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IDENTIFYING AND HANDLING CONTAMINANT-RELATED WILDLIFE MORTALITY/MORBIDITY

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INTRODUCTION

Wildlife biologists have potential for field encounters with wildlife mortality/morbidity incidents as a result of routine monitoring of an area or a call from the general public. Wildlife mortality/morbidity can be due to natural or accidental causes, disease, or exposure to environmental contaminants. Every year, species of wildlife are subject to exposure to a myriad of different chemical contaminants that make their way into the environment. These chemical contaminants include pesticides, metals/metalloids, organics, inorganics, pharmaceuticals, and a wide variety of other compounds in air, soil, sediment, water, plants as well as in wild and domestic animals. If organisms are exposed to contaminants, there may be no resulting visible effects suggesting either there were no effects of exposure, or that if there was a negative effect, it was not apparent. However, there may be visible effects from exposure to chemical contaminants indicating either that it caused sickness or was lethal to wildlife species.

Understanding contaminant impacts on wildlife include parameters such as species (or higher taxa) involved, trophic level of the species involved, chemical(s) involved, route(s) of exposure, signs of intoxication, fate and transport (movement) through the environment, environmental compartment (media), and environmental persistence. Not all classes of contaminants pose the same level of risk to all taxonomic groups of animals. For example, mammals may be relatively less sensitive than birds or reptiles to environmental contaminants due to their evolutionarily more advanced detoxification enzyme system. The physical and chemical properties of the chemical (e.g., lipid solubility, water solubility, environmental persistence, volatility, etc.), differing toxicity, route(s) of exposure, and trophic level of the animal all affect which taxonomic groups may be more susceptible to different classes of contaminants. The trophic level at which the animal feeds is a major factor in contaminant exposure, with higher trophic level animals susceptible to biomagnification of contaminants. Herbivorous species may be less susceptible to the effects of contaminant exposure as they are better adapted for detoxifying foreign chemicals (xenobiotics) because they routinely encounter natural plant toxins (secondary plant compounds) in their diet that require similar detoxification (Vangilder 1983, Ray 1991).

Species that feed on soil invertebrates will be more susceptible to exposure to contaminants such as metals that remain in soil for long periods of time. The behavior of sick or intoxicated animals and where or how many animals are found may be indicative of different classes of contaminants. Once the class of contaminants is identified, characteristics of the class or the specific contaminant will affect what type, where, or how many environmental samples should be collected, and how long after the incident has been discovered the site should be monitored.

Upon discovery of a field mortality/morbidity incident suspected to be caused by environmental contaminants, there generally is little time to plan and conduct a research study of the incident. The available time for collecting evidence such as tissue samples and/or other environmental media (plants, soil, water, sediment, air) is often a matter of hours to a few days. Chemicals degrade, tissues decay/desiccate, and carcasses are readily scavenged, all of which greatly affect time available for sampling.

Box 1. Recommended fish and wildlife mortality/morbidity references.

ADRIAN, W. J., editor. 1996. Wildlife forensic field manual. Second edition. Association of Midwest Fish and Game Law Enforcement Officers, Denver, Colorado, USA.

AMERICAN SOCIETY FOR TESTING AND MEASUREMENT. 1997. Standard guide for fish and wildlife incident monitoring and reporting. Pages 1355–1382 *in* Biological effects and environmental fate; biotechnology; pesticides. ASTM E 1849-96. American Society for Testing and Measurement, Philadelphia, Pennsylvania, USA.

BRIGGS, S. A. 1992. Basic guide to pesticides: their characteristics and hazards. Hemisphere Publishing Corporation, Washington, D.C., USA.

CANADIAN COOPERATIVE WILDLIFE HEALTH CENTRE. 1975. Wildlife disease investigation manual. Canadian Cooperative Wildlife Health Centre, University of Saskatchewan, Saskatoon, Canada.

DIERAUF, L. A., AND F. M. D. GULLAND, editors. 2001. CRC handbook of marine mammal medicine. Second edition. CRC Press, Boca Raton, Florida, USA.

FAIRBROTHER, A., L. N. LOCKE, AND G. L. HUFF, editors. 1996. Noninfectious diseases of wildlife. Second edition. Iowa State University Press, Ames, USA.

FRIEND, M., AND J. C. FRANSON, editors. 1999. Field manual of wildlife diseases: general field procedures and diseases of birds. U.S. Department of the Interior, Geological Survey, Biological Resources Division, Information and Technology Report 1999-001.

HOFFMAN, D. J., B. A. RATTNER, G. A. BURTON, JR., AND J. CAIRNS, JR., editors. 2003. Handbook of ecotoxicology. Second edition. Lewis Publishers, Boca Raton, Florida, USA.

HUDSON, R. H., R. K. TUCKER, AND M. A. HAEGELE. 1984. Handbook of toxicity of pesticides to wildlife. Second edition. U.S. Department of the Interior, Fish and Wildlife Service, Resource Publication 163.

MEYER, F. P., AND L. A. BARCLAY, editors. 1990. Field manual for the investigation of fish kills. U.S. Department of the Interior, Fish and Wildlife Service, Resource Publication 177.

SMITH, G. J. 1987. Pesticide use and toxicology in relation to wildlife: organophosphorus and carbamate compounds. U.S. Department of the Interior, Fish and Wildlife Service, Resource Publication 170.

STROUD, R. K., AND W. J. ADRIAN. 1966. Forensic investigational techniques for wildlife law enforcement investigations. Pages 3–18 *in* A. Fairbrother, L. N. Locke, and G. L. Huff, editors. Noninfectious diseases of wildlife. Second edition. Iowa State University Press, Ames, USA.

U.S. DEPARTMENT OF THE INTERIOR. 1998. Fish and wildlife mortality incident information workshop. U.S. Department of the Interior, Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA.

———. 1999. Wildlife mortality database resource directory. U.S. Department of the Interior, Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA.

———. 2002. Environmental contaminants: field and laboratory techniques. U.S. Department of the Interior, Fish and Wildlife Service, National Conservation Training Center, Shepardstown, West Virginia, USA.

WORK, T. W. 2000*a*. Avian necropsy manual for biologists in remote refuges. U.S. Department of the Interior, Geological Survey, National Wildlife Health Center, Hawaii Field Station, Honolulu, USA.

———. 2000*b*. Sea turtle necropsy manual for biologists in remote refuges. U.S. Department of the Interior, Geological Survey, National Wildlife Health Center, Hawaii Field Station, Honolulu, USA.

The objective of this chapter is to provide guidelines for field biologists to assess wildlife mortality/morbidity incidents and sampling techniques useful in detection and documentation of environmental contaminants impacting wildlife. Presently, there is difficulty in finding published procedures for handling wildlife mortality/morbidity incidents. Few specific criteria are available for conclusive diagnosis of wildlife poisoning other than correlation of effects with chemical residues in critical tissues. We include safe, proper field techniques for collecting, handling, and preserving environmental samples for biological assays or chemical analyses as well as where to look for assistance with wildlife mortality/morbidity (Box 1). Because time is short and field data and samples are critical, assistance from others with experience in handling contaminant-related issues can be important to a full understanding of the entire incident. Careful documentation of the mortality/morbidity incident is necessary including appearance of affected individuals, species involved, and likely scenarios leading to the incident.

ENVIRONMENTAL CONTAMINANTS

Human activities have resulted in the pervasive and dynamic nature of contaminants in our environment. Although environmental contamination increased sharply with the rise of the Industrial Revolution in the mid- to late 1800s, the presence of contaminants in the environment has accelerated greatly since the 1940s. For example, pesticide use in the United States increased more than 10-fold, and chemical and mining industries continued high productivity during the post-war economic growth. The United States is the major producer, user, and exporter of pesticides in the world. In 1999, 2.27 billion kg of active ingredient of toxic chemicals were used as pesticides in the United States (Donaldson et al. 2002). The United States is also the major producer, user, and exporter of organic and inorganic chemicals. In 1995, the amount of the top 50 chemicals produced by the chemical industry in the United States was over 340 billion kg (Chemical and Engineering News 1996). The United States produces or imports about 3,000 different organic chemicals of >454,000 kg each on an annual basis; 43% of these chemicals have no data on basic toxicity and only 7% have a full set of basic toxicity data (U.S. Environmental Protection Agency 1998*b*). The United States is also a major world producer of metals and minerals. In 2001, there were 13,925 active mines in the United States, and the annual per capita consumption rate of newly mined metals and minerals reached 20,870 kg in 2002 (National Mining Association 2004). The high level of production of these industries in the United States resulted in 2.8 billion kg of toxic chemicals released into the air, land, water, and underground in 2001 (U.S. Environmental Protection Agency 2003), 34,360 chemical and oil spills in 2001 (U.S. Department of Transportation 2004), 1,240 Superfund hazardous waste sites (U.S. Environmental Protection Agency 2004), and an estimated 450,000 contaminated commercial/industrial sites across the United States (U.S. Environmental Protection Agency 1996). Because of this great potential for chemical contaminants in the environment, it is inevitable that individuals of a variety of different wildlife species will be exposed and some will become sick and either recover or die.

The U.S. Environmental Protection Agency reports that state wildlife agencies in the United States annually receive about 3,800 reports of pesticide-related fish, wildlife, and plant incidents (American Society for Testing and Measurement 1997). These reports indicate that pesticide

use can pose considerable risk to nontarget species, particularly birds and fishes. Most reported incidents are a result of exposure to insecticides and rodenticides, and not herbicides, fungicides, and other pesticides. The greatest number of wildlife mortality/morbidity incidents has been reported for anticholinesterase (organophosphorus and carbamate) insecticides and anticoagulant rodenticides. There is evidence from field investigations that many pesticides (mainly anticholinesterase insecticides) still on the market today have caused confirmed bird mortalities and that avian mortality occurs regularly and frequently in agricultural fields across North America (Mineau 2002).

In Europe, a large investigation of terrestrial wildlife mortality incidents involving pesticides in 18 countries was conducted from 1990 to 1994 (deSnoo et al. 1999). There were a high number of wildlife mortality incidents in France, The Netherlands, and the United Kingdom, all countries with intensive agricultural programs. Most reported incidents were due to deliberate abuse of pesticides with few mortality incidents reported for normal agricultural use (deSnoo et al. 1999). Their conclusion, that reporting of wildlife mortality incidents was not a reliable tool for obtaining an understanding of the occurrence of wildlife mortality incidents from agricultural pesticide use, is most likely valid worldwide.

Deleterious effects of pesticides on wildlife include death from direct exposure and secondary poisoning from consuming contaminated prey; reduced survival, growth, and reproductive rates from exposure to sublethal dosages; and habitat reduction through elimination of food resources and refuges. In the United States, approximately 3 kg of pesticide/ha are applied to about 160 million ha of land annually (Pimentel et al. 1997). With a large portion of the land area subjected to large quantities of pesticide applications, the impact of pesticides on wildlife could be predicted to be substantial (Pimentel et al. 1992). However, few attempts have been made to estimate the overall magnitude of pesticide impacts on wildlife species over a large geographic scale. An existing estimate for impacts on birds is substantial (Pimentel et al. 1992) but does not include such factors as bird losses caused by poisoning of invertebrate prey, eggs or chicks left to die when adults are killed and those birds suffering neurological effects that move from the area to places where they cannot reproduce or survive their exposure(s). The occurrence of these effects has been documented (Pimentel et al. 1992, Hill 1999) following pesticide application, but their importance to a population at the species or regional level has not been quantified.

In addition to pesticides, exposure to other chemicals can also serve as major sources of wildlife mortality and morbidity. These chemicals include metals/metalloids (Fairbrother et al. 1996, Goyer and Clarkson 2001, Hoffman et al. 2003), organic chemicals (Friend and Franson 1999, Bruckner and Warren 2001, Rice et al. 2003), cyanide associated with gold mining (Henny et al. 1994, Eisler et al. 1999), white phosphorus associated with military use (Sparling 2003), pharmaceutical drugs (Friend and Franson 1999, Oaks et al. 2004), and natural plant/animal toxins (Norton 2001, Russell 2001).

The full extent of wildlife mortality from contaminants is difficult to assess because wildlife species are often secretive, camouflaged, highly mobile, and live in dense habitat. Typical field studies of the effects of pesticides often obtain low estimates of mortality because carcasses disappear rapidly, well before they can be found and counted. Field studies rarely account for animals that die away from treated areas and many individuals often hide and die in inconspicuous locations. Studies have demonstrated that only 50% of dead or moribund birds are recovered even when their location is known (Mineau and Collins 1988). Carcass searches are rarely done and, even more rarely, done properly. Most carcasses disappear within 24–48 hours post-spray, making documentation difficult (Vyas 1999). When known numbers of bird carcasses were placed in identified locations in the field, 62–92% disappeared overnight due to scavengers (Balcomb 1986). Kostecke et al. (2001), using remote cameras, documented heavy scavenging of experimentally placed bird carcasses by mammals, particularly striped skunks (*Mephitis mephitis*), and to a lesser extent by birds. This study demonstrated the potential hazard of secondary poisoning and the need for careful searches for wildlife mortality/morbidity following pesticide applications.

The full extent of wildlife morbidity from contaminants can be even more difficult to assess because sick animals may move from the area of exposure or otherwise disappear (i.e., fly from the area, retreat to a burrow), may not demonstrate visible signs of morbidity, and/or may become more vulnerable to predation or other mortality factor as a result of exposure. Sublethal effects, those that serve to debilitate an exposed organism, are often subtle, and exposure to chemical contaminants can impact all internal body systems (i.e., biochemical, physiological, immunological, etc.), which in turn can reduce the fitness and/or survival of exposed individuals. For example, many chemical contaminants including pesticides, pharmaceuticals, and even natural plant chemicals that have the ability to disrupt normal endocrine function in animals are of particular concern and can have major implications for reproduction in wildlife species (Yamamoto et al. 1996, Gross et al. 2003). Although some sublethal effects can be apparent (i.e., tumors, developmental malformations), many are not and animals suspected of sublethal poisoning often require close examination and laboratory testing. A formidable problem in identifying and understanding sublethal effects is that baseline data for normal (unexposed) individuals are largely unavailable (Hill 1999).

CLASSES OF CONTAMINANTS

Metals/Metalloids

Metals are natural substances and, in most cases, only become significant contaminants where human activity such as mining and smelting releases them from the rocks in which they were deposited during volcanic activity or subsequent erosion and relocates them into situations where they can cause environmental problems (anthropogenic enrichment). Metals are nonbiodegradable and, unlike organic compounds, cannot decompose into less harmful components. Detoxification consists of "hiding" active metal ions within a protein (e.g., metallothionein) or depositing them in an insoluble form in intracellular granules for long-term storage or excretion in feces. The term "heavy metals" generally has been used to refer to metals that are environmental contaminants. However, true heavy metals have a density relative to water >5, which excludes some important contaminants such as aluminum.

The term metalloid is used for elements such as arsenic and selenium, which are transitory in nature between metals and non-metals. In the environment, metals and metalloids occur as organic or inorganic complexes; there are several factors that affect which form is more toxic than the other. For example, inorganic arsenic compounds generally are more toxic to wildlife than organic arsenic compounds, whereas the opposite is true for mercury and lead where the organic forms are more toxic than inorganic forms.

Essential Metals/Metalloids

All animals require 7 major metal minerals including sodium, calcium, phosphorus, potassium, magnesium, and sulfur for ionic balance and as integral parts of amino acids, nucleic acids, and structural compounds. All animals also require 12 trace metals/metalloids that are essential micronutrients including zinc, copper, manganese, iron, selenium, chromium (Cr^{+3}), nickel, cobalt, molybdenum, vanadium, and silicon as essential components of enzymes, enzyme cofactors, and other biochemical functions. Presently, there is insufficient information available to ascertain whether metals such as silver, tin, aluminum, lithium, and boron are essential. All essential metals/metalloids can be toxic to wildlife species if sufficiently concentrated. Selenium is an excellent example of an essential metalloid that has caused notable toxicity problems in wildlife species in the United States.

Selenium.—Major environmental sources of selenium are coal-fired and other fossil fuel-burning power plants, and mining and smelting operations. Selenium is a naturally occurring component of soils (Ohlendorf 2003) and an essential micronutrient for wildlife and an integral component of the glutathione detoxifying enzyme system. However, there is a fine line between selenium deficiency and selenium toxicosis. Selenium can become concentrated at relatively high levels from mining activities and agricultural runoff. Although occasionally implicated in mortalities of adult animals, it is more likely to produce sublethal effects, such as developmental abnormalities, or embryonic death (Eisler 1985*b*, Ohlendorf et al. 1986). It has been shown to be highly teratogenic (producing malformations) in aquatic birds, causing widespread reproductive failure through decreased egg weight, decreased egg production and hatching success, anemia, and a high incidence of grossly malformed embryos with missing or distorted eyes, beaks, wings, and feet (Eisler 1985*b*). Excess selenium also causes behavioral modifications, intestinal lesions, chronic liver damage, and impacts the immune system (Eisler 1985*b*). Signs of selenium poisoning include vomiting, lethargy/weakness, diarrhea, increased urination, panting, central nervous system depression, paresis (partial paralysis), and prostration, and death can result due to respiratory failure (Osweiler et al. 1985). Selenium is readily bioaccumulated in aquatic and terrestrial food chains, but is not biomagnified through food chains. In the early to mid-1980s at Kesterson National Wildlife Refuge in central California, selenium was the causative agent in numerous cases of waterfowl and wading bird nesting failure (Ohlendorf et al. 1986). In this situation, selenium from irrigation drain water accumulated in waters of Kesterson where it caused massive reproductive failure through embryonic mortality and developmental abnormalities of aquatic birds (Ohlendorf et al. 1986). Selenium was deposited in eggs and caused severe developmental abnormalities in chicks. Mean selenium concentrations in livers and kidneys were about 95 ppm dry weight, about 10 × higher than levels in birds from a reference area (Eisler 1985*b*, Ohlendorf et al. 1986).

Nonessential Metals/Metalloids

Some metals have no known biological function and serve to replace essential metals of like valance in animals. These metals include mercury, lead, cadmium, chromium (Cr^{+6}), and arsenic. All tend to be highly toxic and may exert toxicity through the induction of deficiencies of essential metals through competition at active sites in biologically significant molecules. Examples include lead replacing calcium in bone and arsenic replacing phosphorus in DNA. Metals/metalloids with no biological function tend to be those of greatest environmental concern, particularly if they are anthropogenically concentrated in a given area.

Mercury.—Major environmental sources of mercury have been chlor-alkali (plastics) manufacturing; mining and smelting operations; mercurial seed dressings; mercury-based fungicides; coal-fired power plants; thermometer, battery, and fluorescent bulb manufacture; switches; paints; pulp and paper industry; and dental amalgam (Wiener et al. 2003). The use of mercury in agriculture has been largely curtailed in the United States; sources related to energy production and mining are now of greatest concern. About 25–30% of total atmospheric mercury is anthropogenic (Eisler 1987*a*). Under certain environment conditions (e.g., anoxic sediments), inorganic mercury can be readily transformed by anaerobic bacteria into methylmercury, which is extremely toxic.

Mercury deposition since industrial times (mid-1880s) and subsequent biotransformation to methylmercury in aquatic systems has created areas where mercury poses a relatively high risk to wildlife, particularly long-lived, piscivorus species (Henny et al. 2002, Wiener et al. 2003). Methylmercury readily crosses biological membrane barriers whereas inorganic mercury does not. However, once absorbed, both forms of mercury are highly cytotoxic (toxic to cells), causing histopathological lesions in tissues of the nervous, hepatic, renal, and immune systems (Heinz 1996). The most observable sign of organo-mercury poisoning is central nervous system dysfunction leading to respiratory stress and lack of coordination. Other common signs of mercury poisoning in wildlife species include anorexia (and resulting emaciation), ataxia (loss of coordination), progressive paralysis, tremors/spasms, and loss of sight (Heinz 1996, Wiener et al. 2003).

Mercury is readily bioaccumulated in wildlife and biomagnified through food chains. For birds and mammals that regularly consume fish and other aquatic organisms, total mercury concentrations in prey items should not exceed 100-µg/kg fresh weight for birds and 1,100 µg/kg for small and medium-sized mammals (Eisler 1987*a*). In wildlife, concentrations of mercury >1,100-µg/kg fresh weight of tissue (liver, kidney, blood, brain, hair/feathers) should be considered as presumptive evidence of an environmental mercury problem (Eisler 1987*a*). Although mortality/morbidity from mercury is more of an insidious event involving scattered individuals, a substantial number of mercury-related wildlife mortality/morbidity incidents have been reported. Many of these have involved mortality in grebes (Podicipedidae) in the western United States (Eisler 1987*a*), common loons (*Gavia immer*) and turkey

vultures (*Cathartes aura*) in Canada (Friend and Franson 1999), and reproductive impairment in bald eagles (*Haliaeetus leucocephalus*) in the United States (Friend and Franson 1999).

Lead.—Major environmental sources of lead have been leaded gasoline, paints, pesticides, batteries, mining and smelting operations, metal finishing, petroleum refineries, and hunting, fishing, and shooting sports (e.g., trap, skeet, target shooting) as well as firearms training activities (Pattee and Pain 2003). Although leaded gasoline, paints, and pesticides are not as prevalent now, lead from these sources continues to persist in the environment. In animals, <10% of dietary lead is absorbed, but >90% of that absorbed is retained in bones. Lead causes anemia and inhibition of the enzyme D-ALAD (D-aminolevulinic acid dehydratase), and has been demonstrated to cause severe neurotoxic effects in young animals and humans (Pattee and Pain 2003). The exposure and effects of tetraethyl lead, an antiknock agent formerly added to gasoline, have been examined along highways (Grue et al. 1984) while lead shot deposition has also been examined, particularly in wetlands (DiGiulio and Scanlon 1984), around trap/skeet shooting ranges (Stansley and Roscoe 1996), and at firearms training facilities (Lewis et al. 2001).

Lead poisoning is most commonly observed in birds, particularly waterfowl. The first documented report of lead poisoning in waterfowl came from Texas in 1894 and Bellrose (1951, 1959) reported widespread waterfowl mortality and illness associated with ingestion of lead shot in the 1950s. In the United States, an estimated 1.6–2.4 million ducks, geese, and swans died annually as a direct result of lead shot ingestion before widespread use of nontoxic shot in the early 1990s (Pattee and Pain 2003). Sanderson and Bellrose (1986) and Beyer et al. (1998) reviewed the problem of lead poisoning in waterfowl. Signs of lead poisoning include gross lesions, impactions of the upper gastrointestinal tract, submandibular edema (accumulation of fluid), myocardial necrosis, and biliary discoloration in the liver (Friend and Franson 1999). Field signs include inability/reluctance to fly, weak and/or erratic flight, poor landings and, as conditions worsen, birds become flightless and hold their wings in a characteristic "roof-shaped" position that progresses to wing droop as birds become more moribund (Friend 1987). About 95% of waterfowl diagnosed with lead poisoning had liver lead concentrations of at least 38 ppm (dry weight) (Friend and Franson 1999).

Ingestion of lead shot by both predatory and scavenging raptors feeding on hunter-killed carcasses has been reported for bald and golden eagles (*Aquila chrysaetos*), red-tailed hawks (*Buteo jamaicensis*), vultures (turkey and black [*Coragyps atratus*] vultures, and California condor [*Gymnogyps californianus*]) (Janssen et al. 1986, Craig et al. 1990). Vultures and eagles appear to be highly susceptible to poisoning from ingesting small quantities of lead shot (Eisler 1988*b*). In addition to lead shot, lead fishing sinkers have contributed to lead-caused mortalities in a number of aquatic birds and mammals, particularly common loons (Pokras and Chafel 1992, Scheuhammer and Norris 1996, Stone and Okoniewski 2001, Sidor et al. 2003). Lead is readily bioaccumulated in wildlife, but does not appear to be biomagnified in food chains. At least 6 endangered or formerly endangered species, including bald eagle, peregrine falcon (*Falco peregrinus*), California condor, brown pelican (*Pelecanus occidentalis*), Mississippi sandhill crane (*Grus canadensis pulla*), and whooping crane (*G. americana*) have been victims of lead poisoning (Friend and Franson 1999).

Cadmium.—Major environmental sources of cadmium include electroplating, zinc and lead mining and smelting, paint and pigments, batteries, plastics, coal-fired power plants, and municipal wastewater and sewage sludge. Cadmium is a known teratogen and affects calcium metabolism causing excess calcium excretion, which negatively impacts both skeletal and cardiovascular systems (Eisler 1985*a*). In addition, growth retardation, anemia, and testicular damage occur in cadmium-exposed wildlife (Eisler 1985*a*). Cadmium is readily bioaccumulated and data are available suggesting that it is biomagnified through food chains (Larison et al. 2000). White-tailed ptarmigan (*Lagopus leucura*) in Colorado were poisoned by cadmium due to biomagnification (hyper accumulation) in willow (*Salix* spp.), a primary food plant for these birds (Larison et al. 2000). Cadmium residues in vertebrate kidney or liver that are >10 ppm fresh weight or 2 ppm whole body fresh weight should be viewed as evidence of probable cadmium toxicity; residues of 200 ppm kidney (fresh weight), or >5 ppm whole animal fresh weight are indicative of cadmium poisoning. Wildlife, especially migratory birds, which feed on crops growing in fields fertilized with municipal sewage sludge, may be at considerable risk from cadmium toxicity (Eisler 1985*a*).

Chromium.—Major environmental sources of chromium include production of stainless steel (ferrochrome) which includes electroplating and metal finishing industries, pigments (paint, ink), tanning leather, wood preservatives, coal-fired power plants, municipal incinerators and publicly owned treatment plants, cement-producing plants, and from anticorrosives in cooling systems and boilers. Chromium is most frequently found in the environment as trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) forms. The biological effects of chromium (Cr^{+6}) is thought to be related to reduction to Cr^{+3} and formation of complexes with intracellular macromolecules that, if it occurs in genetic material, leads to mutagenesis. Chromium (Cr^{+6}) is toxic to embryos, teratogenic, and causes alterations of blood and serum chemistry, liver and kidney lesions (including acute renal tubular necrosis), and ulcerations in mucous membranes. In wildlife, tissue levels in excess of 4.0 mg total chromium/kg dry weight is presumptive evidence of an environmental chromium problem, although the significance of tissue chromium residues is not known. Chromium is readily bioaccumulated in wildlife, but concentrations are usually highest at the lower trophic levels and it is not known to be biomagnified in food chains (Eisler 1986*a*). Wildlife mortality/morbidity as a result of chromium exposure generally is infrequent (Eisler 1986*a*).

Arsenic.—Major environmental sources of arsenic include copper, zinc, and lead mining and smelting; glass and chemical manufacturing, particularly wood preservatives and arsenic-based herbicides; and coal-fired power plants. There are many different arsenic compounds and their environmental chemistry is quite complex, but trivalent (As^{+3}) and pentavalent (As^{+5}) forms predominate and both organic and inorganic forms are common. Arsenic is a teratogen and can traverse placental barriers and produce fetal death and malformations in wildlife (Eisler 1988*a*). It is highly cytotoxic, affecting mitochondrial enzymes and

impairing tissue respiration. Chronic exposure leads to neurotoxicity of peripheral and central nervous systems, liver damage, and peripheral vascular disease (Eisler 1988*a*). Arsenic is bioaccumulated by wildlife but is not biomagnified in food chains. Despite its high toxicity, wildlife mortality/morbidity as a result of arsenic exposure generally is infrequent (Eisler 1988*a*).

Organic/Inorganic Chemicals

Organic Chemicals

Organic chemicals are based on carbon–hydrogen pairs that range from single carbon chains to multiple aromatic rings. Organic chemicals can be released from refineries, oil/gas spills, incinerators, sewage effluent, wood treating, chemical plants, military sites, and other industrial sites. Many pesticides are organic chemicals; pesticides are treated separately and this section pertains only to nonpesticide organic chemicals. Generally, organic chemicals are more hazardous to wildlife than are inorganic chemicals. A number of organic chemicals are of concern to wildlife including organic solvents, petroleum products, polychlorinated biphenyls (PCBs), dioxins, and furans.

Solvents.—Organic solvents generally are refined from petroleum and are used to dissolve, dilute, or disperse other chemicals (including pesticides) that are not soluble in water. They are used widely as degreasers and as constituents of paints, varnishes, lacquers, inks, aerosol sprays, dyes, and adhesives. They are also used as intermediates in chemical synthesis and as fuels and fuel additives. Organic solvents include widely-used chemicals such as chlorinated hydrocarbons (e.g., trichloroethylene, perchloroethylene, methylene chloride, carbon tetrachloride); aromatic hydrocarbons (e.g., benzene, toluene, xylene, styrene, ethylbenzene); alcohols (e.g., ethanol, methanol); aldehydes (e.g., formaldehyde); ketones (e.g., acetone); glycols (e.g., ethylene glycol, propylene glycol); glycol ethers; phenols (e.g., phenol, chlorophenol); carbon disulfide; and fuel and fuel additives (e.g., gasoline, methyl tertiary-butyl ether [MTBE], jet fuel, kerosene). Because of their widespread use, organic solvents are ubiquitous in the environment (Bruckner and Warren 2001). Generally highly lipophilic, extremely volatile, and of relatively small molecular size and lacking charge, organic solvents are rapidly absorbed across lungs, gastrointestinal tract, and skin. The most notable negative effect of this group is central nervous system depression (Bruckner and Warren 2001). Other negative effects include carcinogenesis and damage to the hematopoietic system (bone marrow), liver, and kidney (Bruckner and Warren 2001). Organic solvents tend to readily bioaccumulate but are not known to biomagnify through food chains.

Ethylene Glycol.—A major ingredient in antifreeze and de-icing solutions, ethylene glycol is responsible for numerous wildlife deaths in the United States and Canada each year (U.S. Environmental Protection Agency 1998*a*). It is an oily liquid with a mild odor and a sweet taste, which makes it attractive to wildlife. Puddles of antifreeze or brake fluid can accumulate on roads or parking lots, and their color and smell attracts many wildlife species. The vast majority of ethylene glycol is released directly into the environment as airport and runway runoff from de-icing activities. An annual release of over 26 million kg of ethylene glycol occurs during icing conditions at the 17 busiest airports in the United States (U.S. Environmental Protection Agency 1998*a*). Ethylene glycol is also used in polyester compounds and as a solvent in the paint and plastics industries, photographic developing solutions, hydraulic brake fluids, and inks.

Wildlife poisoned by ethylene glycol appear intoxicated; signs including depression, ataxia, and reluctance to move appear as soon as 2 to 4 hours following exposure (Stowe et al. 1981). Ethylene glycol metabolizes to oxalic acid and binds to calcium to form calcium oxalate crystals that block renal tubules with death resulting from acute kidney failure (MacNeill and Barnard 1978, Stowe et al. 1981). Kidneys should be collected from carcasses if ethylene glycol poisoning is suspected. Canids and felids are particularly susceptible to ethylene glycol; as little as 4–5 ml/kg is lethal to domestic dogs and 2–4 ml/kg is lethal to domestic cats (Osweiler et al. 1985). Waterfowl, vultures, and birds of the Family Corvidae (jays, crows, ravens, magpies) occasionally are victims of ethylene glycol poisoning. There is at least one record each of a California condor (Murnane et al.1995) and a polar bear (*Ursus maritimus*) (Amstrup et al. 1989) being lethally poisoned by ethylene glycol.

Petroleum Products.—Petroleum products, including crude oil, diesel, gasoline, kerosene, and others are ubiquitous in the environment. Every year, an average of 53 million liters of oil from more than 10,000 accidental spills flow into fresh and saltwater environments in the United States (Friend and Franson 1999). However, accidental releases account for a small fraction of all oil entering the environment; most oil is introduced through intentional discharges from normal transport and refining operations, industrial and municipal discharges, used lubricant and other waste oil disposal, urban runoff, river runoff, atmospheric deposition, and natural seeps (Eisler 1987*b*, Jessup and Leighton 1996, Albers 2003). Wildlife exposed to petroleum products can be impacted both externally and internally. Oil contamination of hair and feathers disrupts their normal structure and function, resulting in a loss of insulation and waterproofing (Eisler 1987*b*). Birds and mammals can also ingest, inhale, and absorb petroleum products when exposed during spill events while preening/grooming contaminated feathers/hair. In birds, hatching success is reduced when adults are exposed to fuel oil during incubation and transfer oil to their eggs (Jessup and Leighton 1996).

Polycyclic aromatic hydrocarbons contribute heavily to the toxicity of crude and refined petroleum products, but amounts of these compounds in petroleum products vary widely. Polycyclic aromatic hydrocarbons are semivolatile, and occur in the environment from many sources in addition to the petrochemical industry, including from natural sources. Lower molecular weight polycyclic aromatic hydrocarbons exhibit significant acute toxicity and other adverse effects to wildlife but are not carcinogenic. However, high molecular weight polycyclic aromatic hydrocarbons usually are less acutely toxic, but may be carcinogenic, mutagenic, or teratogenic to a wide variety of wildlife (Eisler 1987*b*). Polycyclic aromatic hydrocarbons, although highly lipid soluble, generally are rapidly metabolized and tend not to bioaccumulate in wildlife; there is little evidence for biomagnification in food chains. Polycyclic aromatic hydrocarbons such as benzo(a)pyrene, naphthalene, anthracene, styrene, and others have been investigated for

wildlife impacts (Eisler 1987*b*). There are no specific regulations regarding the protection of wildlife species from polycyclic aromatic hydrocarbons other than laws governing petroleum products (Eisler 1987*b*). There is little evidence to indicate that polycyclic aromatic hydrocarbons are likely to produce large numbers of wildlife deaths or sicknesses except when associated with oil spills.

Polychlorinated Biphenyls.—Polychlorinated biphenyls (PCBs) were introduced in 1929 for use in dielectric (insulating) fluids. They were used extensively in the electricity generating industry as insulating or cooling agents in transformers and capacitors. Although their manufacture was banned by the U.S. Environmental Protection Agency in 1977, products containing PCBs made prior to that date can still be found. PCBs are still released from hazardous waste sites, illegal or improper disposal of industrial wastes and consumer products, leaks from old electrical transformers, burning of some wastes in incinerators, and aquatic sediments (Eisler 1986*c*, Eisler and Belisle 1996). The estimated environmental burden of PCBs from these sources is almost 400 million kg (Tanabe 1988, Eisler and Belisle 1996). PCBs bind strongly to organic particles in soil and sediment-forming PCB sinks where local concentrations can be high. PCBs are transported globally through atmospheric and oceanic processes. There are 209 different PCB congeners (forms), but only 100–150 are represented in PCB formulations (Eisler 1986*a*, Rice et al. 2003).

Some PCB congeners are of greater environmental concern than others. In general, PCB congeners with high K_{ow} (a physical characteristic of a chemical correlated with lipid solubility) values and high numbers of substituted chlorines in adjacent positions constitute the greatest environmental threat to wildlife. This includes planar PCBs, a group of about 20 PCB congeners that closely resemble dioxins (Eisler and Belisle 1996, Rice et al. 2003). PCBs cause a wide variety of biological effects including death, developmental abnormalities, reproductive failure, liver damage, tumors, and a wasting syndrome (Eisler and Belisle 1996). Effects on reproduction, endocrine and immune systems, and behavior may have the greatest impacts on wildlife populations. Mink (*Mustela vison*) are one of the most susceptible species to PCBs and dietary levels as low as 100 µg PCBs/kg fresh weight cause reproductive failure and death (Aulerich and Ringer 1977, Aulerich et al. 1987). Signs of PCB toxicity in mink include anorexia; bloody stools; disrupted molting patterns; and thickened, elongated, and deformed nails (Aulerich and Ringer 1977). In birds, total PCB levels (µg/kg fresh weight) of 3,000 in the diet, 16,000 in the egg, or 54,000 in the brain were associated with PCB poisoning (Eisler 1986*c*). PCBs have been shown to have severe effects on avian reproduction, mainly decreased productivity and hatching success (embryo mortality), and abnormal breeding behavior (Eisler 1986*c*, Eisler and Belisle 1996, Rice et al. 2003).

PCBs are highly lipid soluble and readily bioaccumulated in wildlife and biomagnified in both aquatic and terrestrial food chains. Some wildlife species such as long-lived fishes and common snapping turtles (*Chelydra serpentina*) can bioaccumulate and store high levels of PCBs in their tissues posing a potential hazard for predators, particularly avian piscivores (Eisler 1986*c*, Eisler and Belisle 1996). While much of the environmental burden of PCBs is localized, PCBs continue to represent a considerable hazard to exposed wildlife species (Eisler 1986*c,* Tanabe 1988, Rice et al. 2003). However, continuing impacts of PCBs on wildlife are likely to be related to reproductive impairment and other sublethal effects. Mortality from chronic exposure is unlikely except in sensitive species with high risk feeding habits (e.g., piscivores) (Eisler and Belisle 1996).

Dioxins and Furans.—Dioxins and furans have no commercial use and are released into the environment as contaminants from combustion, incineration, synthesis of phenoxy herbicides and wood preservatives, and industrial and municipal processes such as paper manufacturing (Bradbury 1996, Rice et al. 2003). There are approximately 75 different forms of dioxins with tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) being most prevalent in the environment and of most concern to wildlife. There are approximately 135 different forms of furans. Most dioxins and furans are resistant to environmental and biologic degradation and, once formed, disperse throughout the atmosphere, soil, and water. Environmental dioxins and furans have resulted in deaths of many wildlife species and domestic animals (Bradbury 1996, Rice et al. 2003).

Exposure to dioxins and furans can result in a wide range of negative effects, from acute and delayed mortality to teratogenic, histopathological, immunological, and reproductive effects (Rice et al. 2003). Exposure to even minute quantities of 2,3,7,8-TCDD has been shown to result in reproductive failure in mink (Hochstein et al. 1988), wood ducks (*Aix sponsa*) (White and Seginak 1994) and ring-necked pheasants (*Phasianus colchicus*) (Nosek et al. 1992). Signs of dioxin toxicity include a "wasting syndrome," subcutaneous edema, alterations in lipid metabolism and gluconeogenesis, reproductive effects (teratogenicity, fetotoxicity), decreased immunocompetence, and thymic atrophy (Bradbury 1996). As with PCBs, dioxins and furans are highly lipid soluble and readily bioaccumulated in wildlife and biomagnified in both aquatic and terrestrial food chains. Wildlife that bioaccumulate and store high levels of dioxins and furans in their tissues pose a potential hazard for predators, particularly avian and mammalian piscivores. It is recommended that 2,3,7,8-TCDD concentrations in water should not exceed 0.01 ppt (parts per trillion) to protect aquatic wildlife species or 10–12 ppt in food of terrestrial wildlife (Eisler 1986*b*). Currently, there are no regulations governing dioxins and furans to protect wildlife (Eisler 1986*b,* Eisler and Belisle 1996).

Inorganic Chemicals

Inorganic chemicals are a diverse group that includes those that do not have carbon and its derivatives as their principal elements. This includes 4 general groups: alkalis and chlorine, industrial gases, inorganic pigments, and industrial inorganic chemicals. Examples of industrial inorganic chemicals include acids; bases; metallic compounds; catalysts; ammonia; and salts derived from sodium, phosphorus, potassium, and sulfur. Inorganic chemicals generally are disposed in hazardous waste streams and do not pose a great threat to wildlife. However, some chemicals are used in processes such as mining and military activities and can leak or spill from storage where they can occur in large volumes in the environment and pose substantial hazards to wildlife. Two inorganic chemicals that pose a particular hazard to wildlife include cyanide and white phosphorus.

Cyanide.—Cyanides are highly toxic chemicals widely used in mining and other industrial processes. Cyanide levels tend to be elevated in the vicinity of metal processing operations, electroplaters, gold-mining facilities, oil refineries, power plants, and solid waste combustion facilities (Eisler 1991). The most common form of cyanide is hydrocyanic acid, which is used in electroplating and for fumigation. Other chemical forms include sodium cyanide, used in extracting precious metals from raw ore and for predator control (M-44 ejector device), potassium cyanide, and calcium cyanide. Cyanides are readily absorbed through oral, dermal, and inhalation routes and are distributed throughout the body via the blood. Cyanide is a potent and rapid-acting asphyxiant, inducing tissue anoxia through inactivation of cytochrome oxidase causing cytotoxic hypoxia (lack of oxygen) in the presence of normal hemoglobin oxygenation. Diagnosis of acute lethal cyanide poisoning is difficult because symptoms are nonspecific and numerous factors modify its toxicity. The most consistent changes in acute cyanide poisoning are inhibition of brain cytochrome oxidase activity and changes in electrical activity in heart and brain.

Birds, mammals, and other wildlife in the vicinity of gold mining operations are particularly prone to cyanide exposure. Cyanide associated with gold mining activities in Nevada leached into nearby ponds and killed large numbers of migratory birds (Henny et al. 1994) and mammals (Clark and Hothem 1991). In a sampling of Nevada mines, more than 90 avian species (mainly waterfowl, shorebirds, and passerines), 28 mammalian species (mainly rodents, bats, and lagomorphs), and several reptilian and amphibian species were reported poisoned by cyanide solution ponds between 1986 and 1991 (Henny et al. 1994). For birds and bats, most mortality incidents associated with exposure to cyanide at mining operations are reported in spring and fall during migration (Clark 1991, Clark and Hothem 1991, Henny et al. 1994). Eisler et al. (1999) reviewed the specific environmental hazard for wildlife species at gold mining operations. In addition to mining, cyanide is used in M-44 predator control devices mainly in the western United States where mammalian (mainly coyotes [*Canis latrans*]) and avian (mainly golden eagles) predators are subject to cyanide poisoning. From 1986 through 1995, more than 3,000 cyanide-related mortalities involving about 75 species of birds representing 23 Families were reported to the National Wildlife Health Center in Madison, Wisconsin. Waterbirds and passerines were the 2 groups of birds most impacted by cyanide.

White Phosphorus.—White phosphorus (P_4) is a highly toxic, incendiary munition extensively used by the military for marking artillery impacts (target practice) and as an obscurant. Areas in and around active (and inactive) military artillery and bombing ranges can concentrate white phosphorus which can runoff into surface waters and move to areas away from military ranges. White phosphorus caused the death of an estimated 1,000 to 2,000 migrating dabbling ducks (*Anas* spp.) and 10 to 50 swans (*Cygnus buccinator* and *C. columbianus*) per year in the late 1980s and early 1990s at Eagle River Flats, a 1,000 ha estuarine salt marsh at Fort Richardson, Alaska used for artillery training by the U.S. Army (Racine et al. 1992, Sparling 2003). Signs of white phosphorus poisoning observed in wild waterfowl include lethargy, repeated drinking, and head shaking and rolling with convulsions prior to death (Racine et al. 1992). While no direct mortality of predators at Eagle River Flats was found, secondary exposure and poisoning of predators and scavengers such as bald eagles, herring gulls (*Larus argentatus*), and common ravens (*Corvus corax*) was noted (Roebuck et al. 1994). White phosphorus has been shown to cause significant changes in a wide range of blood parameters in mallards (*Anas platyrhynchos*) (Sparling et al. 1998) and mute swans (*Cygnus olor*) (Sparling et al. 1999), and to cause secondary poisoning in American kestrels (*Falco sparverius*) (Sparling and Federoff 1997).

Pharmaceuticals

There is a wide diversity of pharmaceutical drugs, hormones, and other related organic wastewater contaminants present in waterways of the United States that pose a potential hazard to wildlife. In 1999–2000, a U.S. Geological Survey monitoring effort found 82 of 95 different pharmaceutical drugs tested for in water samples from a network of 139 streams across 30 states (Kolpin et al. 2002). A wide range of residential, industrial, and agricultural drugs and chemicals was found in 80% of all streams tested. Little is known about the potential impact of these drugs/chemicals on wildlife, particularly the potential interactive effects that may occur from complex mixtures of these and other chemicals in the environment. Numerous wildlife mortality/morbidity incidents occurring from widely used pharmaceutical drugs such as sodium pentobarbital and diclofenac provide evidence of the hazard posed by this group of chemicals.

Sodium Pentobarbital.—Sodium pentobarbital and related barbiturates are used extensively in veterinary medicine, especially for euthanasia of domestic animals, and result in the deaths of numerous wildlife species across the United States and Canada each year (Friend and Franson 1999). The use of highly concentrated solutions for euthanasia of domestic animals (e.g., cats, dogs, horses, etc.) is routine practice in veterinary medicine. Carcasses that are not incinerated or otherwise properly disposed are subject to scavenging by wildlife, which can result in exposure to this family of chemicals. Any wildlife species that scavenges food potentially is at risk from these chemicals. Mortality of wildlife from bald and golden eagles to grizzly bears (*Ursus arctos*) has been reported from landfills and other improper burial sites where animal carcasses were either left in the open or not disposed of properly. In recent years, the National Wildlife Health Center in Madison, Wisconsin and the National Fish and Wildlife Forensic Laboratory in Ashland, Oregon had verifiable reports of at least 133 eagle deaths resulting from secondary pentobarbital poisoning, most likely only a fraction of the real total.

Diclofenac.—Diclofenac is a nonsteroidal, anti-inflammatory drug used extensively in veterinary medicine and is administered to livestock and other domestic animals for pain and arthritis in many countries around the world. Diclofenac was identified as the most likely cause of a mass mortality of 3 species of vultures in Pakistan (Oaks et al. 2004). Vultures consuming dead livestock containing diclofenac were exposed to high levels of the drug in livestock tissues. Necropsies revealed that exposed animals had visceral gout and histopathological lesions including acute renal tubular necrosis and uric acid crystal formation

in the kidneys and other tissues, which led to acute kidney failure and death. Populations of the 3 species of vultures, Oriental white-backed vulture (*Gyps bengalensis*), long-billed vulture (*G. indicus*), and slender-billed vulture (*G. tenuirostris*) were decimated by as much as 95% in some cases (Oaks et al. 2004). Although this incident occurred in Asia, it clearly demonstrates the potential hazard of pharmaceutical drugs to wildlife.

Pesticides

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. The term pesticide is a generic name for a variety of agents classified more specifically on the basis of the pattern of use and organism killed. Pesticides include chemicals designed to kill specific groups of organisms, such as insecticides, herbicides, fungicides, rodenticides, miticides, acaricides, larvicides, and molluscicides. They also function as attractants (pheromones), defoliants, desiccants, plant growth regulators, repellents, and fumigants for purposes of reducing numbers of pest species.

Pesticides are a unique category of environmental contaminants as they are intentionally released into the environment. Thus, regulations for monitoring pesticide usage and the likelihood of detecting pesticide-related mortality events are enhanced. Insecticides are among the most acutely toxic contaminants in the environment and can produce dramatic mortality and morbidity incidents. Target species selectivity of pesticides is not well developed and nontarget species frequently are affected because they possess physiological and/or biochemical systems similar to those of the target organisms. Specific classes of pesticides of major concern to wildlife include insecticides, herbicides, fungicides, fumigants, and vertebrate pest control chemicals such as rodenticides and avicides.

Insecticides

Most chemical insecticides in use today are neurotoxicants and act by poisoning the nervous system of the target organisms. The central nervous system of insects is highly developed and not unlike that of the vertebrate central nervous system. Generally, insecticides are not selective and affect nontarget as readily as target organisms. Target sites and/or mechanism(s) of action may be similar in all species; only the level of exposure (dosage and duration of contact with toxic receptors) affects the intensity of biological effects. Four distinct groups of insecticides, including chlorinated hydrocarbons, anticholinesterases (organophosphorus and carbamate), synthetic pyrethroids, and other botanicals are discussed as they pose a significant threat to wildlife.

Chlorinated Hydrocarbons.—Chlorinated hydrocarbon (organochlorine) insecticides are a diverse group belonging to 3 distinct chemical classes: dichlorodiphenylethanes (e.g., DDT, dicofol, methoxychlor), cyclodienes (e.g., heptachlor, dieldrin, aldrin), and chlorinated benzenes (e.g., lindane) (Smith 1991, Blus et al. 1996, Blus 2003). DDT was used extensively in all aspects of agriculture and forestry, in building and structural protection, and in human health situations from the mid-1940s to the early 1970s. The chemical properties of chlorinated hydrocarbon insecticides (e.g., low volatility, chemical stability, lipid solubility, slow rate of biotransformation and degradation) that made them effective brought about their demise due to persistence in the environment and bioaccumulation and biomagnification within food chains. Registration for DDT was cancelled in the United States and several other countries in 1972, and cancellation/restriction of registration for other chlorinated hydrocarbon insecticides followed. Despite the ban on their use in North America and Europe, chlorinated hydrocarbon insecticides are used extensively in developing countries. This occurs because they are inexpensive to manufacture, highly effective, relatively safe, few substitutes are available, and the risk-benefit ratio is highly weighted in favor of their continued use for control of insects causing devastation to crops and human health (Smith 1991, Blus et al. 1996, Blus 2003). The ramifications of continued heavy use of chlorinated hydrocarbons are that they become airborne and are transported globally in the atmosphere with deposition occurring on a global basis, particularly at high latitudes (Bidleman et al. 1990).

Definitive studies both in wildlife and laboratory species have demonstrated potent estrogenic and enzyme-inducing properties of chlorinated hydrocarbon insecticides, which interfere directly or indirectly with fertility and reproduction in wildlife. In avian species, this interference due to DDE exposure is related to steroid metabolism and the inability of the bird to mobilize calcium to produce sufficiently strong eggshells to withstand incubation (cracking allows bacteria to enter and kill developing embryos) (Blus et al. 1996, Blus 2003).

Chlorinated hydrocarbons act as diffuse stimulants or depressants of the central nervous system. Signs of acute toxicity occur within minutes to a few days following exposure, usually within 24 hours, may be progressively severe in nature, and can include muscle spasms, seizures, loss of coordination, abnormal walking/posturing, and excess salivation (Osweiler et al. 1985). Exposed animals may become comatose and remain so for several hours prior to death or may regain consciousness and fully recover. Pathologic changes associated with acute poisoning by chlorinated hydrocarbons usually are minimal and nonspecific, and include pulmonary congestion, hemorrhages, and edema, particularly in the central nervous system (Osweiler et al. 1985). Chronic exposure to chlorinated hydrocarbons results in alteration of hepatocytes (liver cells) (Osweiler et al. 1985).

The highly lipid-soluble nature of chlorinated hydrocarbon insecticides results in crossing of the normally protective placental and blood-brain barriers in mammals, leading to direct embryonic/fetal and central nervous system exposure. It also results in these chemicals being sequestered in body tissues (liver, kidney, nervous system, and fat tissue) having a high lipid content where the residues either elicit some biological effect or, as in the case of adipose tissue, remain stored and undisturbed until mobilized. Elimination rate and depletion of body storage sites may be enhanced by fasting resulting in mobilization of fat depots and any insecticide contained therein. However, with a high-chlorinated hydrocarbon body burden, there is a possibility of enhanced toxicity from the circulating agent being redistributed to target organs. The most serious effects, such as mortality, reduced reproductive success, population decline, and even extirpation occurred in birds, particularly raptors, seabirds, and waterbirds in the orders Strigiformes, Falconiformes, Pelecaniformes, Ciconiformes, and Podicipediformes (Blus et al. 1996, Blus 2003).

Anticholinesterases.—Organophosphorus and carbamate insecticides are commonly grouped together and referred to as anticholinesterases (anti-ChE's) (Mineau 1991, Hill 2003). These insecticides have a common mechanism of action, inhibition of the neurotransmitting enzyme cholinesterase (Baron 1991, Gallo and Lawryk 1991). However, they arise from 2 distinctly different chemical classes: the esters of phosphoric or phosphorothioic acid and those of carbamic acid. Currently, there are some 200 different organophosphorus and about 50 carbamate pesticides (mainly insecticides) on the market, formulated into thousands of different products (Hill 2003). Anti-ChE insecticides are applied mainly on terrestrial landscapes but are also used extensively in wetlands and coastal areas for mosquito control. The mechanism by which these insecticides elicit toxicity is identical and is associated with inhibition of the neurotransmitting enzyme acetylcholinesterase. This enzyme is responsible for the destruction and termination of the biological activity of the neurotransmitter acetylcholine. With accumulation of free, unbound acetylcholine at nerve endings of all cholinergic nerves, there is continual stimulation of electrical activity. Following lethal exposure, death results from acute respiratory failure (Hill 2003).

Organophosphorus and carbamate insecticide intoxication has become more complicated in recent years with recognition of additional and persistent signs of neurotoxicity not previously associated with acute exposure to these chemicals. One condition, an "intermediate syndrome," is a potentially lethal paralytic condition of the neck, limbs, and respiratory muscles. The other condition, where neuropathic conditions persist indefinitely, is referred to as organophosphorus-induced delayed neuropathy (Ecobichon 2001, Hill 2003).

Most widely used anti-ChE insecticides are highly toxic but relatively short-lived in the environment (usually 2–4 weeks) and are readily metabolized and excreted by birds and mammals (Hill 2003). Carbamates are direct cholinesterase inhibitors that do not require metabolic activation for full potency. Many organophosphorus insecticides are known to become more toxic as a result of metabolism (e.g., chlorpyrifos, diazinon, parathion, etc.) because the metabolites (the "oxon" form) are more potent inhibitors of cholinesterase (Matsumura 1985, Smith 1987). Thus, there may be some delayed toxicity (and onset of signs) associated with organophosphorus insecticide poisoning. Dietary toxicity experiments have shown that birds that die from carbamate insecticide poisoning do so within a few hours of exposure but mortality from organophosphorus insecticide poisoning may extend over 5 days (Hill 2003).

Organophosphorus and carbamate insecticides are responsible for more reported wildlife mortality/morbidity incidents than any other category of environmental contaminant. However, only a relatively small number of these pesticides are responsible for the majority of large-scale incidents of wildlife mortality/morbidity. Birds are highly sensitive to most organophosphorus and carbamate insecticides, and are particularly susceptible to granular formulations. As few as one granule (0.1–5 mg/kg) of some anti-ChE insecticides such as carbofuran may be lethal in <5 minutes to waterfowl and songbirds (Hill 2003). Extensive records of bird mortality/morbidity from exposure to organophosphorus and carbamate insecticides exist (Smith 1987, Sheffield 1997, Friend and Franson 1999, Mineau et al. 1999). One of the most notable mass mortalities in recent years involved the death of upwards of 10,000 or more Swainson's hawks (*Buteo swainsoni*) on their wintering grounds in the pampas of Argentina in the mid-1990s (Goldstein et al. 1996, Goldstein et al. 1999). In this case, hawks were poisoned through consumption of grasshoppers and other prey items in alfalfa fields sprayed with the organophosphorus insecticide monocrotophos. Although mammals generally are less sensitive than birds to organophosphorus and carbamate insecticides, many mammalian mortality incidents have also been reported (Smith 1987). Intensive field research with mammalian exposure to organophosphorus insecticides has documented reproductive and other sublethal effects at environmentally relevant levels (Sheffield and Lochmiller 2001, Sheffield et al. 2001).

Signs of acute exposure to anti-ChE insecticides include lethargy, excess salivation, lacrimation, urination, and defecation; vomiting may occur along with muscle fasciculation (brief spontaneous contractions of a few muscle fibers) and weakness, dyspnea (difficulty breathing), excessive bronchial secretion, and bradycardia (slowed heart rate) (Fairbrother 1996). In severe cases, prostration and convulsions precede death. These signs are useful when sick animals are found on or near an area of recent anticholinesterase insecticide application. However, these signs are not uniquely different from poisoning by other neurotoxic chemicals. Inhibition of brain cholinesterase activity by 20% (i.e., activity at 80% of normal) is considered diagnostic of sublethal poisoning and dead birds with a >50% reduction in activity generally is diagnostic of anti-ChE poisoning. Activity reductions of 70–95% are commonly reported for birds killed by organophosphorus insecticides (Hill and Fleming 1982, Hill 2003). Conclusive diagnosis depends on biochemical and chemical analyses for brain cholinesterase activity and organophosphorus residues in the carcass (Hill 1999, Hill 2003).

A wide diversity of sublethal effects has been documented to occur following exposure to anticholinesterase insecticides, including biochemical, physiological, behavioral, and others that impact survival and fitness of exposed animals (Mineau 1991, Hill 1999, Hill 2003). Many of these effects may be lethal, but may also mask pesticide exposure as the cause of mortality. For example, a group of exposed animals that has become disoriented and less vigilant may become more susceptible to predation or other mortality factors.

Anti-ChE insecticides generally do not bioaccumulate in organisms and do not biomagnify in food chains (Hill 2003). However, prey items such as arthropods and animal carcasses can contain sufficiently high levels of anticholinesterase insecticides to cause secondary poisoning in predatory and scavenging birds (particularly raptors) and mammals (Sheffield, 1997, Mineau et al. 1999, Shore and Rattner 2001). Bald eagles and red-tailed hawks in British Columbia were found poisoned by consuming unabsorbed pesticide in the stomachs of dead animals up to 6 months following its application (Elliott et al. 1996).

Synthetic Pyrethroids.—Synthetic pyrethroids are the newest major class of insecticides, entering the market in 1980. By 1982, they accounted for about 30% of the worldwide insecticide usage. These synthetics arise from

a much older class of botanical insecticides, pyrethrum, which is a mixture of 6 insecticide esters extracted from dried pyrethrum or *Chrysanthemum* flowers (Ray 1991). The increasing demand for pyrethrum has exceeded the limited world production. This led chemists to focus attention on synthesis of new analogs with better stability in light and air, better persistence, more selectivity to target species, and low mammalian and avian toxicity. In addition to extensive agricultural use, synthetic pyrethroids are components of household sprays, flea dips and sprays, and plant sprays for home and greenhouse use. Studies on intact animals have not yielded conclusive, fundamental information concerning the mechanism of action of pyrethroids (Ray 1991, Ecobichon 2001).

Synthetic pyrethroids alter sodium channels in nerve membranes, causing repetitive (sensory, motor) neuronal discharge and a prolonged negative after-potential with the effects being similar to those produced by DDT. Other impacts noted for synthetic pyrethroids include inhibition of Ca- and Mg-ATPase, the effect of which would increase intracellular calcium levels accompanied by increased neurotransmitter release and post-synaptic depolarization (Matsumura 1985, Ray 1991).

There have been relatively few reports of wildlife mortality/morbidity as a result of synthetic pyrethroid exposure and little is known about their sublethal effects on wildlife. The available evidence suggests that synthetic pyrethroids elicit little chronic toxicity to wildlife. In addition, there is little storage or bioaccumulation of pyrethroids because they are readily biotransformed by the mixed-function oxidase system. However, piperonyl butoxide (an inhibitor of cytochrome P-450s which is an important family of detoxification enzymes) is a synergist added to many synthetic pyrethroid formulations for increased toxicity (10- to 300-fold increase in toxicity) (Ray 1991, Ecobichon 2001).

Other Botanical Insecticides.—Nicotine and rotenone are among the more widely used botanical insecticides. These compounds are natural plant alkaloids whose toxic properties have been recognized for hundreds of years (Ray 1991). Nicotine usually is obtained from the dried leaves of the tobacco plant (*Nicotiana tabacum*) and rotenone is derived from the roots of the derris (*Derris* spp.) (South America) and Cubé (*Lonchocarpus* spp.) (southeast Asia) plants. Used as an insecticide and piscicide, rotenone is extremely toxic to aquatic vertebrates, particularly fishes. Because use of rotenone as a piscicide is so widespread, there is concern about the potential negative effects of rotenone on amphibian (frogs, salamanders) and aquatic reptile (turtles, snakes) species, particularly neotenic salamanders that use aquatic respiration (Fontenot et al. 1994). The most frequent signs of rotenone poisoning in wildlife include vomiting, anorexia, dermatitis, irritation of the gastrointestinal tract, and lack of coordination, muscle tremors, and convulsions with death occurring through respiratory failure (Osweiler et al. 1985).

Herbicides

Herbicides are any compound capable of either killing or severely injuring plants and may be used for elimination of plant growth or killing of plant parts. Many of the early herbicides contained forms of arsenic and were difficult to handle, highly toxic, relatively nonspecific, or phytotoxic to crops as well as undesirable plants. However, currently used herbicides generally have a much lower hazard to wildlife than those used earlier and are more likely to result in sublethal effects rather than cause wildlife mortality/morbidity (Stevens and Sumner 1991).

Over the past 2 decades, herbicides have represented the most rapidly growing section of the agrochemical pesticide business due in part to (1) monoculture practices where risk of weed infestation has increased because fallowing and crop rotation are no longer standard practices, and (2) mechanization of agricultural practices (planting, cultivating, harvesting) due to increased labor costs. The result has been a plethora of chemically diverse compounds rivaling the innovative chemistry of insecticides. The goal of herbicide application has been to protect desirable crops and obtain high yields by selectively eliminating unwanted plant species, thereby reducing competition for nutrients, water, and space (Stevens and Sumner 1991).

There are at least 6 different broad classes and 22 or more different chemical groups of herbicides, including: (1) germination inhibitors such as dinitroanilines (e.g., trifluralin) and chloroacetamides (e.g., alachlor, metolachlor); (2) photosynthesis inhibitors such as triazines (e.g., atrazine, simazine, metribuzin); (3) meristem inhibitors such as sulfonylureas (e.g., chlorsulfuron) and imidazolinones (e.g., imazethapyr, imazapyr); (4) contact action such as bipyridylium (e.g., paraquat, diquat) and arsenicals (e.g., MSMA); (5) auxin growth regulators such as phenoxy acids (e.g., 2, 4-D); and (6) foliar grass killers such as phosphono-amino acids (e.g., glyphosate).

Herbicide classification is based on how and when they are applied. Preplanting herbicides are applied to the soil before a crop is seeded, pre-emergent herbicides are applied to the soil before the usual time of appearance of the unwanted vegetation, and post-emergent herbicides are applied to the soil or foliage after germination of the crop and/or weeds.

The chlorphenoxy (e.g., 2, 4-D; 2, 4, 5-T) and bipyridyl (e.g., paraquat, diquat) herbicides are acutely toxic to wildlife and humans. Paraquat is a contact herbicide and one of the most specific pulmonary toxicants known. Many countries have banned or severely restricted use of these herbicides (Ecobichon 2001). Another group, the triazines, although considered less acutely toxic, are of concern for wildlife due to their widespread and high volume use. There is also evidence of sublethal effects such as endocrine disruption, with subsequent impacts on reproduction and development (Hayes et al. 2003).

Herbicides show a broad range of persistence (Stevens and Sumner 1991). Some, such as paraquat may persist for years while others persist for only days or months. Most herbicides occur either in plants or the soil. Because they are not as persistent as organics such as PCBs or some organochlorine insecticides such as DDT, they tend not to move via the atmosphere to distant locations. However, herbicides such as atrazine and metolachlor with high volume use throughout the midwestern United States can result in high atmospheric concentrations and movement. Most herbicides do not bioaccumulate in animal tissue of any class of animals. Because of the overall limited persistence or tendency to bind to soil particles, there is generally limited movement through the environment. The most frequent signs of herbicide poisoning in wildlife include anorexia, diarrhea, edema, ataxia, inflammation of

the gastrointestinal tract, and congestion of the lungs, liver, and kidneys (Osweiler et al. 1985).

Fungicides

Fungicides are derived from a wide variety of chemicals ranging from simple inorganic compounds, such as sulfur and copper sulfate, through the aryl- and alkyl-mercurial compounds and chlorinated phenols to metal-containing derivatives of thiocarbamic acid. There are at least 36 different chemical groups of fungicides, a direct result of the great diversity of fungi (Edwards et al. 1991, Ecobichon 2001).

There are 3 general types of fungicides: (1) foliar, which are applied as liquids or powders to the aerial green parts of plants producing a protective barrier on the cuticular surface and causing systemic toxicity in developing fungus; (2) soil, which are applied as liquids, dry powders, or granules, acting either through the vapor phase or by systemic properties; and (3) dressings, which are applied to seeds prior to planting and to the post-harvest crop (cereal grains, tubers, etc.) as liquids or dry powders to prevent fungal infestation of the seed and crop (particularly if it is stored under less than optimal conditions of temperature and humidity).

Most fungicides have low acute toxicity to mammals and birds. However, all fungicides are cytotoxic and almost all produce positive results in microbial mutagenicity and animal carcinogenicity tests (Ecobichon 2001). Many fungicides are also teratogenic, embryotoxic, and endocrine disruptors (Edwards et al. 1991). Fungicide groups of current environmental concern to wildlife include benzimidazoles (e.g., benomyl, carbendazim, thiabendazole), dithiocarbamates (e.g., maneb, mancozeb, zineb), aromatics (e.g., chlorothalonil), dinitrophenols (e.g., dinocap), and dicarboximides (e.g., captan, vinclozolin) (Ecobichon 2001). Others, that were heavily used in the past but have largely been discontinued in the United States due to the environmental hazard they pose, include the organo-mercurials, hexachlorobenzene, pentachlorophenol, captafol, and folpet (Ecobichon 2001). The most frequent signs of fungicide poisoning in wildlife include anorexia and weight loss, lethargy and depression, impaired liver function, and reproductive impairment (Osweiler et al. 1985).

Fumigants

Fumigants are agents used to kill insects, nematodes, weed seeds, and fungi in soil as well as in stored cereal grains, fruit, and vegetables, clothes, and other products. They are normally used in enclosed spaces due to high volatility of the compounds. Chemicals used as fumigants include acrylonitrile, carbon disulfide, carbon tetrachloride, ethylene dibromide, chloropicrin, methyl bromide, and ethylene oxide. These can be liquids that readily vaporize at ambient temperatures, solids that can release a toxic gas on reacting with water or with acid, or gases. They generally are nonselective, highly reactive, and cytotoxic. Fumigants of environmental concern include phosphine (used heavily in grains), ethylene dibromide, and 1,2-dibromo-3-chloropropane; the latter 2 are known animal carcinogens (Ecobichon 2001).

Vertebrate Pest Control Chemicals

Rodenticides.—Rodenticides were developed to control pest small mammals (particularly rodents), which cause agricultural damage, carry disease, and are considered by some to be nuisance species. Chemicals used as rodenticides constitute a diverse range of compounds having a variety of mechanisms of action, which are partially successful at attaining species selectivity. Design of some rodenticides has taken advantage of unique physiological and biochemical characteristics of rodents. The sites of action are common to most mammals but advantage is taken of the habits of the pest animal and/or dosage minimizing impacts to nontarget species. A number of inorganic compounds, including thallium sulfate, arsenic oxide and other arsenicals, barium carbonate, yellow phosphorus, aluminum phosphide, and zinc phosphide have been used as rodenticides. A number of insecticides have been used as rodenticides, including DDT. In addition, a number of natural plant toxins, such as strychnine, red squill, ricin, and sodium monofluoroacetate (Ray 1991, Eisler 1995), have been used as rodenticides or to control other mammalian species. Sodium monofluoroacetate (compound 1080) has been used extensively in prepared baits to control rodents and predators, particularly coyotes. Most mammals are fatally poisoned by <1 mg/kg body weight of sodium monofluoroacetate (Eisler 1995). Domestic sheep have experienced toxic effects from wearing compound 1080-impregnated livestock protection collars (Burns and Connelly 1995).

Currently, anticoagulants are the most significant class of rodenticides in terms of wildlife mortality/morbidity incidents. The basis of efficacy of anticoagulant rodenticides is coumadin (warfarin), which was isolated from spoiled sweet clover (*Melilotus* spp.) and acted as an anticoagulant by antagonizing the actions of vitamin K in the synthesis of clotting factors. Warfarin has been in use since the 1920s and some rodent populations developed resistance to it by the 1950s. Since then, the next generation of "super warfarins" has appeared (e.g., brodifacoum, bromadiolone, diphenacoum, diphacinone, and others). These "super warfarins", particularly brodifacoum, have caused a substantial number of wildlife mortality incidents across the United States (Sheffield 1997, Stone et al. 1999). Brodifacoum has been documented to poison nontarget wildlife. Secondary poisoning of raptors (particularly red-tailed hawks and great horned owls [*Bubo virginianus*]) made up 50% of the cases. Gray squirrels (*Sciurus carolinensis*), raccoons (*Procyon lotor*), and white-tailed deer (*Odocoileus virginianus*) were the most frequently poisoned mammals (Stone et al. 1999).

Avicides.—Avicides were developed to control pest birds, particularly species that flock such as European starlings (*Sturnus vulgaris*), blackbirds (Family Icteridae), and rock pigeons (*Columba livia*), which cause agricultural damage or are considered nuisance species. Several chemicals with avicidal properties have been used including avitrol, chloralose, endrin, fenthion, methiocarb, and strychnine. Most of these chemicals are no longer registered for avicidal uses. One currently used avicide, DRC-1339, was developed specifically to kill starlings. Although designed to be specific to starlings, there is evidence that it is nonspecific because it has been shown to pose a hazard to nontarget seed-eating species such as ring-necked pheasants (Avery et al. 1998).

Because of the great potential for these compounds to kill nontarget vertebrates that may come in contact with

them, they were designed to degrade fairly rapidly. Many are unstable and degrade rapidly in water. However, compounds such as avitrol (Kamrin 1997), some anticoagulants, and compound 1080 require months to decompose in soil. Many of these compounds, such as DRC-1339, are stable in water as well (Kimball and Mishalanie 1994). Soil degradation can last from hours to months depending on the compound and climatic conditions. Avitrol degrades slowly in sunlight under dry conditions and in flooded soils, but 2.5 cm of rain will wash it away (Betts et al. 1976). Not only is environmental degradation important, but also persistence within the target species. For example, the half-life of bromadiolone in Norway rats (*Rattus norvegicus*) is up to 58 hours (Kamil 1987) allowing for potential exposure to predators and scavengers.

Secondary poisoning has been documented or considered possible for many vertebrate pest control compounds. Barn owls (*Tyto alba*) are particularly sensitive to DRC-1339, but the residues present in dead birds are usually too low to cause secondary poisoning (Johnston et al. 1999). However, bromadiolone and chlorophacinone have been implicated in secondary poisoning of many predators and scavengers (Berny et al. 1997, McDonald et al. 1998). Avitrol has been shown to be a potential hazard to sharp-shinned hawks (*Accipiter striatus*) and American kestrels (Holler and Schafer 1982).

Vertebrate pest control chemicals include a wide range of compounds with a wide range of behavior in different environmental compartments. Anticoagulants and acute toxicants tend to be nonvolatile whereas fumigants are highly volatile. Fumigants generally are unstable in water, anticoagulants are stable in water, and acute toxicants vary in their water stability. All are fairly stable in dry soil, but fumigants degrade quickly in wet soil. For example, both aluminum and zinc phosphides release highly toxic phosphine gas when in contact with water (Kamrin 1997).

Pest control chemicals vary greatly in mobility in general and specific media alter their mobility. Anticoagulant rodenticides generally are not mobile in any environmental media, while fumigants are mobile in air, but not in water (Kamrin 1997). Acute toxicants are not mobile in air and vary in their mobility in water. Compound 1080 is highly mobile in water because it is highly soluble. However, because of its high adsorption onto soil particles, it does not penetrate deeply into soil (Irwin et al. 1996). Other acute toxicants such as avitrol exhibit moderate water solubility and are not highly mobile in water.

Natural Plant/Animal Toxins

Natural toxins are toxic chemicals produced by living organisms, such as bacteria, blue-green algae, fungi, marine invertebrates and fishes, vascular plants, and poisonous aquatic and terrestrial animal species. Exposure to certain natural toxins, especially natural plant toxins, may have significant impacts on wildlife. There are many different natural plant toxins, also known as secondary plant compounds, which can be highly toxic to wildlife causing mortality and morbidity. Some plant toxins are used as the basis for pesticides (e.g., nicotine, pyrethrum, rotenone, etc.) demonstrating their acute toxicity (Ray 1991). Several factors are involved in exposure of wildlife to natural plant toxins. For example, different portions of the plant (root, stem, leaves, seeds) often contain different concentrations of a chemical. Plant age, climate, soil, and genetic differences within a plant species are also important factors. Examples of natural plant toxin chemical groups that can be highly toxic are alkaloids, tannins, phenols, lectins, glycosides, and terpenes. Generally, wild herbivorous animals have adapted to avoid or efficiently detoxify endemic toxic plants and are not impacted by exposure to these toxins (Vangilder 1983). However, there have been a number of documented cases of poisoning of wildlife by plant toxins (Ray 1991, Wickstrom 1999, Norton 2001).

Three groups of microscopic organisms, bacteria, algae and fungi, are capable of producing some of the most deadly toxins known. Probably the most significant natural toxin in terms of wildlife mortality/morbidity is botulinum toxin from the bacteria *Clostridium botulinum* (types C and E). Type C botulism causes mortality and morbidity in thousands of waterfowl across the United States and Canada each year, while Type E botulism has largely been restricted to causing mortality of fish-eating birds (bald eagles, loons, grebes, gulls) in the Great Lakes (Friend and Franson 1999, Roffe and Work 2005). The botulinum toxin generally is formed under conditions of low environmental oxygen and is considered to be the most toxic substance known. Waterfowl, especially dabbling ducks, are most susceptible to Type C botulism, but American coots (*Fulica americana*), gulls, and shorebirds (Order Charadriiformes) are also commonly killed during an outbreak. In Canada, annual losses of waterfowl in the prairie provinces can reach 100,000–1,000,000 birds (Wickstrom 1999). The neurotoxins produced by *C. botulinum* cause a paralytic effect in birds, which show signs of weakness, dizziness, inability to fly, muscular paralysis, and respiratory distress (Friend and Franson 1999, Roffe and Work 2005)

Blue-green algae (cyanobacteria) blooms commonly occur in fresh and brackish water worldwide. Wildlife that inhabit stagnant, eutrophic, water bodies especially during warm, sunny weather are most susceptible to algal toxins. Algae in the genera *Nostoc*, *Oscillatoria*, *Anabaena*, and *Microcystis* produce hepatotoxic cyclic peptides that disrupt the structure of liver cells, causing massive hemorrhage and necrosis leading to shock and death within hours. Algae in the genera *Anabaena*, *Aphanizomenon*, and some *Oscillatoria* produce potent, rapid-acting alkaloid neurotoxins. Anatoxin-a is a potent cholinesterase inhibitor, which causes permanent depolarization of post-synaptic membranes and disrupts nerve conduction, leading to muscle tremors, rigidity, paralysis, and death by respiratory failure within minutes. Exposure to this toxin could be confounding to analysis of cholinesterase activity due to organophosphorus or carbamate insecticide exposure.

Aphanitoxins, another group of neurotoxins, act by blocking sodium channels, which disrupts nerve conduction leading to muscle tremors, rigidity, paralysis, and death. This group appears to be identical to saxitoxin and neosaxitoxin, the causative agents of paralytic shellfish poisoning in humans. In marine systems, harmful algal blooms produced by phytoplankton containing protozoans (mainly dinoflagellates) together produce some of the most potent toxins known including domoic acid, brevetoxins, and saxitoxins. These compounds are concentrated in shellfish, are highly neurotoxic, and are commonly lethal to mammals at levels of 1 μg/kg (ppb) or less. In North America, harmful algal blooms have been responsible for

the death of wildlife in freshwater and marine systems including waterfowl, colonial waterbirds, and other bird species, wild canids, white-tailed deer, sea turtles, manatees (*Trichechus manatus*), pinnipeds, and whales (Friend and Franson 1999, Wickstrom 1999, Dierauf and Gulland 2001).

Fungi are also known to produce extremely toxic substances collectively known as mycotoxins (O'Hara 1996). Generally, wildlife is exposed to mycotoxins through contaminated feed. Although effects on wildlife can be significant, reports of poisoning by mycotoxins are relatively rare because it is difficult to establish a diagnosis in the field. Aflatoxins, produced by the fungus *Aspergillus flavus* (or *A. parasiticus*), are among the most toxic of the mycotoxins and are common contaminants of corn, peanuts, and other cereal and oil seeds. Wildlife is at risk from eating waste grain, especially during times of restricted access to other feed or forage. The trichothecenes is another group of mycotoxins produced by fungi in the genera *Fusarium*, *Cephalosporium*, *Myrothecium*, and *Trichoderma*. These sesquiterpene compounds include T-2 toxin, diacetoxyscirpenol, and vomitoxin and act to inhibit protein synthesis, targeting rapidly dividing cell types in the skin, intestine, hematopoetic (bone marrow), and lymphoid tissues. These toxins are known to cause anorexia, dermal, oral, and gastrointestinal necrosis and ulceration, hemorrhage, and impairments of the reproductive and immune systems. Other mycotoxins that may have adverse effects on wildlife include fumonisins, zearalenone, ochratoxin A, ergot alkaloids, and sporidesmin. Although data on the role of mycotoxins in wildlife mortality/morbidity are rare, *Fusarium* (trichothecene) mycotoxins on waste peanuts were implicated in a mass mortality of sandhill cranes involving 9,500 birds in New Mexico and Texas between 1982 and 1987 (Windingstad et al. 1989, Friend and Franson 1999). In this case, the most common visible sign was an inability to hold the head erect while standing or flying. Multiple muscle hemorrhages and submandibular edema were the predominant lesions at necropsy (Windingstad et al. 1989).

CONTAMINANT DIAGNOSTICS

Safety

Personal safety is a primary concern in a wildlife mortality/morbidity incident. Field investigators should not handle carcasses, collect environmental samples, or enter the area of the incident until adequate safety precautions have been taken. If the causative contaminant is known, a Material Safety Data Sheet (MSDS) or other Occupational Safety and Health Administration (OSHA) safety publications can provide the level of personal protective equipment required. For pesticides, the product label will provide the necessary information. Since some environmental contaminants may produce cancer, reproductive impairment, or birth defects in humans, which would not become immediately apparent, the results of not adequately protecting investigators can be severe and long lasting.

Field biologists should take safety precautions when investigating possible wildlife contaminant or disease mortality/morbidity incidents. If contaminants are suspected, proper protective clothing for the contaminant type should be worn. As a general rule, impermeable gloves and protective footwear (generally rubber boots) should be worn at incident sites. Some contaminants are readily dissolved in water and can readily penetrate the skin. Therefore, field investigators should keep bare skin protected and should not wade into shallow water. When retrieving carcasses or debilitated animals from water, impermeable gloves and rubber boots should be worn.

Short pants or short-sleeved shirts should not be worn, bare skin should be protected, and dust or fumes should not be inhaled. In wet conditions, waterproof pants may be required. Dust masks or respirators may also be required as well as impermeable clothing (e.g., TYVEK® coveralls or full suit), depending on the situation. In hot or humid weather, this type of equipment can be problematic to the person(s) wearing it, so common sense is needed to prevent heat stress or dehydration. If clothing becomes contaminated, once the contaminant type has been confirmed, it should be washed or discarded. For some contaminants, washing is not sufficient to allow continued wearing.

If disease, rather than contaminants is suspected, caution is still required, but the precautions are not as extensive (Roffe and Work 2005). It must be remembered that many contaminants in the environment are toxic to many different taxa, including humans. Further, some wildlife diseases can be transmitted to humans, but diseases generally are more species-specific than contaminants. This species-specificity may provide some support and clues as to whether an incident was contaminant- or infectious disease-mediated.

Initial Site Reconnaissance

Three rules govern initiation of any wildlife mortality/morbidity investigation: (1) protect yourself and others involved, (2) obtain the best case history possible, and (3) collect the best specimens possible. Handling and collection of specimens in the field will affect what the laboratory can (and cannot) do with them. Whenever possible, notify a wildlife veterinarian or other trained personnel and wait for their arrival before initiating the incident investigation. If this is not practical prior to starting the incident investigation, an initial reconnaissance of the site can direct the subsequent investigation and save time and money. During the initial reconnaissance, it is critical to assume there will be legal implications of the investigation and that the cause may be a highly toxic or contagious agent. Field notes and documentation that begin with the initial stages of the investigation are critically important and impact the entire investigation that follows.

An initial identification of the agent causing the incident should be attempted. (1) Is there reason to believe contaminants are the source? The approach to investigate and collect samples from a disease or contaminant incident differs. (2) Is the incident centralized and is it down slope, downstream, or downwind from a likely point source? (3) Is the incident on or near agricultural lands? In an agricultural setting, the crops in the area would be a starting point for what pesticides might have been applied. Early identification of the contaminant can dictate the safety precautions needed and direct the types of samples that should be collected and how they should be handled. If the source and cause of the incident are not immediately obvious, the field investigator should err on the side of safety and collect samples in the most inclusive manner within the constraints of time and expertise.

As a starting point to decide whether the cause is a disease or contaminant, the species affected should be consid-

ered. If a single species or group of species is affected, it is more likely disease. For example, botulism may be indicated if only ducks are found dead or debilitated while other species appear unaffected. However, if many unrelated species are affected, it is more likely a contaminant. Field biologists should carry an immediate response kit with them at all time. This would include protective (e.g., TYVEK®) coveralls, respirator or dust mask, plastic or rubber gloves, rubber boots, dark glass collection bottles or jars, and plastic bags. This kit could be kept in a waterproof container that can be securely closed to prevent contamination.

Upon initial discovery of the wildlife mortality/morbidity site, the nearest wildlife contaminant or disease expert should be contacted immediately. Experts in these areas may be at a teaching/research wildlife hospital or state or federal agency. In the case of pesticides, a county extension agent may be helpful.

Mortality

Personal safety must be the primary consideration before attempting to collect carcasses, samples, or spending any time at the site of the incident. If an environmental contaminant is present in sufficient concentrations to kill or debilitate wildlife species, it may also pose a health hazard to the field biologist.

Locating carcasses, especially of small, secretive species, can be difficult. Therefore, finding one or a few carcasses should not preclude the possibility that many additional animals could have been poisoned and either removed by scavengers or moved to another area prior to death. Once dead animals are found, the immediate goals are to prevent further deaths and to identify the cause and source of the environmental contaminant(s) involved. It may not be possible to accomplish the former without first determining the latter. An immediate search of the area for intoxicated/sick animals can be instructive in identifying the cause by observing their appearance, movements, and behavior. Detailed observations may also provide an opportunity to provide care for their recovery.

In many cases, exposure to environmental contaminants is obvious. Most likely, dying birds and mammals observed drinking irrigation runoff water from a field recently sprayed with an organophosphorus insecticide were poisoned. Aquatic birds, mammals, or other wildlife species found dead in a containment pond from a cyanide leaching process most likely died from exposure to cyanide. However, no matter how obvious these causal associations may seem, it is imperative that both carcasses and samples of the apparent source of exposure be chemically analyzed for evidence of environmental toxicants. In other cases, exposure to environmental contaminants is not as obvious. A colonial waterbird rookery with almost complete nesting failure the spring following a severe winter may not be due to the colony being exposed to applications of pesticides in the area but to exposure of the adults to organic chemicals remobilized in the environment. This could result from severe scouring of nearby river sediments during heavy winter flows (American Society for Testing and Measurement 1997).

The risk of chemical contaminants to wildlife is dependent on toxicity, concentration, and route of exposure. Acute toxicity of insecticides and vertebrate pest control chemicals (rodenticides, avicides) to wildlife is high, whereas the acute toxicity of herbicides is low. Exposure routes in wildlife include oral, dermal (including ocular, or through the eyes), and inhalation as well as from maternal sources (deposited in eggs, pass through the placenta). For mammals and birds, the most common route of exposure is oral, where contaminants are ingested through the mouth. In addition to consumption of contaminated food items, birds and mammals sprayed directly or exposed to an aerosol suspension of a pesticide would result in oral exposure through preening and grooming behaviors, respectively, which would result in oral ingestion. Secondary poisoning through consumption of contaminated prey items by predatory and scavenging wildlife species is a relatively common occurrence. Mammals and birds can also readily absorb pesticides directly through their feet by standing or perching on a contaminated substrate. This has been shown with red-tailed hawks foraging in orchards during the winter following applications of organophosphorus insecticide dormant sprays (Hooper et al. 1989). Perching behavior in birds has been exploited by avian pest control operators who target perches with toxic chemicals specifically for dermal exposure through the feet. Mortality incidents in birds and mammals through inhalation are difficult to document and relatively uncommon.

Morbidity

Discovering intoxicated or sick (morbid) animals presents the field biologist with a situation where action has to be taken. Species of wildlife that are intoxicated or sick from exposure to environmental contaminants may be able to fully recover. Depending upon the environmental contaminant involved and the concentration, duration, and route of exposure, the negative effects on wildlife may or may not be reversible. However, during a wildlife mortality or morbidity incident, there is the chance that exposed animals have been seriously poisoned and may need to be euthanized (Friend and Franson 1999, Dein et al. 2005).

Treatment or transport of many wildlife species, particularly birds, requires one or more permits. Additionally, a salvage permit is often required to collect dead animals. Before collecting either carcasses or live animals, the necessary permit(s) must be obtained as well as knowledge as to how to transport specimens or animals. It is also important to know where the specimens or animals are to be taken, particularly if the animals are still alive. Treatment of intoxicated or sick animals by wildlife rehabilitators requires specific permits. Most veterinarians are not equipped to accept and treat wildlife species, as they do not have the facilities to hold animals apart from their routine domestic patients. Wildlife rehabilitators generally are registered with state wildlife agencies, which can provide a list of wildlife rehabilitators for a given area. Prior to collecting morbid animals, the destination must be identified and appropriate transport containers obtained that will safely hold the animals and provide comfortable conditions. Allowing animals to die from improper care during transport is not acceptable. It may be better to humanely euthanize an animal than to subject it to unnecessary stress because it is not possible to provide adequate care during transport.

Wildlife species that are intoxicated or otherwise sick from exposure to environmental contaminants invariably demonstrate clinical signs of the poisoning (Table 1). Although many clinical signs from exposure to environ-

Table 1. Overview of clinical signs exhibited by wildlife species by general environmental contaminant group.

Clinical signs	Metals	Organic chemicals	Anti-ChE insecticides	Anti-coagulant rodenticides
Ataxia (loss of coordination)	X	X	X	X
Muscular weakness			X	
Tremors	X	X		
Convulsions		X	X	X
Lethargy	X	X	X	X
Hyperactivity	X			
Reproductive effects	X	X	X	
Developmental abnormalities	X	X	X	
Reduced fertility	X	X	X	
Spontaneous abortions	X	X		
Excretory effects		X	X	
Excess defecation			X	
Bloody feces		X		X
Diarrhea			X	
Spasmodic contraction of anal sphincter			X	
Emesis (vomiting)		X	X	
Anorexia (weight loss/emaciation)		X	X	
Excessive thirst			X	
Nasal secretions			X	
Epistaxis (bleeding from nares)			X	X
Salivation		X		
Edema	X	X	X	
Anemia	X	X		X
Skin lesions		X		
Immunotoxic response		X	X	
Depressed ChE			X	
Behavioral effects		X	X	
Altered behavior			X	
Unkempt appearance		X		
Hypothermia			X	
Coma		X	X	X
Paralysis			X	X
Internal bleeding				X
Dyspnea (labored breathing)		X	X	X
Tachypnea (rapid breathing)			X	
Eye/vision problems	X	X	X	
Blindness	X	X	X	
Contraction of pupils			X	
Dilation of pupils			X	
Ptosis (drooping of eyelids)		X	X	
Protrusion of eyes			X	
Lacrimation (excessive tears)			X	
Head and limbs arched back			X	
Piloerection (erection of contour feathers)			X	

mental contaminants are somewhat general in nature, the suite of responses exhibited in a given situation can be quite useful as a piece of the puzzle in diagnosing the group of contaminants responsible for the intoxication or sickness.

The Wildlife Contaminant Investigation

Circumstances involved in a contaminant-related wildlife mortality/morbidity incident and appearance of exposed wildlife are difficult to distinguish from those caused by disease or natural causes. For example, certain wildlife diseases may resemble wildlife mortality/morbidity caused by contaminants, including botulism, salmonella, trichomoniasis, mycotoxicosis, and duck virus enteritis (American Society for Testing and Measurement 1997). Investigators should rely on a wildlife disease specialist to obtain a definitive diagnosis if disease is suspected (Roffe and Work 2005). Investigations of wildlife mortality/morbidity suspected to be caused by contaminants should proceed as though the cause was unknown. All factors must be checked or eliminated unless there is solid evidence to support specific conclusions.

If only a few carcasses are involved, external examination is necessary to rule out natural (e.g., predation) or

accidental causes. Thus, it is important to be able to differentiate between evidence left by scavenging and true predation. This may not be possible, but should be attempted. It is possible that predation was successful because the animal was impaired from disease or exposure to an environmental contaminant. Thus, overall condition of the carcass can be important. A wasted or unkempt appearance could be indicative of impairment prior to predation. Large numbers of carcasses are likely related to either disease or environmental contaminants, but could be the result of an accidental mortality (e.g., bird collisions with communication towers or other man-made structures, road kills). Therefore, accidents should be considered before assigning the cause to disease or environmental contaminants.

The initial decision as to whether or not a wildlife mortality/morbidity incident is likely contaminant-related is a process of elimination. If there are no other plausible explanations for the incident, the site should be investigated for contaminants or diseases. Locating and contacting someone with experience in differentiating between disease- and contaminant-related mortality is highly desirable. Thus, it is essential to document the incident with detailed field notes and photographs.

The investigator(s) often can obtain a substantial amount of information from the individual(s) reporting the incident, including the extent, whether a field response is necessary, and whether the contaminant(s) may cause more widespread wildlife mortality/morbidity. Important factors in interpretation of the incident scene include location, time and date of incident, species involved, number of dead and/or sick animals, rate of deaths (e.g., did they occur over a short or long period of time), chance of continuing mortality/morbidity, clinical signs observed, climatic conditions (e.g., precipitation, temperature, winds) preceding the incident, and any recent change that has occurred. Recent changes in land use, agricultural practices, insect outbreaks, evidence of recent pesticide applications, or other factor in the area of the incident should be noted as well as other similar incidents in this area and the observations of the person(s) reporting the incident. This information should allow the investigator to decide whether or not the incident warrants a field investigation. A specific case number should be assigned to each investigation and used on all labels, tags, data sheets, photographs, and other records related to the incident. The investigator must rely upon their best professional judgment as to the intensity of the field investigation and the individuals and agencies to contact.

The investigator's interpretation of the wildlife mortality/morbidity incident scene will affect the type, number, and location of samples taken and the analyses performed. The first few hours after arrival on the scene are most critical and information should be collected as soon as possible. This is especially important when an incident occurs in association with flowing waters of ditches and streams. One reason is that some chemical contaminants, such as most organophosphorus and carbamate insecticides degrade relatively quickly and chemical and diagnostic signs present at the site (e.g., sick or dying animals and water conditions) may rapidly disappear.

Wildlife mortality/morbidity incidents may be a result of illegal activities, such as a pesticide applied to intentionally kill wildlife and, thus, have the potential to become a legal case. In any investigation, chain-of-custody documentation is required to demonstrate that evidence can be accounted for at all times (American Society for Testing and Measurement 1997). Chain of custody is defined as the witnessed, written record of all individuals who have maintained unbroken control over the evidence since acquisition. The chain of custody begins with the collection of an item of evidence and is maintained until its final disposal. Each individual in the chain of custody is responsible for items of evidence to include care, safekeeping, and preservation while under their control. Because it is possible that any item or specimen acquired during the investigation of a wildlife mortality/morbidity incident may have value as evidence, it is important to treat all specimens as evidence and follow chain-of-custody procedures.

FIELD PROCEDURES

Sample Documentation and Transport

It is critical that samples collected in the field are handled properly to ensure that useable information can be obtained for the best understanding of what may have caused the wildlife mortality/morbidity incident. All samples should be double bagged with a label on the inner bag or placed between the bags. By labeling the inner bag, if the label somehow becomes detached, the outer bag will keep the label with the sample. If adhesive labels are not available, the information can be recorded on notebook paper and included between the bags. Double bagging will help reduce dehydration and protect against loss of a sample should a bag inadvertently open during shipping or storage. Each specimen should be labeled with sample type, for example tissue type, species, plant, soil, etc. The sample location (both overall site name and location within the site), sample date and time, and the person's name that collected the sample must be included. This information is extremely important for subsequent follow-up and interpretation of the sample analysis.

Labels should be written clearly with indelible felt-tipped pens or other ink that will not smear when it comes in contact with water. Field biologists commonly use pencils for field notes because a lead pencil does not smudge when wet. However, when samples are being tracked for possible litigation, pencil is not acceptable as permanent labeling is required for all sample logs and sample labels. If permanent ink is not available for field records, it is best to make a photocopy of the sample log as soon as possible.

Samples should be placed on ice in the field as some contaminants can degrade quickly, for example in hours, and tissues or carcasses can deteriorate quickly at warm field temperatures. Once the samples are taken from the field, they should be hard frozen. When multiple specimens are available, some samples should be placed on ice for preliminary pathology analysis while the remaining specimens are frozen. The only exception would be animals that may have succumbed to disease. These should be cooled and shipped to a pathologist within 48 hours of collection (Box 2). Samples for contaminant analysis should be transported frozen or on dry ice. It is important that samples not thaw during shipment, because this may compromise subsequent contaminant or disease analyses.

Handling

The manner of handling field-collected samples can differ according to the likely contaminant type. Metals gen-

Box 2. Recommended laboratories for fish and wildlife mortality/morbidity incidents.

USA

New York State Department of Environmental Conservation
Wildlife Resources Center/Wildlife Pathology Unit
108 Game Farm Road
Delmar, NY12054
T 518-478-3032; http://www.dec.state.ny.us/webvsite/dfwmr/habitat/wpu/htm

Southeastern Cooperative Wildlife Disease Study
Wildlife Health Building
College of Veterinary Medicine
University of Georgia
Athens, GA 30602
T 706-542-1741; FAX 706-542-5865
http://www.uga.edu/scwds

U.S. Department of Agriculture
Animal and Plant Health Inspection Service (APHIS)
National Wildlife Research Center
4101 La Porte Avenue
Fort Collins, CO 80521
T 970-266-6000; FAX 970-266-6032
http://www.aphis.usda.gov/ws/nwrc

U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
1315 East-West Highway
Silver Spring, MD 20910
T 301-713-2332; FAX 301-713-0376
http://www.nmfs.noaa.gov/strandings.htm

U.S. Department of the Interior
Geological Survey
National Wildlife Health Center
6006 Schroeder Road
Madison, WI 53711
T 608-270-2400; FAX 608-270-2415
http://www.nwhc.usgs.gov,
http://www.nwhc.usgs.gov/best/index.html

U.S. Department of the Interior
Fish and Wildlife Service
National Fish and Wildlife Forensic Laboratory
Ashland, OR 97520
T 541-482-4191; FAX 541-482-4989
http://www.lab.fws.gov

U.S. Environmental Protection Agency
National Health and Environmental Effects Research Laboratory (NHEERL)
109 TW Alexander Drive
Durham, NC 27709
T 919-541-4577; FAX 919-541-1831
http://www.epa.gov/nheer, http://www.epa.gov/ecotox

CANADA

Canadian Cooperative Wildlife Health Centre
Western College of Veterinary Medicine, Veterinary Pathology
University of Saskatchewan
Saskatoon, SK, S7N 5B4 Canada
T 306-966-5099, 800-567-2033 (Canada);
FAX 306-966-7439
http://wildlife1.usask.ca/ccwhc2003

Environment Canada
Canadian Wildlife Service
National Wildlife Research Centre
Wildlife Toxicology Division
Carleton University
Ottawa, ON K1A 0H3 Canada
T 819-997-2800, 800-668-6767 (Canada);
FAX 819-953-2225
http://www.cws.ec.gc.ca/nwrc-cnrf/toxic/index_e.cfm

erally do not tend to adhere to plastics, nor will storage in plastics interfere with the analysis by the chemist. It is acceptable to use polyethylene bottles for sample shipping and storage with a 1 L bottle an appropriate size. It is important to acidify water samples to prevent degradation, but only when it is known that metals are the contaminant. Acidification can make other water sample types useless.

Organics, including pesticides, can readily adsorb onto or absorb into plastic and plasticizers can leach from the container into a water sample confounding the subsequent chemical analysis. Thus, it is best to use glass when sampling organics, including pesticides. Depending on the type of organic contaminant present, at least 40 ml and up to 2 L should be collected. If freezing without damaging the container is not possible, the sample should be cooled to 4ºC for storage and shipping. At least 186 g of soil should be collected and frozen. Some pesticides may have a tendency to migrate down through soil. If this is considered likely, a soil core of up to 1 m in depth should be collected.

When animal tissues are collected, great care should be taken to prevent cross-contamination either from other samples or sources. Thus, only individuals experienced in dissecting animals for subsequent chemical analysis should do so. If the incident is legally contested and untrained individuals dissect the samples, damage can be done to the legal acceptability of the sample analyses. It is best to freeze the samples and allow specialists to perform the dissections.

Tissues can be placed into plastic bags or small glass sample jars that have sterile interiors and larger samples can be placed in zip-lock bags. Smoky-colored (dark) glass sampling jars should be used for soil, water, and sediment samples, particularly if pesticides or organics are involved. Using dark-colored glass is especially important when handling chemicals that undergo photodegradation. Plastic

containers should be avoided for samples that could contain pesticides or organics, as they tend to adsorb onto the plastic. Sampling equipment should be cleaned between processing and collecting samples to prevent cross-contamination. Gloves should be changed between samples or between groups of samples of similar contamination levels to present cross-contamination.

Record Keeping

A field log will be useful to make entries regarding each sample collected for analysis. Entries should include the sample identification number, type of sample collected, site name where collected, date, and the name or initials of the sample collector. These entries provide backup identification in case sample labels are damaged or lost, or if confusion ensues over when and where certain samples were taken.

Accurate record keeping is critical in documenting wildlife mortality/morbidity incidents. Detailed incident reports (Appendix) are essential to identification and confirmation of ecological risks associated with a particular chemical contaminant. Over time, incident reports provide information regarding those chemicals or agricultural practices that are involved most often in wildlife mortality/morbidity incidents as well as identifying species that are particularly sensitive to certain chemicals. Incident reports can also identify geographic areas or landscape variables most frequently impacted by specific chemicals. The more detailed information provided on the field data sheet, the better the chances the investigator(s) of the incident will be able to understand what happened. The importance of detail in the field data sheet, both to enhance accurate diagnosis and to assure that appropriate information is provided for forensic purposes, cannot be overemphasized. It is imperative to learn if the contaminant threat is still present and if there is a continuing threat of wildlife mortality/morbidity.

Sample Collection

In addition to wildlife tissue samples that are critical for identifying the cause of the mortality/morbidity incident, other environmental samples are also critical. Some contaminants may be metabolized quickly within an animal and the environmental samples may be the only place where the unaltered contaminant will be found. It is also possible the contaminants were encountered in a location some distance from where the carcasses were discovered. If the exposure occurs off-site, the actual contamination source must be located. Depending on the specific conditions of the situation, soil, water, and vegetation should be sampled. If possible, advice should be pursued on the proper sampling techniques for different sample types and for different contaminants.

Environmental samples should be collected from the immediate area of where dead or debilitated animals are found. Additionally, samples should be collected from areas where the contamination may have moved or have originated. It is possible that dead or debilitated animals are first found in a highly visible location, but that contamination may be greater elsewhere. Those experienced with site and contaminant types can provide advice on number of samples required and how far the samples should be collected from the original site.

Many contaminants act as an emetic when ingested. If vomitus or regurgitated material is found with the specimens, it should be collected. This will often contain high concentrations of the contaminant, possibly higher than in the carcass or gastrointestinal (GI) tract. In acute poisonings, contaminant residues are usually higher in the anterior GI tract than in the post-absorptive tissues.

Animal Tissues.—Animal tissues can be taken directly from necropsies while vomitus, urine/feces, blood, and hair/feathers can be collected at or around the mortality/morbidity site. Collecting samples from carcasses becomes more difficult as time elapses. Time since death is a critical factor as the onset of rigor mortis, decomposition, and scavenging by predators make tissue samples more difficult to obtain. Ideally, whole, fresh carcasses available for sampling tissues would be present at the incident scene, but this frequently is not the case. For instances where the whole carcass cannot be submitted and evidence suggests specific causes may be involved, tissue samples can be strategically taken and preserved during necropsy (Table 2). The best materials for establishing oral exposure to an acute toxicant are in the GI tract (crop and gizzard/stomach in birds, stomach in mammals and other wildlife species). Liver tissue, lipid (fat) deposits, and brain tissue generally are considered best for identifying the presence of toxic levels of many lipid-soluble contaminants such as organochlorine insecticides and PCBs, and for trace metals (lead, mercury). Brain tissue also is important for diagnosing anticholinesterase insecticide poisoning through measurement of cholinesterase activity. Keratin structures (hair, feathers, scales) are often used as nonlethal samples to detect chronic exposure to heavy metals, which may be contributing to an overall decline in fitness of the animals making them more susceptible to disease or other environmental conditions. Analysis of samples from other environmental media can also assist in establishment of routes of exposure and to identify occurrences of exposure to multiple toxic chemicals. In the case of predators and scavengers, it may be necessary to collect local prey species or scavenged carcasses to examine possible exposure.

If the animals are not dead but are intoxicated or otherwise sick, nondestructive techniques can be used to collect tissue samples, specifically blood, hair/feather/scale, and biopsies or other types of samples, such as foot washes (Fossi and Leonzio 1994). Waste materials such as urine, feces, and vomitus can be collected from debilitated animals found at the site by holding them in clean, ventilated containers for a period of time. Fecal and urinary products are useful for analysis of contaminants and can be evaluated for disease as well. For living birds, a foot wash with methanol or isopropyl alcohol and analysis of feathers can be useful for establishing exposure to an aerosolized chemical application, such as a pesticide. Typically, a foot wash must be performed within 48–72 hours of exposure to detect the presence of the chemical. After that time, most or the entire chemical will have been absorbed through the skin (Fossi and Leonzio 1994, Friend and Franson 1999, Millam et al. 2000).

If the cause of the incident is unclear or if causes in addition to contaminants are possible, animal carcasses need to be handled in different ways. Freezing animal tissues can cause damage to tissues that make disease identification by a pathologist difficult or impossible. However, failure to freeze tissues for contaminant analysis may allow the contaminant to degrade to the extent they will not appear to be present.

Table 2. Sample selection and preservation from necropsy when whole carcasses cannot be submitted and evidence suggests specific causes may be involved (adapted from Friend and Franson 1999).

Sample	Suggested tests	Preservation	Comments
Lesions	As appropriate	Frozen	Lesions are abnormal-appearing tissues; a portion of each lesion should be saved frozen; fixed tissue important.
Lesions[a]	Specimen is fixed, sectioned, and stained for microscopic study	10% formalin	A portion of each lesion should be saved frozen.
Liver	Metals, organics	Frozen	Entire liver of birds and small mammals, selected portions from larger species; fixed tissue important.
Liver[a]	Specimen is fixed, sectioned, and stained for microscopic study	10% formalin	Specimen portions should not exceed 6 mm in thickness.
Kidney	Metals	Frozen	Entire kidneys from birds and small mammals, selected portions from larger species; fixed tissue important.
Kidney[a]	Specimen is fixed, sectioned, and stained for microscopic study	10% formalin	Specimen portions should not exceed 6 mm in thickness.
Stomach contents	OP and carbamate insecticides, plant toxins, mycotoxins, strychnine, cyanide	Frozen	Save entire contents; samples to be checked for cyanide or other toxic gases must be placed in airtight containers.
GI tract[a]	Specimen is fixed, sectioned, and stained for microscopic study	10% formalin or Bouin's stain	Small piece of stomach at the ileocecal junction, piece of duodenum (near pancreas), and colon.
Brain	Cholinesterase activity, OC insecticide residues, organomercuric compounds	Frozen	For chemical analysis of brain, it must be wrapped in clean aluminum foil and placed inside a clean glass bottle; fixed tissue important.
Brain, nervous tissue, eyes[a]	Specimen is fixed, sectioned, and stained for microscopic study	10% formalin	Divide brain in half (sagittal); place ½ in formalin, save other ½ frozen.
Blood	Lead, cyanide, H_2S, nitrites	Frozen	Samples to be checked for cyanide or other toxic gases must be placed in airtight containers.
Gonads[a]	Specimen is fixed, sectioned, and stained for microscopic study	10% formalin or Bouin's stain	Specimen portions should not exceed 6 mm in thickness.
Lungs	Cyanide, H_2S	Frozen	Samples to be checked for cyanide or other toxic gases must be placed in airtight containers.
Heart, lungs, skeletal muscle, lymph nodes, spleen, thymus[a]	Specimen is fixed, sectioned, and stained for microscopic study	10% formalin	Specimen portions should not exceed 6 mm in thickness.

[a] Histopathological examination (microscopic).

If there are many specimens, some should be frozen (with dry ice) and others kept cool (with ice or refrigeration). Those set aside for disease evaluation should be kept cool and transported to a trained pathologist within 48 hours or less of collection. If transportation will require more than 48 hours, it is best to freeze all specimens. It is best to freeze carcasses that are already deteriorating or have become putrid to prevent contamination. None of the carcasses should be dissected prior to sending them to the pathologist.

Plant Tissues.—Plant residues may be important in identifying how exposure may have occurred and the extent of the contamination. Some contaminants may accumulate in plants via uptake from the roots; however, many others may be present primarily as surface residues. For those contaminants that are most likely to be deposited on plant surfaces, care must be taken not to dislodge the residues from plant surfaces during collection. Contamination during collection of plant samples is a greater concern if surface residues are present.

When collecting plant samples, the plants should be handled as little as possible to prevent dislodging any contaminant residues. If possible, the entire plant including the roots should be collected as they may contain the highest residues making identification of the contaminant more likely. Samples should be collected from both on- and off-site areas. Cross-contamination among samples can be reduced by starting in the least contaminated areas. Consideration should be given to separating animal food items, such as seeds or fruits, from leaves and stems if it will help with the follow-up investigation. Samples should be frozen as soon as possible and remain frozen during shipping and storage until contaminant analysis.

Soil.—Soil samples can be useful for measuring the extent and levels of environmental contamination. Samples should be collected from the immediate vicinity where dead or debilitated animals were found. Depending on the specifics of the incident, soil should be collected at different distances from the site. Samples should be collected off-site if movement is possible, particularly up or down hill (or up or down wind). Some contaminants have a tendency to move down through soil and may contaminate groundwater. If possible and appropriate, collect soil core samples to a depth of 1 m. Samples should be collected first from areas thought to be least contaminated and then in those areas of highest contamination. It is surprisingly easy to contaminate samples from sampling equipment and even clothing and footwear. All soil samples should be frozen, if possible, at time of collection. If prompt freezing is not possible, the soil should be placed on ice and frozen as soon as practical.

Water/Sediment.—Water samples are useful for identifying the extent and levels of environmental contamination. Glass containers should be used to sample water as some contaminants adhere to or absorb into plastics. Samples should be protected from light and glass should either be brown or wrapped in aluminum foil. Samples should be collected from the immediate incident area (e.g., pond) and up or downstream. Samples can be collected from nearby surface water as appropriate. However, care should be taken that water samples contain no soil or other debris. Containers should be about half-full to prevent cracking from expansion during freezing, labeled, and placed in a plastic bag. Samples should be frozen immediately, if possible, but can be cooled to 4 C for shipping. During freezing, glass containers should be stored upright. Containers should be shipped upright, and frozen or cooled to 4 C.

Air.—Air might be the most difficult environmental factor to sample in the field. For soil, water, or vegetation as long as adequate sample amounts are collected, only portions of the sample are required for subsequent analyses. It is impractical to collect a sample of the air to provide to a chemist for analysis. Since air cannot be taken from the field, contaminants must be extracted from the air or measured during a field visit.

The concentration of a contaminant in the air is measured from a known volume of air sampled in the field. This requires a calibrated air pump or detector. Faulty calibration or leaks will produce inaccurate measurement of the volume sampled and inaccurate reporting of the concentrations of contaminants. Monitoring equipment must be checked for air leaks and proper calibration prior to monitoring for contaminants in the field.

Direct measurement of aerial contaminants in the field requires an instrument capable of detecting the presence and concentration of the specific contaminant of concern. If the contaminant of concern is not known before attempting air monitoring, selection of the proper detector will be difficult. Also, some detectors are designed for human health and safety and report only if a contaminant exceeds safe levels for humans. This might not be helpful when the level harmful to wildlife often is not known. Other detectors are designed for monitoring organic compounds and might not detect inorganics well and vice versa.

It is also possible to extract the contaminant from the air and provide the media to a chemist for analysis. Since concentration is based on the volume of air sampled, it is critical that the volume be accurately measured and recorded by using a pump calibrated to move a known volume of air during a specific time period (e.g., ml air/second) for a known period of time. The air being sampled must be pushed or pulled through a filter or liquid capable of extracting the contaminant. For many organic compounds, bubbling air through a solvent like hexane can be an effective sampling procedure. Filters also are available to remove many organic or inorganic compounds. However, the filter must be capable of capturing all the contaminant from the sampled air. The concentration will be under reported if the capacity of a filter is exceeded. Assistance from someone with experience in air quality monitoring will likely be necessary to ensure that measured air concentrations are accurate.

Chemical Residue Analysis.—Residue analysis is expensive and there are many aspects to consider including detection limits, quality assurance and control, how to read and evaluate a laboratory chemical analysis report, and how to interpret the toxicological data. There are 2 types of detection limits to be considered: instrument and method detection limits. Differences between instrument detection limits are a result of detector sensitivity, chromatograph system that precedes it, and the injection method. Method detection limits represent the best performance consistently achievable from a method in a particular laboratory with a given set of instrumentation. Method detection limits are a function of the clean-up and extractive procedure and, thus, more closely allied to the chemist's standard operating procedures and technical abilities. Standard operating procedures vary by detector and chemical based on the relative polarity of the chemical and the environmental media in which it is found.

Interpretation of residue analysis data can be frustrating. Overall, we know relatively little about how body residue levels of environmental contaminants correlate to corresponding effects seen in wildlife species. One excellent source of information on interpretation of residue analysis data is Beyer et al. (1996). This is the first major attempt to make sense of residue analysis data as related to accompanying effects.

SUMMARY

A wide variety and substantial volume of chemical contaminants as well as natural plant and animal toxins are present in the environment and frequently have been shown to have negative impacts on wildlife. As a result, wildlife mortality and morbidity incidents will occur. Thus, there is a strong need for field biologists to be able to adequately identify and handle these incidents. Few biologists receive

training in the field of environmental or wildlife toxicology as this area of interest is relatively specialized. Thus, it is important that field biologists understand and have a source for standard operating procedures for successfully handling wildlife mortality/morbidity incidents. The goal of this chapter is to provide wildlife biologists with guidance on understanding wildlife toxicology and procedures that should be followed when confronted with a wildlife mortality/morbidity event. It is also important for biologists to have additional sources of information as well as locations of wildlife mortality/morbidity incident databases.

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(Appendix on next page.)

APPENDIX

Sample Wildlife Mortality/Morbidity Incident Field Data Sheet (modified from Friend and Franson 1999).

Date: _____

Submitter's name: _____

Submitter's affiliation: _____

Submitter's contact information: _____

Date collected: _____

Method of collection:_____
(found dead, euthanized; if euthanized, technique used)

Incident scene biologist: _____

Incident location: State: _____ County: _____ Lat/Long: _____

Specific incident location: _____

Incident area description: _____
(land use, habitat types, etc.)

Environmental factors at incident site: _____
(climatic conditions, description of waterbodies, evidence of chemicals, etc.)

Time of onset of incident (date and time): _____
(best estimate)

Species affected: _____

Species that appear unaffected (if known): _____

Age/sex of species affected: _____

Number known dead of each species: _____

Mortality/Morbidity ratio: _____
(#dead/#sick)

Estimated dead: _____
(consider scavengers, other removal)

Clinical signs: _____

_____

Species at risk: _____

Additional information and observations/comments:_____

_____