# Sublethal Effects of Exposure to Cholinesterase-Inhibiting Pesticides

## Humans and vertebrate wildlife

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## Introduction

Synthetic and toxic chemicals (of anthropogenic origin) are ubiquitous in the environment at generally low but measurable levels. Pesticide use throughout the U.S. has resulted in the presence of pesticides in surface and ground water supplies (Kolpin et al. 1998; Hopkins et al. 2000), and agrochemicals have been identified as a primary cause of water quality loss nationally (USGS 1999).

Current pesticide regulations in the environmental and human health fields are designed to protect human and wildlife communities from large-dose exposures to pesticides and prevent acute disease symptoms and mortality. However, little protection is currently afforded to humans and wildlife to prevent low-level exposures and sublethal effects (RESOLVE 1994). With improved field monitoring techniques, scientists are producing a growing body of literature documenting in wildlife subtle, adverse effects of low-level chemical exposure on some of the most sensitive physiological processes (e.g., reproduction, development, cognition, and behavior) (reviewed in Grue et al. 1997).

Sentinel animals have alerted humans to chemical hazards in the environment for centuries (van der Schalie et al. 1999). Important breakthroughs in public and environmental health have been made in the last several decades as a result of physiological studies of birds and eggshell formation during the DDT era (Albers et al. 2000) and, since then, of developmental abnormalities due to endocrine disruption from exposure to a wide variety of chemicals (Myers et al. 2003). An integrated examination of the parallels between human and wildlife health with respect to exposure to organochlorine chemicals yielded greater insights, greater awareness, and modified public policies, plus increased activity to mitigate adverse effects.

This proven strategy for advancing environmental protection through integrating wildlife and human toxicity studies has not been extended to one of the most important classes of chemicals actively applied to the environment—the cholinesterase (ChE)-inhibiting organophosphate (OP) and carbamate pesticides. Of all pesticides used, 10% or 122 million pounds of active ingredient are insecticides (US EPA 2004). Approximately 95% of the insecticides applied in the U.S. are these "second generation" compounds (Aspelin and Grube 1999) which replaced organochlorine pesticides (such as DDT) which were found to have intolerable adverse effects due to persistence and biomagnification. Although organophosphates and carbamates are relatively less persistent, they are more acutely toxic, so environmental protection efforts have focused on preventing acute effects.

Several comprehensive reviews of the effects literature are available. A review of lawn and garden pesticide effects by Vanderlinden et al. (2002) provides a good overview of effects from herbicides, insecticides, and fungicides primarily used in Canada. Sanborn et al. (2004) provide a rigorous systematic assessment of chronic human health effects from pesticides. Rolland and Patrick (2000) provide a summary of human and wildlife health threats from environmental chemicals, however, as mentioned above, a characterization of human and wildlife effects specific to cholinesterase-inhibiting pesticides is lacking.

We evaluated relevant studies from the wildlife and human health literature and characterized current knowledge

of adverse effects from non-acute exposures specifically to organophosphate and carbamate pesticides. This product provides a current synthesis and interpretation of the relevant scientific information concerning sublethal effects in humans and vertebrate wildlife from exposure to cholinesterase-inhibiting pesticides. What follows are methods and an abstracted summary of key findings from each review.

## Methods

The objective of this literature review was to characterize the effects to humans and wildlife resulting from low-level exposure to cholinesterase-inhibiting compounds. Review papers pertaining to the neurological, genotoxic, immunotoxic, carcinogenic, reproductive, metabolic, respiratory, dermatological, ecological, and miscellaneous effects on human and wildlife were obtained and reprints of published peer-reviewed review papers and primary literature were examined. Literature searches were conducted through ISI Web of Science®, the National Library of Medicine's PubMed and TOXLINE, and through Web-based search engines. Gray literature resources from Toronto Public Health were utilized for further insights to the primary literature (Vanderlinden et al. 2002; Sanborn et al. 2004). Studies were limited from 1980s to present, although for some outcomes older studies are reviewed for completeness. An attempt was made to include all studies conducted in the United States and Canada. Most studies from other countries are included although the review may not be complete.

Laboratory studies to support human health effects were included only to provide context and are not comprehensively reviewed.

## Ecotoxicological Terminology

A complex nomenclature has developed to describe chemical exposure and effects in humans and wildlife. Although exposure and effects have their own distinct attributes (such as object, timing, and magnitude), they are often defined in the literature in relation to each other (e.g., sublethal exposure; see examples in Brown and Brix 1998). Furthermore, identical descriptors are frequently used to characterize both exposure and effects (e.g., acute exposure; acute effects). Because imprecise use of non-standardized terms can result in a lack of clarity in communicating research findings, we attempted to use consistently specific terms for interpreting and describing the ecotoxicological findings reviewed in this paper. Terms were selected that offer the most precise meaning for describing exposure and effects. In addition, redundant terms were eliminated and terms used to describe both exposure and effects (e.g., acute) were limited to one context.

***Mode of Action.*** Target and non-target exposure is used in the wildlife literature to identify wildlife targeted for pesticide action (i.e. the pests) as opposed to biota exposed collaterally. Occupational/therapeutic/bystander exposure in the human health literature similarly describes the context in which humans are exposed to chemicals. Dermal/oral/inhalation are precise terms that describe the route of exposure in humans and animals. Direct and indirect effects are used throughout the wildlife literature to describe toxic assaults directly on the organism of interest as opposed to toxic impacts to the habitat (including prey base) the organism of interest utilizes. This distinction, and the use of primary versus secondary poisoning to describe the food chain dynamics of toxic exposure, are less helpful than identifying "direct" effects as toxicological and "indirect" effects as ecological.

***Timing of Exposure and Effects.*** Several identical terms are used to describe the timing elements (onset, frequency and duration) of exposure and effects. Exposure and effects may have immediate or delayed onsets, short- or long-term duration, and frequencies of single or multiple events (within a given duration; e.g., acute or chronic exposure or effects). The most problematic of these is "acute" which is simultaneously used to describe the timing and magnitude of effects. Although "acute" is used to describe an exposure that generally results in an immediate and severe effect, providing a quantitative description of the latency and magnitude of effect would be more instructive. Similarly, "subchronic" is another term of limited value because it is non-intuitive and introduced in the literature as a result of regulatory jargon.

***Magnitude of Exposure and Effects.*** More clarity is available from the terms typically used to describe the magnitude of exposure and effects. However use of the term "sublethal" is confusing. Sublethal is used to describe both exposure and effects (i.e. a sublethal exposure is one which results in sublethal effects). A further complication is that "sublethal" implies the magnitude of *immediate* effects since these low level exposures have been shown to result in mortality of exposed animals, although not necessarily within a short time of exposure. More helpful would be the adoption of quantified terms to describe small/large doses, low/high level exposure, and mild/severe effects. The focus of the current paper is on morbidity or "sublethal" effects although, as noted, effects to animals that do not result immediately in death often have profound consequences to animal vigor, including death which may occur at varying times after exposure.

## Summary

### ■ Neurological effects

**Humans.** Neurological and neurobehaviorial effects have been described in studies investigating chronic exposure to anti-ChEs in sheep farmers, agricultural, greenhouse, and orchard workers, and pesticide applicators. The neurological effects noted in the literature include increased prevalence of self-reported symptoms such as sleep problems, fatigue, dizziness, gastrointestinal upset, and loss of strength in the extremities; decreased sensory nerve function; decreased motor function; symptoms of parkinsonism; and changes in brain and muscle electrical activities. Effects tend to be more pronounced in workers with the highest exposure. However, most of the results are inconsistent and exposure measurements either do not exist or the method of measurement varies and therefore comparisons between studies are difficult.

Neurobehavioral effects resulting from an acute episode or long-term exposure to anti-ChEs include increased depressive

disorders and anxiety. Deficits in cognitive function were observed in workers with varying levels of exposure and in some studies, long-term deficits were detected. Reported symptoms include memory disturbances, poor concentration, anger, fatigue, tension, and confusion.

**Vertebrate Wildlife.** Vertebrate wildlife exhibit a broad spectrum of neurological signs when exposed to low and high doses of anti-cholinesterase pesticides. Signs include clinical signs of intoxication such as vocalization, salivation, rapid heart beat, rapid breathing, tremors, and incoordination in mammals; decreased singing, hypothermia and gastrointestinal distress in birds; tremors and convulsions in reptiles; paralysis in amphibians; and muscle paralysis, loss of equilibrium, tetany and convulsions in fish. Behavioral dysfunction has been documented in most vertebrates including impacts to learning in mammals, birds, and fish; hyperactivity in mammals and birds sometimes followed by behavioral "slumps" and lethargy in mammals, birds, amphibians and fish; and, impacts on memory in mammals and birds. Studies show that all vertebrate classes experience disruption of feeding when exposed to cholinesterase-inhibiting chemicals either through pesticide-induced anorexia, prey-avoidance, altered aggressive behaviors and feeding hierarchies, and/or impacts to vision, learning and memory. Increased risk of predation as a result of pesticide exposure has also been documented in most vertebrate classes (mammals, birds, fish) either because of disrupted predator-avoidance behaviors or other behavioral dysfunctions. Studies of mammals and reptiles indicate that males, with higher baseline cholinesterase levels, may be less sensitive to pesticides than females.

## ■ Genotoxic effects

**Humans.** Effects of exposure to anti-ChE compounds include increased aneuploidy in sperm genetic material and increased chromosomal aberrations and fragile sites in lymphocytes. One study reported no change in micronuclei frequency with low exposure to malathion, however, numerous studies indicate an increased frequency of micronuclei with pesticide mixtures that include anti-ChEs. While effects tend to be increased in workers with higher exposure, cytogenetic effects have been observed in workers with low exposure to organophosphates and pesticide mixtures containing anticholinesterases.

**Vertebrate Wildlife.** Very little information is available on the genotoxic effects of cholinesterase-inhibiting chemicals in wildlife. Studies on mammals, amphibians and fish show that carbofuran, carbaryl and malathion cause DNA strand breakage in some vertebrates.

## ■ Immunotoxic effects

**Humans.** Epidemiological data revealed immune function impairment associated with long-term exposure to anti-ChEs in pesticide applicators, agricultural workers, persons ingesting contaminated groundwater or living adjacent to agricultural lands, and organophosphate production workers. Decreases in immune system markers, changes in T-cell ratios, and neutrophil dysfunction indicate humoral and cellular dysfunction. Evidence of elevated autoantibodies suggests possible autoimmune effects. Elevated biomarkers for oxidative stress are also reported.

**Vertebrate Wildlife.** Laboratory mice have been shown to undergo disruptions in immunoglobulin concentrations as a result of *in utero* or lactational exposure to anti-cholinesterases. No information is available on the immunotoxic effects of pesticide exposure in wild vertebrates.

## ■ Carcinogenic effects

**Humans.** In studies that have discerned pesticide types, odds ratios ranging from 1.5 to 7.1 for risk of non-Hodgkins lymphoma have been associated with exposure to Ops, such as diazinon, malathion, chlorpyrifos and to the carbamate, carbaryl, in lawn pesticide applicators and agricultural workers. Increased risk for leukemia has been reported in both adults and children after exposure to OPs and carbamates. Increases in breast tissue lesions that may act as biomarkers for breast cancer were found in women greenhouse workers exposed primarily to anti-ChE compounds and to a lesser extent, triazines and other herbicides. Risk for breast cancer was also increased in farm women who did not directly handle the compounds. Increased risk for prostate cancer with anti-ChEs and increased risk for small lymphatic lymphoma or lung cancer in farmers handling OPs has also been observed. While little evidence exists for risk of brain cancer in adults, several studies have associated exposure to pet flea collars, maternal pesticide use, and home pesticide

application of anti-ChEs with childhood brain cancer. Studies also suggest that risk increases when exposure occurs during critical developmental periods in early childhood.

**Vertebrate Wildlife.** No information is available on the potential carcinogenic effects of cholinesterase-inhibiting pesticides on wildlife.

## ■ Reproductive effects

**Humans.** Occupational studies have shown significant associations for maternal as well as paternal exposure to pesticides and adverse reproductive outcomes. Specifically, anti-ChE compounds have been implicated in the following adverse outcomes: changes in hormone levels, such as adrenocorticotropic and follicle-stimulating hormones; impaired semen quality and concentration; increased risk of spontaneous abortion and congenital defects resulting in fetal death; and altered birth parameters such as low birth weight and birth length with home and agricultural exposure to OPs.

**Vertebrate Wildlife.** Reproduction integrates a number of physiological systems in vertebrates and impacts to reproductive performance as a result of pesticide exposure may result from biochemical, histological, physiological and/or behavioral alterations. Reproductive hormones, including luteinizing hormone, follicle-stimulating hormone, and testosterone in mammals and luteinizing hormone in birds, are adversely affected by exposure to pesticides. Other effects include alterations to testes and sperm, altered sperm capacitation, infertility, maternal weight loss, decreased birth weight, increased stillbirths and decreased litter size documented in mammals; reduced egg-laying, decreased nest attentiveness, decreased hatching success, decreased fledge weight, and increased time to fledging in birds; and decreased egg production, inhibited ovarian development, decreased egg hatchability, and reduced fry production in fish. Exposure to an organophosphate (malathion) has been shown to adversely affect morphogenesis and cause skeletal deformities in amphibians. An organophosphate (parathion) has been found to bioconcentrate in the eggs of lizards.

## ■ Metabolic effects

**Humans.** Contrary to wildlife, hyperthermia is a common effect in humans exposed to poisoning doses of anticholinesterases. With lower dose exposures, the interaction of anticholinesterases with thermoregulatory system functions may affect the ability to dissipate heat while working or exercising.

**Vertebrate Wildlife.** Impact to thermoregulation has been identified as one of the most important outcomes of pesticide exposure in homoiothermic mammals and birds. A hypothermic response is typical in mammals other than humans, and in birds. Hypothermia may reduce metabolic rate and therefore reduce the activation of toxic compounds and metabolites, however, hypothermic birds and amphibians show greater vulnerability to cold stress.

## ■ Respiratory effects

**Humans. Decreased** pulmonary function and increased incidence of asthma was reported in three studies on OP manufacturers and farmers exposed to OPs and carbamates.

**Vertebrate Wildlife.** Very little information is available on respiratory effects of pesticide exposure in wildlife. Clinical signs in fish include gill muscle paralysis, increased amplitude of respiration, and asphyxiation.

## ■ Dermalogical effects

**Humans.** Cases of allergic dermatitis or erythema are common in workers with high and frequent exposure to organophosphates, however, the incidence of these effects was found to be rare in adult populations exposed to low doses of mosquito control pesticides. Increased incidence of dermatological effects in children suggests that more research regarding subpopulations sensitive to OP exposure is needed.

**Vertebrate Wildlife.** No information from studies on mammals, birds, or fish, however, both reptiles and amphibians have shown dermatological sensitivity to cholinesterase-inhibiting chemicals. Phosphamidon has been shown to cause shedding of body scales and color change in agamas, and a number of organophosphate and carbamate pesticides produce damage to melanophores, blisters, negative effects on palate and gill epithelium, and pigmentation effects in amphibians.

## ■ Miscellaneous Effects

**Humans.** Paraoxonase polymorphisms resulting in decreased paraoxonase activity were associated with increased symptom reporting, decreased sperm quality, and decreased fetal growth parameters. Increased chronic fatigue symptoms were found with farmers at the highest level of exposure associated with sheep-dipping tasks. Changes in bone formation and decreased bone density were also found in farmers exposed to sheep dips.

**Vertebrate Wildlife.** Documented effects in mammals include muscle necrosis. Studies show amphibians may exhibit a reduction in red blood cell numbers, edema and liver cell abnormalities as a result of exposure to cholinesterase-inhibiting pesticides.

## ■ Ecological effects

**Vertebrate Wildlife.** Impacts to wild mammal communities include inhibited reproduction, population size reduction, and increased population turnover rates. Causal mechanisms include not only physiological effects to mammals, but also impacts to populations of plants and animals comprising prey and other habitat components. In addition, dominance relationships can be impacted by differential effects of pesticides on mammalian members of communities. Documented impacts to birds include reduced population size as a result of reproductive effects.

## The Avian Incident Monitoring System

The Avian Incident Monitoring System (AIMS), a cooperative program between American Bird Conservancy (ABC) and EPA, is a centralized source for field data on lethal and sub-lethal effects of pesticides on birds. Although capuring a fraction of incidents, AIMS provides valuable pesticide effects information. For more information, contact American Bird Conservancy, P.O. Box 249, The Plains, VA 20198, 540-253-5780, www.abcbirds.org/aims.

### Top Ten Pesticides in the AIMS Database

| Pesticide | Class | # of Incidents | Use | Regulatory Status |
|---|---|---|---|---|
| Carbofuran | Carbamate | 990 | Insecticide | In use |
| Diazinon | Organophosphate | 602 | Insecticide | In use |
| Famphur | Organophosphate | 221 | Insecticide | No registered uses |
| Chlordane | Organochlorine | 204 | Insecticide | No registered uses |
| Fenthion | Organophosphate | 170 | Insecticide, bird poison | No registered uses |
| Brodifacoum | Coumarin | 168 | Rodenticide | In use |
| 4-aminopyridine | Pyridine compound | 155 | Bird poison | In use |
| Strychnine | Botanical | 143 | Rodenticide | In use |
| Dieldrin | Organochlorine | 126 | Insecticide | No registered uses |
| Parathion | Organophosphate | 119 | Insecticide | In use |

## Conclusions

A compilation and interpretation of the scientific literature investigating sublethal effects of exposure to cholinesterase-inhibiting pesticides in humans and wildlife revealed a body of knowledge relatively advanced in some areas, and undeveloped in others. An extensive literature has developed on the neurotoxicity, carcinogenicity and reproductive effects of pesticides on human health. Other physiological endpoints have been much less studied. Neurophysiological, behavioral and metabolic pathways, especially as they impact foraging, reproduction, and survival, have received the greatest attention from wildlife scientists. The wildlife literature is dominated by studies of birds, but increasing attention is being focused on amphibians and reptiles. Information on wild mammals is surprisingly sparse. The areas of greatest overlap in the human health and wildlife effects literature are neurotoxicity and effects to reproduction.

Several reported neurotoxicological symptoms are similar between humans and wildlife such as fatigue and lethargy, gastrointestinal distress, dizziness and loss of equilibrium, and possibly anxiety and hyperactivity. Behavioral effects on mood and memory tend to be present in both humans and wildlife exposed to anti-cholinesterase compounds, while potential similarities in effects on learning are not as evident.

Exposure to cholinesterase-inhibiting pesticides is associated with adverse effects to reproductive performance in both humans and wildlife. Alterations to reproductive hormones, sperm quality, reproductive organs, and reduced production of offspring and offspring viability have been widely reported in the human and wildlife literature. In addition, genotoxicological studies show evidence of chromosomal aberrations in both humans and wildlife.

Finally, our synthesis and analysis reveal two significant areas of impact that are somewhat distinctive in the human and wildlife literature. A research focus on the carcinogenicity of pesticides in long-lived humans has provided evidence that exposure to cholinesterase-inhibiting compounds may be linked to certain lymphatic and blood cancers. Studies of wild mammal and bird populations have shown significant effects to the highest levels of biological organization (i.e. population, community, ecosystem) as a result of the toxicological effects of pesticides on animals and their habitat components.

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**Editor's note:** *This research article is an excerpt of the summary section of a lengthier literature review. The complete article can be obtained from the Manomet Center for Conservation Sciences, P.O. Box 1770, Manomet, MA 02345, 508-224-6521, parsonsk@manomet.org. The Center conducts original research on natural systems and wildlife. See www.beyondpesticides.org/documents/wildlife.pdf for bibliography.*