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Review

ADVERSE EFFECTS OF PESTICIDES AND RELATED CHEMICALS ON ENZYME AND HORMONE SYSTEMS OF FISH, AMPHIBIANS AND REPTILES: A REVIEW

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Summary: The enzyme and hormone disrupting capabilities of pesticides and related chemicals are suspected to be some of the factors contributing to the decline of fish, amphibian, and reptile populations. Globally frogs and other amphibians have been disappearing at an alarming rate. In most cases, the cause or causes are unknown, but are assumed to result from man-made pollutants in the aquatic environment. Some current reports have indicated that many pesticides in the aquatic environment are capable of disrupting the endocrine systems of animals. Some pesticides and related chemicals are persistent in the environment and are accumulated in the fatty tissue of organisms and increase in concentration as they move up through the food web. These chemicals are substances that can cause adverse effects by interfering in some way with the body's hormones or chemical messengers. A recent study indicated that the atrazine effected the sexual development of frogs, even at extremely low doses. Some pesticides reduce the cholinesterase activity of amphibians and reptiles. Some chemicals may cause disease and reproductive failure in fish populations, because they bioaccumulate in the higher trophic levels. Therefore, brown trout exposed to environmental pollutants have been shown to have decreased egg size and low growth rate of the larvae.

Keywords: Pesticides, enzymes, hormones, amphibian, reptile, fish

Introduction

Pesticides and related chemicals destroy the delicate balance between species that characterizes a functioning ecosystem. Pesticides produce many physiological and biochemical changes in freshwater organisms by influencing the activities of several enzymes. Alterations in the chemical composition of the natural aquatic environment usually affect behavioral and physiological systems of the inhabitants, particularly those of the fish [1].

Hormone-disrupting effects in biota as a result of chemicals are caused by a wide variety of mechanisms. Pesticides and related chemicals are substances that can cause adverse effects by interfering in some way with the body's hormones or chemical messengers. These substances are therefore called hormone disruptors or endocrine disruptors, as it is the endocrine glands that secrete the hormones. Hormones play a crucial role in guiding normal cell differentiation in early life forms, and so exposure to endocrine disrupting substances in the egg or in the womb (mammals) can alter the normal process of development. Lately, most attention has been focused on estrogens; natural or synthetic compounds that elicit a feminizing effect by binding to the cellular estrogen receptor in organisms. The interaction between an estrogenic compound and its receptor causes a number of

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reactions and development. Environmental problems with estrogenic compounds seem to occur primarily in the aquatic environment, like feminization of male fish [2]. Adult animals can also be affected, but it is the embryonic stages that are especially vulnerable. Exposure at this sensitive time may cause effects in mammalian systems that are not evident until later in life, such as effects on learning ability, behavior, reproduction and increased susceptibility to cancer and other diseases.

The pesticides and related chemicals originating from human activity or agricultural farming are discharged directly or indirectly into the receiving waters. The presence of these chemicals in the environment has become a global issue. Field studies have shown that the reproduction, growth and development of wildlife species, including invertebrates, amphibians, reptiles, fish, birds and mammals may have been impacted by chemicals that interact with the endocrine system. Pesticides at low concentrations may act as blockers of sex hormones, causing abnormal sexual development, abnormal sex ratios, and unusual mating behavior. Pesticides can also interfere with other hormonal processes, such as thyroid and its influence on bone development [3]. This paper reviews the adverse effects of pesticides and related chemicals on enzyme and hormone systems in fish, amphibians and reptiles.

Effects on fish

Fish species are sensitive to enzymic and hormone disruptors. Chronic exposure to low levels of pesticides may have a more significant effect on fish populations than acute poisoning. Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behavior and physiology that impair both survival and reproduction [4]. Biochemical changes induced by pesticidal stress lead to metabolic disturbances, inhibition of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organism [5]. Liver, kidney, brain and gills are the most vulnerable organs of a fish exposed to the medium containing any type of toxicant [6]. The fish show restlessness, rapid body movement, convulsions, difficulty in respiration, excess mucous secretion, change in color, and loss of balance when exposed to pesticides. Similar changes in behavior are also observed in several fishes exposed to different pesticides [7].

The Great Lakes fish are contaminated with chlorinated organic compounds such as PCB and dichlorodiphenyl dichloroethene, pesticides such as mirex and dieldrin, and trace amounts of metals such as lead and mercury [8]. Lake trout, which became extinct in the Great Lakes in the 1950s, has been shown to be very sensitive to dioxins and (polychlorinated biphenyls) PCBs when exposed as embryos. Several species of salmon introduced into the Great Lakes have severely enlarged thyroid glands, which is strong evidence of hormone disruption. Salmon in the Lake Erie show a variety of reproductive and developmental problems, for example, early sexual development and a loss of the typical male secondary sexual characteristics, such as heavy protruding jaws and red coloration on the flanks.

Some agrochemicals can indirectly affect fish by interfering with their food supply or altering the aquatic habitat, even when the concentrations are too low to affect the fish directly. Other agricultural chemicals are capable of killing salmon and other aquatic animals directly and within a short period of time. For example, in 1996 the herbicide acrolein was responsible for the death of approximately 92,000 steel-head, 114 juvenile coho salmon, 19 resident rainbow trout, and thousands of non-game fish in the Bear Creek, a tributary of the Rogue River [3]. Several laboratory experiments show that sublethal concentrations of agrochemicals can affect many aspects of salmon biology, including a number of behavioral effects [3].

Under experimental conditions, rainbow trout exposed for 18-34 days to a combination of 0.05 mg/l of the organochlorine endosulfan and 0.5 mg/l of the organophosphate disulfoton showed changes in the ultrastructure of hepatic cells, with irregular nuclei, and alterations to the lysozomes and rough endoplasmic reticulum [9]. Some pesticides such as organochlorine, organophosphates and carbamates are known to cause morphological damage to the fish testis. These also affect female fish in the same way. They cause delayed oocyte development and inhibition of steroid hormone synthesis [10]. Experimental exposure of fish to them has been shown to depress protein values in brain, gills, muscle, kidney and liver. In the kidney and the liver there is evidence of significant decrease in the protein content due to stress in elimination and also in metabolism [11].

Interference with endocrine hormones affects reproduction, immune function, development, and neurological functions in several species of wild animals. In fish, endocrine disruptors interrupt normal development and cause male fish to have female characteristics. These outward symptoms of developmental disruption are accompanied by reduced fertility and even sterility in adults, as well as lower hatching rates and viability of offspring. Many studies show a direct relationship between concentrations of pesticides and related chemicals in fish tissues and depressed hormone concentrations. Disruption of the balance of endocrine hormones during development of young fish can also cause defects of the skeletal system, resulting in deformities and stunted growth [3,12].

The common pesticide synergist piperonyl butoxide increases carbaryl toxicity (Carbaryl is a neurotoxic carbamate pesticide). In fish, acute toxicity of a carbaryl- piperonyl butoxide mixture was over 100 times that of carbaryl alone [13]. In addition, carbaryl increases the acute toxicity of the phenoxy herbicide 2,4-D, the insecticides rotenone and dieldrin (an organochlorine) as well as the wood preservative pentachlorophenol [14]**.** Sublethal effects of the organophosphate insecticide phenthoate are also synergized by carbaryl in fish, resulting in AChE inhibition [15] and both morphological and behavioral changes [16]. While the toxicity of combinations of chemicals is rarely studied, the ability of carbaryl to interact with a large number of chemical classes is striking.

Effects on amphibians

Concern over the decline of amphibians globally has highlighted the importance of using this group as a bioindicator of environmental contamination and climate change. Since 1989, there has been a growing realization that amphibian populations have been declining at an alarming rate. The present data show significant declines all over the world. Nearly 600 amphibian populations studied in Western Europe show 53% decline beginning in the 1950s [17]. In North and South America, 54% and 60% of the populations, respectively, have shown significant declines. In Australia and New Zealand, as much as 70% of the amphibian populations studied have declined. According to the 2004 IUCN Red List [18], there are 20 countries with the highest number of threatened amphibians (Table 1). The numbers of threatened species are increasing in all taxonomic groups (Table 2) due to environmental pollution (including pesticides and related chemicals), habitat destruction as well as climate change. During the year 2000 and 2004, the number of extinctions in the fish, amphibian, reptilian, mammalian and avian groups has increased further. There are 338 species which are considered extinct and another 22 species are considered extinct in the wild (Table 3).

Table 1.
Countries with highest number of threatened amphibians [18].

Rank	Country	Threatened Species
1	Colombia	208
2	Mexico	191
3	Ecuador	163
4	Brazil	110
5	China	86
6	Peru	78
7	Guatemala	74
8	Venezuela	68
9	India	66
10	Costa Rica	61
11	Madagascar	55
12	Honduras	53
13	Panama	52
14	USA	51
15	Cameroon	50
16	Philippines	48
17	Australia	47
18	Cuba	47
19	Haiti	46
20	Malaysia	45

Amphibians are important components of aquatic habitats, especially in tropical regions of the world [22]. The mechanisms responsible for the decline of amphibian populations include chemical pollution from pesticides and fertilizers and global climate change [19]. The health of amphibians can suffer from exposure to pesticides [20]. Because of their semipermeable skin, the development of eggs and larvae in water, and the position in the food web, amphibians are prone to adverse effects of waterborne and airborne pollutants in their breeding and foraging habitats [21]. Pesticides may affect amphibian populations in a number of ways [27]; they may kill individual amphibians directly [28] or indirectly through alterations in immune or neurological function [29]. Pesticides may also affect recruitment in amphibian populations by disrupting normal growth and development of the young or by impairing adult reproduction [27]. An extensive research study conducted in Quebec, Canada, shows that hind limb deformities are commonly observed in transformed bullfrogs, green frogs, northern leopard frogs, and American toads [23]. Deformity rates tend to be higher at agricultural areas, suggesting that herbicides and pesticides are the likely causes.

Table 2.
Numbers of threatened species by major taxonomic group [18].

Taxonomic group	Number of described species	Number of species evaluated	Number of threatened species in 2004	Number threatened as % of species described	Number threatened as % of species evaluated
Fish	28,500	1,721	800	3%	46%
Amphibians	5,743	5,743	1,856	32%	32%
Reptiles	8,163	499	304	4%	61%
Mammals	5,416	4,853	1,101	20%	23%
Birds	9,917	9,917	1,213	12%	12%

Table 3.
Numbers of extinct (EX) and extinct in the wild (EW) species by taxonomic group in 2004 [18].

Taxonomic group	Extinct (EX)	Extinct in the wild (EW)	Total
Fish	81	12	93
Amphibians	34	01	35
Reptiles	21	01	22
Mammals	73	04	77
Birds	129	04	133
Total	**338**	**22**	**360**

Because deformities of frogs do not always occur in these areas, a number of other factors may be involved, including the incidence or abundance of certain diseases or parasites [24]. Fifteen amphibian species have been designated as endangered, threatened, or of special concern by the Committee on the Status of Endangered Wildlife Canada due to some threats including chemical contamination [24].

Amphibians are known to be vulnerable to pesticides that are cholinesterase inhibitors [25]. Anticholinesterase pesticides function by binding with this enzyme in animals and disrupting nervous system activity, usually causing death by respiratory failure. Decreased cholinesterase activity can indicate exposure to some commonly used pesticides and can be harmful to wild animals [26]. The anticholinesterase effects of two other pesticides, Lambda cyhalothrin (a pyrethroid) and monocrotophos (an organophosphate), on *Rana cyanophlyctis* (Skittering frog) have been observed in the liver, kidney and brain. About 34.6 - 46.3%, 25.08 - 57.1% and 31.64 - 50.7% of the cholinesterase activities in the liver, kidney and brain, respectively are reduced following exposure to cyhalothrin. For monocrotophos treatment, cholinesterase decreases about 37.7 - 57.7%, 57.5 - 67.5% and 47.6 - 65.9% in the liver, kidney and brain, respectively [38]. The effect of two pyrethroids, Lambda cyhalothrin and Permethrin, on the cholinesterase activity of amphibian *R. cyanophlyctis* and *Rana tigrina* have also been compared. The cholinesterase activities of cyhalothrin treated frogs are decreased 34.6 - 46.3%, 25.08 - 57.1% in the liver and kidney. Permethrin treatment decreases cholinesterase activity 23 - 29% and 6.76 - 35% in the liver and kidney, respectively [39]. Total protein content also decreases in non-target amphibians after pesticide treatment, indicating pesticide-produced changes in the biochemical systems of non-target organisms [40]. The brain cholinesterase activity of *Rana cyanophlyctis* decreases upto 4.10 and 13.84 % under the effect of sandaphos and 5.16 and 23.28% under the effect of b-cypermethrin, respectively [41].

Some pesticides, herbicides, and nematocides are documented to have endocrine-disrupting effects [30]. To date, there are no reports linking endocrine dysfunction with amphibian malformations. However, it is well established that pesticides and related chemicals can be major ecological threats to fish and aquatic wildlife by diminishing productivity and fecundity [30]. The normal growth and development of amphibian larvae rely on functional and uncontaminated aquatic systems. Water sources are particularly at risk to contamination by pesticides because of the accumulation and distribution of contaminating substances in sediments of rivers, lakes, and ponds. Potential sources of EDCs that impact bodies of water include municipal sewage [31], and agricultural runoff (pesticides and herbicides) [32]. Thus, the EDCs that accumulate in aquatic systems may adversely affect amphibian reproductive processes.

Exposure of amphibians to dimethoate, carbofuran and chlorpyifos can alter vitamin A levels [33] and reduce melanogenesis [34]. Carbaryl, a short-lived carbamate that acts through acetylcholinesterase inhibition, may serve as a model chemical for neurotoxins (i.e., carbamates and organophosphates). The effects of sublethal concentrations are more relevant to amphibian communities because they may directly affect time of and size to metamorphosis, or indirectly affect survival [35].

Some studies have reported that the tadpole stage of *Rana* spp. is sensitive to herbicides, and various types of deformities in the tadpoles serve as possible indicators of such sensitivity. Tadpoles are also highly sensitive to organochlorine pesticides, and toxic effects of these pesticides are evident during metamorphosis [29], a period of marked endocrine change and reduction. In Minnesota, USA, some pesticides or their degradation products have been detected in water and sediment samples in very small quantities [36]. Despite the current documentation of amphibian declines and malformations, there are only few reports on the use of amphibians as models for abnormalities of reproductive processes by exposure to EDCs. In one study, the interactions of gonadal steroids and pesticides (DDT, DDE) on gonoduct growth in larval tiger salamanders, *Ambystoma tigrinum*, were examined [37]. The salamanders were immersed in a solution of DDE, DDT, or injected with estradiol or dihydro-testosterone. Essentially all the compounds tested had some adverse effect on the gonoduct growth in this species of salamanders.

Effects on reptiles

Very rapidly deteriorating status of freshwater turtles and tortoises in Southeast Asia has resulted in an increasing number of these species being listed as threatened in the IUCN Red List; globally 42% of turtle and tortoise species are threatened [18]. The decline in the population of alligator in the Lake Apopka, Florida (USA), is contaminated by organochlorine pesticides that emanate from a chemical spill. Here, a number of disturbing abnormalities were recorded in hatchlings and juvenile alligators, including modifications of enzyme activity, concentrations of sex hormones, abnormal ovarian morphology and unusually small phalluses [42,43]. Because these chemicals are known to be weak androgen receptors, the hypothesis that the individual and the population level effects observed in the alligators are due to chemical disruption of endocrine function seems reasonable [44].

The common snapping turtle (*Chelydra serpentina*) is the largest freshwater turtle occurring in Canada. Snapping turtle eggs from the Great Lakes contain high concentrations of fat-soluble contaminants which are absorbed while food is being digested. These include PCBs, dioxins, furans and organochlorine pesticides. Abnormal development, such as incidence of unhatched eggs or deformed animals, occurs at the highest rates in the sites which are the most contaminated [45]. In addition, a correlation between contaminated eggs and reduced developmental success has also been indicated [46].

The anticholinesterase effects of the phytopesticide, biosal (neem based formulation), on Indian garden lizard (*Calotes versicolor*) have been observed in the kidney and liver. About 13.60 - 18% and 39.52 - 52.61% of the cholinesterase activities in the kidney and liver are reduced following exposure to biosal [47].

Conclusion

This review shows that pesticides and related chemicals are capable of blocking the action of hormones in fish, amphibians and reptiles and causing reproductive dysfunction and abnormal development. They act on target tissues through hormone receptors or nonreceptors, may influence hormone secretion or its clearance from the body. In the last years, a number of deformed frogs have been found in the eastern US and Canada. The cause of mass deformities of transforming frogs remains elusive, but various factors have been implicated, including pesticides and related chemicals. The role of pesticides and related chemicals in amphibian malformations may be of concern due to the high deformity rates associated with sites where agricultural chemicals have been used. These chemicals can be a major threat to fish, amphibians and reptiles, and aquatic environment by diminishing productivity and fecundity. Further research is needed to evaluate the effectiveness of alternative pesticides and related chemicals to reduce the effects on fish, amphibian and reptilian populations. Globally, studies need to simultaneously consider the benefits to both agricultural and conservation communities; scientists from both communities should provide input to make realistic and informed decisions about the protection and conservation of aquatic biodiversity within agricultural landscapes.

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