ECOTOXICITY, GENOTOXICITY, AND CYTOTOXICITY OF PESTICIDES AND THEIR DEGRADATION PRODUCTS

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Summary

Ecotoxicology is a branch of toxicology that concerns itself with understanding the adverse effect chemicals and, in this review, pesticides have on wildlife populations, microorganisms, ecosystems and communities. Understanding the physical-chemical properties of a pesticide, distribution and transport in a specified system and the ultimate fate of a pesticide is a necessary factor in the equation for defining the impact a pesticide has on an ecosystem. Other factors necessary to completely predict with any certainty the impact includes the responses at the organism level, the hazards presented by a pesticide and the environmental concentrations that are expected after typical usage of the pesticide.

Pesticides are formulated to deliver minimum levels of active ingredient with maximum efficacy but since many pesticides act via an interaction with the nervous system, they have the potential to also adversely affect nontarget organisms. Pesticides have the ability to cause lethality, reproductive abnormalities, and genetic alterations and, although these effects are at the cellular or organismal level, they ultimately can impact a population or community. In particular, pesticides that may be genotoxic, by direct interaction with the genetic material of a living organism, whether microbial or wildlife, can impact the species populations for subsequent generations. Alterations of genotypic

frequency within a population can impact survival adaptation or capability. Several scenarios based on studies performed in the laboratory and in practical ecosystems will be discussed while attempting to understand the impact pesticide or chemical exposure may have on the health of wildlife communities.

1. Introduction

The use of pesticides by humankind has been prevalent for more than 3000 years. Throughout history, societies have continuously increased their understanding of the value of naturally-occurring, and subsequent synthetic, chemicals that could impede, prevent or eradicate pestilent, disease-causing organisms, decrease the economic impact of agricultural and decrease stored-product losses from pests. Although there has been a long history of usage of natural pesticides (toxins), there is the corollary understanding that pesticides or toxins have numerous non-selective adverse effects.

Ecotoxicology is concerning with understanding the relationships between the biota and abiota of environmental systems and the hazards associated with the introduction of organic and inorganic molecules into that system. It is usually quite difficult to assess the entire ecosystem under study. For example, an aquatic ecosystem has multiple inputs (atmospheric, terrestrial, sedimental), multiple resident stressors (light energy, solids release), innumerable targets (biota and abiota) and various outputs (photosynthetic, degradation and zooplanktonic products). Because of these multiple inputs and outputs, and the dynamic forces that sustain an ecosystem that is never really in steady state or static, subtle disturbances, either in magnitude or temporal length, may be difficult to discern.

Many study methods have been validated to assess the adverse outcomes of toxic compounds. The most relevant, and most difficult, method is the study of systems at the microcosmic and mesocosmic levels of an ecosystem. In this type of study system, all components and processes are accounted for in the assessment of system variables. In fact, because of the difficulty in the evaluation of ecosystem-level effects of pesticides, the United States Environmental Protection Agency (USEPA) has withdrawn the requirement for this type of testing for registration of pesticides. It is easier to assess whole animal and mechanistic considerations of a toxic chemical's effects with laboratory-based methods. These studies utilize biochemical techniques to determine the cellular and molecular mechanisms involved in cytotoxicity. Utilizing these and other studies, each technique provides an assessment of a particular segment of the system and taken as a whole provide an overall perspective of the effects of a chemical.

Because the effects of pesticides on the environment are too numerous to discuss within one review, the focus of this article will be adverse effects of major pesticides with respect to modes of action and chemicals' effects on important nontarget organisms.

2. Pesticide Usage and Distribution

Prior to the early 1990's, nearly all pesticide production was centered on a few select insecticides, such as DDT. The production and introduction of new pesticides into the marketplace occurred in several phases, with the introduction of newly synthesized

pesticides first to market followed by an increase in fungicides and then herbicides. Not until the mid-1900's was there an increase reported in the number of new pesticides available on the worldwide markets. In the period 1940-1965, the availability of new classes of insecticides nearly quadrupled and in subsequent decades increased by nearly 50% during each of those periods.
Organophosphates, developed in Germany in the 1930's, were introduced commercially in the 1940's and gained wide acceptance in various markets due to their relative non-persistence and rapid degradation. However, because of their lack of species specificity, they can be acutely toxic to target organisms as well as nontarget organisms. Despite the issue of toxicity, organophosphates continue to be utilized as acaracides and insecticides in agricultural, industrial and commercial settings. In 1988, nearly $2.3 billion of the reported $6.2 billion international market for insect control was from organophosphate usage, with chlorpyrifos and terbufos the most widely utilized for agricultural purposes. In 1994, chlorpyrifos, an organophosphate, was used at >25 million pounds for both agricultural and nonagricultural uses. On a similar note for other acetylcholinesterase-inhibiting pesticides, more than 100 types of carbamates are currently used in the marketplace with treated acreage exceeding 200 million acres per year.

Although no concise data exists for reporting of worldwide usage of pesticides, recent national information collected by USEPA indicates that more than 1 billion pounds of conventional and pesticide chemicals were used in 1997. If biocides, antimicrobials and preservatives are figured into the usage data, more than 4.5 billion pounds of pesticides were used. Thus, within one industrialized nation, the United States, the use and release of pesticides at such tremendous levels presents unique concerns for their effects on the environment and nontarget organisms.

The challenge is ensure the proper use of deliberately released pesticides by trained individuals and the design of pesticides in formulations and applications that are appropriate for its purpose. Pesticide formulations, which are founded on the physicochemical properties of the active ingredient's chemistry, determine transport, degradation and ultimate fate in the environment.

There are several classification schemes for the categorization of pesticides, including modes of action, affected organ system, target flora/fauna or class of chemistry. Examples of classification of pesticides are organophosphates, carbamates, pyrethroids and organochlorines and examples of target species include insects, fungi or weeds. Although each of these groups are technically classified as pesticides under various international regulatory agencies, *i.e.* United States Environmental Protection Agency's Federal Insecticide, Fungicide and Rodenticide Act (USEPA FIFRA), for the purpose of this review, insecticides as a subset of pesticides will be the primary focus.

Organophosphates and carbamates, the most widely used class of insecticides, are classes of pesticides that target the nervous system by inhibiting the enzyme acetylcholinesterase (AChE) by irreversible phosphorylation or reversible carbamylation, respectively. Six major OP comprise >50% of OP and three carbamates comprise >50% of carbamates utilized.

Pyrethroids, synthetic analogues of the naturally derived pesticides pyrethrum and pyrethrins derived from the pyrethrum flower, genus *Chrysanthemum*, affect their function by inhibiting the closing of activated neuronal sodium channels. Organochlorines, such as dieldrin, endosulfan and dichlorophenyltrichloroethane (DDT), have some effects on cellular respiration but have also been shown to decrease ATP levels and inhibit photosynthesis. In general, chlorinated hydrocarbon pesticides have been banned or restricted since the 1970s but use has continued in many developing countries for more than a decade. Several Central American and Southeast Asian countries still use DDT for the control of malaria, and nearly 200,000 pounds of heptachlor or lindane are still used for agricultural purposes.

Herbicides have several mechanistic effects, including inhibition of electron transport, reactions in photosynthesis and microtubule assembly mechanisms. When herbicides are applied, vegetation is destroyed and there are concomitant effects on plants, release of nutrients and stored organic carbon, all which lead to effects on the aquatic and terrestrial systems.

Pesticides have the potential to enter the water column, soil or bodies of water via several mechanisms such as direct application to water, surface runoff, air transport, volatilization from soil, dislodgeable foliar residue release, household use and inappropriate disposal of pesticide waste. Studies have shown that mixtures of pesticides used under different scenarios are easily detected in public and monitoring waters (Table 1). The principal source of water contamination occurs from agricultural use runoff. Agricultural uses of pesticides account for nearly 70% of pesticide use, followed by industrial and residential home and garden uses.

No. of samples in which combination was detected						
No. of constituents in combination	Two or more		10% or more		20% or more	
	MW	PSW	MW	PSW	MW	PSW
2	50	29	10	17	5	6
3	76	40	7	19	2	2
4	54	30	2	9	0	0
5	16	12	0	1	0	0
6	1	2	0	0	0	0
Total	197	113	19	46	7	8

Table 1. The number of unique combinations of pesticides detected in samples from the public supply and monitoring wells. MW = monitoring wells PSW = public supply wells.

3. General Factors of Ecotoxicology

There are numerous factors that influence toxicity of a pesticide, some factors that are well characterized and others that have not received as much attention. Important factors in the consideration of pesticide toxicology include duration of exposure, dosage, organism biology, general health of and stressors on organismal populations and environmental factors.

For example, pesticides that are applied directly to water sources or vast plots of land have the greatest potential for acute or chronic toxicity. Pesticides that are persistent and do not readily breakdown under standard environmental conditions have the highest risk for adverse outcomes. As a general guideline, nonpersistence is degradation or removal with 2-4 months, moderate persistence is up to 2 years and persistence is greater than 2 years in the environment. Pheromones and insect growth regulators (IGR) are not persistent, OP and carbamates are moderately persistent and organochlorines are highly persistent.

3.1. Fate and Transport

Those pesticides that make their way into the environment may pose acute and chronic risks for organisms. In addition to the direct impact on nontarget organisms, pesticides have the potential to bioaccumulate or bioconcentrate in human food sources, creating the risk for ultimate bioconcentration in humans. Hence, those pesticides that are biologically persistent and are resistant to breakdown pose the greatest concern to wildlife. There are several pesticides, *i.e.* DDT, that are no longer used in developed countries and released into the environment but are still detected in surface water. These pesticides may continue to pose a threat to flora and fauna in ecosystems due to resuspension in land or estuarine environments by dredging and tidal flow actions.

The chemical structure of pesticides is important in the stability and persistence of these molecules in the environment. Pesticides that have the potential for bioconcentration in terrestrial and aquatic systems have high lipophilicity and resistance to degradation. Sediment adsorption, stability and lipophilicity are related and associated with bioaccumulation. Increased lipophilicity is associated with increased sediment adsorption. The octanol-water partition coefficient (K_{ow}) has been utilized to estimate the physico-chemical behavior of pesticides in the environment. Octanol-water partitioning, as a function of the ratio of the concentration of a compound that distributes between octanol and water, is a result of the polarity of the compound, with more polar compounds partitioning into the water phase. In addition to K_{ow}, water solubility, vapor pressure and Henry's law constant are utilized as predictors of behavior in the environment (Table 2).

Compound	**Water solubility (ppm)**	**Vapor pressure (mm Hg)**	**Soil adsorption (K_{oc9}, ml/g)**
Parathion	12	4×10^{-5}	4800
Diazinon	40	7×10^{-7}	251
Chlorpyrifos	1	2×10^{-5}	8753
Chlorpyrifos-methyl	3	4×10^{-5}	3300
Fonofos	16	2×10^{-4}	5105
Fonofos oxon	> 2600	-	-
EPN	-	1×10^{-8}	1327
Malathion	143	4×10^{-5}	280
Dimethoate	25,000	8×10^{-6}	27
Terbufos	6	3×10^{-4}	842
Terbufos sulfoxide	> 1100	-	-
Terbufos sulfone	408	-	-
Isofenphos	22	4×10^{-6}	-

Dichlorvos	10,000	1×10^{-2}	-
Ethoprophos	750	3×10^{-4}	26

Table 2. Environmentally significant properties of organophosphate insecticides

Actual concentrations are dependent on several factors, including application rate, interception by target vegetation, water depth, amount of suspended solids, whether water is stagnant or subject to current, *i.e.*, in a stream.

Differences in fate and transport of pesticides are tantamount, especially when there is acute dosing of a system, rapid movement throughout the system and minimal dissipation or increased persistence of the pesticide. Chemicals that move maximally and rapidly in the system possess the potential to reach far and wide and affect numerous organisms at various trophic levels. If a chemical does move seamlessly through an ecosystem, there is also greater potential for it to degrade more rapidly if its physicochemical properties allow this pathway. If a pesticide is relatively immobile and dissipates only minimally over time, there could be chronic, high dosing of particular areas within the ecosystem.

When a pesticide is dispersed in water, it can quickly move throughout the water column and be introduced into the food chain rather effectively. If there is minimal adsorption of the pesticide to available matrices, and exposure precedes a rainfall, there is also a high potential for movement of the pesticide throughout the system. Ecosystems that are especially diverse in their species population have the greatest potential to withstand chemical and pesticide assault. Homogeneous systems have a higher potential for all species to be affected and, ultimately, not recover. A heterogeneous species population has the potential to deal more effectively with the multiple pathways that a stressor(s) can inflict on an ecosystem.

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Biographical Sketch

David F. Crawford, Ph.D. is Manager of Toxicology for Wellmark International in Chicago, Illinois, USA. He currently engaged in understanding the human health and environmental effects of pesticide active ingredients and formulations and developing risk assessments of these chemicals. He has over 10 years experience in the toxicology and risk assessment fields.

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