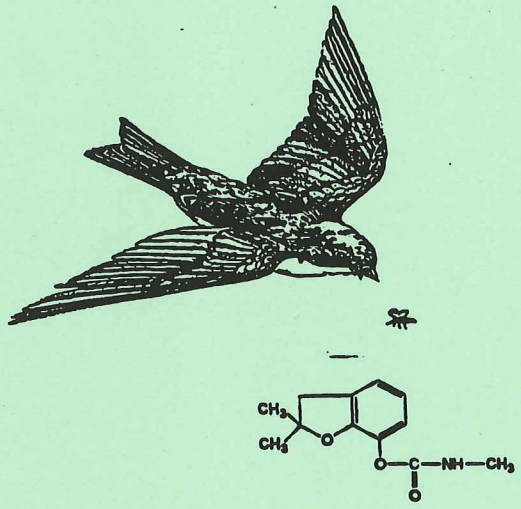

WILDLIFE TOXICOLOGY

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Is Toxicology Important for Wildlife Populations?

Judit Smits

The field of wildlife ecotoxicology arose from a combination of wildlife ecology and wildlife management, rather than from the biomedical professions (as is the case in other branches of toxicology). This still is very evident today as our wildlife agencies generally take on the lead role in the field when it comes to environmental pollution.

In the discipline of wildlife toxicology, there are two main goals: 1) we want to determine if an apparent die-off, population decline, or other problem in wild species is due to exposure to an environmental contaminant or toxin; and 2) we want to be able to predict what environmental concentration of a certain chemical or toxin is likely to result in exposure to, and adverse biological effects in, certain wildlife. Also it is valuable to understand the pathways of such exposure, i.e., is exposure likely to be through the air? food chain? water? Both goals require a combination of field and laboratory studies to describe a cause and effect relationship with any certainty. Chemical analysis are often used to identify the contaminants, but it may not be possible to determine what to ask for, or, in the case of mixtures, it may not be particularly relevant, or won't produce an unequivocal answer.

Wildlife species are important natural resources, as well as indicators of environmental health. We can define wildlife as including a huge array of plants and animals, but for our purposes we'll consider birds, mammals, reptiles, amphibians (and fish) - which restricts us to a mere 2166 species in North America! Couple this with the differences in the life history, physiology, life span, ecology, and sensitivity among animal species, and the challenge in wildlife toxicology becomes apparent.

There are several factors that effect the level of exposure to contaminants that certain species may experience:
1. different geographical ranges (migration corridors)
2. habitat (wetlands, boreal forest)
3. diurnal activity patterns (use of farmed areas and early morning pesticide application)
4. feeding habits and trophic level (bioaccumulation & biomagnification)

Physiological differences in animals will have a great impact on how any particular contaminant will affect them:
1. different mechanisms for detoxification
2. different amounts of the contaminant that would be absorbed through gut or skin
3. how animals get rid of some of their body burden of contaminants
4. different life stages may have greatly varying sensitivity
5. reproductive differences

Besides the complexity of evaluating effects of contaminants in wildlife, there is also a great range of the types and mixtures of xenobiotics (chemicals that are foreign to animals' bodies) in any particular "area of concern"; e.g., industries of Hamilton Harbour *vs* agrochemicals of the prairies *vs* radioactive compounds associated with uranium mining. . .

The beginning of wildlife (eco)toxicology

The Great Depression of the 1930s was the inadvertent beginning of this discipline. The combination environmental / economic disaster lead to desperate agricultural practices that resulted in severe depletion of wildlife populations. This was recognized by both the US Fish and Wildlife Service and the Department of Agriculture, which worked together to develop and support new farming methods that would not only be economically viable, but conserve soil, water and wildlife populations. One of the "great developments" of this time was the agricultural saviour, the newly released insecticide, DDT. It took 30 years to being the understand the impact of his compound, and although it was banned in the developed world since the early 1970s it is still used in many poor countries because it's cheap and effective against malarial mosquitoes for example. DDT and other persistent organochlorine compounds that are currently found in polar bear and seal tissues including milk, and in the milk of Inuit mothers in Canada's north, originate largely in the tropics.

Among the first reports of anthropogenic (human made) environmental contaminants affecting free-ranging wildlife was during the industrial revolution of the 1850s. In Germany, fallow deer which were inhabiting an area downwind from a silver foundry, were dying at an unusually high rate. They were being poisoned by the arsenic in the emissions coming from the foundry. At about the same time in the "new world", there were large die-offs of birds and mammals around an oil Field in Texas. The killer in that case was hydrogen sulfide fumes coming from the wells. Even in the last (19^{th}) century in the United States there was convincing evidence that mortality in waterfowl and pheasants was due to spent lead (Pb) shot.

A new generation of environmental toxins

The new generation of synthetic organic pesticides was one of the products of World War II 'research'. As mentioned above, DDT and spin-off chlorinated hydrocarbons were among the first and most notorious of those compounds introduced during the war. Unfortunately, unlike the dramatic die-offs seen with more acute (more directly lethal) poisons, these compounds had subtle ways of causing damage, which is why it took decades to recognize what was happening and why. There was documentation of the population declines in the 1950s and 60s in song birds, birds of prey, and brown pelicans in Europe and North America. Charles Broley, a banker from Winnipeg and a devoted and brilliant naturalist was one of the first to document the population decline in Bald Eagles. He began banding birds in 1939, on the Gulf coast of Florida. In 1940 he recorded 140 active nests in a certain area, and banded 150 eaglets from 105 nests there. In 1952, in the same area he banded a total of 15 eaglets in 11 nests. There were still many adult birds around, but they were not reproducing. He also observed reproductive failure at his cottage area on eastern Lake Ontario. He was the first to make the connection between eagle population declines and DDT.

Rachel Carson's book, Silent Spring (1962) which was based on wildlife ecotoxicological studies, was a major force in alerting the world to real and potential hazards of synthetic chemicals in the environment. Modern pesticides which have been responsible for huge numbers of deaths in creatures other than the intended insect pests, include organophosphates (OPs) and carbamates, carbofuran (burrowing owls on prairies), organochlorines (OCs) and the list continues.

In the western United States extensive contamination of wildlife resulted, indirectly, from the agricultural practice of subsurface irrigation drainage. Drainage of irrigated fields was done to prevent salt and water build-up. Unfortunately, as the water percolated through the soil and into the wetlands of the Kesterton National Wildlife Refuge and Reservoir, it mobilized selenium, arsenic, molybdenum and other elements which then were available for accumulation in the food chain. Some of these compounds reached toxic concentrations in the wetlands and the wildlife and fish on the preserve. There were dramatic reproductive effects observed as embryonic deaths or deformities in numerous waterfowl species, as well as other less obvious problems in other avian and mammalian species.

The federal wildlife toxicology programs that have been put in place since these events, ensure the screening and testing of industrial, agricultural, and chemical compounds that may be released into the environment. Many of the things I have mentioned will be dealt with in greater details through the rest of this course.

It is not only human activity that can lead to poisoning of wildlife. There are many natural toxins that may cause devastating losses in some populations. Natural toxins that are familiar to us in Canada include botulism toxin from the bacteria *Clostridium botulinum*, blue green algae, mycotoxins which are metabolic products of fungi, and there are many toxic plants also. These will be discussed in later presentations.

micrograms/kg for rabbits, and up to 5,000 micrograms/kg for hamsters. Avian wildlife exhibit a similar range in sensitivity, with oral LD_{50} doses of 15 micrograms/kg for bobwhite quail, > 108 micrograms/kg for mallard ducks, and > 810 micrograms/kg for ringed turtle doves. Therefore, the magnitude of the risk posed by exposure to TCDD varies significantly with the species exposed.

TCDD is globally distributed, persistent in the environment in soil and sediment, highly lipid soluble, and difficult to metabolise. As a consequence, it accumulates in animal tissue, and trace amounts are readily measured in fish, bird eggs, and other biota worldwide. In spite of its high toxicity and widespread occurrence, environmental concentrations are generally too low to cause acute toxicity in wildlife. Potential sublethal effects of chronic low dose exposure, such as endocrine disruption, are unknown.

Phthalate Esters

Phthalates are polycarboxylic acids and esters that have been used for many years in the manufacture of soft plastics. Phthalate plasticizers soften otherwise brittle plastics, such as polyvinyl chloride (PVC), and make them pliable. The concern surrounding phthalates stems from the fact that they are lipophilic liquids, and are not chemically bonded to the plastic. As a consequence, they are able to migrate out of the plastic matrix. The acute toxicity of phthalates is quite low, but chronic exposures to high concentrations has been shown to cause liver and testicular damage in mammals. The recent decision by Health Canada to ban the sale of certain soft vinyl (PVC) plastic chew toys for young children was based on the (very small) risk of chronic exposure to di-isononyl phthalate ester (DINP), which may migrate out of the toys over time. Studies in laboratory animals indicated a potential risk of liver and kidney damage with high chronic exposure.

Phthalates are used in a wide variety of products, and are very environmentally persistent. As a consequence, they are now ubiquitous in air and surface water. There is no evidence of significant wildlife toxicity associated with this class of compounds at ecologically relevant concentrations. However, high concentrations cause mortality in aquatic invertebrates. Perhaps more importantly, phthalates appear to be possible endocrine disruptors, having both antiandrogenic and estrogenic effects. Therefore, it is premature to conclude that they pose no risk to wildlife populations.

Cyanide

Cyanide is one of the oldest, most rapidly acting toxic agents known to man. It is a natural compound, found in many different plant species (including important human and livestock foods) in the form of cyanogenic glycosides. Cyanide poisoning from consumption of cyanogenic plants is a common problem in domestic livestock, but rare in wild species, since many of the most hazardous plants are food and forage crops. Examples of common plants that may contain high concentrations of cyanogenic glycosides (i.e., > 50 mg CN/kg wet weight) include sorghum, millet, flax, elderberry, and pits of *Prunus* sp. (plums, cherries, apricot, peach, almond). However, anthropogenic sources of cyanide have proven hazardous to aquatic and terrestrial wildlife.

Cyanide salts, especially sodium, potassium, or calcium cyanide, are widely used in industries such as metal electroplating, steel making, agricultural and horticultural pest control, cement making, dyeing and printing, road deicing compounds, and precious metal extraction. All of these industries present potential for occupational exposure and risks to human health, but the use of sodium cyanide to extract gold, silver, and other metals from ore is without doubt the most significant source of cyanide poisoning to wildlife.

In the microgold and silver mining industries, highly concentrated cyanide solutions are percolated through ore heaps in order to leach the metal from low grade ores. Solution ponds, mill tailings ponds, heap leach pad sprinklers, and puddles associated with these operations can be attractive sources of surface water to wildlife, especially in arid regions. Cyanide use in microgold and silver mining has resulted in hundreds of toxic ponds contaminated with millions of kg of cyanide worldwide.

Water containing > 100 mg of free CN/L is a serious threat to wildlife, and mortality has been reported at some mines with concentrations as low as 20–50 mg CN/L. Migratory birds, especially waterfowl (38% of all species recovered), are most at risk from exposure to cyanide leaching operations. However, more than 100 different species of vertebrates, from passerines and rodents to mule deer have been killed at gold mines in Nevada alone. Species sensitivity to sodium cyanide varies from 2.7 mg NaCN/kg in mallard ducks, to 4.1 mg NaCN/kg in coyotes, to 18 mg NaCN/kg in white-footed mice. Carnivores (mammalian or avian) are generally more sensitive than herbivores, due to lower gastric pH, which enhances the release of complexed cyanide, increasing the available dose of free toxicant.

Cyanide spills associated with accidents at heap leach mining operations are relatively rare events, but when they occur they can have significant ecological effects, especially if the discharge is into surface water. Fresh water fish are quite sensitive to cyanide. Short-term exposures to concentrations of < 100 micrograms HCN/L is lethal to most species. However, though cyanide will act as a biocide in aquatic systems, the effects are transient. The toxicant is subject to precipitation, volatilization, and photodegradation, and is therefore not persistent in surface water. Complete ecosystem recovery is expected.

Cyanide acts by binding to cytochrome oxidase, a mitochondrial enzyme in the electron transport chain that is involved in cellular energy production. The result is cessation of cellular energy metabolism. Typical clinical signs in birds and mammals exposed to cyanide include rapid breathing, staggering, muscle tremors, weakness, recumbency, coma, and death from respiratory paralysis. Death can occur within minutes. Animals that survive more than 3–4 hours usually recover rapidly, with no chronic effects.

Cyanide does not accumulate in tissue. It is metabolized and excreted rapidly. Therefore, acute toxicity is the principal hazard to wildlife — animals either succumb or recover, and carcasses of poisoned animals do not pose a significant risk of secondary poisoning to scavengers. In addition, application of exogenous cyanide does not cause cyanide to accumulate in plants, so forage plants exposed to cyanide from a spill will not cause toxicosis in herbivores.

Media coverage of cyanide spills associated with mining operations tends to be highly sensationalised, and often inaccurate, especially with respect to long-term risks to human and environmental health.

Radioactivity and Wildlife: An Overview

Patricia Thomas

Radioactivity refers to unstable isotopes (or radionuclides), which emit three basic types of ionizing radiation: alpha particles, beta particles and photons (either gamma rays and/or x-rays). When these radiations are absorbed by a person or an animal from either internal or external sources, a radiation dose can be calculated.[1]

Internal radiation exposure to people and animals results from the inhalation of radon gas and the ingestion of radionuclides with food and residual soil particles. External exposure results from gamma exposure from radionuclides in rocks and soils as well as cosmic rays. Humans receive a greater dose from radon than animals because they live in enclosed indoor spaces. However, animals receive greater doses from radionuclides in food items because they ingest more soil with food items. By living outside, they are also exposed to more cosmic radiation and external gamma irradiation.

Naturally Occurring Radionuclides

The principle naturally occurring radionuclides, which expose people and animals, are uranium and thorium decay products and potassium-40 (0.01% of all potassium). The widespread and short-lived polonium isotopes of the uranium series deliver the greatest doses via the inhalation of radon gas and the ingestion of animal meat and organs, particularly caribou.

Elevated levels of polonium-210 are found in caribou because of their lichen diet. As radon gas is transported in the atmosphere across the continent, it decays to lead-210, which is deposited with rainfall. Lead-210 is absorbed efficiently by lichens and decays to the alpha-emitting polonium-210. Thus, when caribou ingest lichens and people ingest caribou, they receive an elevated radiation dose. Most caribou meat ranges from 10-20 Bq/kg of polonium-210 but is an order of magnitude higher in liver and kidney. Concentrations go up as one moves east across the continent in both North America and Eurasia because of the prevailing westerly winds.

Small mammals can also receive elevated radiation doses, due to greater radon inhalation in underground burrows and greater ingestion of soil radionuclides with food items and grooming. Particularly high concentrations of radium-226 have been found in voles, resident near uranium tailings facilities in northern Saskatchewan. Similarly, benthic invertebrates downstream of uranium mill effluent may receive greater doses due to their ingestion of radionuclides deposited in sediment. As soil and sediment are the environmental sink for most radionuclides, soil and

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[1]Dose refers to the amount of ionizing radiation absorbed per mass of body tissue. Doses are measured in units of Gray (Gy), where 1 Gy = 1 Joule/kg of body tissue. As the dose increases, the absorbed energy ionizes molecules in the tissue, which can damage DNA and other cellular structures leading to death, cancer or impaired function. In general, doses of 10-30 Gy will kill people whereas an acute dose of 1 Gy gives a probability of fatal cancer of 1 person in 10 so exposed. Lower doses result in lower risks, especially if the radiation damage can be repaired.

sediment dwellers often receive greater radiation doses.

Artificial Radionuclides

The artificial radionuclides, which deliver doses to people and animals, are the byproducts of nuclear fission from reactors and bombs. The most important radionuclides from this source are the fission products: iodine-131, strontium-90 and cesium-137. Iodine-131 has an 8-day half-life and represents an internal hazard shortly after an accident, due to its accumulation in thyroid. Immediately after fallout events, there are a plethora of beta emitters released, most of which have decayed away within five years. Strontium and cesium have 28-30 year half-lives and thus persist in the environment. All populations carry scesium-137 and strontium-90 body burdens, due to the 1,000 megatons of nuclear explosive released into the atmosphere between 1945 and 1963.

Cesium-137 is a particular hazard as it biomagnifies in food chains by a factor of 2-3, particularly in environments poor in potassium, such as tundra. Its accumulation in northern food chains during the weapons testing period was instrumental in accomplishing the 1963 Atmospheric Test Ban Treaty. Its accumulation in Scandinavian reindeer after the Chernobyl accident led to bans on reindeer consumption and slaughter of some animals. Levels reached 29,000 Bq/kg in some reindeer meat (relative to a limit of 300-600 Bq/kg set by some European nations) and up to 1,100 Bq/kg in caribou from northern Quebec. Northern Saskatchewan caribou had levels of 370 Bq/kg in 1995, well above the Canadian arctic average. The concern here was not the health of the animals but the human risk of cancer from consuming such meat.

Wildlife Monitoring

My approach to radionuclide monitoring has been to measure the radioactivity in different ecosystem compartments (soil, water, plants and animals) at one point in time, calculate the food chain transfer from one compartment to another via concentration ratios, determine the dose to animals from tissue measurements and compare the dose to experimental no observed effect levels (NOEL). In the most sensitive species (mammals), NOELs are estimated at 1 mGy/day (360 mGy/year) for reproductive effects and 10 mGy/day (3600 mGy/year) for mortality effects (IAEA 1992). The environmental doses, which I have measured in caribou, moose, fish, small mammals and calculated for benthic invertebrates, are generally 1-2 orders of magnitude below these limits.

Areas of Controversy

Methods of dose calculations differ for humans and animals. In humans, the absorbed dose (in Gray) is converted into Sievert (Sv) by applying two types of weighting factors: 1) a radiation weighting factor of 20 for alpha radiation to account for its greater biological effect versus beta and gamma radiation in causing human cancer and 2) tissue weighting factors for particular tissues, which are more likely to develop cancer. Such radiation weighting factor are unknown for animals, particularly since radiation protection for animals has focussed on protecting populations from excess mortality rather than from the risk of carcinogenesis.

As a result, anthropogenic sources, such as uranium mining and nuclear power plants cannot impose more than a 1 mSv/year dose increment on members of the public. In contrast, limits of concern for animal populations are 360-3600 mGy/year with no firm understanding of how to account for the effects of alpha versus beta and gamma radiation or different species and tissue sensitivities.

U-238 Decay Series Radionuclides	Type of Radiation(s) Emitted	Half-life
Uranium-238	alpha	4.5 billion years
Thorium-234	beta, gamma	24.1 days
Protactinium-234	beta, gamma	114 minutes
Uranium-234	alpha	235,000 years
Thorium-230	alpha	80,000 years
Radium-226	alpha	1,622 years
Radon-222(gas)	alpha	3.85 days
Polonium-218	alpha	3.05 minutes
Lead-214	beta, gamma	26.8 minutes
Bismuth-214	beta, gamma	19.7 minutes
Polonium-214	alpha	0.00015 seconds
Lead-210	beta, gamma	22.2 years
Bismuth-210	beta, gamma	4.97 days
Polonium-210	alpha	138 days
Lead-206 (stable)		

Decay series of the primordial radionuclides, ^{232}Th, ^{238}U and ^{235}U; α denotes radionuclides with >50% alpha decay; adapted from Cember 1983, pp.82-85.

Radionuclide		Half-life	Radionuclide		Half-life
Uranium-238	α	4.5×10^9 y	Thorium-232	α	1.4×10^{10} y
Thorium-234		24.1 d	Radium-228		6.7 y
Protactinium-234		114 m	Actinium-228		6.13 h
Uranium-234	α	235,000 y	Thorium-228	α	1.91 y
Thorium-230	α	80,000 y	Radium-224	α	3.64 d
Radium-226	α	1,660 y	Radon-220(gas)	α	52 s
Radon-222(gas)	α	3.85 d	Polonium-216	α	0.158 s
Polonium-218	α	3.05 m	Lead-212		10.64 h
Lead-214		26.8 m	Bismuth-212		60.5 m
Bismuth-214		19.7 m	Polonium-212	α	3.04×10^{-7} s
Polonium-214	α	0.00015 s	Thallium-208		3.1 m
Lead-210		22.2 y	Lead-208		(stable)
Bismuth-210		4.97 d			
Polonium-210	α	138 d			
Lead-206		(stable)	Plutonium-241		13.2 y
			Americium-241	α	462 y
Uranium-235	α	7.1×10^5 y	Neptunium-237	α	2.2×10^6 y
Thorium-231		25.64 h d	Protactinium-233		27.4 d
Protactinium-231	α	3.43×10^4 y	Uranium-233	α	1.62×10^5 y
Actinium-227		21.8 y	Thorium-229	α	7.34×10^3 y
Thorium-227	α	18.4 d	Radium-225		14.8 d
Francium-223		21 m	Actinium-225	α	10.0 d
Radium-223	α	11.68 d	Francium-221	α	4.8 m
Radon-219(gas)	α	3.92 s	Astatine-217	α	0.018 s
Polonium-215	α	0.00183 s	Bismuth-213		47 m
Lead-211		36.1 m	Polonium-213	α	4.2×10^{-6} s
Bismuth-211	α	2.16 m	Thallium-209		2.2 m
Polonium-211	α	0.52 s	Lead-209		3.32 h
Thallium-207		4.78 m	Bismuth-209		(stable)
Lead-207		(stable)			

Persistent Organic Pollutants: Polychlorinated Biphenyls (PCBs)

Judit Smits

Polychlorinated biphenyls (PCBs) are environmental contaminants very familiar to anyone who reads the paper or listens to the radio. These chemicals have been used since the 1930s as heat transfer fluids, flame retardants, lubricants, and particularly as insulating fluids in electrical transformers and capacitors. The same qualities that make them so useful technically, are unfortunately the qualities that contribute to their environmental persistence and stability. By the late 20th century, PCBs have been detected in almost every component of the global ecosystem from the Antarctic to the Arctic. The ubiquitous presence of PCBs in the environment, in the food chain and in humans, as well as in many wildlife species, led to banning the use of PCBs in the early 1970s, and their production ended in 1977. In spite of these controls, there have been continuing concerns about the negative effects that these compounds are having on wildlife, and of course, on people. Because of their chemical structure, they fit into the so-called endocrine disrupting substances that have been the source of great concern, especially in the last decade.

PCBs can enter the atmosphere and have ended up everywhere in the world. Waxy surfaces of plants, and surface water interact with these Atmospheric PCBs which is the main way they get into the food chain. There are "hot spots" recognized in various parts of the world, where body burdens of PCBs are so high in fish and wildlife, that consumption advisories are posted. Lake Michigan is one of these problem spots. There have been large epidemiological studies of fertility problems in fishermen and their families around the Great Lakes. Piscivorous fish, waterfowl and marine mammals, as well as other fish-eating species, have been of particular concern because of very high accumulation of PBCs. Generally, older animals will have higher levels than young ones, because PCBs (and any other contaminants that persist in the environment) will accumulate in fat tissue. An interesting thing to recognize is that when those fat stores are used up, during migration, or breeding, or starvation, or gestation, lactation, or egg laying, the PCBs will be mobilized and then are able to damage susceptible organs. In the case of reproduction, the contaminants will be transferred to the offspring.

Generally PCBs are in concentrations too low to cause acute toxicosis. Mortality as an endpoint even for chronic exposure is very unlikely. However, effects on reproduction, immune status, hormonal function and behaviour have been described, and are the most likely area to see effects on wildlife populations. We will consider terrestrial, freshwater and marine ecosystem wildlife that have been affected. The risk to people who depend on the land for subsistence was one of the main driving forces behind the great research effort into PCB toxicology. Because PCBs break down very slowly, residues in the tissues of game animals pose health threats to top predators (e.g., nonvegetarian humans). A grave incident of human poisoning occurred in Taiwan in 1979, through cooking oil that was contaminated with PCBs. Children exposed during gestation were stunted, had abnormal teeth, gums, skin, nails and lungs, and short term memory damage has also been described.

Studies in the Great Lakes Basin concluded that both mink and otter populations had been affected.

In the late 1980s otters were extinct in the Netherlands where much of the research was being done, with a similar situation happening in the UK. The toxic effects on mink have probably been the most widely publicized. Mink are more sensitive to PCBs than many other species, and in more contaminated areas have been threatened at population levels. Besides their sensitivity, they are semi-aquatic top predators which expose them to the bioaccumulated PCBs that have been described in aquatic food chains. Reproductive problems that occur with chronic exposure at low enough doses that there is no obvious disease in adults, have included total reproductive failure, low birth rates, and high mortality of the kits as they continue being exposed through their dams= milk. Other effects of higher levels of PBCs on adult mink are anorexia, stomach ulcers, bloody stools, fatty livers and kidney degeneration. Affected animals become emaciated and die. Although many species detoxify some of the PCBs, mink do not seem able to do this. The highest PCB residues that have been found in the tissues of polar bears have been in northern Norway. Coincidentally (or perhaps not), there have been two sets of cubs that are female hermaphrodites. This is being studied in more depth at the moment, since there are similar reports in bears in Canada.

The marine environment is probably the ultimate sink for persistent xenobiotics, and it covers 70% of the earth. Marine mammals, some of which are at the top of the food chain, and are universally appealing, have been closely watched for negative effects of environmental contaminants. Population declines in seals, sea lions and walrus, have been linked to PCB biomagnification through the food chain. These animals have large amounts of blubber which accumulate these contaminants. Males always have higher levels because the females mobilize the toxins during gestation and lose some to the offspring during lactation. There is suspicion of PCB-induced neurotoxicity which has led to increased abandonment of calves or pups by their mothers.

The massive epizootic of seals in and around the North Sea and the Baltic Sea in the late 1980s, was ultimately due to Phocid Distemper Virus (PDV-1) (related to canine distemper), and killed off about 20,000 harbor seals and several hundred grey seals. There were two interesting features of this devastating disease. The virus probably had been around before the outbreak occurred, so what made the animals so vulnerable? Also, significantly more males than females died during the outbreak. There is a substantial body of evidence that although the virus killed these animals, contaminant-related immunotoxicity contributed greatly to the severity and extent of the epizootic. A 2 2 year study was conducted using semi-captive seals fed on either Baltic Sea fish (contaminated), or Atlantic fish (relatively uncontaminated). Results showed that various aspects of immune function were suppressed, and the suppression was associated with the levels of contaminants in their diets. A major portion of the pollutants were PCBs. Without normally functioning immunity, the seals were at increased risk of infection and dying of PDV-1. Males have higher body burdens of PCBs for reasons described above, so it is reasonable to assume they were more immunosuppressed than the females.

In Canada the St Lawrence River is highly polluted because of the heavy industrialization on Lake Ontario where Monsanto discharged PCBs until the late 1970s. Beluga whales from this estuary have high rates of cancer and other disease problems that are well correlated with their tissue levels of PCBs.

Birds, piscivores in particular, like cormorants and gulls, have also been negatively affected by PCBs. There is little mortality, but severe effects are seen on reproduction. Fewer eggs are laid and embryo mortality is increased. The Great Lakes Embryo Mortality and Edema Syndrome (GLEMEDS) has been blamed on various pollutants including PCBs. There is no specific pathology to diagnose PCB toxicity, but a group of lesions including embryo and chick mortality, growth retardation, developmental deformities, edema (fluid in the abdomen and under the skin), and liver enlargement, make up GLEMEDS and PCB toxicity is a main consideration for causality. Some effects are thought to be due to endocrine disruption. Abnormal breeding behaviour such as poor incubation, and female - female pairs have been reported at higher than normal rates. colonies, so adult rather than site factors were associated with poor hatching. Bald eagle populations around the Great Lakes have been declining, and although DDE played a major role in the past, PCB levels in tissue and eggs in these populations are correlated with poor reproduction. Alligators in Florida have made headlines recently, because of contaminant-related (PCBs in particular) abnormal gonad development and altered sex hormone levels.

Large predatory fish species from the Great Lakes (especially Michigan), the St Lawrence River, the Baltic Sea and the Hudson River in the US, all have high PCB levels. Salmonid egg survival to hatching is about 25% lower in L. Michigan compared with Lake Superior. Other species have shown fin abnormalities which are proportional to PCB burdens. As with other animals, the body burden of PCBs increases with age and size in the male fish, but not so in the females because of the high lipid content of roe through which they eliminate much of these compounds.

In summary, PCB contamination is a global problem. There is little concern about acute toxicity associated with exposure. However, because of the accumulation of these compounds through the food chain, because humans and wildlife are being exposed, and because we know these contaminants are going to around for a long time yet, we should be aware of the types of effects PCBs can have on wildlife. Wildlife personnel should be aware of the regions within their jurisdiction, which are "hot spots" of PCB contamination, and work together with government agencies and research institutions whenever problems suspected of being related to PCB pollution may occur.

Petroleum Oils and Wildlife

Ted Leighton

Sources of Petroleum Oil

It has been estimated that the some 3-4 million metric tons of petroleum oil contaminate the world's oceans annually. Most oil pollution occurs along marine coastlines, but spills also occur inland. There are many sources from which petroleum oils can reach the environment. Most result from human activity, but there also are natural sources. The largest source of petroleum contamination is discharge from routine shipping operations, for which petroleum-derived oil is the main fuel. Accidental spills associated with shipping or with off-shore oil production facilities have lead to the largest oil spills. Western Saskatchewan and Alberta have vast numbers of oil wells, storage facilities and pipelines. Many of these are in or near wetland habitat. Thus, there is potential for contamination of prairie aquatic environments by oil .

Petroleum contamination of the environment occurs in two very different forms. Most petroleum arrives in the environment from numerous small sources, none of which would be classified as a significant "spill". Only up to about 15% of annual petroleum contamination occurs in the form of "spills" - large volumes of oil released into the environment from a single source. Practically nothing is known about the environmental effects that may be due to diffuse, low-intensity oil pollution. Almost everything that is known about the impact of petroleum pollution has been learned in the context of real or simulated oil spills. Thus, the information presented here pertains to the effects of classical oil spills.

What is "oil"?

Petroleum oils include all liquids derived from crude petroleum. The environmentally-important petroleum oils are those produced and transported in large quantities which can, thus, be spilled in large quantities. These include crude oil (the stuff that comes out of the ground), and major fuels produced by distillation of crude oil: gasoline, kerosine/jet fuel, diesel fuel, heating oil (fuel oil No 1) and bunker c oil (fuel oil No 6, the major fuel of marine shipping).

Crude oils usually are named after the geographic location of the oil well: South Louisiana, Hibernia, Kuwait, Prudhoe Bay. These names say nothing about the chemical composition of the oil. Crude oils vary markedly in chemical composition and even the oil from one well may differ in chemical composition over time. The fuel oils produced form crude oils also differ greatly in chemical composition. Thus, two No 1 fuel oils can be totally different chemically, although their combustion properties will be virtually identical.

Petroleum oils are complex mixtures of chemicals: hydrocarbons and hydrocarbon-like chemicals that include atoms of nitrogen, oxygen, or sulphur (often called N-S-O compounds). There also are metals in oil, such as vanadium and mercury. Hydrocarbons occur in two major chemical subdivisions: those made up of benzene rings (called aromatic hydrocarbons) and everything else

(aliphatic hydrocarbons). Every petroleum oil consists of hundreds of different hydrocarbons and N-S-O compounds, and no two oils are the same in chemical composition. For example, some may contain 30% or more of aromatic hydrocarbons and other virtually no aromatic hydrocarbons at all.

Some aspects of the toxicity of petroleum oils depend on the chemical composition of the oil. Where this has been studied, most such toxicity has been found associated with the aromatic compounds in oil and not in the other fractions. Note that "aromatic" means "composed of benzene rings"; it does not mean "easily evaporates" or "volatile" or "of low molecular weight". In fact, where this has been studied, it has been the larger, non-volatile aromatic compounds that have been the most toxic compounds found in petroleum oil.

Oil Changes After It Is Spilled - The Process of "Weathering"

When oil is spilled into the environment, its chemical composition immediately begins to change. The rate of change varies with environmental conditions. The processes by which oil changes in composition after it is spilled referred to collectively as "weathering". Weathering is a continuous process that, on average, is completed in about one year. Initial changes in the spilled oil occur fairly quickly, and the rate of change then slows over time. The end result of weathering of oil spilled into water is conversion of the original oil into hydrocarbons dispersed in the air or water and to masses of residual asphalt called tar balls, which are cast onto the shore or sink.

The main processes of weathering are evaporation, formation of emulsions of oil-in-water or of water-in-oil (this latter produces and stick mess fondly called a "mousse", and dispersion of those emulsions, the dissolving of oil components into water, photo-oxidation of compounds, sedimentation, and biological conversion of oil compounds to carbon dioxide and water. At any point in the process, the oil may become buried in soil or sediments, and the weathering process may be suspended for years until the oil is re-exposed when the beach or sediment is disturbed, as by a storm.

The concentration of potentially poisonous chemicals in oil is most likely to increase during weathering, especially during the first few weeks. Volatile components evaporate quickly, leaving behind the less volatile molecules. The less-volatile aromatic hydrocarbons and related N-S-O-compounds are the group of chemicals in petroleum oils most likely to include serious poisons. It often is implied in papers and reports that weathered oil is less toxic than is fresh oil. There is no scientific basis for this assumption, and the opposite is more likely to be true.

Impact of Spilled Oil on The Environment

Petroleum oils spilled into the environment can affect organisms by direct physical coating, by altering essential elements of the habitat and by direct toxic effects of chemicals in the oil. The environmental affect of spilled oil always is a complex mixture of these effects applied to all living organisms in the affected area.

Oil is an unusual pollutant. When spilled into water, it does not quickly become diluted but remains in a concentrated mass on the surface which is only slowly changed and degraded by the process of weathering. Thus, oil has its most pronounced affects on organisms that make use of the water surface or that inhabit shorelines. This effect extends to benthic organisms when large quantities of oil are incorporated into sediments.

Impact on Plants

High mortality in marsh grass, mangrove, and intertidal plant communities have occurred due to spilled oil, and may require one or two decades to recover. Floating algae may be killed or may grow more abundantly in response to oil, depending on conditions and on the concentration of the oil. Variable responses to oil by plants can result in major shifts in the relative abundance of plant species in polluted environments. Terrestrial plants also may be killed by oil or suffer reduced growth and reproductive rates due to combinations of physical coating, altered soil chemistry and toxic effects of petroleum components.

Impacts on Invertebrates

Invertebrates in intertidal zones affected by spilled oil often are virtually totally eliminated. Again. the affects of oil include physical smothering, loss of food and direct chemical toxicity. Invertebrates may be eliminated from sediments contaminated by oil for many years, since the oil can persist in sediments for long periods of time.

Impacts on Fish

Eggs and larvae of fish are very sensitive to toxic chemicals in oil. Pacific Herring and Pink Salmon larvae suffered high mortality and high rates of physical deformity when exposed to concentrations as low as 0.4 to 1.0 part per billion of non-volatile aromatic hydrocarbons derived from weathered oil. Such levels can occur in water in oil-spill areas. Adult fish are less sensitive and generally can avoid oil; they are exposed only to droplets of oil dispersed into the water column or to dissolved compounds. In shallow waters, adult fish can be exposed to higher concentrations of oil and oil-derived chemicals, and fish kills have occurred in such circumstances. Fish also can be affected by altered food resources and habitat, especially in near-shore areas, streams and estuaries.

Impacts on Birds and Mammals

Birds often are killed in large numbers in oil spills. Aquatic birds inhabit the water surface and are exposed directly to concentrated oil that is spilled into their habitat. Birds often are the most evident victims of oil spills and much effort is expended in cleaning and releasing oiled birds. The impact of oil on marine mammals generally is much less than on birds, with the exception of mammals such a Sea Otters and other species that rely on fur, rather than blubber, for thermal insulation. The impact of oil on these latter, fur-dependent species can be severe and similar to that on birds.

Physical Contact with Oil: . Physical contact is the effect of oil that kills large numbers of birds and fur-dependent aquatic mammals and is by far the most important effect of oil on these animals. Birds rely on their feathers for water-proofing, buoyancy, insulation and aerodynamic contours. Water is excluded by feathers because the narrow slits between feather barbules are too narrow to permit water, with its high surface tension, to pass through. Oil has a low surface tension and readily passes through feathers. Feathers thus become matted by oil immediately upon contact, and all the protective and functional properties of feathers are lost. Birds on cold water quickly die of cold; others are prevented from feeding or leaving the oil spill area, and die of exposure, starvation and dehydration. Similarly, the fur of aquatic fur-bearing mammals excludes water because the spaces between guard hairs are too small to admit water. Oil penetrates such fur easily and the fur becomes matted with oil. Death from cold and exposure follows quickly, particularly in animals who must live in and feed in cold water, such as Sea Otters.

Oil as a Poison: Petroleum oils contain potentially poisonous compounds. Animals may be exposed to these by inhalation or ingestion, and bird embryos can exposed to oil compounds when the external surface of egg shells is contaminated with oil.

Inhalation of Volatile Components - During the earliest stages of an oil spill, evaporation may result in high concentrations of volatile compounds in the local atmosphere. This could lead to suffocation and/or to inhalation of toxic concentrations of volatile components such as hexane and benzene. These high atmospheric concentrations do not last long, perhaps a few hours. There is one report of birds dying under conditions that suggested the cause was inhalation exposure. Probably this is not a major cause of death or illness in wildlife.

Ingestion of Oil - Animals contaminated externally with oil will attempt to clean themselves by licking and preening, and will ingest oil in the process. Oil also can be ingested when their water or food is contaminated. It is virtually impossible to separate out the effects of external, physical oil exposure and the effects of ingested oil when animals are exposed to by external contamination, as in real oil spills. The effects of ingested oil are known only from experiments in which animals were fed oil without also being contaminated externally. The results of such experiments over the past 40 years are quite confusing, since many different oils fed to many different species at many different doses and frequencies have been reported. The chemical composition of the oils used in these experiments seldom was determined. Nothing helpful is known about the toxicity of ingested oil to relevant mammalian species. Three toxic effects of oil ingestion by birds have been well-documented. The first is that ingestion of petroleum oil appears to induce a stress response that is additive, perhaps synergistic, with other stress-inducing stimuli to which birds are exposed at the same time. This may be of great importance in the wild, where multiple stresses occur and affect survival in multiple ways. The second effect is a negative impact on reproduction. Several different oils fed to several different species have resulted in reduced reproduction with effects on both male and female birds. The third effect is damage to red blood cells, with anemia as the result. This has been documented for only one crude petroleum oil which a high content of aromatic compounds and was ingested at the high end of expected doses in oil spill situations.

Oil on Bird's Eggs - Tiny quantities (a drop or less) of oil applied to birds' eggs during the first half of incubation can cause almost 100% mortality of the embryos inside. This high sensitivity of avian embryos to compounds present in petroleum oils has been documented in the field as well as the laboratory. Adult birds with lightly-contaminated feathers can deliver lethal doses of oil to their eggs when then return to the nest to incubate. Fortunately, this period of high vulnerability is limited to the few short weeks of the breeding season for most bird species.

Responding to Oil Spills

Wildlife agency personnel may be required to respond to oil spills. The aim of such responses usually is to limit damage to the environment and to do something with wild animals coated with oil. Often there is intense public interest in the welfare of oil-contaminated birds and mammals. Environment Canada and most provinces have emergency response plans that include oil spills. Part of any response will involve handling oil-contaminated wildlife. The rehabilitation of such animals may be expected by government and by the public, and agencies should be fully prepared to respond. Rehabilitation of oiled wildlife is best left to organizations that do this on a professional basis. The basic process of washing oiled wildlife is simple, but the logistics of doing it effectively with large numbers of animals are horrific. Failure to do the job properly will make the work totally ineffective and also can rightly be considered inexcusable given the experience and expertise available through professional oil spill response organizations. One such organization that can provide such assistance, and also can recommend other experienced groups, is listed below.

Tri-State Bird Rescue and Research, Inc.
110 Possum Hollow Road
Newark, Delaware 19711 USA
Phone (302) 737-9543
Fax (302) 737-9562
Website: http://www.tristatebird.org/information.html

The State of California has an extensive oil spill response program. The program office can recommend actions and organizations competent to assist in an oil spill response.

Office of Oil Spill Prevention and Response
Department of Fish and Game
1451 Shaffer Road
Santa Cruz, CA 95060 USA
OSPR 24-hour Communications Center (916) 445-0045
Website: http://www.dfg.ca.gov/Ospr/

Suggested Reading

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Natural Toxins and Wildlife

Mark Wickstrom

Natural toxins are poisonous substances produced by living organisms, such as bacteria, blue-green algae, marine invertebrates and fish, fungi, vascular plants, and venomous arthropods (spiders, insects, scorpions), amphibians, and reptiles. Exposure to some of these toxins may have significant effects on various species of free-ranging wildlife.

Bacterial Toxins

Probably the most important natural toxin in terms of demonstrated wildlife mortality is botulinum toxin. Botulinum toxin is produced by the bacteria *Clostridium botulinum* (type C), under conditions of low environmental oxygen. This toxin is a high molecular weight protein, and is usually considered to be the most poisonous substance known. Lethal doses as low as 0.01 micrograms/kg (> 100,000 times more toxic than strychnine) have been reported. It acts by blocking the release of the neurotransmitter acetylcholine at neuromuscular junctions, leading to muscle paralysis.

Waterfowl, especially dabbling ducks, are most at risk from botulism, but coots, gulls, and various shorebirds are also commonly killed during an outbreak. Exposure to botulism toxin results in the death of large numbers of waterfowl in North America every year. Annual losses in the Canadian prairie provinces alone have been estimated to range from 100,000 to 1,000,000 birds. Clinical signs in ducks progress from inability to fly to difficulty walking and holding their head erect, to prostration, total paralysis, and death from respiratory arrest.

Botulism outbreaks typically occur in late summer/early autumn, when environmental conditions in wetland sediments are optimal for bacterial spores, which can persist in soil or sediment for years, to germinate, and produce toxin. The toxin is inadvertently ingested by birds when they feed. Rotting carcasses of birds containing fly maggots (which contain high levels of toxin, but are not affected by it) are thought to be the major source to toxin for perpetuation of the outbreak. Outbreaks do not occur at every site containing bacterial spores every year. The biotic and abiotic conditions required to trigger an outbreak are not completely understood, and are the subject of continuing research.

Blue-Green Algal Toxins

Blooms of toxic blue-green algae (cyanobacteria) occur commonly in fresh and brackish water worldwide. Stagnant, nutrient enriched (eutrophic) water bodies are most at risk, especially during warm, sunny weather. Domestic animals and occasionally wildlife are exposed when prevailing winds concentrate the bloom near shore.

Algae in the genera *Nostoc*, *Oscillatoria*, *Anabaena*, and especially *Microcystis* produce hepatotoxic cyclic peptides. These toxins act to disrupt the structure of liver cells, causing massive haemorrhage and necrosis, leading to shock and death in a matter of hours. Microcystin is the most

common of the hepatotoxins in toxic blooms. It is very potent in some species, with an LD_{50} of < 100 micrograms/kg (20 times more toxic than strychnine) in mice. However, there are large differences in sensitivity between species, especially when natural bloom material is ingested.

Algae in the genera *Anabaena* and *Aphanizomenon* (and some *Oscillatoria aghardii* blooms) produce potent, fast acting alkaloid neurotoxins. Anatoxin-a acts by causing permanent depolarization of post-synaptic membranes, disrupting nerve conduction and leading to muscle tremors, rigidity, paralysis, and death by respiratory arrest within minutes. Anatoxin-a (s) is a potent cholinesterase inhibitor that resembles organophosphorus insecticides. It is the only natural cholinesterase inhibitor known. The final member of this group of neurotoxins, the aphanitoxins, act by blocking sodium channels, which disrupts nerve conduction. These toxins appear to be identical to saxitoxin or neosaxitoxin, the causative agents of paralytic shellfish poisoning in humans.

Toxic algal blooms have been associated with the death of numerous wildlife species, including white rhinoceros in Africa, and white-tailed deer and wild canids in North America. Waterfowl deaths may be seen in association with blue-green algal blooms, but controlled studies indicate that ducks are quite resistant to *Microcystis* toxins when given orally, so other causes of death need to be ruled out in order to confirm the diagnosis when hepatotoxic algae are involved. In contrast, ducks are known to be moderately sensitive to the neurotoxic algae, and outbreaks of *Anabaena* and *Aphanizomenon* poisoning have caused severe losses of waterfowl and other birds in North America and Europe.

Microcystins have recently been implicated as the cause of large fish kills in farmed salmon in marine coastal areas. These fish have lesions consistent with liver failure. However, most aquatic organisms, including various species of fish, tadpoles and adult frogs, crayfish, and cladocerans are apparently unaffected by blue-green algal toxins.

It has been estimated that about 50% of blue-green algal blooms involve toxogenic species. However, the concentration, composition, and distribution of toxin within a toxic bloom varies greatly over time, and between different portions of the bloom at the same time. Therefore, establishing a cause-and-effect relationship between wildlife losses and algal blooms can be challenging.

Mycotoxins

Mycotoxins are poisonous substances produced by fungi; usually growing on or in feedstuffs. Consumption of mycotoxin-contaminated feed is a major cause of disease and decreased performance in domestic animals. Effects on free-ranging wildlife can also be significant, although wildlife mycotoxicoses are relatively rarely reported due to difficulties in establishing the diagnosis in the field. There are a few reports of acute mycotoxicosis resulting in death of wild species, but the sub-lethal effects of chronic, low level exposure (including reduced growth , impaired reproduction, and immune system compromise) may be more significant at the population level.

Aflatoxins

These toxins, produced by the fungus *Aspergillus flavus* or *A. parasiticus*, are among the most prevalent and toxic of the mycotoxins, causing malignant neoplasms (cancer) and/or severe liver disease in many species. Chronic exposures have been associated with immunosuppression and increased susceptibility to infectious diseases.

Aflatoxins are common contaminants of corn, peanuts, and many other cereal and oil seeds. Wildlife are at risk from eating waste grain, especially during times of restricted access to other feed or forage. Heavy mortality has been reported in natural exposures of bobwhite quail (*Colinus virginianus*) to aflatoxin. The LD_{50} for bobwhite quail is about 6.0 ppm in the diet. Aflatoxin concentrations in waste grain at these levels are relatively common, especially under warm, humid conditions. Ring-necked pheasants (*Phasianus colchicus*) are significantly more sensitive to aflatoxin than bobwhite quail (LD_{50} about 1.5 ppm), and are therefore likely to be at greater risk.

Ducklings are reported to be the most sensitive animals to the acute effects of aflatoxin, with an LD_{50} of only 0.36 mg/kg. Acute aflatoxicosis in ducklings is characterised by anorexia, depressed growth, ataxia, and sudden death. Mass waterfowl mortalities in mallard ducks (*Anas platyrhynchos*) and snow geese (*Chen caerulescens*) in Texas have been attributed to aflatoxin exposure, with estimated losses of 500 and 7,000 birds, respectively. Aflatoxin exposure has also been implicated as a possible cause of the high rate of neoplasia in endangered Mississippi sandhill cranes (*Grus canadensis pulla*).

Although there are numerous anecdotal reports, to the author's knowledge there are no confirmed cases of acute mycotoxicosis leading to death in wild mammals. In a controlled study with captive animals, white-tailed deer fawns (*Odocoileus virginianus*) exposed to 0.8 ppm in the ration for 8 weeks demonstrated mild hepatic injury, with reduced body weight and feed consumption. These findings indicate that young deer with access to waste grain are potentially at risk from sublethal effects of the toxin.

Trichothecenes

This is a large and diverse group of about 150 sesquiterpene compounds produced by fungi of the genera *Fusarium*, *Cephalosporium*, *Myrothecium*, and *Trichoderma*. The most important toxins in this group are T-2 toxin, diacetoxyscirpenol (DAS), and deoxynivalenol (DON, or vomitoxin). These toxins generally act to inhibit protein synthesis, and target rapidly dividing cell types in the skin, intestine, bone marrow (hematopoietic), and lymphoid (immune system) organs. As a group, these toxins are known to cause feed refusal, dermal, oral and gastrointestinal necrosis and ulceration, coagulopathy (haemorrhage), decreased reproductive performance, and immune system dysfunction.

Although data on wildlife exposures are scarce, trichothecene mycotoxins (possible T-2 toxin) have been implicated in a mass mortality event involving sandhill cranes in New Mexico and Texas in

which an estimated 9,500 birds died.

Other mycotoxins

Other mycotoxins of importance to domestic animal producers and veterinarians which may have adverse effects in wild species under certain conditions include fumonisins (from *Fusarium moniliforme*), zearalenone (from *Fusarium roseum*), ochratoxin A (*Aspergillus* and *Penicillium*), ergot alkaloids (from *Claviceps purpurea*), and sporidesmin (from *Pithomyces chartarum*).

Phytotoxins

Phytotoxins are a large and diverse group of hundreds of toxic compounds produced by vascular plants. Worldwide, they represent a multi-billion dollar concern for the livestock industry, especially in areas where rangeland and pasture grazing are practiced. Wild herbivores, which have adapted physiologically and behaviourally to endemic poisonous plants, and which have the ability under most circumstances to avoid toxic species, are seldom adversely affected by exposure to these toxins. However, there have been a number of documented cases of plant poisoning in free ranging wildlife.

Locoweed poisoning has been reported in elk (*Cervus elaphus*) in Colorado and New Mexico. The animals were feeding on plants in the genus *Astragalus*, which are among the first green plants to appear on spring ranges. Pyrrolizidine alkaloid poisoning has been documented in deer. Sika deer (*Cervus nippon*) have been poisoned by essential oils when drought conditions forced them to consume the needles and bark of jack pine trees (*Pinus banksiana*). In Missouri, white-tailed deer grazing in sorghum fields developed cyanide toxicosis. Koala (*Phascolarctos cinereus*) have been reportedly poisoned by cyanide from leaves of eucalyptus trees not commonly eaten by koala. Moose (*Alces alces*) feeding on bog asphodel (*Narthecium ossifragum*) in southern Norway developed severe, degenerative renal disease. In the United States, wild ducks have been reportedly poisoned by exposure to ricin, a highly toxic substance in castor beans (*Ricinus communis*).

Overgrazing, range deterioration and fencing may enhance the risk of poisoning of wild herbivores as well as livestock. About 250 pronghorn antelope in Texas died from exposure to an undescribed hepatotoxic compound in tarbrush (*Fluorensia cernua*), when construction of new netted fencing prevented their migration to areas of fresh browse during drought conditions. Similarly, wild herbivores confined to game ranches in South Africa have suffered mortality from consumption of toxic, tannin-containing plants that would normally be avoided.

One particular class of phytotoxins which is the subject of recent research interest are the phytoestrogens. Phytoestrogens are estrogenic compounds produced by plants that have the potential to affect reproduction and fertility in animals by mimicking the action of normal sex hormones (i.e., estradiol). These compounds may be structural analogues of normal sex hormones that bind directly with and stimulate endogenous estrogen receptors (e.g., isoflavones, coumestans, lignans and resorcyclic acid lactones), or they may act indirectly by increasing or decreasing the

synthesis or degradation of sex hormones. They are widely distributed across many plant families, and are therefore available to wildlife.

Examples are limited, but there are several documented cases where exposure to phytoestrogens appears to affect fertility and reproductive success in wild species. Reproductive success in California quail (*Callipepla californica*) is negatively correlated with the presence of isoflavone phytoestrogens in common forage plants. During dry years, these forage plants are smaller and contain higher concentrations of estrogenic substances than during normal years. In montane voles (*Microtus montanus*), phenolic compounds such as cinnamic acid isolated from dietary plants were found to decrease uterine weight, inhibit follicular development, and decrease litter size, indicating significant antiestrogenic effects. Phytoestrogens in forage plants from a cypress plantation were found to decrease litter size in Japanese voles (*Microtus montebelli*), compared to populations in adjacent habitats without exposure to these toxins.

Pesticides and Birds – A Practical Approach

Pierre Mineau

[2]

1. INTRODUCTION

This short course is aimed at anyone who is in a position to investigate firsthand the potential impact of pesticides on birds. This person may be a field biologist, veterinarian, agronomist or even a volunteer responding to a bird kill.

Why birds? First of all because they are an important and visible part of our environment. They have been used for a long time as sentinels of general environmental quality and a large body of literature exists on avian toxicology. In North America, most species are federally protected from unlicensed taking or kill, to the level of the individual. Birds are extremely mobile, and it is therefore more difficult to exclude them from areas that have been treated with pesticides. Groups such as raptors (hawks, eagles and owls) are often brought to clinics or centres for rehabilitation, and these centers can become a valuable source of information and samples. Some bird species are attracted to agricultural pests, and many are economically important for control of insect pests. Finally, birds, as a group, are particularly sensitive to some of the more toxic classes of pesticides such as the organophosphorus and carbamate insecticides, and their reproduction has been found to be vulnerable to a wide range of pesticides. New pesticides developed in part for their relative safety to humans have been found to be especially toxic to birds.

Unfortunately, this focus on birds has resulted in virtual ignorance of the effects of pesticides on most other groups of nontarget organisms. Biologists around the world are sounding an alarm about the state of reptile and, especially, amphibian diversity and population numbers. These groups have been largely ignored in most countries.

2. THE SCOPE OF THE PROBLEM

Birds ingest pesticides through their food or through preening or grooming. Despite being feathered, they absorb pesticides through their skin, encountering droplets directly or by rubbing against foliage and other contaminated surfaces. Birds are also exposed through their feet. Finally, they have a very high ventilation rate and inhale vapor and fine droplets. The degree to which each of these routes of exposure contributes to the total dose depends on the crop being sprayed, the chemical, the species exposed and environmental factors. The ecology of the species (i.e., feeding preferences, behavior) along with the characteristics of the chemical (i.e., its persistence, tendency to bioaccumulate, toxicology) and the intended use go a long way in determining the nature and the

[2] **I wish to acknowledge Dr. Nimish Vyas of the U.S. Geological Survey for assistance with portions of this text, notably the section on legal considerations. The text also benefited from the comments of Alain Baril and the editorial pen of Susan Burns, both with the Canadian Wildlife Service.**

scope of the impacts on wildlife.

2.1. Substances that bioaccumulate in wildlife

Substances that are poorly metabolized and excreted by birds and that accumulate in some tissue, such as in fat in the case of lipophilic substances, are usually a problem whether or not they are acutely toxic. The most notorious and best documented examples are a number of persistent organochlorine pesticides, such as DDT, dieldrin, heptachlor and chlordane. Even pesticides with moderate ability to bioaccumulate may cause problems if toxic doses can be accumulated gradually over the course of several days, e.g., coumarin-type rodenticides or the new pyrrole insecticide chlorfenapyr.

2.2. Direct mortality

Mortality of wildlife in and around treated fields and forests is one of the most visible signs of a pesticide impact. More than 30 pesticides registered in North America or Europe have been known to result in kills of wild birds or mammals even when used according to the relatively stringent instructions in force in those countries. Insecticides are usually more toxic to birds than are herbicides or fungicides. Two groups in particular, the organophosphorus and carbamate insecticides which affect the nervous systems of both insects and vertebrates, cause most of the deaths. Because birds are free to move in and out of treated areas, they run the risk of being exposed to a very high dose simply by being in the wrong place at the wrong time. Although the use of a toxic insecticide does not necessarily mean an impact on the wildlife present, some pesticides are so toxic to birds that it is difficult to use them under any circumstance without causing mortality.

2.3. Secondary poisoning

Secondary poisoning occurs when predators, such as hawks or owls, consume prey contaminated by pesticides. Such predators are few because of their position at the top of the food chain. Therefore, the death of one predator may constitute a significant reduction in the local population of that species. Furthermore, predatory birds are important agents of control for a number of species considered to be pests, such as many rodents.

Historically, researchers have associated secondary poisoning with persistent organochlorine insecticides and other substances that are not readily metabolized and that therefore accumulate in tissue. We now know that other currently registered pesticides, even those that are readily metabolized, can cause secondary poisoning under the right conditions -- namely when the predator encounters the pesticide in a high concentration on the surface or in the gastrointestinal tract of its prey. And predators capture birds debilitated by insecticides much more easily.

Other birds that often suffer secondary poisoning are the scavengers that feed on carcasses of dead or dying poisoned animals, or on baits used for vertebrate control.

2.4. Sublethal effects and delayed mortality

Many pesticides can affect the normal functioning of exposed individuals at doses insufficient to kill them directly. At high doses, the organophosphorus and carbamate pesticides previously

described cause respiratory failure and death. However, wild birds exposed to these agents in lesser amounts have experienced impaired coordination, hypothermia and loss of appetite. Also, sublethal exposure to these agents may result in birds spending less time at the nest, providing less food for their young, being less able to escape predators and being more aggressive with their mates.
It is difficult to assess how much environmental factors combine with sublethal exposures to cause delayed mortality. Exposure to some pesticides makes wildlife more vulnerable to predation. It also causes weight loss and an inability to maintain body temperature, both of which increase the chances of a small bird dying in inclement weather. Finally, exposure to pesticides and resistance to disease may be linked.

2.5 Effects on reproduction

Notable effects of sublethal pesticide ingestion also include reproductive failure through reduced hatching or fledging success. A high proportion of pesticides currently registered have the potential to affect reproduction, although the extent to which this actually happens is not known. A few products cause embryonic mortality when sprayed directly onto eggs.

2.6. Impacts on critical habitat

Impacts of pesticides on plants and invertebrates, whether target or nontarget, may secondarily affect birds, mammals and other vertebrate wildlife. Impacts on wildlife can occur when a pest, which may also be food or shelter for another species, is eradicated or heavily controlled. To make pest control programs compatible with the preservation of wildlife, pesticides that are as target-specific as possible must be chosen and, ideally, unsprayed areas should be provided as refuges. Using a more target-specific product minimizes impact on desirable nontarget species, such as beneficial arthropods, both in the crop and in adjacent areas.

As agriculture becomes increasingly intensive, birds and other wild species are pushed into remnants of natural habitats alongside cropland, for example, small wetlands or woodlots in the middle of cultivated fields, drainage ditches, fencelines, hedgerows or even small rock piles. Impacts inadvertently occur in these habitats either through the direct application of pesticides or by spray drift or runoff. Birds and other species also enter the crop area to feed.

3. MAIN ROUTES OF EXPOSURE OF BIRDS TO PESTICIDES CAUSING ACUTE POISONING

3.1. Abuse and Misuse

Deliberate attempts to poison wildlife, or abuse, usually involve baits of some kind. The only limit is the imagination of the perpetrator. Typically, liquid insecticides are poured or injected and applied to seed, bread, meat, etc. Granules can be sprinkled or mixed into a paste.
The choice of chemical reflects availability and toxicity. Pesticides typically used in deliberate poisoning attempts include carbofuran, aldicarb, monocrotophos, parathion, mevinphos, diazinon and fenthion. The main problem of course is that the baits are often indiscriminate in the species that they kill. Secondary poisoning is also frequent when predators or scavengers take dead or

debilitated prey with a highly concentrated bait in their gut.

In the United Kingdom, as well as in several European countries, officials estimate that deliberate bird kills due to pesticides outnumber cases where label instructions were strictly followed. Between 1978 and 1986, officials in the U.K. estimate that, on average, 71% of incidents were the result of abuse. For birds of prey alone, over 90% of cases recorded between 1985 and 1994 in the U.K. were abuse cases. For raptors in the United States during the same period, kills involving labeled uses of pesticides were almost as frequent as abuse cases. This difference appears to be wholly attributable to the high toxicity of insecticides used in the U.S., especially the insecticide carbofuran in liquid or granular formulations. In Canada, the proportion of raptor kills resulting from labeled uses seems to be higher still. However, the Canadian tally is heavily biased toward the West Coast where there is a problematic overlap between high wintering populations of Bald Eagles and intensive agriculture.

The term misuse refers to a pesticide application that is not exactly as specified by the label. This may be an application at a rate which is higher than specified, or to a crop or pest other than those listed. Pesticide misuse is difficult to establish, especially after the fact. Also, in some cases, it becomes very difficult to distinguish a misuse from a normal agronomic use when the label contains instructions that are difficult or impossible to follow. Examples are labels that warn against using a product in "*areas frequented by wildlife*" or labels that require that no granular insecticides be "*left on the soil surface*". Both of these requirements are impossible to follow.

3.2. Persistent bioaccumulating substances

The use of most of these products (DDT, aldrin, dieldrin, heptachlor, chlordane, HCB, HCH, mirex) has been canceled or significantly curtailed in most countries. However, some products like DDT continue to be a concern because of continuing local contamination in areas of high historical use, and because of high residue levels in birds that migrate to areas where these products are still used. These birds in turn pass the residues along the food chain, where they hamper the full recovery of populations of species such as the Peregrine Falcon (*Falco peregrinus*) in North America.

3.3. Grazing

Grazing birds are particularly vulnerable to spray applications of pesticides. Kills have been recorded with several pesticide sprays: e.g., diazinon, carbofuran, dimethoate and triazophos. Grazers typically include geese, ducks and coots. These birds eat large quantifies of foliage because they do not digest cellulose. Fertilized areas are particularly attractive to grazing species. Grazers can detect the high nitrogen levels. Golf courses attract grazers because the turf is cut frequently, watered and fertilized, and courses often have other attractions such as ponds and drainage streams.

Over 100 cases of waterfowl mortality were recorded due to the use of diazinon on turf before the pesticide was withdrawn from golf courses and sod farms in the U.S. Other well documented problems are kills of ducks and geese in alfalfa fields treated with carbofuran and of Sage Grouse (*Centrocercus urophasianus*) feeding on alfalfa crops treated with dimethoate or on potato foliage

and weeds in potato fields sprayed with metamidophos.

3.4. Species that feed on sprayed insects

Bird species that feed on agricultural pests such as grasshoppers, leatherjackets (larvae of the crane fly), grubs and cutworms are at high risk of poisoning. Kills of these species is all the more unfortunate because they are beneficial to agriculture.

Some species are particularly vulnerable because they specialize in insect outbreaks. These birds take advantage of pest control operations that result in insects becoming either debilitated or more visible, as when soil organisms come to the surface following treatment. In a recent case in Argentina, approximately 20,000 Swainson`s Hawks were poisoned within the span of a few weeks after feeding on grasshoppers sprayed with monocrotophos. Fortunately, that situation was corrected following the intervention of the Argentine government, the manufacturers and several other cooperators. However, a review of the available data on monocrotophos indicates that this insecticide is responsible for frequent and unavoidable poisoning cases where it is still in use, especially in developing countries. As with carbofuran, the extreme toxicity of this product means that it is difficult to find use patterns that do not result in bird kills.

There are other avian consumers of grasshoppers. Poisoning incidents that involve the large species that are likely to come in flocks (gulls, partridges, grouse, tinamous, hawks, owls, ibises, egrets and herons) will be easier to detect. More difficult to find will be the small insectivorous birds which may be feeding on the same insects, or feeding them to their young.

3.5. Granulars

Granular insecticides were designed for convenience and the safety of the person applying the insecticide and also to provide timed release of the chemical; yet for birds, many have proven to be disastrous. Several granular products are reported to kill birds -- for example, aldicarb, parathion, carbofuran, fensulfothion, phorate, terbufos, fonofos, disulfoton, diazinon and bendiocab. Granular insecticides come up time and time again as a source of wildlife pesticide mortality. Many cases have been reported in several countries.

Poisoning cases can occur through direct exposure, because several bird and small mammal species are attracted to granular formulations. Exposure can also occur via invertebrates, especially earthworms, or secondarily through predators and scavengers that eat their prey whole or ingest their gastrointestinal tract contents. In Canada and the U.S., we have seen cases of poisoning of waterfowl foraging in puddles in fields more than six months after pesticide application. Out-of-season poisoning cases are therefore possible with these formulations.

The high risk associated with granulars is a result of (1) the high toxicity of several registered products, (2) current agricultural machinery which ensures that granulars are left exposed on the soil surface and (3) the attractiveness of granules to birds.
Despite a lot of research, we still do not completely understand what exactly attracts birds to

granules and under what conditions. The use of granules as grit is known to occur. Normally, grit consists of sand or small rocks swallowed by birds, especially seed-eaters, to help in digestion. Granules made of the dried and granulated cob of corn (maize), or other organic substances, are probably taken as food or mistaken for waste grain. The most dangerous granules are those made of sand (silica) or dried corn. Somewhat less dangerous are clay, gypsum or coal granules. There is insufficient information on paper granules but, like corn, they float, which may present a problem in the case of puddling. Granules that are friable and break down quickly in or on the soil are best for birds, but they are the products least convenient to farmers. Regardless of the type of carrier, a pesticide granule is likely to be a problem if a lethal dose can be obtained in a few granules only. No one, however, has been able to work out what "few" actually is.

To date no agricultural machinery or application technique can achieve complete incorporation of the granules into the soil. Birds have also been known to probe the soil for granules or to pull up germinating seeds with granules attached. The worst applications are those made above the soil surface and in a band (a wide strip over the seed furrow) rather than in the seed furrow. In carefully controlled engineering trials, between 6 and 40% of applied granules were left on the soil surface. Side dressing (when granules are applied to either side of the seed furrow after germination) also leaves most granules exposed. There is a great variability in the types of applicators and how they are used. The same equipment can achieve radically different soil incorporation when used by different individuals under different conditions.

Recently, we were able to compare the number of granules left on the surface after controlled engineering trials of seeding equipment with the number left by working farmers. For precision air seeders, the farmers left 10-20 times more granules on the surface than the engineers. The average number of granules found on the surface in 32 oilseed rape fields was 7-8 per square metre or about 6% of the quantity applied. These were corn cob granules mixed with the seed.

Another less recognized problem is that under wet, acid conditions the toxicity of granules can persist several months. We have on record many kills of eagles and hawks that scavenged dead waterfowl poisoned in wet fields half a year after granular pesticides were applied. Ducks find the granules by sifting through sediments when fields flood.

3.6. Treated seed

Treated seed presents a similar engineering problem. Historically, seed dressings were one of the main sources of bird exposure to organochlorine and mercurial compounds. Poisoning incidents with seed dressings are still relatively frequent, especially in Europe where there is a heavy use of cholinesterase-inhibiting pesticides for this purpose. Lindane (which is a non-inhibitor) is the main seed dressing chemical in North America, a situation which may change soon because of pressures to remove it from the marketplace and an announcement by a major canola growing association that they were phasing it out.

Several bird species make heavy use of waste (or even planted) grain in fields. The exposure associated with treated seed is therefore always high. The size and type of seed dictate which bird

species are at risk. Since use of organochlorines and organomercurials has declined, kills have been recorded with carbophenothion, chlorfenvinphos, bendiocarb, furathiocarb and, to a lesser extent, fonofos. Some kills have been recorded with new insecticides as well, e.g., imidacloprid although it is not yet known how serious or frequent a problem this is. The acceptability of any chemical as a seed treatment depends on a consideration of the seed type, the planting equipment, conditions under which the seeds are planted and the species likely to be interested in the seed.

As with granules, more seeds are left on the surface in turn areas at field borders. Spills can occur anywhere depending on topography and soil conditions. The number of seeds will diminish over time as they are consumed. The speed of disappearance may provide some indication of the risk of exposure of wildlife species.

Systemic insecticides (those that are taken up and translocated within the crop plant) applied either in granules or treated seed can be present in sufficient quantity in a germinating plants to lead to primary or secondary toxicity. In a well-documented case in the U.S., Swainson's Hawks have died after eating insects that were feeding on cotton seedlings, the seed having been treated with the insecticide disulfoton.

3.7. Vertebrate control agents and veterinary products

Rodent control

Rodenticides as a rule are not specific to their intended targets and cause direct impacts to nontarget species. Only a detailed knowledge of the habits of the target species and use of specific baiting locations or specialized bait holders can reduce kills of nontarget species. More problematic is secondary poisoning. Unfortunately, the trend has been for the more recent, more efficacious "single feed" anti-coagulants to present a greater hazard to predators than the older products. Compounds such as difenacoum, brodifacoum, bromadialone, flocoumafen, difethialone and other similar "super" coumarin-type products should not be used in a situation where the target species is likely to be predated or scavenged. Residues may be very long lived and the risk of poisoning cumulative. Generally, this means that the use of these products should be restricted to human dwellings and grain storage areas. The use of thallium and endrin to control rodents has also been shown to have disastrous consequences on raptors. Other strategies should be tried for field-control of rodent pests.

It might be safe to use products with the potential to cause secondary poisoning under conditions that restrict the availability of the target to predators or where the predator, through its normal feeding practice, discards the dangerous body part(s). Strychnine for instance is probably only dangerous if the predator consumes the gastrointestinal tract of the target.

Bird control

Fenthion, which is used to control pest birds in Africa (*Quelea* species) and in North America (e.g., by means of the Rid-a-Bird™ perch system), has given rise to frequent secondary poisoning. Secondary poisoning is also very likely following the use of toxic organophosphorous or carbamate

products in grease for the control of Monk parakeets in South America.

Veterinary drugs

Many poisoning cases result from the use of organophosphorus pesticides for the treatment of ectoparasites and endoparasites in livestock. The most interesting case is that of famphur. Famphur remains one of the leading causes of eagle poisonings in the American Southwest. It persists on the hair of cattle up to 100 days after treatment. Magpies are poisoned when they eat the hair, and eagles when they scavenge the magpies. Fenthion can also give rise to problems when used this way.

Recently, problems have been documented when treated animals contaminate water bodies. Medicated feed at livestock feed yards is another high exposure situation. Sparrows, starlings and other birds pick up the feed and subsequently are scavenged by hawks and eagles.

3.8. Forestry insecticides

Insecticides applied to large tracts of forest for the control of defoliators, if at all toxic to wildlife species, are bound to be problematical. In a forestry situation, critical wildlife habitat is sprayed directly, and a large number of individuals of many species are exposed to the chemical. For that reason, forestry products should be more stringently reviewed than agricultural products. In Canada, the forestry insecticides phosphamidon and fenitrothion were canceled after impacts on birds were judged unacceptable. Although fenitrothion is not as acutely toxic as a number of other anti-cholinesterase insecticides used in agriculture, its use in forestry leads to severe and widespread inhibition of brain acetylcholinesterase in a number of songbird species. Similar levels of inhibition have been associated with serious sublethal effects as well as mortality. Research suggests that fenitrothion is readily absorbed through the skin, and this may help explain the impact on birds when it is applied as a fine aerosol.

4. STRATEGIES TO STUDY AND MONITOR THE IMPACT OF PESTICIDES ON WILDLIFE

Frequently, when a pesticide is initially submitted for registration, comprehensive environmental field testing with the product has not been done. Thus hazard is frequently estimated on the basis of toxicity values to a few test species and the projected use of the product (see Appendix 1 for a discussion of current methodology for wildlife risk assessment of pesticides). This hazard assessment often follows a "quotient" method, in which the levels that cause toxicity and/or mortality of test species are compared to predicted levels of exposure. In theory, safety factors are introduced in this calculation to allow for errors in estimation or extrapolation.

In practice, however, the level of uncertainty is so high that most of the acutely toxic pesticides, such as organophosphorus and carbamate insecticides, cannot be assessed adequately without field testing. If done at all, either as part of the regulatory process, or following a regulatory decision, field testing will follow one of two directions: (1) directed studies where the experimental conditions are controlled and the questions asked are very specific, or (2) incident monitoring, where the intent

is to put in place a network of competent observers in order to be able to investigate reported problems or carry out spot checks of operational pesticide use.

4.1. Field testing -- active monitoring

Carrying out a field study to measure the impact (or lack of impact) of a specific pattern of pesticide use usually consists of the surveillance of individual birds and/or avian populations prior to, during and after the application of the pesticide according to label instructions. Researchers observe or count individuals of one or more species within and outside the treated area and record behaviors and activities. Sometimes they capture birds to ascertain the health of individuals or to collect samples, for example, blood for biochemical assays or feathers for residue determinations. Less commonly, they find and monitor nests or band or mark individuals in order to assess turnover rates and help locate sick and dead birds. Often, they search for carcasses in order to record mortality and determine the cause of death.

No single strategy is adequate for all situations. Rather, it is often best to start with testable hypotheses and then devise ways that will allow one to prove or disprove these hypotheses. In probabilistic terms, type I errors (or concluding that there has been an effect from a pesticide when in fact there have been no effects) are uncommon. For example, when one encounters instances of wildlife mortality in a treated field, the probability that this mortality is unrelated to the application is small for the simple reason that it is uncommon to witness random wildlife mortality under normal circumstances. On the other hand, the probability of type II errors (or failure to detect an occurring problem) is much higher. It is easy to delude oneself that one would see an impact if it occurred. A good example of this is carcass searches conducted on treated fields when it has not been ascertained whether affected wildlife are likely to die locally or whether they are likely to leave the area and die further afield.

Field studies of a pesticide may rely on controlled application of the product by the experimenters or they may rely on normal operational use of the product, either with or without the knowledge of the user. Again, the question being asked should dictate which strategy is followed. Of course, considerations of cost and logistics often weigh heavily in this consideration. The answer obtained will be interpretable in different ways depending on whether the application was done under controlled or operational conditions.

4.2. Crop and use-pattern inquiry

It is not always feasible to investigate the effects of a single pesticide on wildlife. In a number of cropping situations, several pesticides are used in quick succession making the identification of compound-specific impacts difficult. On other farmlands, the mosaic of treated fields can be so complex as to make it difficult to assess exposure to any one pesticide. Two approaches then suggest themselves: (1) treated sites or landscapes are compared to non-treated areas provided those can be found and (2) the "severity" of treatment (the *a priori* expectation of toxicity) for any given site is used as a variable against which a number of different parameters (such as reproductive success) are regressed. Great care must be taken in comparing treated to non-treated areas because

they are likely to differ greatly in several ways, making the interpretation of the results difficult. In Canada as well as elsewhere, these approaches have been used to look at the impact of multiple sprays on the bird fauna of fruit orchards and in comparing the number and diversity of wild species on conventional farms and on "organic" farms or farms where synthetic pesticides or fertilizers are not in use.

4.3. Population enquiry and baseline wildlife surveys

Data from regional or national surveys of wildlife population levels are rarely adequate to demonstrate pesticide impacts. However, with some particularly damaging compounds (e.g., DDT, dieldrin), it has been possible to document regional and near-global population effects. Systematic survey data are not available in many countries. However, local lore and knowledge of the local fauna by the growers and field hands should not be overlooked. The bulk of pesticide application is by hand in developing countries, and field hands develop a rather intimate knowledge of the field and surrounding areas. Field workers should be encouraged to report any mortality, abnormal behavior or disappearance of wildlife species. Thought should be given to the setting up of standardized surveys to estimate wildlife abundance and diversity in intensively farmed landscapes. Rare, vulnerable or ecological keystone species should be used as indicator species wherever relevant.

In order to carry out wildlife monitoring in treated areas, it is necessary to have a good knowledge of the normal complement of species for the area of concern and to be able to assess the vulnerability of each of these species during and after pesticide treatments. Investigators should keep in mind that the diversity or abundance of species may already be affected by past pesticide uses. Some sort of quantification of the use of cropland by wildlife is a necessary part of any well- designed impact assessment program.

4.4. Baseline agricultural surveys

Our ability to define wildlife impacts is often hampered by a lack of knowledge of prevailing agricultural practices. Such information may be exceedingly difficult to obtain when the growers are not willing to cooperate with the investigators. It is especially important to persevere in those cases where support from the local community is lacking, because it may indicate a failure to comply with existing pesticide regulations. Realistically, however, no amount of policing can replace a good grower education system and the full participation of landowners and field workers in promoting sound environmental protection.

It is often necessary to conduct engineering or residue monitoring studies to define the extent of the hazard to the local wildlife population. An example of such an approach is the measurement of the proportion of granular insecticides or treated seed remaining on the soil surface after typical "incorporation" procedures with existing agricultural machinery. Similarly, the level of residue remaining on leafy surfaces after application of a liquid spray, and at various times before harvest, can be useful for determining exposure to wildlife feeding in the crop.

4.5. Incident monitoring schemes -- passive monitoring

Even if a pesticide has been studied extensively under controlled conditions, unforeseen problems and situations often arise following the commercialization of the product. Incident monitoring refers to the capacity of competent authorities to investigate reported problems or conduct spot checks of use conditions. An absence of incident reports does not necessarily mean there are no problems but, conversely, well-investigated incidents can reveal unforeseen aspects of a pesticide or reinforce a suspicion that arose in the course of field testing. An incident monitoring scheme will require a network of individuals trained in carrying out pesticide investigations and in proper handling of carcasses and tissue samples, as well as access to a laboratory equipped to do chemical and biochemical analyses.

Why incident monitoring is important?

Registration decisions are made on the basis of very limited information. The following factors contribute to uncertainty surrounding risk assessments:

A: Most of the information submitted by pesticide companies applies to human health. A limited number of studies are relevant to wildlife species and only a few wildlife species are ever tested.

B: There are large differences in toxicological and ecological vulnerability among species (see Appendix 1 on risk assessment).

C: The ways in which wildlife species are exposed to pesticides are varied and difficult to predict or study.

D: The behavior of pesticides depends on local conditions. Their mobility and persistence are soil and weather dependent. Pesticides are often tested under standardized conditions only -- they cannot be tested under all possible soil types and climatic extremes encountered in a vast country.

E: The outcome of exposure is also much more variable in the wild. Pesticide exposure can interact with weather, the condition or health of the animal, etc. A good example is control of *Quelea* species with fenthion: most birds die of starvation -- not of the acute effects of the pesticide.

All of these considerations increase substantially the uncertainty associated with predictions of risk. Whether or not pesticides are routinely field tested to look for environmental impacts, it is essential to have a good incident monitoring system in place. An incident monitoring system can also be useful to warn manufacturers if their products are abused or used incorrectly.

Inclusion of incident monitoring information in the decision-making process

The usefulness of an incident monitoring scheme will grow as the quality, reliability and coverage of reports increase. Incident monitoring data can be used to:

- verify whether registration decisions were appropriate: i.e., confirm a risk predicted from lab data, or identify a risk not predicted from lab data
- trigger more systematic field studies
- improve label directions
- allow recommendations on the "best" product to use under some circumstances
- trigger a regulatory review

- ensure that products are being used correctly
- provide data for potential legal action

Existing pesticide incident reporting systems -- good and bad points

There are biases in any reporting system; it is important to understand and recognize those biases. The biases will depend on how the incident monitoring system is set up and on the persons/organizations responsible. Some biases can be reduced over time, but others are unavoidable. Common biases relate to body size and color of the casualties, numbers and density of the species in any given area, "status" of the species and individual and institutional interests and sensitivities. The most important ingredient is the commitment and interest of the participants. Honesty and openness of the system with adequate feedback to the participants is crucial.

A quick assessment of existing systems:

- U.K.: the best model because of stable funding from a tax on pesticide sales and because it was designed for pesticides. Also, heavy involvement of nature groups
- France: emphasis on game and strong disease component
- The Netherlands: strong emphasis on disease
- U.S. EPA: effort variable in time and space -- relies on State-based systems
- U.S. FWS: emphasis on selected species and protected areas; also strong emphasis on disease
- Canada: strong emphasis on disease; use of raptor rehabilitation centers; uneven coverage

Most schemes have a heavy bias to cases of pesticide abuse. It is important to recognize the difference between approved use, normal use, misuse and abuse. However, it is often difficult to categorize pesticide use into "proper" and "improper" because of difficulties with label interpretation, permitted latitude in pest control, inadequate machinery or instructions that are impractical or impossible to follow.

5. INTRODUCTION TO CARCASS SEACHING

5.1. Difficulty of finding carcasses

Carcasses and poisoned wildlife can be very difficult to find for several reasons:

- the majority of kills consist of very few widely dispersed small birds
- many species are cryptically colored
- poisoning may be delayed and occur away from the site of intoxication
- poisoned individuals will often find cover
- carcasses quickly disappear because of scavengers

5.2. Importance of the search effort

Few poisoning incidents are detected: of those detected, few are reported. The small number of incidents involving carbofuran granular on record in the U.S. is a case in point. The product is one of the most toxic to birds, was used on a large scale and is now known to kill birds almost anytime it is used.

There is a large difference between casual searching of fields and a well organised intense search of an area. Again, carbofuran offers a good example: low effort searching (single untrained observer, three visits per field within 48 hours after application) produced 20 carcasses/1000 ha. Intensive searches (several trained individuals, transects, visits every two days after application) of other fields with the same formulation produced 200-9000 birds / 1000 ha. The most important factor under your control is the motivation of the searchers.

Even a well-organized search effort can be affected by uncontrollable variables. In one study, rain reduced the probability of finding carcasses by 30-45%. Scavenging is one of the most important factors. Typical rates of carcass removal by scavengers are 40-90% in the first 24 hours.
Every carcass counts. In one recent exercise in Canada, 32 fields were treated with a granular insecticide and searched the next day and again three days after application. Seven carcasses were found with residues. The maximum number of birds per field (64 ha) was two. Based on measured scavenging rates, this represented about 300 birds in the search transects. Extrapolated to the fields where the compound was used, the total kill was estimated (albeit with impossibly large confidence intervals) to be between 100 000 - 1 000 000 for the five species found dead. Very few carcasses can indicate a sizable impact.

5.3. General considerations to conduct a successful investigation and carcass search
The following are questions you should be keeping in mind as you begin your investigation. However, you must be careful not to ignore the unexpected.

A good investigation involves:
- adequate resources and equipment
- keen observation and an inquisitive mind
- familiarity with the literature on birds and bird kills
- knowledge of resources and operational and administrative procedures

Keep the following in mind:
- A pesticide may not necessarily be the cause of the mortality; the mortality may be the result of other chemicals, metals, disease, trauma, weather, etc. Conversely, pesticide intoxication may be a causal factor in a kill visibly caused by something else -- e.g., intoxicated birds hitting fences, cars or buildings
- Is a specific chemical or class of chemical suspected based on the interviews?
- Is there a history of similar problems in the area?
- Is there a perceived problem of wildlife as pests in the area?
- What is the crop/landscape like -- are you likely to be concentrating your efforts in a single crop/field or over a wider area?
- Has there been unusual weather or rainfall that might affect pesticide exposure?
- Are there features that may concentrate wildlife after they are exposed: hedgerows, trees, waterbodies etc.?
- What exposure routes are likely ?

- What species are present at this time of the year?
- What species may have been poisoned based on feeding habits? Are they more likely to be concentrated in the field edges or in the crop area?
- What is the time of the year? Are the birds migrants or residents? Are they likely to be in flocks or solitary?
- What will be the most suitable strategy for carrying out the carcass searches: transects, distance sampling, etc. The choice of the appropriate strategy will depend on the size of the kill, the habitat, the time available for the search, etc.

5.4. Legal considerations

The possibility exists that the incident may become important in a regulatory or legal case and the findings may be scrutinized. Therefore, an investigation should be:

- carefully planned
- properly conducted
- legally defensible

Getting the most of an incident report:

Upon receiving notification of an incident, an investigator can obtain significant amount of information about the kill. Questions to ask the informer, landowner or applicator to clarify the nature of an incident:

- Where has the event taken place?
- When did the incident occur and where were dead or disabled birds observed?
- What species were affected?
- How many of each species have died?
- Did the deaths occur over a short or long period of time?
- Is mortality ongoing?
- At what rate are the individuals dying?
- What are the signs of sick or dying animals?
- Have there been any deaths of livestock or other domestic animals?
- What were the climatic conditions (precipitation, wind, temperature) preceding the incident and have they recently changed?
- Has there been a recent pesticide application or changes in land use practices?
- What were the details of the application -- product, rate, tank mix concentration, machinery and configuration?
- Has anything similar occurred before in this locality?
- Any opinion as to what may have caused the incident?
- Have any carcasses been collected already and how are they stored?

Based on these questions, if an investigation is warranted, a specific case number should be assigned to the investigation and used on all labels, tags, data sheets, photographs, and other records related to the incident.

Preparation for an investigation

Before entering a site:

- the hazard to the investigator should be assessed
- the equipment needed should be checked and inventoried
- The sample identification system should be in place before field collection
- The same identification system should be used by all parties involved
- There should be communication between the field crew and laboratory about sample size, type, identification system, collection protocols, preservation methods, chain of custody requirements and analysis required

Initiating an investigation

Three rules:

- Protect yourself and others
- Get the best case histories
- Get the best specimens

Handling and collection of specimens will determine what the laboratory can do with them. Each sample should be uniquely identified and related in time and space to the incident, associated to the primary incident number, and accompanied by a chain of custody record. A field log with the information on the sample label is necessary on each sample in case the sample label is destroyed.

Documentation

Written record of an investigation should include:

- forms to record data
- names and telephone numbers of persons to be contacted
- names and telephone numbers of other departments and laboratories involved
- map of the incident area
- sample logbook (catalogue of specimens collected)
- maintenance check sheet
- chain of custody materials
- log of phone conversations (party, phone number, date, time, conversation)
- photographs or video of site
- description of the general site (especially including unique geographical markers)
- positions and location of victims (place your identification or some marker next to victim)

Handling evidence

Treat every case as a legal case!

When handling evidence consider issues of:

- Accountability
- Contamination
- Alteration
- Real evidence vs. circumstantial evidence

Chain of custody: the witnessed, written record of all individuals who have maintained unbroken control (custody of) the evidence since its acquisition. The chain of custody begins when an item is collected and is maintained until final deposition. This is the documentation that protects you, the investigation, and the case from attacks on the credibility of the evidence. It demonstrates that the evidence can be accounted for at all times. For example, you could be asked the following questions:

- Where did you keep the birds?
- What else was in the freezer?
- How were the bird carcasses protected from contamination?
- Did the same person handle different specimens?

Keep the chain of custody as short as possible. A sample is considered in custody if it is in actual possession, it is in view after physical possession, or it is placed in a secure area (accessible by or under scrutiny of authorized persons only). At the end of all diagnostic analyses, the original chain of custody forms should be maintained by the responsible agency with other case-related records. The individual responsible for storage should maintain accurate records documenting the evidentiary items and the dispensing of the items when transferred out of the facility. All forms must be completely filled out.

Labeling

A label should have:

- Unique case number and/or identification number
- Species name
- Date of collection
- Location of collection
- Collector's name, affiliation, phone number
- Name of person to receive the specimens

Evidence Identification Tag: Identification for each item seized or collected. One tag per item (item does not have to be biological).
Evidence Seizure Tag: Summary of Evidence Identification Tag. To place on the entire package/box.
Evidence Submittal Form: For submitting samples to laboratory
Located Evidence List: Form for refreshing memory
Shipping evidence:

- Check with transporter first
- Proper shipping and handling of specimens is necessary to ensure that all specimens arrive at the laboratory in good condition.

Considerations for shipping:

- Prevent cross contamination of specimens
- Prevent decomposition of specimens

- Do not allow specimen fluids to leak
- Properly identify each specimen
- Properly label the package being shipped
- Carcasses and tissues should be double-bagged in plastic and sealed
- Breakable containers should be protected
- Specimens must be kept cool during shipping
- Blue ice can be used or if ice is used, it should be contained to prevent leakage (e.g., plastic jugs with frozen water).
- Use insulated containers (e.g., coolers with walls at least 2.5 cm thick) for shipping
- Line the shipping containers with a plastic bag and then place samples
- Place case history and chain of custody materials in either in a sealed waterproof bag in the shipping container or in an envelope taped to outside of cooler
- Coolers should be placed in cardboard boxes or wrapped in cardboard to prevent breakage during shipping
- Use crumpled newspaper to fill in gaps
- Seal entire package with strapping tape and ship overnight
- Before shipping, ensure that someone is available to accept the package
- If the package contains biological specimens, it should be marked on the outside as "Wildlife Diagnostic Specimens"
- Containers may be boldly marked "EVIDENCE" and "TO BE OPENED ONLY BY _____"

5.5. Personal safety

General principles

For the purpose of personal safety, investigators should presume that the event is a result of a contagious disease until proven otherwise. Investigators must take every precaution to ensure that disease is not spread to humans, other wildlife, or domestic animals. Safety of the investigator and public should be the primary concern.

Skin contact with all unknown materials and solutions should be minimized by wearing appropriate clothing. This is also true when entering treated areas, washing equipment, putting hands in unknown solutions or soils that might be contaminated, or handling contaminated plants or animals. Information on toxicity of the material to humans, recommended handling procedures, and chemical and physical properties of the materials should be studied before handling if possible.

Remember to:

- Wear protective clothing and disinfect yourself, clothing, and equipment before leaving the site, or bag clothing and equipment for cleaning later
- Wash clothes and sun dry
- Wash hands with soap and water (especially before going to bathroom)
- Wash boots and equipment

Re-entry interval

- Varies by chemical
- Requires PPE (Personal Protective Equipment). You may want to use PPE beyond the re-entry interval

Anthrax

- Deserves special mention because very infectious and high mortality rate
- Appearance of carcasses may be very similar to cases of poisoning by anti-coagulants
- Avoid handling bleeding carcasses in areas where anthrax is present

6. METHODS FOR FIELD COLLECTION OF ENVIRONMENTAL SAMPLES: VEGETATION, SOIL AND WATER

6.1. General aspects:

Why:

- To determine potential dietary exposure in grazers or browsers, or dermal exposure
- Applicator may not know, may not tell you or may give wrong information on the identity of chemical
- May help determine if an application rate was exceeded

Where:

- crop or treated area if looking for highest residue possible; depending on the situation, samples can be randomly collected or taken preferentially from an apparent "hot spot"
- edge habitat to look for evidence of drift
- sampling decisions will vary with method of application e.g., ground cover at drip line of fruit trees

How:

- >10 grams per sample; a sample of at least 40-50 g is recommended; one of 100 g is usually sufficient
- for water, this depends on size of the body of water; e.g., if from a pond take one litre
- 10 samples collected and kept separate; may be pooled at a later date
- clean jars (chemical cleaning if used once before). Glass is the usual recipient; linear polyethylene bags (whirlpacks™) or jars acceptable as well and better for metals
- chemically-cleaned scissors, clippers, trowel or corer; alcohol/acetone wash needed in field to clean tools between samples; if manual picking is used, change gloves between samples
- samples to be placed on ice for transport and frozen as soon as possible; keep away from light (either dark-coloured containers or foil)

6.2. Vegetation

- When: for applications of systemic pesticides or any pesticide in liquid form.

6.3. Soil

- When: for almost any situation; especially recommended for seeding or granular application, or when vegetation not available e.g., pre-emergence sprays or soil injection
- How: surface scrapings recommended to maximize probability of finding residues; taking sample from known surface area would help establish if application rate exceeded
- Sample obvious spills of seeds and granules; label as such

6.4. Water or water film

- when: puddles or drainage pond present, appears to be source of intoxication

6.5. Spray solution

- 1 litre samples adequate

Extra care needed when handling samples of concentrated pesticide

6.6. Others

- toxic baits or treated seed: 100-200 g adequate; any amount may be sufficient because of high concentration

7. METHODS FOR FIELD COLLECTION OF ENVIRONMENTAL SAMPLES: INSECTS AND OTHER INVERTEBRATES

7.1. General aspects:

Why:

- to determine potential dietary exposure in insectivores

Where:

- in crop area or feeding on crop plants

When:

- the insects are recognised prey items

How:

- in order of preference: hand collection (tweezers) of dead/dying insects >> sweep net from sprayed vegetation >> pit fall traps
- for earthworms, grubs and cutworms: collect from surface if present; use shovel otherwise
- quantity as above

8. SAMPLING CARCASSES

8.1. General considerations

- avoid contamination of your samples
- carry out dissections away from the field area, if possible
- use a clean surface to work on for each carcass handled

- if many carcasses available, choose whole and fresh carcasses
- if many birds available, keep some whole and refrigerated for pathological analysis; ship whole carcasses to a veterinary centre
- collect as many as feasible; the size of bird and space available for proper storage are factors.
- clean instruments beween carcasses

8.2. Tissue collection for chemical or biochemical analysis:

1) **Brains**

- heads can be severed, placed in whirlpacks and frozen immediately
- or... if liquid nitrogen tank is to be used, best to dissect out complete brain (including cerebellum), use cryovials or wrap in double aluminium foil keeping identification tag away from direct contact; parafilm is recommended as a layer between the brain and foil
- keep some brains in storage -- don=t send all to lab at the same time
- an option is to do sagittal section (right and left hemisphere) of the brain and reserve one half. No other type of division is acceptable

2) **Gastrointestinal (g.i) tracts**

- most important clue is the identity of the gut contents; look also for staining from bait material
- remove entire g.i. tract and place on clean surface for dissection
- if many animals available, concentrate on those with full oesophagus/proventriculous/crop (upper g.i.)
- if upper g.i. contains food material -- take only this area for sample
- if all g.i. tracts empty, take entire g.i. tract (oesophagus to cloaca)
- samples to be placed on ice for transport and frozen as soon as possible

3) **Rest of the carcass**

- bag and freeze for possible future analyses
- one of these analyses may be extraction of residues from feet and feathers

9. THE USE OF CHOLINESTERASE MEASUREMENTS IN WILDLIFE SURVEILLANCE

In any incident monitoring system, poisoning cases involving cholinesterase inhibitors (organophosphorus and carbamate pesticides) are frequent. It is essential to be able to diagnose them. The test has the advantage of being economical and relatively easy to carry out.

It provides useful information in many cases but is not infallible and the results need to be interpreted carefully. For example, carbamate poisonings are often harder to diagnose. At higher temperatures especially, evidence of inhibition disappears spontaneously. This could be a serious problem when carcasses are not immediately sampled. For purposes of a pathological examination, carcasses must not be frozen. Yet, waiting for the necropsy to be completed before taking brain samples for measuring acetylcholinesterase levels means that evidence of a carbamate exposure may be lost. The solution: When several specimens are available, some (or at least their heads) should be frozen as soon as possible without waiting for necropsy results.

Not every analytical method currently available is equally well suited. For example, the use of a plate reader for measuring samples inhibited by carbamates is probably not a good idea because of the high degree of sample dilution required and, therefore, the high risk of spontaneous sample reactivation.

Furthermore, certain pesticides do not cross easily into the brain, especially when death is rapid, which is often the case with carbamates. A large proportion of birds lethally dosed with methiocarb in a laboratory and dying within a 2-hour period show no significant level of cholinesterase depression. For the insecticide fenamiphos, it has been reported that brain cholinesterase measurements are meaningless.

In birds, blood cholinesterase activity is entirely in the plasma fraction and results from a mixture of acetyl and butyril cholinesterase, the proportion of which varies between species. The efficiency of the various substrates used in measuring the enzyme activity is therefore variable. Blood cholinesterase levels are more variable than brain and tend to indicate exposure rather than a life-threatening intoxication.

When an animal survives the intoxication and is kept under surveillance, the sequential measurement of blood cholinesterase levels (e.g., daily) offers the most reliable indication that there was exposure to a cholinesterase inhibitor.

The chemical or spontaneous reactivation of a sample is also a powerful tool. Often, failure of a sample to reactivate is due to the presence of insecticide. When the sample is filtered in an exclusion chromatographic column to strip the insecticide out, it is often more easily reactivated. Failure to spontaneously reactivate but a positive reactivation with the oxime 2-PAM, indicates the presence of an organophosphorus pesticide. This test is not foolproof, however, because reactivation may be prevented by either remaining pesticide or chemical "aging" of the sample. In summary, although cholinesterase measurements are useful, they are not infallible and must be interpreted carefully considering all the ancillary evidence and chemical analyses when available. When there is any evidence of cholinesterase depression, or even where there is no clear evidence but where circumstances are highly suspect, one should proceed with the chemical analysis of the gastrointestinal tract contents. This is usually (but not always) where the highest pesticide levels are noted. In some cases, it is recommended to also rinse the feet and feathers of birds suspected of having been dermally exposed.

The level of a pesticide measured in a carcass is extremely variable. One may think that criminal abuse cases are characterized by high residue levels because concentrated baits are often used. In practice, however, the extensive overlap in residue levels between abuse cases and poisonings occurring in the case of labeled pesticide use means that residue levels cannot be used to separate those two situations.

Similarly, brain cholinesterase depression levels correlate poorly with the residue concentration found in the gut. Therefore, when the presence of a cholinesterase inhibitor is confirmed through a cholinesterase assay, any finding of a cholinesterase inhibiting pesticide, regardless of its concentration, should be considered significant. Failure to find a pesticide agent chemically is not absolute proof that intoxication did not take place.

The involvement of cholinesterase inhibitors or other pesticides should always be considered unless specifically ruled out. There are several examples of birds showing signs of trauma (collision, electrocution) with an underlying exposure to an anticholinesterase agent or an anticoagulant before their accident. This seems to be especially true for raptors which are often found far from where they were poisoned. Available data suggest that cholinesterase inhibitors reduce motor and sensory capabilities of affected animals making them more susceptible to accidents.

The measurement of cholinesterase levels, because of its low cost and because of the frequent involvement of cholinesterase inhibitors in poisoning cases should be routine in agricultural areas even when an alternate diagnosis (e.g., trauma) is indicated. Several methods and approaches make the collection and assay of samples quite simple.

A detailed description and analysis of the stomach contents of poisoned animals remains one of the most important tools in understanding why an animal was poisoned. Unfortunately, this information is often missing or not detailed enough.

10. PRACTICAL CONSIDERATIONS FOR THE USERS OF PESTICIDES AND THE REGULATORY AUTHORITIES CONTOLLING THE USE OF PESTICIDES.

In conclusion, here is a list of recommendations for environmentally-friendly pesticide use that does not impact bird populations.

* Use the least toxic and the least persistent product available for the use required. Consider whether spraying is absolutely essential. Assume that the product is as toxic or more to birds and other wildlife than it is to humans.

* Avoid using products that are known to move away from the area of application through vapor drift or runoff.

* If you have a choice, use a spraying time which does not coincide with the breeding season for wildlife species in your area. Avoid spraying near nests, dens or burrows.

* Follow label instructions scrupulously. Take heed of any special warnings concerning fish or wildlife and abide by specified buffer zones.

* Avoid the use of granular formulations of acutely toxic insecticides. If you must use these

products and are applying them with mechanized equipment, shut off delivery before you reach the ends of the rows if you can and avoid any spills over bumps, in turn areas and at loading sites. Cover any visible spills. If you are applying pesticides by hand, ensure that a mechanism is in place to ensure the best possible incorporation of the granules into the soil.

* Protect valuable wildlife areas by staying well away from field edges, woodlots, wetlands (even if temporarily dry), ditches, hedges, fencelines, rockpiles, etc. Do not allow pesticide spray to drift onto these habitats.

* Inspect your field areas carefully. Avoid the repeat use of any product that causes any wildlife mortality. Experience shows that you are only seeing the "tip of the iceberg.".

* Treat and dispose of empty containers as directed. Where the necessary programs are available to you, recycle them.

* Avoid contamination of any body of water, whether permanent or temporary in nature.

* Never wash your spray equipment in lakes, ponds or rivers. If drawing water from these areas, use backflow devices.

- If carrying out a vertebrate poisoning program, ensure that bait placement minimizes exposure to nontarget species. Locate and remove all carcasses so as to avoid scavenging. Avoid using products of high secondary toxicity if it is at all likely that the target species will be at some risk of predation or scavenging.
- Protect all species of raptors. These often fall prey to farmers who hold the misguided views that they represent a threat to their livestock. Their benefits to agriculture almost always outweigh their occasional taking of livestock.

* Report any incident of wildlife mortality to competent authorities. Only through such feedback will it be possible to minimize wildlife impacts in the future.

Appendix 1. RISK ASSESSMENT IN WILDLIFE: SOURCES OF UNCERTAINTY

The following is intended to give the reader a brief introduction to some of the difficulties in accurately predicting the possible impacts of a pesticide on wildlife on the basis of laboratory-derived results with emphasis on those factors that might cause one to underestimate the hazard.

A1.1. Laboratory data available for hazard assessment

Table 1. gives a list of the usual (Tier 1 and some Tier 2) environmental data requirements currently required by the U.S. Environmental Protection Agency (EPA). Because of the importance of the U.S. pesticide market and because a U.S. registration is highly desirable to pesticide manufacturers, these data are usually available for most of the pesticides currently registered worldwide. Canada

currently accepts all data submissions pertaining to environmental toxicology generated to U.S. specifications. Other data are assessed on a case-by-case basis.

Table 1. Environmental toxicology required for the majority of field-use pesticides at the Tier 1 and Tier 2 level.

Vertebrate tests:

- Avian single-dose oral LD50
- Avian dietary LC50
- Wild mammal toxicity test (seldom available -- data on laboratory rodents used instead)
- Acute toxicity test for freshwater fish
- Avian reproduction test
- Fish early life-stage test

Note: Under the OECD umbrella, many of the avian and mammalian tests are being re-assessed or modified.

Invertebrate tests:

- Acute toxicity test for freshwater invertebrates
- Honey bee -- contact LD50 and toxicity of foliage residues
- Aquatic invertebrate life-cycle test

Note: Supplementary tests on estuarine and marine fish and invertebrates are also requested where relevant.

Plant tests:

- Some nontarget plant data are available. This is in a state of flux.

The way in which these data are used by the U.S. EPA to generate hazard scenarios is currently under revision. The goal is to assess the risk that the pesticide in question will cause mortality of exposed wildlife and hence the need for field work to either confirm or negate this assessment. Indirect impacts through habitat modification are poorly assessed at this time.

A1.2. Application rates and expected residue levels

Under operational conditions, considerable variation in application rates of pesticides can be expected. This is generally not recognized as a problem by the user of pesticides, because all pesticides benefit from a relatively wide margin of safety in terms of both efficacy and safety to the crop. Exact application of a pesticide according to label instructions would require accurate measuring out of the various tank mix components, perfect calibration of the equipment being used, faultless technique on the part of the applicator and finally, ideal weather and terrain.

Even under the highly regulated and mechanized conditions in effect in industrialized countries, these requirements cannot be met. Rather, it is more reasonable to expect that the rate of the pesticide delivered to the crop follows a broad distribution about the desired application rate. This is especially true for pesticide use in parts of the world where the bulk of the spraying is done

by means of back pack sprayers and hence, is even more vulnerable to human error.
There are other situations that give rise to a higher-than-intended rate of application of a pesticide. Drift is a major problem with either ground or aerial application. One of the important aspects of drift from the point of view of hazard assessment is that, following multiple-swath applications of a pesticide, the additive nature of droplet drift associated with each swath can give rise to high application levels in the downwind parts of the field and beyond.

Exposure of wildlife species, if primarily through the consumption of contaminated foods, is only approximately related to the amount of pesticide delivered to the crop and to nearby non-crop areas. In currently accepted risk assessment procedures residue levels on foodstuffs are estimated on the basis of standard factors which assume that the rate of application and the area of the impacted surfaces are the only factors having a bearing on the resulting residue levels. A recent analysis of residue data on turf grass prompted by widespread mortality of waterfowl caused by the use of the insecticide diazinon showed just how difficult it is to accurately predict residue levels on plant surfaces. Residue values on grass blades following a 1.1 kg active ingredient per hectare mechanized application of diazinon ranged between 17 ppm and 181 ppm over the course of six different studies. Yet, well tended turf is as uniform and structurally simple a "crop" as one is ever likely to encounter in any hazard evaluation.

The relationship between the labeled rate and the likely exposure of wildlife species is therefore complex and difficult to predict accurately even if the route of exposure is known. This is a serious problem for acutely toxic products with low margins of safety.

A1.3. Field persistence of modern pesticides

Most non-organochlorine pesticides are relatively short lived, at least in plant and animal tissue. They are more likely to persist in abiotic components of the environment such as soil, aquatic sediments or groundwater. The rapid disappearance and lack of bioaccumulation of organophosphate and carbamate insecticides, for example, is what made these classes of insecticides so attractive when the problems associated with organochlorine insecticides became widely known. A recent compilation of residue dissipation rates from plant surfaces gave average half lives of 3.0 days and 2.4 days for organophosphates and carbamates respectively. However, there are site specific examples of long environmental half lives so that, here again, generalizations are not always adequate. For example, the relatively lipophilic organophosphate fenitrothion was found to persist at low levels bound to the waxy epicuticle of conifer needles.

A proper evaluation of the persistence of a pesticide requires an exact knowledge of the conditions under which the product will be used. For example, the persistence of carbofuran is 7-10 fold greater in acidic soils (pH 4.3 - 6.8) than in alkaline soil (pH 7.9). The stability of carbofuran granules under acidic conditions has been a factor in large kills of waterfowl in Canada. Carbofuran granules were found to remain toxic to waterfowl throughout the autumn and winter following a spring application.

A1.4. Formulation-specific concerns.
It is usually unclear to what extent widlife species are exposed to the technical pesticide and to what extent the various elements of a pesticide formulation remain with the active ingredient over time. Some additives are known to enhance the toxicity of the product, whereas others have the opposite effect.

Predictably, granular formulations of organophosphate and carbamate insecticides are as toxic or less so than the equivalent technical grade pesticide. Unfortunately, the availability and attractiveness of these formulations to birds more than makes up for any reduced toxicity. Liquid formulations on the other hand are typically of higher toxicity than the parent material.

A1.5. The community at risk
The first step in a pesticide evaluation is a knowledge of the biological communities that are potentially at risk in the area of pesticide use. In a large country with several distinct physiographic regions or for countries with incomplete faunal surveys, this can be a formidable challenge in itself. Species may change their food habits in relation to an overabundant supply such as during an insect outbreak, and therefore even an intimate knowledge of the "normal" ecology of a species may not suffice.

Also, the extent to which the use of the pesticide itself modifies the propensity of wildlife to use the field or adjoining areas for feeding is another question that has not received much attention. The presence of a given array of species in the general area of pesticide use does not necessarily mean that those species will be exposed.

Methods to quantify the use of fields by birds exist. An approach made necessary by the complexity of ecosystems has been to focus the evaluation on a few indicator species. These should be chosen not so much for their inherent physiological susceptibility to the pesticide (this is not usually known) but for the likelihood that their life habits will lead to maximum exposure. Unfortunately, the choice of indicator species has often been made on the grounds of cost, logistics and overall feasibility rather than on the grounds of more scientifically desirable criteria. This can give rise to a false sense of security when the indicators give a misleading signal.

A1.6. Routes of exposure
Knowledge of nontarget species likely to be exposed goes hand in hand with an understanding of exposure routes. Traditionally, ingestion of contaminated food has been identified as the most likely route of intoxication for wild birds or mammals and this is still the only route which is commonly assessed in standardized hazard assessment procedures. It is now known that, under the right circumstances, dermal exposure can be more important than the oral route for birds in treated fields or forests.

A1.7. Assessing dietary exposure
It is a well known fact that the food intake of a small organism is greater than that of a larger one

when expressed as a ratio of its body weight. It therefore follows that smaller species tend to be more vulnerable to taking in a lethal dose of pesticide. Also, small birds have an inherently higher susceptibility to acute dosing.

Depending on the time of the year, wildlife species may have higher energy requirements, and hence food intakes, than at other times. For example, a bird feeding young at the nest, may be expected to have energetic requirements far and above its normal needs at rest.

Climatic conditions and condition factors, such as nutritional status, disease, and parasite load also exert an influence, directly on the toxicity of the pesticides to the organism, and indirectly through their influence on food consumption. These are yet additional reasons why predicted exposure and hazard may be in error.

A1.8. Representativeness of the test species.
Evaluators assessing the safety of pesticides to humans have the benefit of being able to study several surrogate species in order to extrapolate to the single species of interest. By contrast, wildlife evaluators are constrained to look at a few species and predict impacts on a diverse fauna. They do have the benefit, in some cases, of being able to work directly with their species of interest, but for practical reasons, interspecies extrapolation is the rule. There are an estimated 10 000 species of birds living in the world today. Testing all possible species for likely pesticide impacts is impractical as well as unethical.

Phylogenetically related birds do not necessarily show a similar sensitivity to any given pesticide although there are family relationships for some groups of pesticides. Some species do appear to show an inherent susceptibility or resistance to a wide range of environmental toxicants. One cannot rely on the toxicity values obtained in the Mallard (*Anas platyrhynchos*) and Bobwhite Quail (*Colinus virginianus*) (the two most common test species) and assume these values can be used in risk quotients to protect all bird species. Safety factors are now available that can be applied to those data.

Metals As Threats To Wildlife

Mark Wayland

Metals are naturally-occurring substances to which plants and animals have been exposed since the beginning of life on earth. Over the ages, living things have evolved numerous, complex mechanisms to utilize, detoxify or eliminate metals. Therefore, under normal conditions, metals pose little threat to wildlife. In fact, several species of wildlife carry heavy burdens of certain metals, seemingly with no ill effects, probably as a result having adapted on an evolutionary time scale to high exposure to metals.

However, despite the numerous defense mechanisms that living things have evolved to protect themselves, recent trends in environmental concentrations of several, metals suggest that they should be a source of concern for wildlife and environmental biologists and managers. The industrial revolution sparked dramatic increases in environmental concentrations of several metals around the world. Cores from glaciers which provide an historical record of atmospheric events dating back thousands of years indicate that metals such as zinc, cadmium, copper, lead and the trace element, mercury, have increased several-fold since the beginning of the industrial revolution. These increases are the result of numerous industrial activities, the predominant one being the burning of fossil fuels. Evidence of increasing metal pollution in remote polar ice caps is testimony to the widespread nature of this phenomenon. Fortunately, during the past 20 - 30 years, global pollution by metals, with the exception of mercury, has decreased, mainly because of technological advancements in pollution control, and in the case of lead, because of the ban on the use of leaded gas in most industrialized countries. In addition to global pollution by metals, their bioavailability (potential for uptake by living things) may be enhanced at specific sites where certain human activities occur, for example near smelters and at other sites that have been strongly influenced by human activities.

In Canada, and throughout most of the world, metal-induced wildlife mortality is rare. Indeed, there have been only 60 cases of confirmed lead poisoning, four of mercury poisoning and one possible case of copper poisoning reported in the Canadian Cooperative Wildlife Health Centre's records of wildlife mortality in Canada since 1990. This out of a total of more than 10,000 mortality records. Although incomplete and perhaps under-representative of certain types of wildlife, particularly fish, their database suggests that mortality from metals poisoning, with the possible exception of lead, is an unimportant source of wildlife mortality in Canada.

While it is apparent that wildlife mortality from metal poisoning is rare in Canada, it is unclear to what extent sublethal exposure to metals are affecting wildlife populations. However, information has been accumulating recently that suggests that metals do exert sublethal effects on wildlife. Coupled with other environmental stressors such as adverse weather, food shortages and predation,

it is possible that sublethal exposure to metals could affect wildlife at the population level by interfering with physiological processes that normally enable wildlife to cope with those stressors.

Metals encompass a large group of trace elements, some of which are essential for life, such as zinc and copper, and others of which are not required, for example, lead and cadmium. All metals are potentially toxic. Other trace elements, for example, mercury and selenium, are not true metals but are nevertheless conveniently grouped with metals in most discussions of the effects of metals on wildlife. The metals, or, more properly, trace elements of greatest concern and interest to wildlife toxicologists include lead, mercury, cadmium, selenium and arsenic. In freshwater systems, zinc, copper and aluminum are also a concern for aquatic biota. In the paragraphs that follow, brief summaries of sources, bioaccumulation potential, and toxicology of lead, mercury, selenium, and cadmium will be discussed.

Of all the toxic trace elements, mercury has probably received the greatest amount of attention. Human activities, primarily the burning of fossil fuels, contribute > 50% of the total mercury in the environment. After inorganic mercury in the atmosphere is deposited in aquatic ecosystems, it undergoes many chemical changes. The most important change is the conversion of inorganic mercury to organic, methylmercury. Methylmercury is the most toxic form of mercury and the most bioaccumulative. It is also the dominant form of mercury in fish. Lake acidification and the flooding of terrestrial landscapes enhance the conversion of inorganic mercury to methylmercury. This results in an increase of total mercury in fish in such habitats. In fact, in highly acidic lakes and in recently-flooded reservoirs, mercury in fish often surpasses the 0.5 ppm (parts per million) level, which is considered to be the maximum "safe" mercury level in fish for human consumption. Mercury may also increase in sediments of lakes near metal smelters. Such increases have been recorded in lakes near the metal smelter at Flin Flon, MB. Museum collections provide evidence for temporal increases in mercury in wild birds. Mercury levels in the feathers of sea birds from the North Atlantic Ocean were higher in the 1980s than they were during the period 1850-1950. Furthermore, mercury in eggs of sea birds nesting in the Canadian arctic increased between 1975-1998. There is evidence for food chain biomagnification of mercury in aquatic ecosystems, suggesting that predatory species, especially fish-eating birds and mammals, may be at greatest risk from increases of mercury in the environment. In birds and mammals, a large proportion of the total body burden of mercury occurs in feathers and hairs, respectively. Dietary mercury as well as that stored in liver, kidney and other organs can be deposited in growing feathers and hairs. This is an important "detoxification" pathway, because once mercury is deposited in feathers or hairs it is inert and non-toxic. Mercury toxicity manifests itself in several ways. At high concentrations, mercury can damage kidneys, cause abnormal development of reproductive organs, result in neurological impairment and altered behaviour, decrease egg hatchability, result in abnormal development of embryos, impair the immune system and change aspects of the endocrine system with consequent disruption of regulatory mechanisms for many physiological processes. Decreased

reproductive success is probably the most sensitive endpoint for assessing the sublethal effects of mercury on wild birds and mammals. In freshwater birds and mammals, total mercury concentrations associated with death from mercury poisoning are in the range of 20 - 60 ppm wet weight for liver, 20 - 40 ppm for kidney, > 10 ppm in diet, 24 ppm in brain and 35 ppm in mammalian fur. Methyl mercury concentrations may provide a better indication of toxicity and are generally above 14 ppm in brain, 17 ppm in liver and 29 ppm in kidney of otter. Sublethal effects have been demonstrated in non-marine birds when mercury concentrations exceed 0.5 ppm in eggs or diet. This dietary value is based on studies demonstrating lower reproductive success in common loons whose diets exceeded approximately 0.5 ppm mercury. Total mercury levels as low as 6 ppm wet wt in birds' livers have been associated with a greater incidence of chronic disease, although, at present, it is unclear whether the elevated mercury increased the susceptibility to disease by damaging the immune system or whether it was a simple artifact of tissue weight loss that normally accompanies prolonged disease. Fish may also be susceptible to mercury intoxication. Fish with muscle mercury in the range of 6-24 ppm wet weight were "enfeebled" and emaciated at two locations where severe mercury pollution occurred. In experimental exposures, muscle or whole body mercury generally must exceed 5 ppm before sublethal effects occur. These levels are far higher than those seen in most fish throughout Canada, even at sites suffering from relatively high mercury pollution. However, it should be noted that whole body mercury levels of as low as 0.7 ppm, which often occur in fish in Canada, were associated with diminished predator avoidance in experimentally-exposed mosquitofish. In general, levels of aqueous mercury required to elicit toxic effects in fish are much higher than those occurring in most polluted waters in Canada. Reproduction and behaviour are two of the most sensitive toxic endpoints that have been examined to date.

Living organisms have the capacity to detoxify mercury. The process by which mercury is mobilized into growing feathers and hair has already been mentioned. In addition, it is apparent that birds and mammals can demethylate methyl mercury in liver. The inorganic mercury formed by this process of demethylation is both less toxic and more readily excreted than methyl mercury. In addition, selenium is known to co-accumulate with mercury in animal tissues and to protect against mercury toxicity. Thus, the accumulation of high selenium levels together with mercury may protect an animal against the toxic effects of the latter. While the protective effect of selenium is generally true, it should be noted that at least one study found that selenium actually increased mercury toxicity.

Lead has accounted for the highest proportion of metal-induced wildlife deaths in North America. By the late 1950s, it was estimated that 2-3% of all waterfowl in North America died annually from lead poisoning. These estimates were somewhat refined in later years and ranged from 1 - 4 million waterfowl per year. Canadian estimates for losses of waterfowl ranged from 200,000 - 400,000 per year. Unlike the global pollution caused by mercury, the source of lead causing this mortality was

very specific: lead shot used by waterfowl hunters and deposited in wetlands where waterfowl feed. This metallic lead, once ingested by birds, erodes in the gizzard, enters the circulation and causes toxicity. In addition to waterfowl, several other avian species, most notably water birds, game birds and raptors have died from lead poisoning. Lead poisoning accounts for 10 - 15% of known mortality in raptors in western Canada and has been attributed primarily to secondary ingestion of lead shot in the tissues of prey animals, principally waterfowl. Interestingly, in the prairie provinces, lead-poisoned bald eagles were more likely to be found in areas of high waterfowl hunting than their non-poisoned counterparts. However, the opposite trend is true for golden eagles: waterfowl hunting activity was lower in areas where lead-poisoned golden eagles were found than it was where their non-poisoned counterparts were found. It is possible that lead shot used by waterfowl hunters is not the major source of lead ingested by golden eagles. Indeed, several authors have commented that lead bullets and lead shot embedded in tissues of upland gamebirds and mammals may be ingested by raptors. Lead fishing sinkers and jigs have also been implicated in lead poisoning in water birds, particularly swans in Great Britain and loons in North America. In eastern Canada, 50 of 215 dead common loons examined during the mid-1990s had been lead-poisoned. Nearly all had lead sinkers or jigs in their digestive tracts. Fewer loons have been examined in the prairie provinces. To date two of 28 loons examined from this area have been lead-poisoned. Lead from trap and skeet ranges may also pose a hazard to wildlife. When pickerel frog tadpoles were placed in water from a wetland at a trap and skeet range, mortality was nearly 100%. After a chelating agent that binds lead was added to the water, mortality was reduced to 10 - 20%, indicating that lead was responsible for the high mortality in the untreated water. Small mammals living at trap and skeet ranges had tissue lead levels from 2 - 1000 times higher than at nearby control sites. Unlike the situation with waterfowl and other birds that directly ingest leaded ammunition, lead exposure in tadpoles and small mammals was the result of lead leaching from the ammunition into the environment. In the case of the tadpoles, the lead leached directly into water while the mammals were exposed to lead from ingesting soil particles and plants that had accumulated lead leached from the ammunition. Such leaching occurs predominately in acidic environments. Under alkaline conditions, lead is less likely to leach from ammunition. Lead deposited in riverine and wetland sediments near lead mines may also pose a risk to water birds. Liver lead levels in waterfowl collected on the Couer d'Alene River in Idaho near an old lead smelter were much higher than in those collected from reference areas. Several lead-poisoned birds were found in the lead-polluted area of the river. It was determined that the source of lead was the tailings-contaminated sediment. In Canada, there have been no cases reported of lead poisoning in wildlife in the vicinity of lead smelters.

Lead has wide-ranging effects on animals, including effects on the nervous system, the immune system and on the synthesis of heme, a precursor of hemoglobin. Lead-poisoning is often a slow-acting, debilitating disease. Digestion is impaired in affected birds which eventually cease to feed and become emaciated and weak. Green diarrhea stains feathers around their vents. Movement

becomes impaired, followed eventually by death. While lead accumulates over the short term in liver, kidney and blood, bone is a long-term repository for lead. Thus, the first three tissues can provide information about recent exposure to lead while bone can provide information about lifetime exposure to lead. Blood lead from 0.2 - 3.0 ppm, 0.5 - 5 ppm, and 1 - 10 ppm represents subclinical exposure (no obvious adverse effects), clinical poisoning, and death from lead poisoning, respectively, in waterfowl, raptors and gallinaceous birds. Similarly, liver lead levels compatible with the above-named lead exposure categories range from 2 - 6 ppm, 6 - 15 ppm and > 3 ppm (wet weight basis). Kidney lead from 2 - 20 ppm, 3-15 ppm and >5 ppm represent subclinical exposure, clinical poisoning and death from lead poisoning, respectively. In waterfowl, bone lead from 10 - 20 ppm (dry weight) represents subclinical or clinical poisoning while levels > 20 ppm represent severe lead poisoning. In general, gallinaceous birds are least sensitive to lead, waterfowl are somewhat more sensitive and raptors are the most sensitive of these three groups. Selenium is an essential but potentially toxic trace element with a narrow margin of tolerance in animals. Several human activities result in increased selenium levels in the environment. Perhaps the best example is that of irrigation drain water in central California and throughout many of the western states. Irrigation drainwater leaches selenium from seleniferous soils and carries it to terminal wetlands and evaporation ponds. Fly-ash contaminated effluent from coal-fired power plants is another source of selenium in some aquatic ecosystems. Selenium levels are high in certain coal deposits. Thus, selenium levels could be elevated in ecosystems in the vicinity of coal mines. There is at least one such example in Canada from southeastern BC where selenium is elevated in an aquatic ecosystem near a coal mine. Emissions and effluents from some metal smelters may also contain high selenium levels. Another possible source of elevated selenium is liquid waste from feedlots where selenium is used as a dietary supplement. If such waste is routed to outdoor holding ponds that are accessible to wildlife, then selenium contamination of wildlife could occur.

Only in the most extreme cases of selenium poisoning such as occurred at the Kesterson National Wildlife Refuge in California during the late 1970s and early 1980s, is selenium likely to poison adult animals. In contrast, developing bird and fish embryos are highly susceptible to selenium poisoning. Dietary selenium that is only 2-3 times higher than normal may be sufficient to cause developmental problems and death in bird and fish embryos. Like mercury, selenium bioaccumulates through food chains. Thus, while it is normally < 1 ppb (part per billion) in water, concentrations in biota are usually in the range of 2 - 5 ppm in invertebrates, fish and birds in unpolluted environments with the highest levels at the top of the food chain. Therefore, insectivorous and piscivorous birds and fish are normally at greater risk from selenium pollution than plants or herbivorous wildlife. To date, adverse effects on embryos have been seen in several species of ducks, coots, grebes and shorebirds (black-necked stilts and American avocets) as well as in several species of fish. Normally, birds' eggs or livers are used to determine selenium levels, while, for fish, whole bodies or livers are used. Selenium levels greater than 4 ppm in the diet (dry weight), 4 ppm in fish whole bodies or 8 ppm in birds' eggs are associated with toxic effects. Duck

embryos exhibiting signs of selenium toxicosis may have excess water in the head, and have deformities of the beak, eyes, legs and wings. Larval fish often display spinal deformities and vestigial pelvic fins.

The Canadian water quality guideline for selenium for the protection of aquatic life is 1 ppb. In the Canadian prairie provinces, that level has been exceeded in several water bodies, especially those near heavily-irrigated areas or near metal mines and smelters, such as the zinc smelter at Flin Flon and near uranium mines in northern Saskatchewan.

Cadmium is a toxic metal that has increased in the environment principally because of metal smelting which accounts for 65-85% of the anthropogenic cadmium entering the atmosphere and the burning of fossil fuels which accounts for a further 6-7%. Globally, human activities release roughly 3 - 10 times more cadmium into the atmosphere than natural sources. The bioavailability of cadmium is highly dependent on the form in which it occurs. The free cadmium ion is the most bioavailable form of cadmium. When cadmium forms inorganic or organic complexes or becomes associated with particulate matter or, its bioavailability diminishes. Generally speaking, cadmium bioavailability is reduced in saline environments and is increased in acidic water bodies. Thus cadmium accumulation may be more likely to occur in highly oligotrophic water bodies, especially if they are acidic. In aquatic ecosystems, cadmium accumulates in sediments, acting as a source for benthic biota such as mussels. Birds and mammals that feed on mussels have relatively high levels of cadmium.

Classical signs of cadmium toxicity include kidney damage, testicular lesions, delayed maturation of the testes in male birds and bone degeneration. Bone degeneration was observed in Japanese women eating cadmium-contaminated rice and was referred to as Itai-itai disease. It resulted from the inhibition by cadmium of intestinal calcium absorption. Recently, experimental studies on fish have shown that cadmium can disrupt both the immune and endocrine systems at concentrations much lower than those required to produce the classical signs of cadmium poisoning. An important feature of cadmium accumulation in animals is its tendency to induce and bond with a metal-binding protein called metallothionein. Mercury, zinc and copper also induce and bind to this protein. This occurs primarily in kidneys. It is believed that the binding of cadmium and other metals to this protein serves to reduce their toxicities by rendering them inert and unavailable for interaction with enzymes and cells. As cadmium levels in kidneys increase, so too do metallothionein levels. Differences among species in their abilities to synthesize metallothionein may be important in determining their relative abilities to cope with exposure to cadmium and other metals. Cadmium levels are generally much higher in marine birds and mammals than they are in their terrestrial counterparts. At the same time, marine animals appear to tolerate cadmium much better than terrestrial animals.

In freshwater and marine ecosystems, the lowest aqueous levels of cadmium required to produce toxic effects on animals are from 4 - 470 ng / L and from 1200 to 50,000 ng / L, respectively. Of course, these levels are highly dependent on water salinity. In sediments, cadmium levels in the range of 3 - 1000 ppm (dry wt) in freshwater and above 4 ppm in marine and estuarine habitats may cause toxic effects in aquatic biota. These levels have been exceeded at several sites in Canada including the Columbia River and Slocan Lake in BC, several lakes near Flin Flon and Sudbury, the Detroit and Niagara Rivers and Hamilton Harbour as well as at several sites on the BC coast and at Belledune Harbour, New Brunswick. However, throughout most of Canada, aqueous and sediment cadmium levels are usually much lower than those required to produce toxic effects in aquatic animals.

In mammals and marine birds, cadmium levels in kidneys exceeding 30 and 100 ppm (wet wt.) respectively, may cause kidney damage. There are few examples of marine birds with cadmium levels above the 100 ppm threshold. However, many marine mammals (narwhal and ringed seal) and some terrestrial mammals (mainly moose and elk) have cadmium levels in kidneys that exceed the 30 ppm toxicity threshold. It has not been determined with any certainty whether sublethal effects occur at concentrations below the toxicity thresholds reported above. However in eider ducks, levels of corticosterone, a hormone involved in the stress response and in intermediary metabolism, have been correlated with cadmium levels, suggesting a possible role of cadmium in affecting glucose and glycogen metabolism and storage.

In conclusion, lead poisoning has probably been responsible for the deaths of many birds in Canada. However, other metals rarely cause direct mortality to wildlife. Sublethal effects may occur near certain smelters, metal mines and other highly-industrialized areas and in areas where ecosystems have undergone changes that enhance the bioavailability of metals, for example on acid lakes and recently-flooded reservoirs. Wildlife specimens collected near industrial sites and sites where metals are highly bioavailable, especially those species believed to be at greatest risk from metals intoxication should be autoposied and their tissues should be preserved for analysis of selected metals.

Monitoring Impacts of Contaminants on Wildlife

Judit Smits

In the field of wildlife toxicology we try to assess the hazards of environmental contamination to wildlife populations. Investigations may be conducted to determine whether a population decline, or die-off in a wild species (or several species) is the result of exposure to toxins in their environment. Alternatively, if an unusual chemical, or unusually high concentrations of a known contaminant are detected in an area, there will be investigations into the possible adverse biological effects of these compounds on the wildlife at risk. Because the pathways that chemicals take when moving through the environment to get into wildlife is important and interesting, the area of wildlife toxicology is a subdiscipline of environmental toxicology. That explains the necessary collaboration and communication between biologists, chemists, ecologists, veterinarians, the general public, etc. when studying the impacts of pollutants on wild animals.

Generally, with environmental contamination, we are dealing with complex mixtures. Depending upon the major industries in a given area, there may be a great diversity of xenobiotics (i.e., compounds that are foreign to the body) in the environment. To add to the complexity, animals may be exposed sequentially during a particular season to the same chemicals, as well as having chronically, to deal with exposure to many chemicals.

Traditionally, toxicity testing has always been carried out in laboratories, and has been conducted using one compound, at several predetermined concentrations, using one or more laboratory species. Early tox testing protocols also used lethality as the main endpoint. That is what the terms LD50 (acute oral median lethal dose), and LC50 (5 day median lethal dietary concentration) are referring to. These kinds of tests were usually in place for regulatory reasons, but with the growing issues associated with animal welfare, and the true "biological cost" of conducting animal experiments, it was clear that changes were needed. First procedures were modified to reduce the number of animals used, and adjust the testing as results became clear.

The idea of LD50 is now recognized as having limited value in non-regulatory toxicology. Some of the more obvious drawbacks include the issue of interspecific variation being unpredictable. Another consideration is that the time of test relative to the life stage or age of the animal may not correlate with physiologically relevant periods in real life exposure. These tests were usually of short duration, which would mostly provide information on situations of acute toxicity. We now have become, for the most part, more interested in longer term and subclinical effects; things like reproductive problems, behavioural changes, skewed sex ratios, endocrine modulation, and immunosuppression that would lead to increased disease susceptibility.

Generally now that LD50s are not considered appropriate use of animal life, considerable effort and pressure exists to find sound alternatives.

Some of the guidelines for a new approach include looking for alternates to whole animal testing, techniques that could be nonlethal, and relatively non-invasive techniques. In the case of concerns about specific toxic compounds, monitoring can be much more focussed and, therefore, much less costly than some of the situations described above. In a recent example of monitoring specific "areas" for the presence of pesticides that had previously been responsible for an infamous kill-off (Swainson's hawks), the investigation was carried out over the course of one year. Local landowners were interviewed and became an important and integral part of this program. The owners provided information about the types of pesticides they were using, how often and at what rate they were applied. Information was also collected regarding previous mortality events on their property. Farms over the particular area were observed for the presence of night roosts and active hunting by the hawks to allow for plans for live trapping. Birds, once trapped, had washes taken from their legs and feet, and had a few coverlet feathers and blood samples taken. Analyses for specific enzymes and residues were later carried out in the lab. This kind of monitoring accomplished two valuable endpoints; the level of compliance with the newly instituted ban on the use of certain compounds could be established, and, the landowners - the general public - became part of the solution. Education of the non-scientific community may well be the most important step towards improving the health of the ecosystems over which we have such a great influence!

In the efforts to look at the more likely scenario, that of the presence of an unknown mixture of potentially hazardous chemicals, a much more generic approach needed to be established. Now enter "biomarkers" as indicators of exposure to pollutants in wildlife. The development of biomarkers stems from efforts to identify physiological or biochemical changes in target species, which alert researchers to harmful effects on the species being examined that is due to exposure to environmental xenobiotics. They have become basic tools of environmental monitoring programmes.

The following factors have to be established for a variable to be considered a useful biomarker:
1) a relationship must exist between chemical exposure, the biomarker response and an adverse biological effect
2) responses must be distinguishable from natural sources of variability (e.g., ecological, physiological, species specific differences, individual variation)
3) ultimately want to be able to extrapolate the biomarker response in individual animals to higher-level population level effects
4) animal biomarkers may be useful as surrogates to evaluate potential threats for humans or other species at risk of exposure

The ideal bioindicator is one which indicates a biological response, and is not just a measurement of chemical / metabolite residue!

Some commonly used biomarkers

MFO induction (very general)
These cytochrome P-450-containing monooxygenases are in the liver of every animal species examined. The induction (increased levels in the liver) of MFO enzymes represents one of the major ways that a body can get rid of xenobiotics. These compounds are transformed in the liver so they are water soluble and can be excreted (usually through the urine or bile). Measuring the activity levels of these mixed function oxidases has been widely used as a biomarker of environmental toxicity in spite of some short comings. There are few data regarding a dose-response reaction, and the significance of finding increased enzyme activity as it relates to the health of the animals being tested is unpredictable and debatable. The most useful application of this biomarker is that it **is** a biological response to xenobiotic exposure. It has been extensively monitored in wildlife; fish, birds, mammals.
Porphyrins, which are a type of MFO, are sometime used because they are known to be strongly induced by some PHAHs.

Cholinesterase inhibition
This test is the diagnostic test for organophosphates (OPs) and carbamate toxicity. Some of the most widely used OPs in US are methyl parathione, parathion, terbufos, fonofos, phorate and azinphos-methyl. The major carbamates used in Canada and the US are carbaryl (Sevin), aldicard, carbofuran, thiram (seed dressings), and methomyl and butylate (sprays). The way these insecticides work is by inactivating the enzyme cholinesterase, which is an essential part of the nervous system in insects. The problem is they also are effective through the same mechanisms in vertebrates, which leads to, among other unpleasant symptoms, eventual paralysis of the muscles of respiration.

To confirm that wildlife has been poisoned by OPs, the brain is needed to measure cholinesterase activity. It is important to have concurrent submissions from normal animals to generate control (or reference) values for wildlife species. A >20% reduced activity is diagnostic in live animals; while >50% reduction is proof of OP poisoning in dead animals.

Metallothionein (MT)
This compound can be measured in blood or tissues to confirm heavy metal toxicity.

ALAD (aminolevulinic acid dehydratase)
This enzyme, associated with incorporation of hemoglobin into rbcs, is found in the blood.

Increased activity of this enzyme is very specifically indicative of lead poisoning, which makes it close to the "ideal" bioindicator.

DNA adduct formation
This is a sensitive method for detecting DNA damage, and should be considered an early warning of future biological problems.

Vitamin A - retinol levels
Retinol, the biologically active form of Vitamin A, is essential for reproduction, fertility, embryonic development and tissue repair. It can be considered a nonspecific bioindicator of exposure to environmental contaminants.

Thyroid function
Many environmental contaminants have a negative effect on the thyroid glands which are essential for normal growth, development and homeostasis.

Immunotoxicology

This is a fairly new term that refers to the study of chemicals which alter the immune response. Immunotoxicity may mean immunosuppression, or, immunostimulation (like an allergic response). If there is immunosuppression, the affected animals could suffer from increased incidence or severity of infectious diseases, or perhaps increased incidence of tumor formation.

The functional ability of the immune system cannot be evaluated by a single test, because there are so many ways the immune system can back itself up.

In monitoring populations using immunotoxicity techniques;

1) one tries to identify the presence or absence of immune alteration through basic tests
2) once immune function changes are detected, or suspected because of increased incidence of disease problems, more detailed studies follow.

Commonly used tests in immunotoxicology:
- basic hematology; CBC and differential
- histopathology of organs involved with the immune system
- *in vitro* lymphocyte proliferation
- *in vivo* lymphocyte proliferation (PHA skin tests are often conducted in wild birds)
- antibody (B cell)-mediated immunity

1) hemolytic plaque assay (sheep red blood cell (srbc) assay)
2) ELISA - a technique for measuring the total specific antibody (IgG) in serum

- macrophage phagocytosis
- natural killer cell function

General Reference Texts
for sections by Judit Smits

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