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Handbook of
ECOTOXICOLOGY

Second Edition

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LEWIS PUBLISHERS

A CRC Press Company

Boca Raton London New York Washington, D.C.

Wildlife Toxicology of Organophosphorus and Carbamate Pesticides

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12.1 INTRODUCTION

Organophosphorus (OP) and carbamate (CB) pesticides are used in domestic and natural environments for control of a wide variety of insect pests and disease vectors, other invertebrates, fungi, birds, and mammals; some CBs are used as avian repellents, and others have herbicidal properties. These two pesticidal classes, numbering about 200 OPs and 50 CBs, have been formulated into thousands of products that are available in the world's marketplace for varied applications to wetlands, rangelands, cultivated crops, forests, and rural and urban environs.¹⁻⁵ Except for mosquito control, these products are applied mostly on terrestrial landscapes. When applied according to the label, the active ingredient should be reasonably well contained within the intended treatment area. However, due to drift, runoff, or applicator error, the pesticide or toxic degradates are inevitably detected in water, soils, and vegetation outside the treated area — sometimes in toxic concentrations and for durations well beyond the expected residual life of the product. Off-site contamination of OPs and CBs has resulted in massive episodes of mortality of aquatic organisms,⁶ but the long-term implications of periodic transient mortality on ecosystem productivity has not been thoroughly evaluated. Free-ranging vertebrates have also suffered large-scale mortality from acute exposure to OP and CB insecticides within and peripheral to the treated area.⁷⁻¹⁰ OPs and CBs are acutely toxic to most animals with the potency of a chemical often widely variable among species.¹¹⁻²⁰

Most widely used OP and CB insecticides are highly toxic but relatively short-lived in nature (e.g., 2 to 4 weeks) and are readily metabolized and excreted by homoiothermic animals.^{2,3} These factors and broad-spectrum insecticidal efficacy favored OP and CB pesticides as replacements for the persistent and problematical mercurial and organochlorine compounds.²¹⁻²³ For example, certain organochlorine pesticides and metabolites bioaccumulate in food chains, inhibit proper eggshell formation, and severely jeopardize populations of fish-eating birds such as brown pelicans (*Pelecanus occidentalis*), bald eagles (*Haliaeetus leucocephalus*) and ospreys (*Pandion haliaetus*).²⁴ This type of chronic manifestation has not been demonstrated for OP or CB exposure, and it is not certain that OP- and CB-induced mortalities of nontarget vertebrates have critical effects at the population level. However, there is increasing evidence that mortality of raptorial birds from OP and CB poisoning may be affecting some species at the regional level.^{10,25}

The ecological hazard of OP and CB pesticides to wildlife is primarily from acute anticholinesterase toxicity but also includes species habitat association and foraging preference. Exposure may be directly from the pesticidal application, contact with or ingestion of contaminated water, soil or vegetation, or ingestion of contaminated prey or pesticide impregnated seeds or granules. Other factors also bear on wildlife tolerance of an OP- or CB-contaminated environment. For example, the prey base may be altered and affect foraging success; sublethal exposure may affect critical behaviors such as reproduction and migration; and proper balance between producer and consumer organisms in soil and aquatic systems may be disrupted.²⁶ Fish and other aquatic organisms also vary widely in tolerance of OP and CB exposure depending on inherent sensitivity and factors of water quality, chemistry, and temperature.²⁷

This chapter provides an overview of the hazard of organophosphorus and carbamate pesticides to avian and mammalian wildlife. Attention is given to practical environmental considerations rather than interpretation of laboratory studies that were detailed in the first edition of this book.²⁸ Invertebrates, fish, amphibians, and reptiles are exemplified as ecosystem components or for comparison with birds and mammals, but the toxicology of OP and CB pesticides to these taxa is presented in other chapters. The focus herein is on concepts of ecological toxicology of birds and mammals related to natural systems as affected by pesticidal application in agriculture and public health. The environmental fate of representative OP and CB pesticides, the availability of these pesticides to wildlife, and toxicology as related to ambient factors, physiological cycles and status, product formulations, and sources of exposure are discussed.

12.2 GENERAL TOXICOLOGY

12.2.1 Organophosphorus Pesticides

Organophosphorus chemicals comprise more than one third of the registered pesticides on the world market. Most registrations are for control of a large array of insect pests and disease vectors, but OPs effectively control many animal pests including other invertebrates and terrestrial vertebrates. Over 95% of the OP products presently in production are used in agriculture and mosquito control.²⁹ In the United States about 70 OPs are registered as the active ingredient (AI) in thousands of products, but only 10 to 15 of the chemicals account for over three fourths of the use. Examples of OPs that have been widely used in U.S. agriculture include azinphos-methyl, chlopyrifos, fonophos, malathion, methyl parathion, parathion, phorate, phosalone, and terbufos; OPs used extensively for mosquito control are fenthion, malathion, naled, and temephos. Many of these chemicals have been reviewed by regulatory agencies for environmental and public health concerns and are now classified as restricted-use pesticides in the United States; some uses have been cancelled. OPs registered for outdoor use have label warnings about toxicity to wildlife and application to wetlands.

The main concern about OPs is their acute lethal toxicity. Based on single-dose oral LD₅₀ tests, the above chemicals are among the most toxic OP pesticides to laboratory rodents and wildlife.^{11,13,15,16,30} Of the 12 chemicals, ten are classed highly toxic to birds (LD₅₀, <50 mg AI/kg body mass³¹), and seven are highly toxic to mammals. Only malathion is considered to be of a low order of acute toxicity to both birds and mammals (LD₅₀, >500 mg/kg). Chlorpyrifos and fenthion are moderately toxic (LD₅₀, 50 to 500 mg/kg) to mammals, and naled is moderately toxic to birds and mammals. Some OPs that have had most uses withdrawn or cancelled in the United States (e.g., dimethoate, EPN, monocrotophos, parathion, TEPP)⁵ remain available on the international market in spite of their demonstrated environmental hazard to human health and wildlife. In the U.S.S.R., pesticides with a single-dose oral mammalian LD₅₀ less than 50 mg/kg were banned in the 1960s; a few exceptions were permitted for use of granular formulations in agriculture.³²

The principal toxicity of OP pesticides is based on disruption of the nervous system by inhibition of cholinesterase (ChE; acetylcholinesterase, EC 3.1.1.7, and a mixture of nonspecific esterases) activity in the central nervous system and at neuromuscular junctions with death generally attributed to acute respiratory failure.³³ When OP binds to ChE, a relatively stable bond is formed and prevents the ChE from deactivating the neurotransmitter acetylcholine. This permits buildup of acetylcholine and overstimulation of the cholinergic nervous system. Some of the nonspecific signs following acute OP exposure of small mammals and birds include lethargy, labored breathing, excessive bronchial secretion, vomiting, diarrhea, tremors, convulsions, and death. These toxic indicators are useful when sick animals are found near an area of recent OP application, but the signs are not uniquely different from poisoning by other neurotoxic agents.^{1,16,33} In nature, notation of toxic signs is important in the investigation of a wildlife incident, while conclusive diagnosis depends on biochemical and chemical analyses for brain ChE inhibition and OP residues in the carcass.³⁴⁻³⁶ Biochemical diagnosis of OP and CB exposure is described in Section 12.4.7.

Two additional syndromes of single or very short-term OP exposure have been demonstrated in the laboratory. The first, referred to as an "intermediate syndrome," is a potentially lethal paralytic condition of the neck, limbs, and respiratory muscles.^{37,38} The paralysis from muscle necrosis follows an acute OP exposure by 2 to 3 days and is apparently initiated by depressed ChE activity and calcium accumulation in the region of the motor end-plate.^{38,39} This syndrome has not been identified as such in either laboratory or field studies of wildlife, but it could be an important factor in OP hazard in nature. For example, when flocking birds enter a hazardous OP-treated area, onset of acute toxicity often occurs within a few minutes in some birds, while others appear unaffected. If the intermediate syndrome plays a significant role, mortality away from the treated area could be much higher than generally believed. Examples of OP pesticides demonstrated to induce the

intermediate syndrome in mammals include fenthion, malathion, and monocrotophos.³⁸ Fenthion and monocrotophos have caused large-scale episodes of wildlife mortality; malathion has not. The possibility of intermediate syndrome from malathion has not been investigated.

The second syndrome of single-dose OP exposure is OP-induced delayed neurotoxicity (OPIDN) in which a relatively small dosage (e.g., 1/25 LD₅₀) of OP causes degeneration in the myelin sheath of long peripheral nerves and the spinal cord.⁴⁰ This debilitating malady develops in 1 to 3 weeks, causing a stumbling gait and incoordination. OPIDN has been demonstrated in a variety of laboratory animals including rodents, chickens, and mallards (*Anas platyrhynchos*); mallards do not appear to be as susceptible as chickens to OPIDN.⁴¹ Apparently, OPIDN is not related to anticholinesterase action of OPs.⁴² Most OPIDN-inducing pesticides are no longer on the market (e.g., leptophos and mipafox), but a few remain in use in some countries (e.g., cyanofenphos, EPN, methamidophos, trichlorfon, and trichloronate).^{38,42}

Subacute or subchronic exposure to repeated sublethal doses of OP have been demonstrated to affect birds and mammals in captivity^{28,43,44} and undoubtedly influences critical physiological functions in nature. However, validation of low-grade OP hazard to natural wildlife populations remains elusive. Some of the more likely sublethal effects on wildlife that live in or depend regularly on OP-contaminated forage and water include changes in response to ambient stressors, changes in foraging and reproductive behavior, and possible alteration in migration orientation. Whereas these effects appear related to anticholinesterase insult, other effects may be entirely independent of ChE inhibition. Examples include mutagenicity, carcinogenicity, and organ-specific toxicity to the heart and kidneys.⁴² Putative sublethal effects of OP exposure are discussed in Section 12.5.

12.2.2 Carbamate Pesticides

Fewer than one fourth of the registered carbamates in the world market are insecticides with significant anticholinesterase activity; the others are fungicides and herbicides with little acute hazard to birds and mammals. Of approximately 50 registered CB pesticides, only about eight (aldicarb, carbaryl, carbofuran, formetanate, methiocarb, methomyl, oxamyl, and propoxur) are used for insect control on crops, forests, and rangelands; methiocarb and methomyl are also used as avian repellents. Of these eight, carbofuran, methomyl, and carbaryl account for more than 90% of the use. As with OPs, CB insecticides have label warnings about toxicity to wildlife and application to wetlands.

CB insecticides exert their toxicity through acute ChE inhibition, and all of the above-named except carbaryl are classed highly toxic to birds and mammals.^{12,16} LD₅₀s are generally less than 20 mg/kg for both taxa and as low as 0.8 mg/kg for aldicarb with male laboratory rats and 0.5 mg/kg for carbofuran with male mallards. In contrast, the LD₅₀s for carbaryl are reported as 850 and >2,500 mg/kg for male rats and mallards.

Acute toxicity of CB insecticides including toxic signs are similar to that of OPs, except onset of and recovery from CB is faster than for equipotent exposure to OP insecticides.⁴⁵ The rapid reaction is partly because CB insecticides are direct ChE inhibitors that do not require metabolic activation for full potency as do most OPs. Rapid recovery is a product of near spontaneous reactivation of carbamylated ChE. Thus, an equipotent sublethal exposure of CB is generally less severe than OP exposure, and factors of delayed toxicity described for OPs do not occur with CBs.^{1,42}

12.2.3 Some Considerations

The toxicity of organophosphorus and carbamate pesticides varies considerably among vertebrates (Table 12.1). OPs and CBs do not bioaccumulate in food chains. Cumulative depression of ChE enzyme may occur and persist from repeated exposure to some OPs but generally not from CBs. CB and a variety of OP esters (e.g., acephate, monocrotophos, trichlorfon) are direct ChE inhibitors, but most OP pesticides (e.g., diazinon, malathion, parathion) must first undergo an

Table 12.1 Acute Response of Fish, Laboratory Rats, and Birds for Anticholinesterase Pesticides of Widely Variable Mammalian Toxicity

Pesticide	Rainbow Trout ^a	Bluegill ^a	Laboratory Rat ^{b,c}	Ring-Necked Pheasant ^{b,d}	Red-Winged Blackbird ^{b,e}
Aldicarb	560	50	0.8	5.3	1.8
Phorate	13	2	2.3	7.1	1.0
Carbofuran	380	240	11	4.1	0.4
Azinphos-Methyl	4	22	13	75	8.5
Mexacarbate	12,000	22,900	37	4.6	10
Ethion	500	210	65	1297	45
Methiocarb	800	210	70	270	4.6
Dimethoate	6200	6000	215	20	6.6
Carbaryl	1950	6760	859	707	56.0
Temephos	3490	21,800	8600	35	42

^a LC₅₀ = µg of active ingredient per liter of water calculated to kill 50% of test population during a standard 96-h exposure. Tests were conducted under static conditions (pH 7.2-7.5 at 10-13°C for trout or 20-22°C for bluegills), *n* = 50-60 per test.¹⁴

^b LD₅₀ = mg of active ingredient per kilogram of body mass calculated to kill 50% of test population.

^c Sherman strain male laboratory rats, 3 months old, *n* = 50 to 60 per test; dosage by gavage in peanut oil.^{11,12}

^d Farm-reared male and female ring-necked pheasants, 3 to 4 months old, *n* = 8 to 28 per test; dosage by gelatin capsule.¹⁶

^e Wild-captured pen-conditioned male and female red-winged blackbirds, adult, *n* = 8 to 28 per test; dosage by gavage in propylene glycol.¹⁵

oxidative desulfuration step for maximum anticholinesterase potency.^{2,33} This toxicating step is mediated by mixed-function oxidases (MFO) in the liver of vertebrates and in the fat body, malpighian tubules, and digestive tract of invertebrates. MFO activity varies widely among vertebrates in the following order: mammals > birds > fish.^{46,47} (The place of amphibians and reptiles in this ranking has not been determined.)^{48,49} The same physiologic system responsible for toxication of most OPs also has a primary role in their detoxication. Since OP and CB metabolism occurs primarily in the liver of vertebrates, the portal of entry into the circulatory system is important to acute toxicity. CBs and direct-acting OPs may be more hazardous through inhalation than through ingestion where substances are routed through the liver and first-phase metabolism. The environmental hazard of inhalation toxicity has not been thoroughly evaluated but is generally believed less important than ingestion. Likewise, percutaneous exposure to OP and CB pesticides has not been adequately studied in wildlife, but in nature the composite of inhalation, percutaneous exposure, and ingestion must all be considered in hazard prediction and understanding differences of species' response to OP and CB applications. It is important that OP toxication via oxidative desulfuration also occurs in nature as mediated by microbial metabolism in soils and by phytometabolism, but this process is slow compared to MFO metabolism in vertebrates.

The toxic consequences of OP or CB application to aquatic and terrestrial wildlife usually last only a few days. However, multiple applications of pesticide are the rule during the growing season; this extends the potential hazard to wildlife. In areas where a variety of crops are cultivated, exposure to a variety of pesticides and unexpected hazard may occur, especially to highly mobile wildlife. Other factors of variability must also be considered in hazard assessment of OP and CB pesticides. Predator-prey or competitor balance among invertebrates and aquatic vertebrates may be disrupted. Daily activity patterns, energy budgets, and various behaviors of terrestrial vertebrates may be affected. Repeated application of these biologically labile chemicals may cause cumulative physiological effects without a corresponding accumulation of chemical residues such as occurs for heavy metals and organochlorine pesticides. Recovery from anticholinesterase exposure may differ among pesticidal classes. For example, 1 to 3 weeks may be required for brain ChE activity to recover in vertebrates receiving a single exposure to OPs,^{50,51} but only a few hours may be necessary

to recover from CB exposure.⁵²⁻⁵³ Many of the confounding variables will become evident in the following section on environmental fate and hazard of several representative OP and CB pesticides.

12.3 ENVIRONMENTAL FATE AND HAZARD

The first considerations of environmental fate and hazard of organophosphorus and carbamate pesticides are agricultural crop, products formulation, rate and method of application, likelihood of wildlife exposure, and the biological and physical characteristics of any wetlands likely to receive runoff. OP and CB pesticides are comparatively labile in circumneutral environments and readily degrade under conditions of alkalinity, rain, sunlight, and temperature.^{2,3,54} Neither bioaccumulation nor biomagnification occurs to any important degree in aquatic or terrestrial food chains. The main hazard of OP and CB exposure to wildlife is from short-term, potentially lethal exposure to pesticidal treatment. This may include direct contact via inhalation and percutaneous routes or from ingestion of contaminated food and water. Classic secondary poisoning through biomagnification of highly lipid soluble pesticides, such as chlorinated hydrocarbons, does not happen with OP and CB compounds. Instead, predators are often poisoned by feeding on prey that had been contaminated by pesticidal application. For example, invertebrates and aquatic vertebrates adsorb OP and CB pesticide on their cutaneous surface or mucosal coating, which is then readily dissociated and absorbed when eaten by another animal.²⁸ Predatory birds have also been poisoned from eating insects that had ingested systemic OP and CB while foraging on vegetation.¹⁰ The stomach contents of poisoned birds and mammals may contain large concentrations of OP and CB that have proven toxic to predators and scavengers.⁵²

OP pesticides and anticholinesterase CBs have broad-spectrum toxicity to most animals with a cholinergic-dependent nervous system. OP and CB products are used as stomach and contact poisons for nearly any type of insect control; about 20% of OP and 40% of CB insecticides have systemic activity. They are also used as acaricides, nematocides, rodenticides, avicides, and bird repellents. As mentioned, a few OPs (e.g., butylate, EPTC, molinate) and many CBs have herbicidal properties, and some CBs receive wide use as fungicides (e.g., benomyl, maneb, mancozeb). In spite of their broad toxicity, OPs such as fenthion, naled, and malathion are widely used over wetlands and areas of human habitation for mosquito control.²⁹ ChE-inhibiting CBs are not as extensively applied on natural wetlands as are OPs, and CBs other than carbaryl are not applied extensively near human habitation.

Except for mosquito control, nearly all OP and CB application is on terrestrial landscapes. Nonetheless, pesticidal treatment of farmlands, forests, and cities results in the contamination of adjacent aquatic systems by some of the pesticide and its degradates. It has been theorized that all pesticidal chemicals will eventually enter an aquatic environment and affect a much larger number of species than originally intended.¹⁹ The hazard of OP and CB pesticides to nontarget life is a product of the amount, rate, and form of residual entering the aquatic system as well as the dynamics and chemistry of the system. For example, if runoff from a pesticidal application enters a stream, rapid dilution and dispersal may soon render the contamination ecologically innocuous. In contrast, if the same runoff enters an aquatic sink, such as a farm pond rich with detritus, the ecological consequences may be considerable to organisms at all levels including terrestrial species. In general, the residue levels of OP and CB compounds in natural waters are as follows: closed pond > free-flowing waters > lakes > estuaries > open sea.¹⁹

Contamination of small closed ponds has resulted in large incidents of mortality of waterfowl and predatory birds including northern harriers (*Circus cyaneus*) and golden eagles (*Aquila chrysaetos*) in the northern plains of the United States. In this example a granular formulation of phorate was incorporated into the soil for nematode control. Heavy rains occurred several months after application and facilitated the transport of phorate and degradates into small ponds, resulting in mortality of aquatic and terrestrial animals for 2 to 3 weeks following the storm event. Similar scenarios leading

to mortality from OP and CB leaching from granular formulations have been documented for agricultural application of pesticides such as parathion, diazinon, and carbofuran.⁶ Wildlife have been acutely poisoned by ingesting OP- and CB-impregnated granules and by foraging in areas treated with flowable and emulsifiable concentrate formulations and technical grade pesticide.^{4,6,10,52}

12.3.1 Organophosphorus Pesticides

Phosphorothioic acids contribute more than 90% of the OP pesticide use in the United States. These pesticides require metabolic activation for maximum anticholinesterase effect and vary widely in acute toxicity and chemical stability in the environment. Phosphoric acids, representing nearly all of the remaining OP pesticides, do not require metabolic activation for toxicity and, except for acephate and ethoprop, are presently of little importance in the United States. This is because voluntary withdrawal and regulatory restrictions have reduced the market for some previously widely-used and extremely toxic products such as dicrotophos, mevinphos, monocrotophos, and phosphamidon.⁵ The major phosphoric acid products on the U.S. market are acephate and ethoprop, while the others remain available on other world markets.

As discussed, OP pesticides are labile in natural environments, which generally limits their hazard to only a few days for vertebrates depending on a variety of ambient factors related to method of application and formulation of product. In contrast, a few OPs, such as phorate in the above example, have hazard potential to wildlife for a longer period due to systemic action and toxic degradates.

12.3.1.1 Case Study: Phorate

Phorate is one of a small but widely used group of highly efficacious insecticide-acaricide compounds whose environmental degradates are more toxic and stable than the parent chemical. This feature increases their potential hazard to wildlife but also increases their marketability as broad-spectrum systemic pesticides for foliar application and incorporation into the soil. Examples of these structurally similar OPs include phorate, (EtO)₂ PS.SCH₂ S_{Et}; disulfoton, (EtO)₂ PS.SCH₂CH₂S_{Et}, and terbufos, (EtO)₂ PS.SCH₂ S_{Bu.t55}, are extremely toxic to mammals and birds (LD₅₀, <10 mg/kg).^{12,16} For comparison, the acute toxicity of methyl parathion and carbofuran has been reported as 14 and 11 mg/kg for rats¹² and 10 and 0.5 mg/kg for mallards.¹⁶ These pesticides are also highly toxic by the dermal route of exposure.^{5,12}

This group of pesticides has an additional oxidative pathway of toxication in the environment that is mediated primarily by UV irradiation in water and soil, to a lesser degree by microbial metabolism,⁵⁶ and by phytometabolism in plants.⁵⁷ As with the parent compound, the sulfoxide and sulfone degradates also require the oxidative desulfuration step to increase anticholinesterase potency.

With phorate as the model, several observations on environmental fate are important to the ecological hazard of these unique phosphorothioic acid pesticides. The fate of phorate in water is determined by pH, temperature, and photolysis.² Phorate is stable in water at about pH 5; its half-life in pH 6 water at 25°C is about 7 days.⁵⁸ The rate of hydrolysis increases about tenfold per pH unit under alkaline conditions. Irradiation by UV light oxidizes phorate within minutes to its more potent ChE-inhibiting sulfoxide and sulfone degradates.⁵⁶ Neither aquatic invertebrates nor fish tend to bioaccumulate phorate in model ecosystems,⁵⁹ but phorate is highly toxic to fish. For example, in comparable standardized 96-hour LC₅₀ tests, phorate is more than 100 times as toxic as methyl parathion and carbofuran to an array of both warm- and cold-water game fish.¹⁴

The fate of phorate in soil is affected by pesticide formulation, the method and rate of application, soil type, pH, temperature, moisture content, irrigation and water percolation, vegetation type and abundance, and microbial populations.^{60,61} Surface application of either granular or emulsifiable phorate results in 15 to 20% loss due to volatilization within 1 h; thereafter, nearly all the residual phorate remains bound to the soil particles but undergoes UV irradiation and oxidation to

sulfoxide and sulfone degradates within a few days. Up to 80% of these highly toxic degradates persist in various soil types for 1 to 2 months.⁶⁰ The terrestrial dissipation half-life of phorate in irrigated soils is 2 days in sandy loam and 9 to 15 days in silty loam. For sulfoxide and sulfone degradates, the half-life in sandy loam is 12 to 18 weeks.⁶² Phorate movement is more rapid in summer than in winter, but it is more stable in winter, probably due to lower microbial activity.⁶¹ Phorate is poorly soluble in water (~50 mg/L), and residues do not migrate extensively from the treated area. However, residues may be transported by erosion and runoff from agricultural fields into aquatic systems, where they have caused major episodes of fish and bird mortality.⁶

Soil-incorporated phorate is readily translocated through the roots and stems of plants and provides insecticidal protection to plants for a relatively long time because of the greater persistence of the sulfoxide metabolite.² The initial oxidation to sulfoxide proceeds rapidly in plants, whereas further oxidation to sulfone and desulfuration goes more slowly. Anticholinesterase activity increases as phorate oxidation proceeds to the most toxic sulfone. The oxon analog of each form is even more potent. Therefore, the anticholinesterase activity of phorate assimilated into plants increases for several days and only then gradually loses activity over 2 to 5 weeks.² Because of this systemic activity, phorate poses a potential hazard to herbivorous animals including wildlife and livestock.

In summary, phorate, like most widely used OP pesticides, is highly toxic to vertebrates. However, where most OPs rapidly degrade to biologically inert products, phorate degrades to more stable and potentially more potent anti-ChE products. This contributes to the systemic efficacy of phorate but also extends its hazard compared to other systemic OP insecticides. Examples of other systemic OPs include acephate, dicotophos, oxydemeton-methyl, and phosphamidon; all are direct-acting ChE inhibitors.

12.3.2 Carbamate Pesticides

Carbamates with significant anticholinesterase activity were developed as analogs of alkaloid extracts of the calabar bean (*Physostigma venenosum*). Elimination of the polar moiety of the natural drug enhanced lipid solubility and penetration of the insect cuticle and nerve sheath, leading to broad-spectrum insecticidal activity.^{3,54} Most insecticidal products are phenyl N-methylcarbamates (e.g., carbaryl, carbofuran, propoxur); the others are oxime carbamates (e.g., aldicarb, methomyl, oxamyl).⁵⁵ Carbofuran and most oxime carbamates have significant systemic activity in plants. All CB insecticides are potent anticholinesterases and highly toxic to most invertebrates, and many are highly toxic to vertebrates. Birds and mammals receiving sublethal exposure to CB readily detoxicate the molecule and appear to fully recover within a few hours. There is little evidence that herbicidal and fungicidal CBs pose important hazard to terrestrial wildlife.⁴ For example, none of these CBs have significant anticholinesterase activity, and only one of the widely used products has a mammalian LD₅₀ of less than 1000 mg/kg (molinate, 720 mg/kg for laboratory rats), while most yield LD₅₀s above 2000 mg/kg for birds and mammals. However, some products are highly toxic to fish.¹⁴

Carbamate insecticides are generally short-lived as foliar applications (e.g., 1 to 4 weeks) and require several applications during growing seasons; when applied to soil, significant residues may persist 2 to 16 months.³ Little information is available on the biological hazard of multiple applications or on the importance of comparatively more persistent but less toxic degradates of CBs such as aldicarb and carbofuran. Both of these compounds are highly toxic to terrestrial animals^{3,4,6} and aldicarb is toxic to fish;¹⁴ carbofuran is not nearly as toxic as aldicarb in 96-hour LC₅₀ tests with freshwater fish.¹⁴ Toxic residues of aldicarb and its degradates (mostly aldicarb-sulfoxide and sulfone) have been detected in ground water and farm produce as long as a year after application.^{63,64} Due to extreme mammalian toxicity and hazard to farm workers, aldicarb is a restricted-use pesticide in the United States. Carbofuran is also a restricted-use pesticide, but it is still widely used in spite of regulatory concerns about its environmental hazard.^{6,65}

12.3.2.1 Case Study: Carbofuran

Carbofuran is used mostly in granular formulations for control of soil and foliage insects and agricultural nematodes. Flowable formulations were used extensively in the United States through the early 1970s and resulted in large-scale episodes of wildlife, mostly waterfowl, mortality. Thereafter, granular formulations were favored, but similar incidents of mortality continued.^{65,66} These incidents usually occurred within the first 2 to 3 days following approved pesticidal application to field crops. Additional incidents of mortality of both terrestrial and aquatic wildlife have also been documented subsequent to heavy rainfall for more than 6 months following application.

Carbofuran is the epitome of an acutely toxic environmental poison. That is, a trivial exposure (e.g., 0.1-5 mg/kg) may be lethal in less than 5 min, especially to some waterfowl and passerine birds.^{13,16} Other individuals exposed to the same and even higher dosages (e.g., > LD₅₀) may exhibit intense signs of poisoning within the same time frame and then abruptly recover and become fully alert and ambulatory within as little as 30 min posttreatment.^{45,67} In captivity, survivors of this initial toxicity appeared to recover fully; whether similar recovery would occur in nature is questionable. For example, on the day after an application of carbofuran on alfalfa both dead and living American wigeon (*Anas americana*) were in the field.³⁶ After a brief period of observation, the field was entered, and many of the birds flew to a nearby irrigation canal only to die soon thereafter. It was not known how long the wigeon had been in the field when approached or whether they were experiencing the onset of toxicity or were recovering from toxicity when flushed. However, during initial observations many normal appearing birds were noted feeding on the alfalfa in the same location where others had died.

It has been determined experimentally that birds may continue foraging on potentially lethal carbofuran-treated feed immediately upon recovery from a toxic episode, and without apparent effect.⁶⁸ In 5-day feeding studies with 1- to 21-day-old Japanese quail (*Coturnix japonica*), nearly all mortality and overt toxic signs from all graded exposures subsided within about 6 h of initial presentation of carbofuran. Both recovered, and apparently unaffected birds continued feeding on their carbofuran diets at the same rate and with comparable growth as controls on untreated diet. A few birds died later in the studies at most levels of exposures. Because these birds were tested in groups and not handled during treatment, it was not determined which individuals had previously shown overt toxicity. However, once toxicity occurred on day 3 or 4, the birds showed signs characteristic of acute poisoning and promptly died. In reference to the above wigeon example: (1) wild birds may initially recover from acute carbofuran exposure and then continue foraging on the same diet; (2) it is not certain that such recovered wild birds can also tolerate natural stressors such as fright response, extended flight, or adverse weather in the short term.

As mentioned, the most common hazard of carbofuran to wildlife is foraging in treated fields shortly after pesticidal application to foliage. Ambient factors of wind, rain, and UV irradiation rapidly reduce the hazard of readily available pesticide in its most toxic form within 2 to 3 days, even though systemic insecticidal activity may continue for more than a month.³ The availability of contaminated invertebrate prey diminishes precipitously immediately following a carbofuran treatment. Even for grazing waterfowl, such as the American wigeon and Canada goose (*Branta canadensis*), the hazard of carbofuran poisoning is greater immediately after treatment than after its incorporation into plant tissue. Another hazard that has been substantial to both aquatic and terrestrial life is periodic surges of pesticide into wetlands from runoff of heavy rainfall.⁶ In this situation the source of the carbofuran has often been granular product that had been incorporated in the soil. Though soil incorporation of granular would seem safe, wildlife mortality has occurred within a day following careful application of carbofuran according to the best available technology.⁶⁹ A form of secondary poisoning other than eating contaminated insects has also been documented for carbofuran. Predatory and scavenging birds and mammals have been poisoned by eating the entrails, including unabsorbed carbofuran in the stomachs of dead animals,^{69,70} sometimes as long as 6 months after application of pesticides for control of soil pests.⁷¹

General factors affecting the hazard of carbofuran to wildlife include formulation and application, its water solubility and potential for leaching and transport out of the treated area, and the likelihood of its entrance into wetlands. Carbofuran is soluble in water to about 700 mg/L and essentially stable in acidic medium.³ The fate of carbofuran in water is influenced by pH, photolysis, temperature, and trace impurities.⁷² The half-life of carbofuran in distilled water at 25°C and pH 5.5, 7, 8, and 9 is about 16 years, 1 month, 6 days, and 6 h, respectively.⁷³ The rate of hydrolysis is positively correlated with ambient temperature. Neither aquatic invertebrates nor fish tend to bioaccumulate carbofuran in slightly alkaline model ecosystems.⁷³⁻⁷⁵

The fate of carbofuran in soil is affected by pesticide formulation, rate and method of application, soil type, pH, rainfall and irrigation, temperature, moisture content, and microbial populations.^{3,76} Carbofuran decomposes rapidly in alkaline soil and is stable at pH 5.5; the hydrolytic half-life in soil at pH 7 is about 35 days.⁷³ Temperature and moisture content are positively correlated with degradation; maximum degradation to hydrolytic metabolites occurs at 27°C.⁷⁷ The terrestrial dissipation half-life of carbofuran in irrigated soils is 4 to 11 days in sandy loam, 1 month in loam, and less than 5 months in silty loam.⁶ Carbofuran is mobile and has been found in streams, surface water, and runoff sediments from treated watersheds.⁶ This suggests carbofuran may be quite stable in association with acid precipitation.²⁸ It is also suggested that carbofuran could leach into ground water, where it could persist at characteristically acidic pH, like aldicarb.^{64,65}

Carbofuran degrades within about 7 to 10 days on crops such as alfalfa sprayed with flowable formulations.⁷⁸ In-furrow application of granular formulation is readily translocated through the roots and stems with insecticidal activity continuing in foliage for 2 to 4 months.^{3,79} Even when granules are covered by soil, toxic carbofuran residues may be available to herbivorous animals.

Carbofuran is readily metabolized in living animals and is rapidly excreted. Toxicologically important bioaccumulation of either parent compound or degradates does not occur in either invertebrates or vertebrates.⁷³ Nonetheless, secondary hazard from carbofuran occurs in predatory vertebrates that feed on dead and struggling insects, earthworms, and small birds and mammals. As mentioned above and in the case study of phorate, this source of poisoning is most likely from unabsorbed carbofuran in the gut of the primary subject.

12.3.3 Wetlands

Organophosphorus and carbamate insecticides are generally not permitted for use on wetlands. An exception is a few OP products used for mosquito control, most notably malathion, but even this use is decreasing in favor of synthetic pyrethroids and biological control agents. However, wetlands are routinely contaminated during agricultural use of a wide array of OP and CB products. Drift, direct overspray, and runoff are sources of contamination when farmlands border natural wetlands, irrigation canals, and drainage ditches.⁶ Ground application of pesticide contaminates wetlands through volatilization, migration, and runoff, but drift from this source is not equal to that of aerial treatment. It has been estimated that downwind drift from aerial application is conservatively four times higher than produced by higher clearance ground sprayers.⁸⁰

Small streams and ponds are especially prone to OP and CB contamination from overspray, drift, and runoff. This is partly because they are difficult to avoid during aerial application when crops abut the wetlands. Of more importance, however, is the fact that these small wetlands, often less than a hectare in size and only 1 to 2 m deep, are not fully appreciated for their intrinsic value in regions dominated by expansive farmlands and monocultures. For example, in North America's prairie pothole region of south-central Canada and the north-central United States, about 65% of the original wetlands have been drained and put in cultivation.⁸¹ The remaining wetlands contribute more than 50% of North American's duck production.⁸² A very large proportion of these wetlands are near or surrounded by agricultural fields and are considered of high potential for excessive agricultural pesticide contamination.^{83,84} OP products constitute the majority of the pesticide use in the region.

Agricultural contamination of small and sometimes only seasonal wetlands is probably the most ecologically abusive use of OP and CB pesticides. Many of these wetlands are extremely productive in terms of biomass and wildlife dependence during critical spring and summer periods. These small ponds contain proportionally little volume to surface area for dilution and buffering of toxicity compared to larger ponds and lakes. OP and CB insecticides may seriously offset invertebrate populations for the short-term. However, most OP and CB products are inactivated within a few hours in circumneutral aquatic environments and within a few days to 1 to 2 weeks in more acidic systems. This rapid degradation is ecologically advantageous because most knocked-down invertebrate populations seem to recover rapidly from a single operational OP or CB treatment. The cumulative effects of multiple applications on species diversity, balance, abundance, and total biomass are not well understood for different product formulations or aquatic systems.

The U.S. Fish and Wildlife Service developed a comprehensive research plan in the mid-1980s to evaluate hazard of pesticides to aquatic invertebrates and waterfowl in the prairie pothole region of North America.⁸⁵ The studies focused on an array of "average" potholes of similar size and depth to determine the response of mallard and blue-winged teal (*Anas discors*) to "typical" treatments of parathion and methyl parathion on wheat and sunflowers. In both studies each of five broods was confined to a fenced pothole and permitted to roam throughout the pond and riparian zone to the edge of the treated crop that surrounded the pothole. Each pothole was believed to be of adequate size and quality to provide necessary cover, spatial, and nutritional requirements for simultaneous fledging of several mallard broods. (Details of this research initiative have been summarized in several case studies.⁸⁶)

In the study of spring wheat the first step was to treat all of the fields, including the ten potholes, with an aerial application of the herbicide 2,4-D (0.3 kg AI/ha), which is a standard practice in the region. One month later one half of the fields and ponds were treated by standard operational application of parathion (0.6 kg AI/ha), and other fields and ponds were treated with methyl parathion (0.3 kg AI/kg). All treatments were made from 1.5 to 2 m above the crop, which resulted in a spray swath of about 18 m. Three days prior to the insecticide treatments 10 to 12 3-week-old mallards and 10 to 12 3-week-old teal were released on each wetland. The ducklings were sampled at 2 and 7 days after the treatment, and then at about weekly intervals through 30 days posttreatment. Body mass, tissue residues of OP, and brain ChE activity were evaluated at each sample period. None of the ducklings died from OP poisoning, but within 2 days posttreatment, 27% of the ducklings on parathion-treated ponds and 29% of those on the methyl parathion treatment had significantly depressed ChE activity. Body mass was not affected. Behavioral and population studies did not indicate any differences in the number of wild broods or adults of either species on treated and control fields.

In the study of sunflowers an operational aerial treatment of parathion was evaluated.⁸⁷ The main differences from spring wheat studies were the use of younger mallards (1 to 3 days of age), the release of two broods of 10 to 12 ducklings with their hens in each pond, and near doubling of the rate of parathion treatment (1.12 kg AI/ha). This was the maximum recommended treatment for sunflowers in North Dakota. One half of the study wetlands were oversprayed to test the worst-case scenario. For the other half of the wetlands, the operator attempted to achieve complete crop coverage, while avoiding overspray of the ponds. Overall, more than 90% of the ducklings in the oversprayed ponds were dead within 3 days posttreatment. Brain ChE activity was inhibited an average of 76% compared with ducklings collected on control wetlands. In this study parathion residues were determined in several matrices (water, vegetation, insects, and snails) 1 days prior to spray and 1, 7, 14, and 29 days posttreatment. All prespray samples were clear of parathion. All samples contained parathion (mean residues: water, 0.12 µg/mL; insects, 0.29 µg/g; snails, 9.04 µg/g; and vegetation, 0.42 µg/g) 1 day posttreatment and by 7 days all samples were clear of detectable residues. Ground searching of the oversprayed ponds and their riparian zones yielded more than 30 dead birds with inhibited brain ChE activity. Over two thirds of the dead birds were juveniles of blue-winged teal, American coot (*Fulica americana*), red-winged blackbird (*Agelaius*

phoeniceus), and yellow-headed blackbird (*Xanthocephalus xanthocephalus*). For the wetlands where reasonable effort was made to avoid direct pond overspray while providing complete crop coverage, parathion was not attributed to the death of any of the ducklings. Likewise, there was not evidence of other birds having been acutely poisoned by the treatment. Wetland residues of parathion were also much less than for the oversprayed ponds. The mean residues for key wetland matrices 1 day posttreatment were also much less than for the oversprayed ponds. The mean residues for key wetland matrices 1 day posttreatment were: water, 0.01 $\mu\text{g}/\text{mL}$; insects, 0.13 $\mu\text{g}/\text{g}$; snails, 3.04 $\mu\text{g}/\text{g}$; and vegetation, 0.35 $\mu\text{g}/\text{g}$. The effort to avoid direct overspray of small wetlands eliminated OP-induced avian mortality and reduced parathion in water (> 90% and invertebrates 34 to 45%).

These studies were invaluable in demonstrating that: (1) standard commercial aerial application of flowable OP may be directly lethal to avian wildlife on small wetlands and (2) adjustment in application to avoid direct overspray reduced the inherent hazard of highly toxic OP to avian wildlife and critical components of the pothole ecosystem. Though an inconvenience to the applicator, the benefits of improved precision in broad-spectrum pesticidal application are clearly warranted in protection of North American waterfowl production. Similar studies, generally absent from the literature, are needed to identify practical problems and provide solutions. Even though OP and CB pesticides are not considered persistent in the same terms as organochlorine pesticides, there is ample evidence of wildlife mortality in wetlands as long as 6 months after application of granular formulations, e.g., phorate, fensulfothion, carbofuran.^{4,6,66,71} These mortalities were mostly delayed effects of leaching and pesticide availability in water killing waterfowl, which were then eaten by scavenging predatory birds and mammals. It has not been demonstrated that flowable formulations of either OP or CB insecticides produce the same long-term hazard as granular formulations.

12.3.4 Mosquito Control

Mosquito control is the only major use of organophosphorus pesticides on wetlands in the United States, and carbamates are not used in mosquito control. However, OPs and CBs are used extensively in mosquito control outside the United States. OP use is being supplanted by biological control agents such as BTI (*Bacillus thuringiensis israelensis*) and other subspecies (e.g., *B.t. darmstadiensis*, *B.t. kyushuiensis*, *B.t. galleriae*), fungus (e.g., *Lagenidium giganteum*) and anti-juvenile hormone enzymes.⁸⁸ This transition is in response to pesticides needed for wide-scale application to wetlands and areas of human habitation and is becoming necessary to overcome developing resistance of some mosquito species to chemical control agents.

Mosquito control routinely uses OPs throughout the world for suppression of nuisance mosquitoes and disease vectors. For example, under the threat of encephalitis epidemics following hurricanes and floods, thousands of hectares of contiguous bottomlands and major populated areas of the United States have been aerially sprayed with undiluted OPs, such as malathion, at rates as small as 219 mL/ha.²⁹ These ultra-low-volume treatments are efficient and comparatively safe for use wherever there is a need for rapid application of OP to large or remote lands.⁸⁹ Only a few OPs, such as malathion, naled, and fenthion, have been used extensively for mosquito control as ULV sprays, and none has been implicated in serious human health effects. This does not imply such treatments are without environmental hazard. In May 1969 undiluted fenthion was applied by helicopter at the recommended rate of 95 mL/ha to approximately 600 ha of a portion of the Red River and residential and park areas of Grand Forks, North Dakota.⁹⁰ Over the course of 2 days more than 450 birds representing 37 species were found dead or moribund within the fenthion-treated area. Most of the dead birds were warblers and other species that feed mainly on invertebrates. The mortality was finally estimated at more than 5000 birds in and around the city. In part because of this extreme incident of wildlife mortality from a standard mosquito-control application, many other studies have been conducted on fenthion mosquito control.²⁹ Though most studies resulted in avian mortality, fenthion remains a favored OP for use in mosquito control throughout much of the world.

Mosquito-control agents are normally used in areas of human activity and habitation and should not be either acutely toxic to mammals or induce serious side effects. The oral LD₅₀s for laboratory rats of favored mosquito-control agents in the United States, such as fenthion, malathion, temephos, chlorpyrifos, and naled, vary from about 150 (chlorpyrifos) to 8600 mg/kg (temephos).¹² Thus, LD₅₀s for these OPs are considered only moderately (chlorpyrifos, fenthion, and naled) to slightly (malathion and temephos) toxic to mammals, according to the acute toxicity ranking of Loomis and Hayes.³¹ However, all except malathion are classed highly toxic to birds. Fenthion may also induce potentially lethal "intermediate syndrome" in mammals.^{37,38} This syndrome has not yet been diagnosed in birds or wild mammals.

All of the above mosquito-control agents except temephos are broad-spectrum insecticides that are also widely used in agriculture. Temephos is used almost exclusively for mosquito, midge, and black-fly larvae control in lakes, ponds, and wetland habitats.⁴ All of the OPs are acutely toxic to susceptible invertebrates and fish within a few minutes of exposure but are poorly soluble and rapidly degraded in water (e.g., 2 to 72 h in circumneutral water) and have little potential for bioaccumulation in nature.⁹¹ For reference, except for temephos, all of these mosquito-control agents are generally more toxic to fish and aquatic invertebrates than are parathion or methyl parathion, are restricted-use pesticides in the United States, and are extremely toxic to natural pollinators such as honey bees (*Apis mellifera*).⁵ The potential hazard of these mosquito-control agents to bee pollinators is greater during general aerial application for nuisance and disease vector control than when applied to wetlands for larvae control.

In conclusion, malathion remains the primary OP adulticide for use in event of mosquito-transmitted disease epidemics in the United States, but with developing evidence of OP resistance in many mosquito populations its continued efficacy and large-scale use is being reconsidered. Development of alternative methods is in progress, and these alternative methods are generally believed to be less hazardous when used in wetlands. OP larvicides are likely to soon be replaced by alternative larvicides such as BTI and other biologicals. Such alternatives are expected to be safer in wetland applications.

12.4 FACTORS OF ACUTE HAZARD

Short-term life-threatening exposure is the principal hazard of organophosphorus and carbamate insecticides to aquatic and terrestrial wildlife. Nearly all applications of the more toxic products are on cultivated crops and, depending on the formulation and treatment, may remain potentially lethal to nontarget vertebrates for several days. These same treatments have sometimes contaminated nontarget wetlands. Such aquatic incidents usually involve large numbers and kinds of wildlife, often including birds and mammals. Sometimes aquatic toxicity lasts only a few hours depending on the system's capacity to dilute and degrade the contamination. In contrast, granular formulations, even when incorporated into the soil, have proved toxic to birds and mammals immediately posttreatment and again later when either granules or leachate become available to wildlife.⁷¹

Considering different scenarios of exposure and that multiple applications of insecticides are routine during a growing season, many forms of toxicity could manifest from OP and CB exposure. Perhaps they do, as will be discussed later, but the overwhelming effect is lethality. Most factors contributing to the acute toxicity of OP and CB insecticides in nature have been reasonably understood for at least two decades; therefore, only basic concepts are summarized here.

12.4.1 Comparative Toxicology

Organophosphorus and carbamate insecticides and product formulations vary widely in toxicity to different aquatic and terrestrial vertebrates.^{4,14,48,49,92} Those pesticides that are esters of carbamic acid and phosphoric acid are direct ChE inhibitors that invoke toxicity immediately upon absorption

into the circulatory system. Most OPs are esters of phosphorothioic acid and must undergo an oxidative desulfuration step for maximum anticholinesterase potency, as described earlier under general toxicology. Direct-acting ChE inhibitors may be more hazardous through inhalation and percutaneous exposure than are phosphorothioic acids.

Metabolic responses to OP and CB insecticides are similar for birds and mammals with any difference more quantitative than qualitative.⁹³ Several quantitative enzymatic differences between birds and mammals are important to differential response to acute toxic exposure. Birds generally have lower levels of hepatic metabolizing enzymes and A-esterase activity than do mammals, which tends to make birds more sensitive to acute anticholinesterase poisoning.^{46,47,94} However, the majority of widely used anticholinesterase pesticides are phosphorothioic acids that must be metabolically activated to their most potent analog. This step is mediated by the same hepatic enzymes as detoxication in birds and mammals.² This would suggest that mammals should toxicate phosphorothioic acids more efficiently than birds and be the more sensitive taxa. But this is not so, because activated OP analogs are substrates for A-esterase hydrolysis and are rapidly detoxified in the liver and blood. In a study of 14 species of birds, three species of laboratory mammals, domestic sheep, and humans, plasma A-esterase was at least 13 times higher in all mammals than in any of the avian species.⁹⁴ The importance of these enzymatic differences to birds was demonstrated with dimethoate, a phosphorothioic acid, in which adult ring-necked pheasants (*Phasianus colchicus*) and laboratory rats were compared. The toxic oxygen analog was rapidly formed and accumulated in pheasants but was rapidly degraded to inactive metabolites in the rats.⁹⁵ This undoubtedly contributed to the more than tenfold difference in the acute oral sensitivity of pheasants (LD₅₀, 20 mg/kg)¹⁶ and rats (LD₅₀, 215 mg/kg).¹²

In general, the array of domestic and wild bird species commonly studied in the laboratory are consistently more sensitive to acute oral toxicity of OP and CB insecticides than are laboratory mammals and wild mice (*Peromyscus* spp.) and voles (*Microtus* spp.).^{11-16,30} Though this generalization of differential acute sensitivity is convenient for preliminary risk assessments, it is not reliable for all species and pesticides.²⁶ For example, the acute toxicity of a series of phosphorothioic acid pesticides was compared for standardized LD₅₀ tests of adult male laboratory rats,¹² ring-necked pheasants,¹⁶ and red-winged blackbirds¹⁶ (Table 12.1). Pheasants and blackbirds were used because both species have general feeding habits but represent extreme body mass compared with rats (e.g., blackbirds, ~65 g; rats, ~200 g; pheasant, ~1000 g). The rat LD₅₀s were graded from 2.3 (phorate) to 8600 mg/kg (temephos). By most criteria for ranking acute mammalian toxicity, phorate is classed extremely toxic, and temephos is practically nontoxic. At the one extreme, though phorate is also highly toxic to pheasants (LD₅₀, 7.1 mg/kg), it is about three times more toxic to rats. At the other extreme, temephos is nearly 250 times more toxic to pheasants (LD₅₀, 35 mg/kg) than to rats. The original premise that birds are more sensitive to anticholinesterase insecticides was borne out for red-winged blackbirds, which were 2 to 200 times as sensitive as the laboratory rat.

The limited comparison of phosphorothioic acids has important implications for ecological risk assessment. First, avian mixed-function oxidase activity tends to be inversely related to body mass,^{46,47} and therefore red-winged blackbirds should be more tolerant of OP poisoning than is the ring-necked pheasant, but they are not. This may be partially explained by the fact that red-winged blackbirds (and possibly other small passerines) are particularly deficient in liver-detoxication enzymes, but it also may have been influenced by small birds having a much higher metabolic rate. Second, it was noted that OPs with rat LD₅₀s above 200 mg/kg were usually more than ten times as toxic to pheasants as to rats; but for OP LD₅₀s less than 200 mg/kg, rats were 2 to 20 times more sensitive than the pheasants (Table 12.1). This is especially important because rat LD₅₀s are often used as a primary source for preliminary risk assessments, and LD₅₀s above 200 mg/kg are considered only moderately toxic for wildlife.⁴ This conclusion would be erroneous for OPs such as dimethoate, fenitrothion, and temephos, all of which are classed highly toxic, with LD₅₀s of less than 50 mg/kg for many birds. In nature, fenitrothion applied at recommended rates on rangelands for grasshopper control resulted in bird mortality and decreases in breeding populations.⁴

Based on the foregoing, the laboratory rat is not a good model for prediction of acute anticholinesterase toxicity in birds. In contrast, laboratory rats and mice are conservative predictors of acute toxicity to wild rodents. In a study of the acute oral toxicity of a spectrum of pesticides including OPs to four species of voles and laboratory rats and mice it was determined that laboratory rodents are generally more sensitive to acute exposure than the most sensitive of the voles, (*Microtus canicaudus*).⁹⁶ Laboratory mice were also found to be more sensitive than deer mice (*Peromyscus maniculatus*), but the relationship was not consistent.³⁰

12.4.1.1 Acute Toxicity Testing

Acute exposure is the main hazard of organophosphorus and carbamate insecticides to wildlife, and of all standard toxicological tests, it is best represented by single-dose LD₅₀ study. Response to the dose of anticholinesterase is usually rapid, and, if not fatal, recovery normally occurs within a few hours. This test provides a sound method for quantification of naive sensitivity to OPs and CBs and comparisons such as differences between sexes, age classes, and formulated products. The test also provides characterization of the dose-response curve, which is essential for proper hazard evaluation and risk assessment. However, the acute test provides only an estimate of relative sensitivity based on a single potentially lethal exposure; whereas in the field exposure varies widely from repeated small doses during feeding (the degree of response depends on the level of contamination and susceptibility of the individual) to massive overdose from rapid ingestion of highly contaminated water or bolus of food. Therefore, a second "acute" test has also been developed to check the short-term response of birds and mammals to OP and CB-contaminated forage. The feeding trial is designed, replicated, and analyzed statistically the same as the single-dose acute test but provides graded levels of contaminant for 5 days. The critical statistic is the LC₅₀ (median lethal concentration). This feeding trial was developed in the 1960's as an alternative to the acute test for highly persistent pesticides that were not acutely toxic.⁹⁷ As with the acute test, this feeding trial does not adequately represent field exposure of wildlife to OPs and CBs, but in combination the two tests provide insight into how wildlife may respond. Where the acute test provides information on inherent sensitivity to OP and CBs, the feeding trial provides information on response to repeated chemical exposures as may be encountered in nature. The use of these tests and their value in evaluation of ecological hazard has been critiqued in detail.⁹⁷⁻¹⁰⁰

12.4.2 Acute Environmental Hazard

Low LD₅₀s indicate that most common organophosphorus and carbamate insecticides are extremely toxic to birds and mammals. However, only a small number of these pesticides are responsible for the majority of large-scale incidents of wildlife mortality. This is probably because certain uses are more likely to bring large numbers of wildlife in contact with a few of the more commonly used products. It is also likely that many incidents of wildlife mortality are not detected or reported. Most reported incidents are of three types: (1) a few dead songbirds in a neighborhood park or backyard, (2) an unusual concentration of dead flocking birds, or (3) large conspicuous water birds or special interest species such as raptors. In contrast, sometimes large flocks of birds were found dead and not reported because the discovery was believed to be part of an avian-control program. (Note: OPs and CBs have been used in wildlife damage and hazard control.)¹⁰¹ The prevalence of small mammals may range from common to abundant in many habitats routinely treated with OP and CB pesticides, but these mammals are rarely listed in reports of even large-scale terrestrial mortality. This omission is more likely due to difficulty in detection of small secretive species (e.g., shrews, mice, voles) than to any special tolerance. There has been comparatively little documentation of reptile and amphibian mortality from OP or CB pesticide treatments.^{48,49}

Prediction of acute OP or CB hazard to wildlife is confounded by many factors of anthropogenic and natural origin. A primary factor, and perhaps most easily addressed through limited additional

testing, is the influence of pesticidal formulations on availability and toxicity to wildlife. Regulatory agencies have only recently begun to consider the differential ecological hazard of finished product formulations, and then only after years of reported episodes of wildlife mortality from certain products.²⁶ Some of the other factors that may affect acute OP and CB hazard to wildlife to different degrees include the route, source, and timing of exposure and possible interactions with other chemicals, infectious diseases, and weather stressors.

12.4.2.1 Confounding Variables

Tolerance of anticholinesterase exposure may be affected by growth, maturation, gender, reproductive status, lineage, nutritional status, and exogenous and endogenous stressors. Few rigorous studies have been conducted on the influence of normal physiological factors on the sensitivity of wildlife to pesticides, or whether captive wildlife adequately represents nature. This is a critical uncertainty that must be attended to for reliable hazard and risk assessment. Even species raised commercially, such as quail and ducks, are not well understood, and they serve as the primary models for regulatory hazard assessment in North America and Europe.²⁶ Another uncertainty is the suitability of wild-captured animals for routine toxicity testing. Simple survival and weight maintenance for a few weeks in captivity may not reflect variables, such as nutritional imbalance or stress response, to confinement, isolation, or crowding.²⁸

Maturation alone does not always increase avian tolerance of anticholinesterase pesticides. For example, in a seminal study of acute toxicity with mallards at 1.5 days, 1 week, 1 month, and 6 months of age, LD₅₀s tended to follow different patterns for esters of phosphoric and phosphorothioic acid insecticides.¹⁰² LD₅₀s between 1 week and 6 months decreased by 45 and 53% for demeton and monocrotophos, while they increased by 60 and 190% for parathion and chlorpyrifos. Tolerance patterns for the two CBs tested — carbofuran and aldicarb — were similar to demeton and dicrotophos, both direct-acting OP insecticides. The apparently higher tolerance of young ducklings compared to adults was attributed to an immature nervous system being less sensitive to certain neurotoxic agents. The authors offered no explanation for the differences between direct-acting ChE inhibitors and those requiring metabolic activation for potency.

In contrast to single-dose LD₅₀ tests, dietary LC₅₀s typically increased with growth of precocial birds.^{68,103} The increase is probably due to larger (older) chicks eating less proportional to body mass, which reduces practical exposure to chemicals over the duration of the experiment. Physiological tolerance undoubtedly plays a role, but it was demonstrated that relative food consumption normally decreases about 60% for Japanese quail from hatch through 3 weeks of age.⁶⁸ Food consumption of controls in proportion to body mass averaged 48 g/100 g at 3 days of age, 31 g at 10 days, 24 g at 17 days, and 19 g at 24 days. This decreased energy requirement was correlated with an overall three- to fourfold increase of OP and CB LC₅₀s from hatch to 3 weeks of age. This study included chlorinated hydrocarbon and mercurial pesticides, which also yielded, increased LC₅₀s over age, but the differences between hatch and 3 weeks were consistently less than twofold. These differences indicate the more biologically labile and less hazardous nature of OP and CB pesticides compared to highly lipid soluble and cumulative products.

Historically, the sex of a bird or mammal was not considered important in studies of acute OP and CB exposure, unless the animal was in reproductive condition.¹⁰⁴ Therefore, to conserve resources for testing as many species and pesticides as possible LD₅₀ tests were designed for use of approximate statistical methods^{105,106} and reproductively quiescent animals.^{16,107} It was felt that routine tests reported for laboratory rats provided sufficient insight on sex effects for most hazard assessments.¹⁰⁴ For example, it was known that females in estrus were significantly more sensitive than males to about 30% of technical-grade OP and CB insecticides tested; males were more sensitive about 10% of the time.^{11,12} When sex differences were detected, females were usually 1.5 to 5 times as sensitive as males; when males were most sensitive, the difference was usually less than 50%. These relationships have not been verified for commercially reared mallards and quail

that are easily brought into reproductive status and are routinely used in regulatory decisions on ecological hazard. (Note: In the primary source on acute pesticide toxicity to wildlife, sexes are indicated, but most animals were reproductively quiescent.¹⁶) The need for such testing is critical because OP and CB pesticides are intensively applied throughout breeding seasons of many species. The importance of this variable was indicated by an acute test of fenthion in which ovulating northern bobwhites were over twice as sensitive as males.¹⁰⁸ This disproportional sensitivity to OP and CBs is potentially critical at the population level because females of most species are more important than males in population recruitment.

12.4.3 Routes of Exposure

Wildlife are most often exposed to organophosphorus and carbamate pesticides through ingestion of contaminated food and water. Inhalation and dermal exposure also occur but are believed to be less common and less hazardous to wildlife than is oral ingestion.¹⁰⁹ Accordingly, nearly all studies of anticholinesterase toxicity to wildlife involve oral administration of chemical, but such studies are usually based on technical grade chemical via gelatin capsule, gavage, or dry mash. Little is known about the toxicity of the finished formulated pesticide complexed with natural matrices or alternative routes and conditions of exposure.

As discussed earlier, the toxicity of OP and CB insecticides is primarily a function of hepatic metabolism regardless of the route of entry.¹¹⁰ Thus, esters of phosphoric and carbamic acids are potentially more potent if they enter the circulatory system by routes other than the alimentary canal, which shunts directly to the liver for first-pass metabolism and detoxication. Esters of phosphorothioic acids that require hepatic metabolism for activation (which includes most OP pesticides) are theoretically more toxic when ingested. Usually, however, pesticides are not rapidly absorbed through the skin and scales, the main alternative routes to ingestion. Percutaneous hazard depends on the rate (dose and time) at which chemical penetrates the skin and varies widely among chemical formulations, sites of application, and species.¹¹¹ An array of anticholinesterase pesticides have been tested by application to the wrapped feet (tarsometatarsis, phalanges, and webbing; ~12% of body surface) of adult mallards for 24 h,¹¹² and to a 1 cm² of a featherless skin under the wing joint of small passerine birds.¹¹³ At the same time, companion birds were dosed orally. Percutaneous and oral LD₅₀s were positively correlated in both studies (mallard, $n = 19$, $r^2 = 0.42$, $p < 0.01$; passerine, $n = 17$, $r^2 = 0.72$, $p < 0.01$). The LD₅₀s were consistently highest for percutaneous routes.

Though potentially more important because OP and CB insecticides readily cross the mucus membrane of the lung, inhalation toxicity is considered minor compared with deposition of chemical on the skin.^{109,114} This concept has not been rigorously tested on wildlife species. For example, it is not known to what degree bats are at risk when returning to their roost during morning twilight, the time considered optimal for aerial application of pesticides because wind is usually less of a factor.

12.4.4 Sources of Exposure

Wildlife are exposed to organophosphorus and carbamate pesticides by ingestion of contaminated water, soil, seeds, foliage, invertebrates, vertebrates, and formulated granular particles. All of these sources have killed large numbers of wild birds and mammals under varied environmental circumstances. Water is a common source of exposure that is poorly documented for terrestrial vertebrates. Potential OP and CB hazard is dependent on widely variable factors of ambient water quality, movement, and the solubility and stability of the product. Water soluble formulations usually remain available longest and tend to be the most acutely toxic.^{2,3} How these studies relate to waterborne exposure in nature is not clear because rates of feed and water consumption vary widely among wildlife species at different ages and seasons of the year. Smaller birds and mammals have a much higher water requirement relative to body mass than do larger species under similar ambient conditions.¹¹⁵ However, even closely related birds of similar sizes and feeding habits vary their

rates of free-water consumption from about 15 to 40% of their body mass per day at the ambient temperature of 25°C.¹¹⁶ Though aquatic wildlife undoubtedly contact and ingest more water through feeding, swimming, and wading than do terrestrial species that use water primarily for hydration, terrestrial species may opportunistically ingest large amounts of highly contaminated water, especially in arid regions.

Few studies of OP and CB pesticides in water have been reported for wildlife species. However, as previously mentioned, field applications of technical-grade fenthion in various formulations of 47 to 100 g of active ingredient per hectare over wetlands of various water depths killed a variety of passerine and water birds.^{90,117,118} The authors concluded that contaminated insects were an important source of fenthion in the avian mortalities, but the importance of contaminated water was not dismissed. Mortality of wildlife from puddling and run-off from agricultural fields has been documented for some of the more acutely toxic OP and CB pesticides, as discussed in Section 12.3.3.

OP and CB residues in foliage, whether by topical or systemic soil application, may be hazardous to wildlife in or near the treated area. Treatments to control insects in forests and orchards and on cultivated crops, such as small grains, alfalfa, and turf grasses, have all resulted in excessive mortality over the years. Foliar treatments may be especially hazardous when animals are in the spray zone during treatment and subject to multiple routes of exposure. Turf grass treatment with OPs (e.g., diazinon) has proven particularly hazardous to waterfowl such as American wigeon and the Canada goose.¹¹⁹ This unique hazard to grazing waterfowl resulted in the U.S. Environmental Protection Agency's issuing a cancellation notice for the use of diazinon products on golf courses and sod (turf) farms.¹²⁰

Seeds, like granular pesticides, are an important source of exposure to wildlife when the seeds are treated with pesticides for soil insect control.^{6,121,122} OP- and CB-treated seeds are readily eaten by small granivorous animals in spite of being brightly colored for safeguard of human health. This hazard is not limited to seeds on the surface of the soil or to small animals; large-scale mortality of greylag geese (*Anser anser*) was attributed to the uprooting and ingestion of germinating OP-treated seeds.^{123,124} It is not known whether phytometabolism of systemic OP and CB has contributed to wildlife mortality, but increased hazard to herbivores is plausible because metabolites are more persistent and may be as potent as the parent compound.

Contaminated arthropods have been proven lethal to wildlife after application of OP and CB insecticides such as terbufos, monocrotophos, dimethoate, fenthion, trichlorfon, and carbofuran.^{4,9,10,90,125,126} Such poisonings are most likely from insecticides adsorbed on the cuticle of the arthropods. An adsorbed chemical may be rapidly dissociated in the stomach of the consumer. However, pesticide ingested by arthropods may also contribute to a consumer's exposure. Recently, there was a documented incident of disulfoton-treated cotton seeds passing sufficient insecticide through the plant to grazing insects to be lethal to Swainson's hawks (*Buteo swainsoni*).¹⁰ In another incident as many as 3000 Swainson's hawks died from eating freshly-sprayed insects following monocrotophos treatment for grasshopper control.¹⁰ Monocrotophos is extremely toxic to birds with acute oral LD₅₀s consistently less than 5 mg/kg and as low as 0.8 and 0.2 mg/kg for California quail (*Callipepla californica*) and the golden eagle.¹⁶ Monocrotophos has been cancelled in the United States.⁵

Predatory and carrion-eating birds and mammals are known to have died from eating prey and carcasses contaminated with anticholinesterase pesticides such as monocrotophos, fenthion, mevinphos, phorate, famphur, and carbofuran.^{6,10,127} Most of these secondary poisonings were probably from eating unaltered chemical in the alimentary canal of prey or carcasses. The liver and kidneys may contain some biologically available anticholinesterase residues (e.g., oxons, sulfoxides, sulfones), but other postabsorptive tissues and fluids are not as hazardous as a source of secondary poisoning. For example, in an experiment with barn owls (*Tyto alba*) fed quail that had been killed with oral dosage of famphur, intact carcasses caused significant ChE inhibition in the owls, whereas owls fed quail with the entrails removed were unaffected.¹²⁸ Famphur presented a unique form of secondary poisoning because the product is applied directly on livestock for

vermin and parasite control. American magpies (*Pica hudsonia*) were poisoned by eating contaminated hair of cattle and possibly invertebrates in stockyards.¹²⁷

The potential for secondary poisoning from aquatic vertebrates has also been demonstrated.¹²⁹ Tadpoles exposed to parathion at 1 mg/L of water for 96 h were force-fed to 14-day-old mallards at the rate of 5% of body mass. A single meal was lethal to ducklings within 30 min. Because only parathion, and not its oxygen degradate, was found in the tadpoles and stomachs of the dead ducklings, it is likely that parathion concentrated in the protective outer mucus layer of the tadpoles and was almost immediately available for absorption upon ingestion by the ducklings. The treated tadpoles appeared healthy when fed to the ducklings.

Percutaneous, ocular, and inhalation exposure undoubtedly occur when wildlife are directly oversprayed by OP and CB pesticides, or when entering a freshly-sprayed area. The few studies conducted on these alternative routes of exposure have demonstrated that they may be important to toxicity in some circumstances, but most often ingestion is the primary source of poisoning.¹⁰⁹

12.4.5 Pesticide Formulations

Organophosphorus and carbamate residues on seed grains, vegetation, and formulated pesticide granules have killed large numbers of wildlife under varied environmental circumstances. Some of the kills were due to misuse, but some of the problem was due to general lack of information on the comparative toxicology of pesticidal formulations and hazard associated with various application techniques. Most often, potential hazard or risk to wildlife is estimated by comparison of the theoretical concentration of the active ingredient in a food item to results of standard acute tests of technical-grade chemical with northern bobwhites and mallards but without regard to the differential effects of finished product on absorption, fate, and toxicity.^{28,130}

Only rarely is technical-grade chemical applied in the field, and then only in very low volume, as described earlier for mosquito control. Instead, pesticides are normally applied as a formulated product that may differ substantially in acute toxicity compared to the technical-grade material tested. As a general rule, it has been determined that granular formulations are most often less toxic than technical-grade materials, whereas liquid formulations are usually more toxic than technical-grade products. This relationship was demonstrated with acute tests of northern bobwhites in which emulsifiable concentrates (48% AI) of diazinon and carbofuran were significantly more toxic than either technical-grade (99% AI) or granular formulation (14 to 15% AI).⁵² Sometimes the LD₅₀s among anticholinesterase formulations varied as much as fourfold. Aqueous solutions were consistently more toxic than oil-based ones. In contrast, pesticides were more toxic to mallard embryos when applied to eggs in an oil vehicle than when applied in an aqueous emulsifiable concentrate.^{131,132} Apparently, the oil medium retarded volatilization, increased the time of contact, and facilitated pesticide transport through the shell and membranes.

Though granular OP and CB insecticides may be less toxic in dosing studies than other formulations and are safer to handle during application, their potential environmental hazard is excessive.^{45,133,134} The hazard of granulars in nature depends on whether they are haphazardly or selectively ingested. If ingestion is haphazard, then the application rate is the more critical variable, but if ingestion is selective, then even the most stringent attempts to reduce granule availability may fail to reduce the hazard.⁶⁹ The color, size, texture, granule base, and application rate are all factors for consideration in reduction of granular hazard to wildlife.¹³⁵⁻¹³⁹ The substantial hazard to wildlife of granules leaching into wetlands is discussed in Section 12.3.3.

12.4.6 Toxic Interactions: Chemical and Environmental

Interaction among organophosphorus and carbamate pesticides and between anticholinesterase and other common environmental xenobiotics has not been thoroughly studied for wildlife species.

A few studies have been conducted on subchronic exposure of birds to expected field concentrations of persistent pesticides followed by acute challenge with OP or CB, or studies of simultaneous feeding on combinations of OPs for 5 days. Results of these studies are generally consistent with similar studies of laboratory mammals, but there are some differences that may affect ecological hazard assessment. For example, when laboratory rodents were pretreated with chlorinated hydrocarbon pesticides that increased hepatic mixed-function oxidase activity, their sensitivity to OP insecticides was reduced.¹⁴⁰⁻¹⁴² In contrast, when the chlorinated hydrocarbon DDE was fed to Japanese quail for 3 months, the sensitivity to a single dose of parathion increased significantly.¹⁴³ When the quail were pretreated with chlordane, another chlorinated hydrocarbon, acute sensitivity to parathion was decreased. This latter relationship was also observed in similar studies with mice.¹⁴¹

In general, response of naive birds and rodents is additive when acutely challenged with paired anticholinesterases or anticholinesterases with chlorinated hydrocarbon pesticides.⁵² When more than additive effects are detected, the level of synergy is usually less than twofold, whether exposure is acute by oral dosage¹⁴⁴⁻¹⁴⁶ or short-term dietary presentation.¹⁴⁷ Little information is available on the effects of sequential exposures of wildlife, but the potential hazard may depend on the order in which the chemicals are encountered. When the initial exposure is to CB, some protection from OP may occur.¹⁴⁸ In contrast, when the initial exposure is to an OP, toxicity of subsequent exposure to either OP or CB may be increased.¹⁴⁹ Sequential exposure to different pesticides is a reasonable possibility in nature, especially for birds that are highly mobile and may forage among several crops.

Temperature extremes and season of the year are natural stressors that can affect the acute toxicity of anticholinesterase insecticides to wildlife. Abrupt changes in climate, particularly late spring cold fronts with heavy rains, may profoundly influence avian nesting success. Whether effects of OP exposure on thermoregulation so far demonstrated in the laboratory would exacerbate an already dramatic physiological challenge is not known. At the other extreme, heat stress was suggested as a contributor to dimethoate toxicity in an episode of sage grouse (*Centrocercus urophasianus*) poisoning in Idaho.¹⁵⁰ Environmental factors affecting OP and other xenobiotic toxicity in wildlife have been reviewed.^{44,151,152} Effects of seasonal changes on OP and CB toxicity are difficult to elucidate because many confounding endocrine changes are cued to photoperiod and temperature changes, with the more profound differences usually in females.

12.4.7 Diagnosis of Anticholinesterase Exposure

Organophosphorus and carbamate pesticides have resulted in hundreds of incidents of wildlife mortality from disease vector control and agriculture (including forest and range management) throughout the world. When many dead and moribund animals of mixed species are found in an area of known OP or CB treatment, the casual association may be evident but is not conclusive without biochemical and chemical confirmation. Proper diagnosis is then contingent upon demonstration of brain ChE inhibition consistent with levels indicative of toxicity or exposure and chemical detection of residues of the causative agent. This last step is sometimes difficult because neither OP nor CB residues tend to accumulate in postabsorptive tissues. However, a strong inferential diagnosis is possible by demonstrating depressed brain ChE activity and detection of a known anticholinesterase in either ingesta or tissues.^{34,36,123}

A conservative threshold of about 50% depression in whole brain ChE activity is generally considered diagnostic of death from anticholinesterase poisoning,³⁵ though depression of 70 to 95% is commonly reported for birds and mammals killed in nature by OP pesticides.³⁶ In contrast, when animals are killed in the field by CB pesticides, whole brain ChE activity may vary from near normal to depressions of only 60 to 70%.^{36,153} Apparently high levels of CB exposure kill by systemic neuromuscular blocking before significant penetration of the central nervous system has occurred.^{53,154} Also, lesser degrees of ChE inhibition may reflect spontaneous postmortem reactivation of carbamylated enzyme.⁶⁷ Brain ChE can be determined by many techniques,^{155,156} but either a laboratory norm must be developed for each species or a suitable enzyme reactivation technique

must be used to determine the degree of inhibition. In cases of poisoning by OP compounds ChE activity can be reactivated *in vitro* by the oxime 2-PAM (pyridine-2-aldoxime methyl chloride).³⁴ Carbamylated ChE is much less stable than phosphorylated ChE; therefore, simple *in vitro* heat reactivation will serve as a rapid indicator of carbamate exposure,³⁶ as will dilution techniques.¹⁵⁷ OP and CB reactivation techniques provide important guidance for analytical chemistry.

Blood plasma or serum ChE may be used as a nondestructive technique for detection of OP or CB exposure. As for brain ChE, the species-specific norm must be developed for diagnostic reference. If ChE depression exceeds the lower to end of normality, i.e., more than two standard deviations below the baseline mean, the subject is considered to have received significant exposure to anticholinesterase compound. Again, heat and 2-PAM reactivation may be used as provisional indicators of CB and OP ChE inhibition. These concepts have been reviewed in detail for wildlife including fish.¹⁵⁸

12.5 SUBLETHAL ENVIRONMENTAL HAZARD

12.5.1 Subchronic and Behavioral Effects

Wild birds and mammals are relatively tolerant of low-level exposure to organophosphorus and carbamate pesticides.^{43,44} This is partly because the chemicals are labile and readily excreted by warm-blooded animals. Also, low-grade exposure to anticholinesterase may stimulate changes in synaptic physiology, which may include reduction of both axonal release of acetylcholine transmitter and the density of postsynaptic acetylcholine receptors. This apparent tolerance was determined from laboratory studies that do not involve the rigors of nature that affect free-living animals, such as extreme weather and the ability to capture prey or avoid predation. For example, a single OP or CB dose of about 5% of its LD₅₀ (essentially nonlethal but induces significant brain ChE inhibition) may reduce the core temperature as much as 2°C in homoiothermic animals acclimatized to moderate ambient conditions of 25 to 30°C. If the ambient temperature is abruptly shifted to mimic a cold front, the core temperature may drop as much as 3 to 6°C from the same dose.¹⁵⁹⁻¹⁶¹ However, it has been demonstrated that even under these chilled ambient conditions the core temperature of OP-induced hypothermic animals returns to normal within about a day.⁴⁴ Generally, hypothermia occurs with brain ChE inhibition of about 50%, which is the degree of inhibition often associated with general physiologic deficit and sometimes death. Hypothermia is also associated with reduced metabolic efficiency, which in turn may slow metabolic degradation and excretion of OP and CB, thereby extending contact at receptor sites and enhancing toxicity.

Hypothermic animals are more sensitive to OP poisoning than normal.^{161,162} Young birds may be especially susceptible to anticholinesterase interference with thermoregulation because many species are not fully homoiothermous for 1 to 3 weeks after hatching.¹⁶³ Interaction between OP and ambient chilling was studied with 14-day-old northern bobwhites acclimatized to 35°C and then subjected to 27.5°C for 4 h.¹⁶⁴ Brain ChE inhibition from a single dose of chlorpyrifos in the chilled chicks was depressed about twice as much as in chicks continued at 35°C. Both nestling and precocial chicks could be further compromised because parental care may be affected when the female is exposed to anticholinesterase.^{165,166} Temperatures below 15°C are common throughout the breeding season of birds and small mammals in temperate climates. Mechanisms of thermoregulation and interaction with anticholinesterase have been thoroughly reviewed.^{43,151}

The ability of birds and mammals to capture prey or avoid predation after subacute exposure to OP and CB pesticides has not been properly evaluated. Controlled experiments are difficult to interpret. Treatment levels were usually based on some level of acute dosage that at least initially rendered the subject, predator or prey, critically ill and often immobile. The results tend to be predictable, i.e., predators will not hunt, and prey cannot escape. When levels are tested that do not indicate overt toxicity, predators and prey seem to respond normally. Field studies are hindered

by uncertainty of the subjects' exposure history and inability to follow movements and observe behavior of highly mobile or reclusive species. Radio tracking has provided some insight into effects of OP on northern bobwhite survival but not necessarily cause of death. One hundred ninety-seven wild quail were captured, equipped with a small radio transmitter, dosed once with either 0, 2, 4, or 6 mg/kg of methyl parathion, and their movements were monitored for 14 days.¹⁶⁷ Quail receiving the highest dosage had lower survival than did the controls; otherwise, there were no apparent treatment effects. The authors concluded that reduced survivability was due to increased OP-induced vulnerability to predation, perhaps due to reduced covey integrity. A similar study was conducted with wild northern bobwhites dosed with 0, 3, 5.6, or 21 mg/kg of terbufos and realized essentially the same results as for methyl parathion.¹⁶⁸

Subacute exposure of birds and mammals to various anticholinesterases has been shown to affect an array of behaviors, such as activity level, alertness, aggression, foraging and drinking, learning and memory, navigation, and reproduction.^{43,44,169,170} Though most of these behavioral studies were well planned and have important theoretical implications for survivability, laboratory studies are highly restrictive, and their projection to natural populations is speculative.¹⁷⁰ Or, as aptly stated, whereas behavioral tests seem desirable, there are two fundamental difficulties in the use of such tests: (1) the best-studied and most easily performed and quantified have the least environmental relevance and (2) the most relevant behaviors are the most strongly conserved against change.¹⁷¹

12.5.2 Chronic and Reproductive Effects

The chronic toxicity of organophosphorus and carbamate pesticides has not been extensively studied with wildlife because such chemicals were believed to be too labile in nature to pose a serious sublethal hazard. However, as discussed earlier, some OPs and CBs may remain available in the soil and vegetation for several months, and they may be applied to wildlife habitat several times during the growing season, which may coincide with critical periods of reproduction. Specially designed research is needed to evaluate: (1) intermittent exposure from repeated pesticide application, (2) exposure of naive animals to pesticide application at different stages in the reproductive cycle (e.g., courtship, onset of lay, incubation), (3) exposure to systemic pesticide in plant tissue, and (4) effects of potential interaction of tank mixtures and different anticholinesterase application on nearby crops.

Chronic studies of OP and CB pesticides with wildlife were usually some modification of the standard reproduction trial developed for pesticide registration. These tests were developed for evaluation of more persistent chlorinated hydrocarbons and heavy metals. The studies, usually of mallards or northern bobwhites, expose first-time breeders to constant rate of chemical from several weeks prior to lay through chick hatchability. Chicks were then observed for 2 weeks for evidence of mortality. Main effects noted in these studies included reduced feeding and corresponding weight loss in adults followed by predictable reduction in rate of lay and egg hatchability. Pharmacologic action on the endocrine system has also been demonstrated.^{43,44}

Some of the most acutely toxic OP and CB pesticides may also pose hazards to wildlife that have gone unnoticed. Monocrotophos has been implicated in some of the largest wildlife kills, but trivial residues have also been shown to depress egg production and hatchability in northern bobwhites.^{172,173} In another study with northern bobwhites the objective was to determine how reproductively active birds responded to decreasing concentrations of OP, as would be expected from a single application in nature.¹⁷⁴ Monocrotophos at concentrations of 0.1 to 1.0 mg/kg in diet were provided to breeding pairs. Then, at 3-day intervals the basic concentration was either continued or reduced so that at the midpoint and end of the 15-day study the concentrations were reduced by 50 and 75%. Finally, all birds were fed untreated diet for 2 weeks. Food consumption and egg production was negatively correlated with increased concentrations of OP. Inhibition of oviposition was not permanent, and time to recovery was dose-related. There was no evidence of

monocrotophos effect that could not be attributed to OP-induced anorexia. In studies of methyl parathion on avian reproduction, effects were also mediated by anorexia.^{175,176}

The most important effect of OP and CB pesticides on avian reproduction in nature, other than killing or incapacitating the parents, is the removal of the prey base.¹²² When prey is depleted, birds may abandon nests and leave the treatment area, or at least have more difficulty in caring for their young. Abandonment of the first nesting attempt is especially critical to population success because subsequent attempts are usually less successful.¹⁷⁶ Some of the subtle effects of sublethal parental poisoning have been studied for a variety of free-living birds. Female red-winged blackbirds were captured on their nests and given a single dose of methyl parathion (0, 2.4, or 4.2 mg/kg) and then observed for 5 h to document acute or gross behavioral responses including times spent incubating.¹⁷⁷ Each nest was then monitored through fledging. Females at the highest dose showed classic signs of acute anticholinesterase poisoning, but they all recovered, and there was no apparent effect on nest success. In a similar study¹⁷⁸ European starlings (*Sturnus vulgaris*) were induced to nest in artificial nest boxes. When nestlings were 10 days old, the male parent was eliminated, and the female parent was dosed once with dicrotophos at 2.5 mg/kg, and her activities were monitored at 2-hour intervals for the next 3 days. OP-dosed females made fewer trips in search of food for their young and stayed away from their nests longer than did the controls. Nestlings of treated females lost significant amounts of weight, which could have affected their postfledgling success.

Though the potential for reduced prey availability causing a negative effect on avian reproduction is evident, neither decreased nestling growth nor fledgling success was detected for free-living passerines in spite of 50 to 70% depletion of primary insect prey due to aerial application of fenthion¹⁷⁹ or trichlorfon.¹²⁵ The importance of relative depletion of insect prey probably varies widely depending on prey abundance at the time of pesticide application and the size, mobility, and energy demands of the insectivore.

Studies of nest attentiveness or other breeding behavior in wild birds gave mixed results when subjects were dosed with OPs at rates producing 10 to 50% brain ChE inhibition, but without causing observable signs of toxicity. Sharp-tailed grouse (*Tympanuchus phasianellus*) given a single dose of malathion at 200 mg/kg were less effective in defending breeding territories on leks.¹⁸⁰ One member per pair of incubating laughing gulls (*Larus atricilla*) was dosed once with parathion at 6 mg/kg, and incubation behavior was observed for 10-minute intervals throughout the day for 3 days. No effects on incubation were detected on the day of dosing, but parents dosed with parathion spent less time incubating on day 2 and the morning of day 3; activities appeared normal thereafter.¹⁸¹ This study was motivated by a natural event in which adult laughing gulls gathered parathion-poisoned insects in nearby cotton fields and either died leaving chicks to starve or returned and poisoned their chicks through presentation of parathion-contaminated insects.¹⁸²

OP and CB pesticides are not believed to pass through the parent to the egg in biologically important amounts, but such pesticides may be deposited on the egg from parents' feathers during incubation or from direct contamination by pesticide application. Effects of such topical exposures have been studied extensively with northern bobwhite and mallard eggs at day 3 of incubation. Eggs were immersed for 30 sec in aqueous emulsion or were dosed with a single topical application of pesticide in nontoxic oil. OPs were shown to be as much as 18 times more toxic when applied to the shell in oil than when immersed in water.¹³¹ Embryotoxicity and teratogenicity of OPs and CBs applied to bird eggs have been thoroughly reviewed.¹³²

12.6 CONCLUSIONS AND RECOMMENDATIONS

About 200 organophosphorus and 50 carbamate pesticides have been formulated into thousands of products that are available in the world's marketplace for application to forests, rangelands, wetlands, cultivated crops, cities, and towns. Though most applications are on field crops and other

terrestrial habitats, the chemicals often drift or otherwise translocate into nontarget aquatic systems and affect a much larger number of species than originally intended.

OP and CB insecticides, though acutely toxic to most animals, are readily metabolized by vertebrates and are quite labile in nature. Therefore, OPs and CBs have generally been underestimated as important environmental hazards beyond periodic incidents of short-lived mortality of fish and birds. These incidents, sometimes involving hundreds and even thousands of nontarget animals, were often justified philosophically by resource managers and regulators by equating them with mortality from natural phenomena such as storm fronts and infectious disease. This justification is inappropriate because wildlife populations evolved in coexistence with nature, but not with the extra burden of anthropogenic factors of habitat modification and reduction or application of potentially incapacitating or lethal chemicals during critical periods of reproduction.

OP and CB pesticides have not caused the type of population effects attributed to highly persistent chlorinated hydrocarbon pesticides, but there is increasing evidence that populations of some raptorial birds have been affected at the regional level. This is an important concept because prior to the mid-1980s predatory birds and mammals other than those foraging regularly on large insects were not generally believed to be subject to OP and CB hazard. It has since been determined that predators and carrion feeders are regularly poisoned by foraging on the OP- and CB-contaminated entrails of small birds and mammals, and small insectivorous species are often found dead in OP- and CB- treated habitats.

Granular formulations are another aspect of OP and CB hazard that has been increasingly documented for wildlife since the mid-1980s. Granulars were generally considered safer than flowable products, and controlled release granular formulations were highly efficacious. However, recurring incidents of toxicity were noted in association with rainfall and when granulars used in soil treatment were not properly buried. Sometimes, the hazard persisted as long as 6 months posttreatment.

Despite evidence that OP and CB toxicity to wildlife may persist well beyond the first few days posttreatment and that there are many uncertainties about their environmental hazard, regulatory controls of these widely used pesticides are almost exclusively based on incidents of aquatic and terrestrial wildlife mortality. Little information has been acquired to properly account for sublethal effects of multiple applications during the growing season or effects on ecosystem structure and productivity. Some areas in which aggressive research is needed are listed below:

1. Pesticidal formulations need to be tested for comparison to existing data on technical grade active ingredient. Technical grade pesticide is rarely used other than for emergency mosquito control. Finished-product and technical-grade chemicals are not equally absorbed by vertebrates, and differences of acute toxicity among formulations may exceed threefold.
2. The rate and method of pesticidal applications affect hazards to nontarget animals. Additional field studies of natural populations are essential and are critical to advance concepts of probabilistic risk assessment.
3. The stability and fate of finished product in nontarget habitats is critical. Studies are in progress on aquatic macrocosms, but little information is available for aquatic or terrestrial systems adjoining croplands.
4. OP and CB pesticides are applied at different intervals during the growing season depending on the crop and target pest. These treatments coincide with the reproductive cycle for many species. Information is generally lacking on wildlife behavior and productivity during this critical period.
5. Studies are needed to understand population effects relating to OP- and CB-induced mortality when coupled with natural wildlife mortality from climatic events and infectious diseases.

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