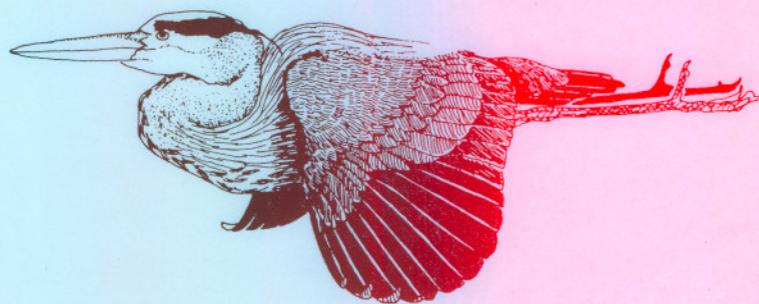


# A REVIEW AND EVALUATION OF THE AMPHIBIAN TOXICOLOGICAL LITERATURE

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A. Harfenist  
T. Power  
K.L. Clark  
D.B. Peakall



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## I INTRODUCTION

The impact of environmental contaminants on amphibians is a subject that has received limited attention. This review is an attempt to present the toxicological data that exists. The majority of the toxicological research conducted using amphibians is centered around the anurans and urodeles. The apodans, due to a lack of knowledge concerning the biology and secretive lifestyle have not been studied in relation to toxic chemicals.

Amphibians, with the exception of a few specialized species, require moisture in some form, whether it be a permanent lake or leaf that has collected dew, in order to complete their life cycle. It is this requirement and their subsequent metamorphosis to the combined terrestrial/aquatic adult stage that is the major route of exposure for amphibians to toxic contaminants. Thus, in order to fully comprehend the possible implications of environmental contaminants on amphibians a brief discussion of their life cycle is necessary.

Amphibians fall into three general categories with respect to their breeding habitats. The first category is composed of those that breed in permanent water; the second breed in temporary or seasonal pools; and the third group breeds out of the water (Porter 1972). The latter group still requires a moist location to deposit its eggs in order to ensure the development of the embryos. Thus all amphibians rely upon seasonal and geographical patterns of precipitation which govern their distribution (Romer 1959, Porter 1972). In general, the amphibians of North America breed in spring or early summer (Romer 1959, Porter 1972). The rate of development of the eggs and embryos is species dependent. Eggs deposited in seasonal pools or other moist areas develop more quickly to avoid dessication. Eggs laid in permanent water bodies need not develop as fast due to the unlikelihood of dessication (Porter 1972).

Anurans congregate in leks in a pond where the males call to attract reproductively active females. The female, upon hearing the mating call of her species, will move in the direction of the call. The male mounts the female and clasps her inducing the deposition of the eggs, which are immediately fertilized. The number of eggs deposited by a female during a breeding season, ranges from a single egg (Genus Sminthillus) to 30,000 or more (Genus Bufo) (Porter 1972). Anuran egg masses are of three generalized types and positions enabling them to maximize oxygen availability in different habitats and water temperatures. One type is deposited as a flattened egg mass at the surface of the water such as those of R. catesbeiana; the second type is globular and attached to submerged vegetation as in P. triseriata egg masses; and the third type is characteristic of the true toad species (Genus Bufo), and are deposited in elongated strings (Otto and Towle 1947, Romer 1959, Porter 1972). Depending upon the species, the eggs take between 2 and 30 days to hatch.

Other species exhibit more specialized breeding behaviour. Some female anurans protect their eggs by carrying them on their backs or under the skin. Others build nests while some lay eggs enveloped in a gelatinous material that hardens and reduces moisture loss. For the most part these specialized adaptations are found in amphibians inhabiting the tropics, while the generalized life cycle described here is more characteristic of the species located in North America (Villemant et al. 1958).

The embryo hatches out as a larva equipped with a well developed tail for swimming. This aids it in foraging for submerged vegetation upon which it browses (Villemant et al. 1958, Romer 1959, Porter 1972). The anuran larvae respire by gulping water and forcing it over the gills located in the future neck region. Some gaseous exchange is also accomplished through the skin (Romer 1959, Porter 1972). The larvae or tadpoles transform to the adult stage via metamorphosis. This occurs two weeks to three years after hatching, depending upon the species (Porter 1972).

Metamorphosis involves radical structural changes of the body: the gills disappear, lungs and limbs develop rapidly (hind limbs first), the tail is absorbed and the digestive tract shortens. Eventually the adult frog becomes an air breather (Romer 1959). Respiration is accomplished via internal lungs as well as via the skin.

The length of the metamorphic period is also species dependent. In the case of the Spadefoot toad (Scaphiopus holbrookii), metamorphosis into a terrestrial toad takes only twelve to thirteen days from fertilization. Spadefoot toads breed in temporary pools and must develop quickly to avoid desiccation (Porter 1972). Some species require a year, (R. catesbeiana) (Cecil and Just 1979), while others require still more time. Several amphibian species are non-metamorphosing and retain both larval characteristics and their aquatic lifestyle (genus Necturus) (Porter 1972).

Adult anurans, unlike the larvae, are carnivorous, consuming almost any moving prey of the right size. This includes worms, insects, other amphibians, reptiles and occasionally mammals. Throughout its life cycle an anuran can occupy several different positions within the food web (Curtis 1968, Porter 1972).

The urodeles also follow a general pattern of breeding behaviour. During courtship the male either clasps or blocks the path of the female and begins lashing with his tail, bumping or rubbing to stimulate the female. The male then deposits a spermatophore (a capsule containing sperm). The female either follows the male or is pulled by him into a position where she can pick up the spermatophore

with the cloaca, where it is stored (Romer 1959). The eggs are fertilized internally and may be deposited in decaying vegetation, logs, pools or other moist environments. The eggs can take up to two months to hatch. Aquatic salamander larvae differ from anuran larvae in that they develop adult appearance quickly, they possess external gills and their diet is primarily insectivorous (Romer 1959). After several months and under the correct environmental conditions the larvae transform into the adult stage and may leave the water. Some species have lungs whereas the Plethontidae, known as the "lungless salamanders", respire via the skin. Another group, the Necturidae, are neotonic, retaining their external gills and aquatic lifestyle even after sexual maturity.

Adult urodeles, like adult anurans, are carnivorous. Their diet consists of arachnids, annelids, insects, amphibians, small fish and their eggs, and in the case of Cryptobranchus, mammals. Adult urodeles inhabit moist locations under logs, rocks, and leaf litter, returning to the water in spring to breed (Porter 1972).

Interruptions of this life cycle by toxic contaminants can have disastrous effects on amphibian populations (Paulov 1977). Amphibians are of significant economic importance to man. Due to their carnivorous adult stage they consume insect pests harmful to crops (Schwabe 1977). Some cultures use amphibian skins for leather goods, amphibian venom for hunting and medicines, while frogs legs (R. catesbeiana) are considered a delicacy by some humans. Amphibians are also the perennial biological specimen in institutions due to their relative abundance and availability (Otto and Towle 1947). Several species have actually benefitted from man's agricultural practices by taking advantage of irrigation ditches and new habitats that have been created, but many more species have been detrimentally affected by habitat destruction and the use of toxic chemicals. Amphibians are not only important to man, but also to other animals within the community. They make major contributions to community biomass (Burton and Likens 1975a, 1975b, Cecil and Just 1979, Debendictis 1974), as well as playing significant roles in competitive and predator-prey relationships (Orser and Shure 1972, Burton and Likens 1975b, Lynch 1979). It is of interest to note that amphibians are the only class of vertebrates that do not include any pests or species harmful to man. Amphibians do not compete for harvests, nor are there any ferocious or destructive species (Porter 1972). Even so amphibians are victimized by man's attempts to control pests with toxic chemicals, primarily insecticides and herbicides (Fashingbauer 1957, Hazelwood 1969, Porter 1972, Cooke 1973a, Curtis 1968).

The toxicity of these contaminants to amphibians in lab bioassays and field experiments as well as their general effects on populations, will be reviewed in order to identify specific substances that pose

potential hazards to amphibians. From this information an evaluation will be made as to whether amphibians can be employed as useful indicators of environmental quality.

## II INSECTICIDES

### a) ORGANOCHLORINES

#### ALDRIN AND DIELDRIN

##### Short-Term Effects

In static bioassays dieldrin was highly toxic to B. woodhousii tadpoles with a 96 hour LC50 of 0.15 mg/L (Sanders 1970) (Table 1).

In static bioassays, complete mortality of adult R. cyanophlyctis (an Asian frog species) occurred within 215 minutes of exposure to 0.006 mg/L aldrin (Rane and Mathur 1978). Although sample sizes were small (2 to 4 frogs per treatment with 6 different treatments) no frogs survived longer than 120 minutes when exposed to 0.125 mg/L (the highest concentration tested) (Table 2). Preceding death, the frogs swam erratically exhibiting a loss of equilibrium. A coagulation of mucous occurred over the body with the belly becoming bloated and the skin turning pale (Ibid 1978). These results indicate that aldrin is very toxic to the adults of R. cyanophlyctis and that the 96 hour LC50 would be much lower than 0.006 mg/L considerably less than the 96 hour LC50 reported for B. woodhousii by Sanders (1970) (Table 1).

Mulla (1962, 1963) found R. catesbeiana tadpoles experienced 100% mortality within 24 hours when maintained in ponds and treated with 0.1 kg/ha dieldrin (Table 3). Since the actual concentrations of dieldrin in the ponds were not measured it is difficult to compare these results with other studies.

##### Long-Term Effects

Short-term exposures to dieldrin can have long-term effects on developing amphibians. When groups of 150 Limnodynastes tasmaniensis (an Australian frog) embryos were exposed to 0.0, 0.01 and 0.1 mg/L dieldrin for 7 hours, the 0.1 mg/L exposure resulted in accelerated growth and abnormalities later in development (Brooks 1981) (Table 2). No mortality occurred during the exposure, but 21 of the tadpoles treated with 0.1 mg/L dieldrin as eggs, exhibited some degree of deformity later in development. Nineteen days after exposure some

## b) TOXICITY TESTING TECHNIQUES

Several species of amphibians are readily available from the field or from commercial distributors. Adult anurans can be housed at low temperatures, without food, until they are to be used. They can be induced to spawn readily (Rugh 1962, Browne and Dumont 1979). Spawn can be collected from the field and developed under laboratory conditions. Development in lab aquaria can easily be observed and amphibians do not generally require a large amount of water or space. Some studies have shown that anurans require less attention during these processes than urodeles. Urodeles require live food and can be difficult to handle, making anurans more attractive as a test organism (Slooff and Baerselman 1980).

Amphibians have several other attributes that make them useful test organisms. A short life cycle which involves physiological, histological and anatomical changes (Cooke 1981), allow observations of the complete cycle over a short period of time (Birge et al. 1975). They respond to environmental contaminants in many different ways and these can be monitored over the life of the organism.

Some concerns that are evident after reviewing the amphibian toxicological literature about methods and techniques that should be evaluated prior to any toxicity testing are as follows:

1. Section of species. There is a certain degree of species variability in response to environmental pollutants (e.g. Sanders 1970, Cooke 1972a,b, Rzehak et al. 1977, Birge et al. 1979) (Table 1). Although it is generally recommended that the most sensitive species be used in any toxicity testing, so little is known about the sensitivity of most amphibians that it is difficult to choose a single indicator species. X. laevis and R. pipiens are often used in toxicity testing. X. laevis does not naturally occur in Canada and may not reflect the sensitivity of native species. Whenever possible a number of species should be tested and results from single species should not be generalized to cover all amphibians until species sensitivities have been more extensively investigated (Hall and Swineford 1980, 1981).
2. Lifestages sensitivity. There is a wide range in sensitivity of lifestages to the same toxicant (e.g. Cooke 1972a,b, Wohlgemuth 1977, Lyons et al. 1976, Hall and Swineford 1980, Davis et al. 1981). In general, adults are more tolerant than either the egg or larval stages. The egg and larval stages exhibit wide ranges in sensitivity (Hall and Swineford 1980, Davis et al. 1981). The sensitive stages vary greatly between toxicants presumably dependent upon the physiological processes that are occurring in the organisms during the developmental stage and the physiological

mechanism causing toxicity (Davis et al. 1981). One process that has not been considered, except with respect to pH, is toxic effects on fertilization and egg cleavage. Schlichter (1981) found fertilization of R. pipiens eggs to be highly sensitive to pH 5.8. Species may be experiencing high mortality during fertilization which is not measured in standard egg bioassays. In all bioassays as many lifestages as possible should be tested and in all instances, the exact stages when exposures were initiated and terminated should be reported.

3. Toxic effect. Mortality, as measured in bioassays, is not the only toxic effect that can be induced by a pollutant. Behavioural, morphological and developmental aberrations that occur during chronic dosing at levels much lower than the LC50, can also be important measures of toxicity (e.g. Cooke 1970, 1973a, 1981, Lyons et al. 1976, Rzehak 1977, Harri et al. 1979, Hall and Swineford 1980). Residues are an indirect toxic effect that should be considered, particularly for pollutants that have little effect on amphibians but that may accumulate and pass up the food web (Rosato and Ferguson 1968, Collins et al. 1973, Baudo 1976).
4. Dilution water. Although dilution waters should vary to represent conditions where the pollutant is a concern, the water characteristics should always be reported, particularly pH, alkalinity and oxygen content. Generalizations cannot be made about the effects in all water qualities. pH is an important parameter that can be toxic by itself (Gosner and Black 1957, Pough 1976) and also can influence toxicity (Hashimoto and Nishiuchi 1981). Dissolved organic carbon levels are also important because some toxicants, such as metals, can interact with organics and modify the toxicity (Baker and Schofield 1980). Hardness or alkalinity is closely linked with pH, but alone it can influence toxicity (Porter and Hakanson 1976). For example, metal toxicity is greater in soft water than in hard water at the same pH (Ibid 1976).
5. Temperature. Toxicity can also vary with temperature during exposures (Licht 1976b, Pough and Wilson 1977). Temperature should always be reported and experiments should be conducted at a temperature comparable to what would be encountered in field conditions.
6. Holding technique. With only a few exceptions, aquatic amphibians inhabit lentic waters, often small temporary pools or ponds with very little water flow. Flow-through experiments may therefore not represent an accurate field situation. To maintain toxicant levels and avoid build up of waste product a flow-through system is often preferred to static bioassays. Hall and Swineford (1981) found

flowing water, with a five hour replacement time, caused moderate mortality to R. septentrionalis tadpoles and in later experiments reduced the flow to a 24 hour replacement (Ibid 1981). In comparison with Sanders' (1970) methods Hall and Swineford (1980) found that continuous-flow methods yielded lower LC50 estimates than static methods. However, flow-through methods with a rapid renewal time do not duplicate conditions in the field. Further investigation is necessary to fully assess the implications of static and flow-through bioassays on different life stages.

7. Rearing density and body size. Rearing density can affect size of tadpoles with high densities (50 tadpoles/L) producing tadpoles half the size of those reared at low densities (10 tadpoles/L) (Cooke 1979). The significance of size is that large individuals can be more resistant to pollutants than small individuals. For example body size was negatively correlated with T. cristatus sensitivity to Maneb (a fungicide) (Zaffaroni et al. 1978) and copper toxicity to R. pipiens tadpoles (Landé and Guttman 1973). Bioassays should therefore include either a random sample of sizes or should be designed to incorporate any effects of body size.
8. Observation period. The observation period to observe lethality after exposure to different chemicals needs to be determined for different groups of pollutants. Hall and Swineford (1980) reported much lower LC50s for toxaphene based on a 30 day observation period than Sanders (1970) did based on a 4 day observation period. Hall and Swineford (1980) attributed their lower LC50 mainly to the longer observation period because most mortalities were observed after the 4 day dosing between 1 and 26 days after exposure.

There are no generally accepted standardized techniques for toxicity testing using amphibians. The wide range of bioassay methodologies used in measuring LC50s makes comparison between studies very difficult. The United States EPA (1975) has published a detailed methodology which is recommended for use in toxicity testing of amphibians. However, it is designed mainly for fish, and to a lesser extent, aquatic invertebrate testing, and it does not fully address amphibian toxicity testing.

#### c) AMPHIBIANS AS MONITORS OF ENVIRONMENTAL POLLUTANTS

Ideally indicator species should be both representative of a specific trophic level within the ecosystem and capable of being used, at reasonable cost, under laboratory conditions. The latter requirement has already been considered under section XI b).

The requirements for indicator species were listed by Moore (1966) as follows:

- (a) The species should be widely distributed, relatively abundant and easy to collect.
- (b) If monitoring is to be carried out by chemical analysis of organs, these should be large enough for adequate samples.
- (c) It should be possible to ascertain the age of the individual.
- (d) The level of residues in the species should be between the limit of detection and the limit of crude toxicological effect.
- (e) If measurements of local changes are required, the species must be sedentary. If the degree of contamination of a large area is to be measured, species with more extensive ranges can be used. In either case the range of the indicator species must be known.

Dumpert and Zietz (1984) considered that the platanna (X. laevis) could be used as an indicator species for determining embryotoxic effects of environmental chemicals. It met their criteria that it was available in sufficient numbers and at an acceptable cost at any time of the year and was adequately sensitive.

Cooke (1972a) has stated that under normal usage patterns that most insecticides would probably not be likely to reach levels high enough to induce toxic effects in the field. Meeks (1968) and Niethammer et al. (1984, 1985) believed that adult anurans would not be useful as indicators of organochlorine compounds as they do not accumulate residues in proportion with environmental levels.

The toxicity and toxic effects of organophosphate insecticides to amphibians varies greatly (Table 1) and due to the low persistence of these compounds in the environment, results are difficult to compare. Pearce and Price (1977) reported that spraying operations using fenitrothion represented little hazard to amphibians when used according to recommended field application rates. In contrast, Johnson and Prine (1976) reported that fenthion even when used at half the usual concentration caused marked physiological changes. These results, the apparent tolerance to cholinesterase inhibitors by amphibians (Potter and O'Brien 1964) and the generally low toxicity of organophosphates (Edery and Schatzberg-Porath 1960), indicates amphibians would not be adversely affected by many organophosphate compounds in the environment.

Arias and Zavanella (1979) presented data indicating the feasibility of using forelimb regeneration in newts as a model for assessing the risks incurred by the use of pesticides. However, the applications of this method may yield results difficult to apply to environmental situations.

Birge et al. (1973, 1975, 1976, 1977, 1979) have studied the effects of metal contamination on amphibians and the possibility of using amphibians as bioassay and bioindicator species based on the sensitivity of some amphibian species. Birge et al. (1975) considered that amphibians are an ideal test organism. The authors noted that due to differences in species sensitivity, that conclusions should be based on results from two or three test species. Birge et al. (1979) noted significant differences between urodele and anuran sensitivity based on individual ecological requirements, and concluded that sensitivity to environmental stress may be related to individual life cycles and requirements.

The sensitivity of amphibians to metallic contaminants may allow environmental monitoring of these compounds using amphibians as indicators, once standardized techniques and representative species have been established (Birge et al. 1975, 1979, Niethammer et al. 1985).

Amphibians can be used to monitor the effects of acidification. The survival of embryos has been related to pH, under both experimental and field conditions (see Section X b)). Fish are widely used monitor species for the impact of acidification. Amphibians could be used to broaden the approach and might be particularly valuable for assessing the impact on temporary water bodies.