groups is better assessed, species of many groups must be tested.

Presently, assurance that preregistration testing leads to conclusions of reasonable confidence would require many types of tests with several species, developmental stages and protocols, for each chemical. Compounding the difficulty is the need to expose subjects in a variety of unconventional ways and the necessity to obtain test subjects from nature, with attendant concerns for quality assurance and conservation.

An alternative to an elaborate array of laboratory tests for registration could be carefully designed and monitored field tests that expose a variety of free-living amphibians and reptiles to each chemical in an environmentally realistic manner, consistent with anticipated use of the chemical and cognizant of life history events that might modify hazard. The relative values of field tests, controlled field experiments (also known as *mesocosm tests*), and controlled laboratory tests were discussed by Hoffman, Rattner & Hall (1990). There are tradeoffs that make it impossible to simultaneously maximize environmental realism, repeatability, and predictive value. Testing schemes that best resemble actual exposure in one field situation may be of least value in predicting effects when the chemical is used in a different situation. Nevertheless, there is a role for careful field testing that cannot easily be filled by any combination of experimental procedures in the laboratory. Furthermore, hybrid testing methodologies that, for example, use caged tadpoles to monitor effects of operational pesticide applications (Cooke, 1981) may optimize advantages of the different kinds of tests.

One solution to the problem of preregistration testing is to perform the research to determine first whether preregistration testing of amphibians and reptiles is necessary and, if required, how the required information can be obtained most efficiently.

A RESEARCH STRATEGY

The goal of this research is to determine what kinds of new information are necessary to protect amphibians and reptiles from the hazards of environmental chemicals.

Research should examine the relative sensitivity of major groups of amphibians and reptiles to the major groups of environmental contaminants. Of primary concern is inherent (taxonomic) variability in responses; for this reason *in vitro* investigations may be preferred. Endpoints need not be lethality, but must be repeatable. Ecological diversity contributes greatly to risk in the field, but is of less immediate concern than basic differences in sensitivity because ecological effects in modifying toxicity can often be projected across species. For example, amphibians with aquatic larvae are probably more susceptible to water-borne pesticides than those that develop on land.

Chemicals with selective toxicity should be examined first. Differences in toxic rank are important because they permit comparison of data from aquatic and terrestrial tests. Data on toxic rank can be determined from a variety of *in vitro* procedures to reduce costs. Research should determine whether abnormalities in embryos or larvae are indicators of general toxicity, or whether they are caused by wholly different mechanisms than those resulting in acute lethality. If teratogenesis is a good general indicator of hazard, it may provide the basis of a low cost routine screening.

If sensitivity of one group of amphibians or reptiles falls outside the range of observed sensitivities in the other groups commonly tested, further investigations should determine to what extent the sensitive amphibian or reptile represents its taxonomic group and to what extent responses of the group could be predicted from data routinely collected on other vertebrates.

Once there is a reasonable base of knowledge on ranges of expected responses of different groups of amphibians and reptiles to different classes of chemicals, it may not be necessary to perform complete series of tests on all chemicals. New chemicals introduced or those undergoing reevaluation can be given one or more spot tests to determine whether responses fall within expected limits. Those that fall within expected limits may not require further testing, while those that exceed expected limits may need detailed evaluation.

Data, statistics, computer models and risk assessments are only of limited value in predicting the hazards that chemicals may pose to natural populations. Chemicals that have appeared safe based on laboratory tests have been implicated in dieoffs in the field. For example, dimethoate and methamidophos were thought to pose little risk to birds, but each chemical caused mortality of sage grouse (*Centrocercus urophasianus*) feeding in treated fields (Blus, Staley, Henny, Pendleton, Craig, Craig & Halford, 1989). Additional research should seek better understanding of the ways in which ecological factors modify chemical risks. For wildlife in general, there is inadequate knowledge of the significance to populations of transient sublethal effects or of subtle changes that may go undetected in the laboratory. In predicting hazards from the kinds of information most often available, there is no substitute for knowledgeable biologists who are experienced with chemical contaminants and who are familiar with amphibians and reptiles as they live in the field. Laboratory investigations, therefore, should provide a guide, but should not obviate the need for well designed field tests and vigilance by biologists after registration. Good field testing and surveillance of chemicals in use can, moreover, improve the quality of testing in the laboratory by providing feedback on the quality of risk assessments that may lead to their improvement.

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