Improving Detection of Pesticide Poisoning in Birds

by Pierre Mineau and Kelley R. Tucker

Birds and Pesticides

Birds are an important and visible part of our environment and serve as sentinels of general environmental health. In North America, and in many other developed countries, most bird species are protected from unlicensed taking or kill of individuals. Yet one estimate of pesticide effects on U.S. farmland—considered conservative by many avian biologists and advocates—states that as many as 72 million birds die each year as a result of pesticide exposure (Pimentel 2001). Our ability to refine this estimate or to predict pesticide impacts more accurately is hampered by the difficulty of gathering data, identifying, and assessing pesticide effects on birds in real-life scenarios. Birds are extremely mobile, and it is difficult to exclude them from areas that are treated with pesticides. Whether in agriculture—where bird species attracted to agricultural pests can be economically important for the control of such pests (Kirk et al. 1996, Tucker 2002)—or in forestry, parks, or backyards, birds and pesticides intersect. Birds suffering from lethal or sublethal pesticide effects often escape the notice of humans. When they are noticed, some are brought in to rehabilitation centers. The wildlife rehabilitation community can become a valuable source of information. Research scientists and cooperators from government and industry are working with academic and nonprofit organizations to provide rehabilitators and wildlife professionals with the tools and resources to improve their knowledge of, and ability to report, pesticide incidents.

This paper will discuss the science behind the effects of pesticides on birds, with special emphasis on cholinesterase inhibition, and provide practical information for rehabilitators and others interested in responding to suspected avian pesticide poisonings. While the focus here is birds, much of the information can easily be applied to other taxonomic groups. Mammals, fish, amphibians, and reptiles have all been reported as casualties following pesticide field trials, and biologists around the world are sounding an alarm about the state of reptile and amphibian diversity and population numbers. Birds clearly are not the only taxon under fire.

Cholinesterase-inhibiting Pesticides

According to the Federal Insecticide, Fungicide, and Rodenticide Act—the governing legislation in the United States for pesticide use and regulation—"pesticide" is an umbrella term for a range of "substances or mixture of substances intended for preventing, destroying, repelling, or mitigating" any organism deemed to be a pest. Pesticides are most often categorized by target pest (e.g., herbicide, insecticide, fungicide, rodenticide, avicide) or by chemical class, a somewhat arbitrary system that groups chemicals according to their known biochemical mode of action and common chemical features (e.g., organochlorine, organophosphorous, or carbamate compounds, synthetic pyrethroids, triazine herbicides, etc).

Pesticides are designed to disrupt vital biological processes of a target organism—for example, photosynthesis in plants or blood clotting in mammals. These processes are often shared by nontarget organisms, thus creating a realm of risk. In the most general terms, risk to any specific nontarget organism is assessed by looking at the toxicity of the substance and the probability and nature of exposure of the organism to the substance. In the end, any estimated risk is weighed against the benefits of that same pesticide's prescribed use. Pesticides can be valuable tools in food or fiber production, habitat restoration, disease control, and indoor pest control—more so when used in the context of a well-planned "integrated management" program that emphasizes monitoring and includes nonchemical alternatives.

The importance to agriculture of the two main classes of "cholinesterase-inhibiting insecticides," the organophosphorous (OP) and carbamate (CB) products, is undeniable. The use of these products, however, has not been without problems. Their toxicity to birds has recently been compiled (Mineau et al. 2001). In North

ABSTRACT: Pesticide effects on birds are multiple yet remain poorly understood. We know that birds are important sentinels of ecological health in every environment. Our knowledge of pesticide effects on birds and other wildlife is greatly improved when avian pesticide incidents are properly identified, reported, investigated, diagnosed, and recorded in accessible standardized formats. Properly informed and with resource support, the wildlife rehabilitation community can become a valuable source of information.

KEY WORDS: avian, pesticides, poisoning, organophosphorous, carbamate, anticoagulant, cholinesterase, neuro-toxicity, synapse, 2-PAM, atropine, clinical signs, measurement, monitoring, reporting

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America, it is estimated that since 1965, about 3% of bald eagles (*Haliaeetus leucocephalus*) examined by the U.S. Fish and Wildlife Service were poisoned by cholinesterase inhibitors (Franson et al. 1995). This is a known underestimate because the measurement of cholinesterase levels, the primary diagnostic tool, did not become routine until the early 1980s. The 12% calculated for red-tailed hawks (*Buteo jamaicencis)* between 1975 and 1992 (Franson et al. 1996) is probably closer to what we can reasonably expect with either species. It has been suggested however, that these types of estimates are all underestimates of the true impact of pesticides (Porter 1993, Mineau et al. 1999). In all diagnostic centers involved in assessing wildlife incidents, the investigation seldom goes further than establishing the primary cause of death. The most frequent causes identified are invariably trauma, often from impacts with static objects or motor vehicles and electrocutions. The possible contribution of sublethal pesticide intoxication to this type of mortality is very real and will be discussed later.

On a regional level, the importance of poisonings caused by cholinesterase inhibitors can be substantial and may account for a large proportion of all raptor intakes at select rehabilitation centers (reviewed in Mineau et al. 1999). Therefore, the diagnosis of intoxication by a cholinesterase-inhibiting agent (OP or CB) is currently one of the most important skills needed for the investigation of pesticide effects on wildlife. This may change in the future as a number of OP and CB products and use patterns are phased out. There are special problems associated with replacement chemicals that will be discussed briefly below. Although the discussion so far has emphasized birds of prey, intoxications with OPs and CBs are clearly not restricted to this group. Large numbers of songbirds are routinely killed when ingesting treated seed, granular insecticides, or contaminated seeds or insects (e.g., Mineau 1993, Friend and Franson 1999), but their small body size means that these incidents may easily be overlooked and/or may rarely involve rehabilitation. Waterfowl are also likely to be poisoned, especially when they graze on treated crops; their larger size means they are more likely to come into rehabilitation centers.

Clinical Signs Associated with OP and CB Poisoning

Although there are well-described clinical signs that are typical of poisoning by a cholinesterase (ChE) inhibitor, clinical signs can be so variable as to obscure diagnosis. A basic understanding of the mode of action of anticholinesterases helps us to understand this variation.

The synapse

Nerve impulses rely on chemical messengers that travel from cell to cell. No chemical messenger is more important than acetylcholine. Acetylcholine-mediated pathways (or cholinergic tracts) occur in the central nervous system (CNS), in the peripheral nervous system, and at neuro-muscular junctions. There are few physiological processes that are not affected by acetylcholine at one point or another. Acetylcholine is released in the synapse, or gap, between the two communicating cells and stimulates the "receiver" cell. The proper functioning of the system requires acetylcholine to be removed quickly from the synapse once it has performed its function. This is accomplished by the enzyme acetylcholinesterase (AChE). If acetylcholine is not removed but allowed to pool in the synapse, the receiver cell, whether a nerve cell or muscle fiber, will be overstimulated. As it tires (because it is unable to repolarize), it may shut down altogether, preventing further impulses to pass. It is in part because of this variation of effect at the synapse (from initial overstimulation to eventual blockage) that clinical signs of poisoning can be variable and at times contradictory.

OP and CB pesticides have a high affinity for AChE and prevent it from acting on acetylcholine. The main difference between OPs and CBs is that, in the case of the former, the bond between the pesticide and the enzyme is very strong and quasi permanent. Recovery of enzyme activity is generally accomplished by synthesis of new AChE, although release of the enzyme from the OP can be hastened with drugs during rehabilitation (see below). The bond between CBs and AChE is weaker and can be broken by a simple reaction with water (hydrolysis). Upon exposure, a CB is every bit as dangerous to a bird as an OP of equivalent toxicity. However, birds surviving an intoxication with a CB make a much faster recovery than those exposed to an OP. The rapid hydrolysis of the CB-AChE bond means that such intoxications are more difficult to diagnose unless samples are taken early and preserved properly (see below).

Clinical signs and why they are so variable

In the somatic nervous system that controls voluntary muscle movement, overstimulation resulting from pooled acetylcholine typically gives rise to tremors, muscle twitches and piloerection, as well as paralysis resulting in ataxia. More rarely, the animal may convulse. Cholinergic tracts are also important to both the parasympathetic and sympathetic autonomic nervous systems but especially to the former, where they conduct impulses from the neural ganglia to a multitude of effector organs such as the heart, endocrine glands, and digestive system. Because the autonomic nervous system is subject to constant adjustment through feedback mechanisms, intoxication with a cholinesterase inhibitor sends the poisoned organism into a veritable roller-coaster ride. For example, individuals may show alternating constriction or dilation of the pupils, speeding up or slowing down of the heartbeat, etc. Also, because the somatic and autonomous systems react to different levels of cholinergic stimulation, some doses of an anticholinesterase may produce apparently opposite signs (e.g., contraction of the striated muscles involved in locomotion and simultaneous relaxation of the smooth musculature leading to a flaccid gut and food impaction). The rate at which the individual was exposed to the pesticide is as important as the dose itself. Typically, gradual exposure allows the individual to compensate and tolerate a higher dose than if the exposure was a single large dose.

Finally, different cholinesterase inhibitors have different properties that may dictate which clinical signs are expressed. Some pesticides are directly active on synapses, others need to be metabolized to the active molecule; some pass readily into the brain (with obvious effects on the CNS), others have difficulty crossing the blood-brain barrier and therefore show more "peripheral'" effects. Porter (1993) cautions that many of the "classic signs'" of parasympathetic stimulation reported from

standard toxicology texts may not be seen in poisoned raptors—and certainly not with any consistency. Shimmel and Snell (1999) similarly comment on the difficulty of arriving at a conclusive diagnosis without chemical or biochemical laboratory backup. Nevertheless, the following list modified from Grue et al. (1991) summarizes the signs noted by Hudson and colleagues (1984) following dosing of various bird species with a variety of cholinesterase-inhibiting agents:

- ataraxia (induced tranquility), lethargy
- ataxia (incoordination of muscular action)
- blindness
- convulsions (particularly just prior to death)
- defecation, diarrhea
- dyspnea (difficult breathing)
- epistaxis (bleeding from the nares)
- exophthalmia (protruding eyes)
- hyperexcitability
- lacrimation
- miosis (contraction of pupils)
- myasthenia (muscular weakness)
- mydriasis (dilation of pupils)
- opisthotonos (heads and limbs arched back)
- paresis (slight paralysis)
- piloerection (erection of contour feathers)
- polydipsia (excessive thirst)
- ptosis (drooping of eyelids)
- slurred vocalizations
- tachypnea (rapid breathing)
- tenesmus (spasmodic contraction of anal sphincter)
- tremors
- vomiting

As emphasized by Franson and Smith (1999), the presence of recently ingested food material in the upper gastrointestinal tract is suggestive of acute poisoning by a cholinesterase inhibitor. Certainly, identifying the gastrointestinal content may provide the best indication that the bird has been poisoned by an agricultural chemical (e.g., the presence of undigested grasshoppers or other insect pests, treated seed, granules of pesticides, etc.). A number of diseases produce signs that could be mistaken for poisoning. These have been thoroughly reviewed in Friend and Franson (1999).

Usual cause of death

Typically, but not always, poisoned birds die of anoxia because of respiratory failure. This is the result of one or a combination of factors: excessive secretion in the respiratory tract, bronchoconstriction, failure of the muscles required for respiration, and/or failure of the respiration center (see Gallo and Lawryk 1991 for a review).

Delayed mortality: Secondary causes

Because of the far-reaching importance of cholinergic tracts, poisoned individuals may be seriously compromised even if they survive the initial exposure—hence the importance of lengthy follow-up of animals in rehabilitation. Some of the most important delayed effects may be as follows (see Grue et al. 1991, 1997; Mineau 1991; and Parsons et al. 2001 for a more complete coverage of "sublethal" effects):

Trauma or other mishaps in the course of intoxication. It has been shown that birds sublethally exposed to cholinesterase inhibitors are more susceptible to predation (Galindo et al. 1985 Buerger et al. 1991, Hunt et al. 1992). There is also reason to believe that sublethal exposure to cholinesterase inhibitors makes animals more vulnerable to collision with objects, both moving (e.g., vehicles) or stationary (e.g., powerlines, fences, buildings). Upon arriving at rehabilitation centers, raptors, for example, are often diagnosed as victims of collision. The evidence of pesticide involvement is two-fold: (1) Anecdotal evidence from rehabilitation centers where cholinesterase measurements have been made on a routine basis (e.g., Porter 1993); and (2) The wealth of human evidence about the various visual and motor effects that affect the safety of workers (see Gallo and Lawryk 1991 for review) following exposure to OPs and CBs. Blurred vision is a common complaint; unequal miosis also can lead to a phenomenon called the Pulfrich Stereo Effect where depth perception and the ability to compute trajectories are affected. Any of these effects in a flying bird would be expected to lead to higher rates of collision.

Poor condition resulting from a reduced ability to feed, as well as a disruption in normal circadian patterns and thermoregulation. Energy deficits are thought to be especially important in explaining mass mortality of exposed wildlife under inclement conditions or when body reserves are low, such as on migration, especially in the case of small birds that cannot endure long fasts because of their high metabolic demands.

Muscular necrosis as a result of transient anoxia. In humans, this results in what has been termed "type II toxicity" or "intermediate syndrome," which typically results in cardiac failure several days after return to normal cholinesterase status. The authors are not aware of any descriptions of this syndrome in birds.

Delayed neurotoxicity. This syndrome is the irreversible dying back of neurons as a result of an enzyme inhibition effect other than the usual anticholinesterase effect. Only a few OPs are reputed to cause this. It is noteworthy that the chicken is the usual test organism for this syndrome, but effects are often seen at dosing levels that would be lethal were the animal not antidoted for cholinesterase inhibition effects, as such lab chickens commonly are. This syndrome has not been reported in wild birds, although we might expect to see it first in animals subject to intensive rehabilitation efforts. Woods and Plumlee (1999) briefly mention possible cases in cage birds from California but provide no further detail.

Treatment of Poisoning Cases

Treatment of wildlife generally follows a four-pronged approach. Atropine is considered to be the most effective antidote for both OP and CB intoxication. By effectively competing with acetylcholine for the muscarinic cellular recep-

tors, it prevents overstimulation of the autonomic parasympathetic system. Most importantly, it helps prevent asphyxia, the main cause of death. In human subjects, it is customary to infuse atropine constantly in order to maintain optimal concentration throughout recovery from the "cholinergic crisis." In wildlife rehabilitation this is impractical, and subjects need to be repeatedly injected as indicated by the reappearance of clinical signs.

The second prong consists of the administration of chemicals that hasten the release of bound OP from the acetylcholinesterase enzyme. This strategy is effective only where intoxication results from an OP pesticide and is recent. (Porter 1993 gives 24 hours as a guideline, but this will vary from pesticide to pesticide.) The most frequently used chemical for this purpose is 2-pralidoxime chloride (2-PAM).

The third prong in the approach is the provision of supportive symptomatic care, especially positive ventilation in case of respiratory arrest. Other supportive measures include a quiet and warm environment, rehydration, prophylactic use of fungicides to prevent aspergillosis, and the use of antiseizure medication such as diazepam.

Finally, it is important to eliminate the source of the exposure. Gastric lavage may be performed where there is evidence of a large food bolus, which may continue to release pesticide over time. Some have found surgical evacuation of the crop to be more effective and less stressful than forced regurgitation, such as in the case of bald eagles having scavenged contaminated waterfowl (K. Langelier, pers. comm.). Others prefer to remove crop contents in such larger species with forceps when circumstances allow. Frazier (2000) advocates the use of activated charcoal. Pesticides absorbed into the bird's subepidermal tissues can be slowly released over time and result in prolonged re-exposure (Henderson et al. 1994). Where dermal exposure is suspected, a vigorous rinsing of the feet with warm soapy water may help limit pesticide entry. Alcohol is used routinely to collect foot rinses from birds thought to have been exposed dermally, and these can be kept frozen for chemical analysis where feasible and warranted. However, there is a concern that applying alcohol to the feet may enhance pesticide penetration.

The exact dosages of atropine and 2-PAM to be administered are currently a matter of debate. The "traditional" approach is based on levels found to be effective in humans. For example, Porter (1993) recommends an injection of 0.5 mg/kg atropine IM (or one-fourth of the total dose given IV) repeated after 15 min if no decline in signs is observed. According to the same source, the recommended dosage of 2-PAM is 20 mg/kg IM. Frazier (2000) recommends 0.1–0.2 mg/kg atropine every 3–4 hours and suggests that 10–20 mg/kg 2-PAM be given concurrently. A far more "aggressive" approach was recently recommended by Shlosberg and colleagues (1997). Their experiments with chickens led them to recommend 25 mg/kg atropine and 50 mg/kg 2-PAM as the best treatment for an unknown cholinesterase inhibitor. These dosages were established empirically; the highest doses not causing obvious toxicity in normal chickens were retained. According to Schlosberg et al., the ease with which each species breaks down atropine varies. Future research should focus on establishing maximum tolerated dosages for atropine or, better still, an atropine + 2-PAM cocktail for those species commonly treated at rehabilitation centers. The best and final judge as to the dose needed, however, is the person performing the treatment. It is much better to "titrate" the clinical signs rather than use a fixed-dose approach.

Other possibilities for treatment exist, but they have not been systematically investigated in wildlife. Injections of glucose and of vitamin C have afforded some protection to small mammals experimentally dosed with various OPs (see Gallo and Lawryk 1991 for review). The ready availability of both make them obvious candidates for further experimentation. Frazier (2000) also notes that diphenhydramine has been used in mammalian intoxications to counter muscle fasciculations and other nicotinic symptoms not resolved by atropine.

When the time for release of recovered birds approaches, rehabilitators might consider the potential for re-exposure on the capture site. When initial capture sites are known to be contaminated or are regularly associated with suspected poisonings, it may be prudent to identify a separate release site. As a check for an individual bird's readiness for release, subjecting the animal to increased stress—for example, by "flushing" them from perch to perch in a large enclosed flight cage, thus forcing sustained, involuntary activity—may allow observation of possible lapse into cholinergic crisis. Many birds that appear fully recovered may again exhibit clinical signs of poisoning when exposed to brief periods of sustained stress, suggesting their normal activity in the wild would be compromised (M. Hooper and the authors, pers. obs.).

Measurement of Cholinesterase Levels

The best diagnosis of intoxication with a ChE inhibitor is the measurement of enzyme levels. The level of acetylcholinesterase in the brain is best correlated with morbidity and mortality, and is often used to diagnose mortality. A rule of thumb is that a 20% decrease of brain acetylcholinesterase is indicative of exposure. Lethal intoxication is usually associated with a 50% decline, although this figure can be lower or higher depending on the situation (see Grue et al. 1991 for a review). If a brain is to be subsampled (a good idea because it allows for repeat testing should anything go wrong and keeps some tissue for other possible tests, such as the determination of organochlorine residues), it is essential to do a sagittal section and test half of the brain. This is because different portions of the brain register a different cholinesterase activity. Where a live bird is to be tested, the test is carried out on a blood sample. In mammals, red blood cells also carry acetylcholinesterase. In birds, all the activity is associated with the plasma. In addition, all vertebrates have a related enzyme in plasma: butyrylcholinesterase. The function of this enzyme is a matter of debate; most likely it offers a first line of defense against cholinesterase-inhibiting substances of which there are a few in nature. This is important because the tests routinely used to monitor acetylcholinesterase levels are, in fact, unspecific and also measure the butyrylcholinesterase titer. Adding a little more complexity is the fact that the ratio of acetylcholinesterase:butyrylcholinesterase in avian plasma is extremely variable among different bird species. Inhibition of blood cholinesterases *per se* may not compromise an individual; however, it is indicative of the presence of a systemic cholinest-

erase inhibitor. In mammals, whole blood is usually tested because red blood cells have a high level of AChE activity. In birds, typically the blood is centrifuged and the plasma only is retained for analysis. Both brain and plasma samples can be frozen for future analysis (a minimum temperature of -30°C is recommended). Repeated freezing and thawing (as can occur in domestic self-defrosting freezers) must be avoided. Recently, the Canadian Wildlife Service Biomarker Laboratory has demonstrated the feasibility of collecting blood as dry spots on filter papers. This avoids the need to freeze the samples and allows them to be sent through the postal system. (Trudeau et al. 1995, Sans Cartier and Trudeau 2000). The laboratory analysis is modified to avoid interference problems with whole blood, although recent work shows that this may not be necessary.

A cholinesterase assay is 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. It is important for wildlife rehabilitators to have a basic understanding of the assay. Even if they do not carry it out themselves, this understanding will allow them to ask the right questions and/or correctly interpret test results. For more detailed information on the cholinesterase assay itself, refer to Fairbrother et al. 1991 and Sans Cartier and Trudeau 2000. It is advisable to submit samples to the same laboratory over time and insist that the assay be rigorously standardized by the laboratory in question. Marden et al. (1994) showed how small interlaboratory differences in procedure could give rise to very different results. Repeat testing of the same species by the same laboratory will more easily paint a picture of what normal and abnormal activity values should look like.

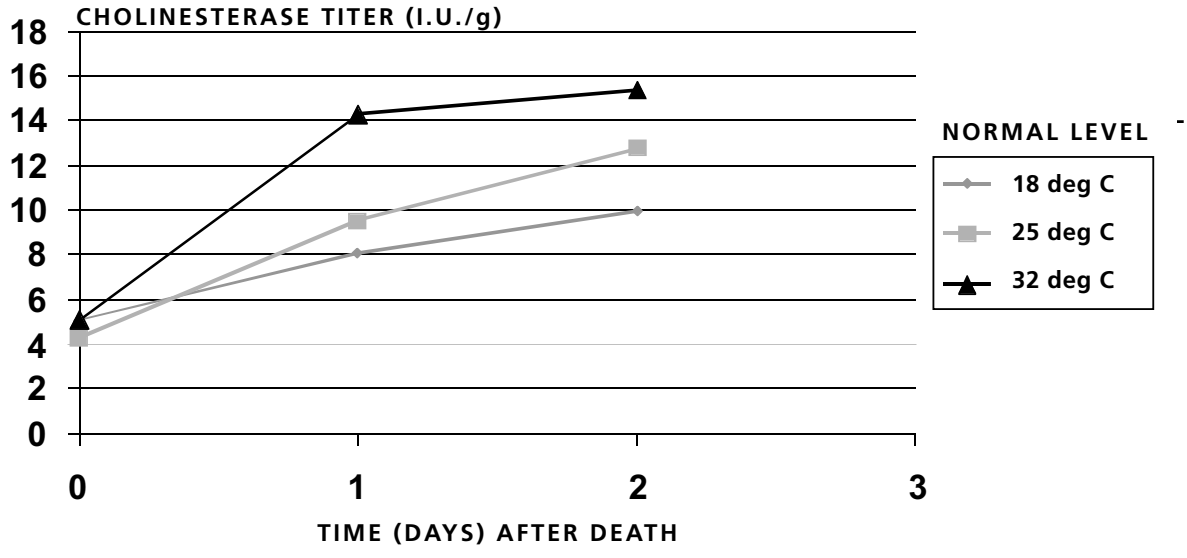
It is clear that CB poisonings are often harder to diagnose than OP poisonings. This is obviously a concern, given the overwhelming importance of some CB insecticides, such as carbofuran, in the avian kill record (e.g., Mineau et al. 1999). The difficulty of diagnosis results from the strength of the pesticide-acetylcholinesterase bond discussed earlier. Figure 1, derived from the data of Hill (1989), shows the effect of temperature on the spontaneous reactivation of brain tissue (hydrolysis of the AChE-pesticide bond as explained earlier) from quail lethally dosed with the CB carbofuran.

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. When several specimens are available, some (or at least their heads) should be frozen as soon as possible without waiting for necropsy results. The reader is referred to Hunt and Hooper (1993) and Padilla and Hooper (1992) for a detailed discussion of cholinesterase measurements following a CB intoxication.

The capacity for CB-inhibited tissue samples to spontaneously recover cholinesterase activity differentiates between OP and CB intoxication (e.g., see Smith et al. 1995). In an analogous fashion, 2-PAM can be used to attempt reactivation of a sample and show involvement by an OP. Because the 2-PAM is added to samples in an aqueous solution, this may also foster recovery of a sample inhibited by a CB. However, a measurable recovery in enzyme activity with 2-PAM and concomitant absence of a recovery with water alone is diagnostic proof that an OP was present in the sample.

Another problem complicating diagnoses is that certain pesticides do not cross easily into the brain, especially when death is rapid, as is often the case with carbamates. Some OPs also exhibit this same characteristic (e.g., terbufos in Hooper et al. 1990), although in some intoxicated birds, brain AChE

FIGURE 1. POSTMORTEM REACTIVATION OF BRAIN TISSUE FROM JAPANESE QUAIL *(COTURNIX JAPONICA)* DOSED WITH THE CARBAMATE INSECTICIDE CARBOFURAN AND EUTHANIZED 1 HR POST DOSE (after the data of Hill 1989)



depression can be extreme with those compounds as well (see carbofuran and terbufos data below).

Figure 2 shows brain cholinesterase analyses performed on five bald eagles from five different poisoning incidents investigated by the Canadian Wildlife Service. These were chosen to illustrate some aspects of cholinesterase analyses.

The first eagle was a carbofuran victim and shows that spontaneous reactivation of the sample is not always successful following intoxication by a carbamate. In this case, the failure of the sample to reactivate was due to the presence of leftover unbound insecticide. When the sample was filtered in a special chromatographic column (which strips small molecules such as insecticides but allows large ones such as the cholinesterase enzymes to pass through—as described in Hunt and Hooper 1993) the sample's cholinesterase activity was easily reactivated, showing that a carbamate was indeed present. The second sample—also carbofuran intoxication—was already reactivated or never showed any inhibition. The cholinesterase measurement was of no diagnostic help here. The third eagle was killed by the OP terbufos. The initial analysis showed a marked depression relative to controls. However, the sample could not be reactivated with the 2-PAM. The ability of 2-PAM to free the acetylcholinesterase molecule declines markedly over time. The fourth eagle shows the result of a positive reactivation following phorate poisoning (another OP). However, the last bird, also killed by phorate, failed the 2-PAM reactivation test. Why the difference? Perhaps it is explained by the presence of the insecticide (in which case a column should have been used to clean the sample) or a longer period elapsed since intoxication in the latter bird.

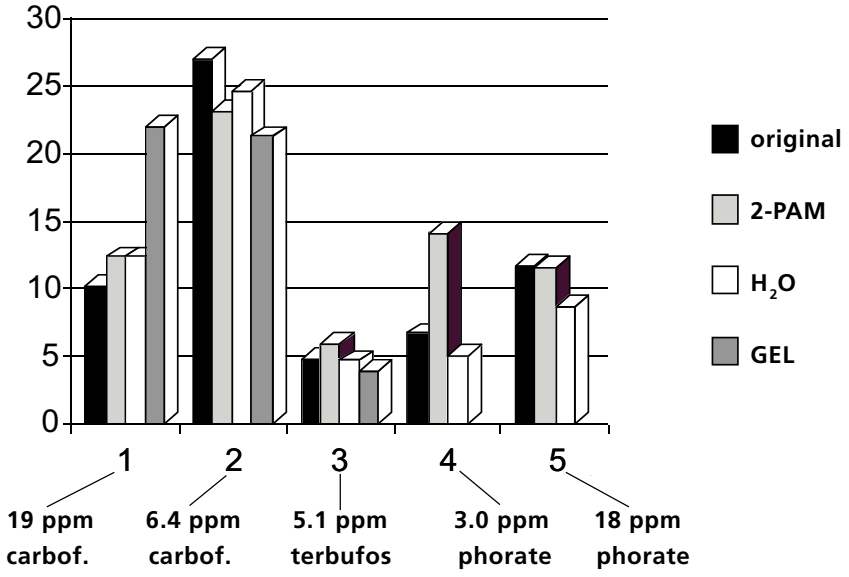
As noted earlier, cholinesterase activity in avian blood is entirely in the plasma fraction and results from a mixture of acetyl- and butyrylcholinesterase, the proportion of which varies between species. Most diagnostic centers associated with hospitals or veterinary clinics are well equipped and aware of the procedures associated with mammalian blood, but they generally are not familiar with analyzing bird samples. Avian plasma can be analyzed by the same techniques commonly used for mammalian whole blood samples. Blood cholinesterase levels are more variable than those of the brain and tend to indicate exposure rather than a life-threatening intoxication. For a live animal under care, repeated measurement of blood cholinesterase offers the most reliable indication of exposure to a cholinesterase inhibitor when serial samples show a recovery to normal levels over time (e.g., see Elliott et al. 1997 for an example of eagles poisoned by phorate).

In the end, while cholinesterase measurements are useful, they are not infallible and must be interpreted carefully, considering all the ancillary evidence and chemical analyses when available.

Beyond Cholinesterase: The Residue Angle

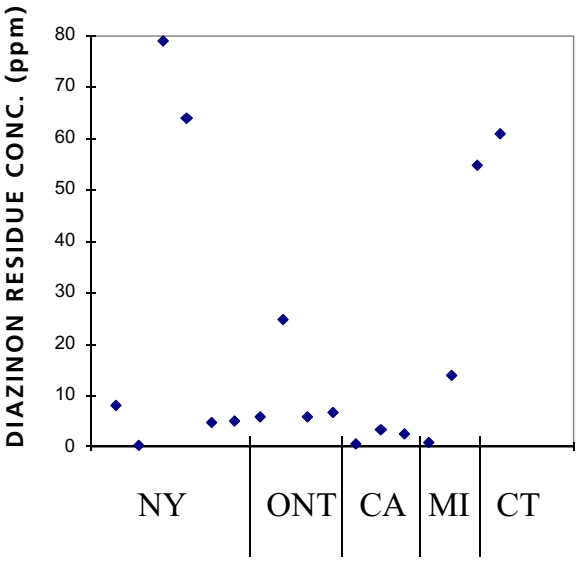
When there is evidence of cholinesterase depression, or even where there is no clear evidence but where circumstances are highly suspect, one should proceed with the chemical analysis of the gastrointestinal tract contents. This is usually—but not always—where the highest pesticide residues are found. Before gut contents are sent off for analysis, it is extremely important that they be well described. Often, gut contents offer the best clue to explain a pesticide kill. In some cases, it is

FIGURE 2. BRAIN AChE ANALYSES FOR FIVE BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*) RECEIVED AT BRITISH COLUMBIA REHABILITATION CENTERS MATCHED WITH RESIDUE ANALYSIS OF GASTROINTESTINAL TRACT CONTENT



Data from cases reviewed by Mineau et al. 1999; analyses provided by S. Trudeau, Canadian Wildlife Service Biomarker Laboratory. See text for details of sample treatment.

FIGURE 3. DIAZINON RESIDUES IN GRASS SAMPLES RETRIEVED FROM THE UPPER GASTROINTESTINAL TRACTS OF POISONED DUCKS OR GEESE



Data from Stone and Gradoni 1985, Stone 1987, and Frank et al. 1991. Reported cases are from New York State (NY), Ontario, Canada (ONT), California (CA), Michigan (MI), and Connecticut (CT).

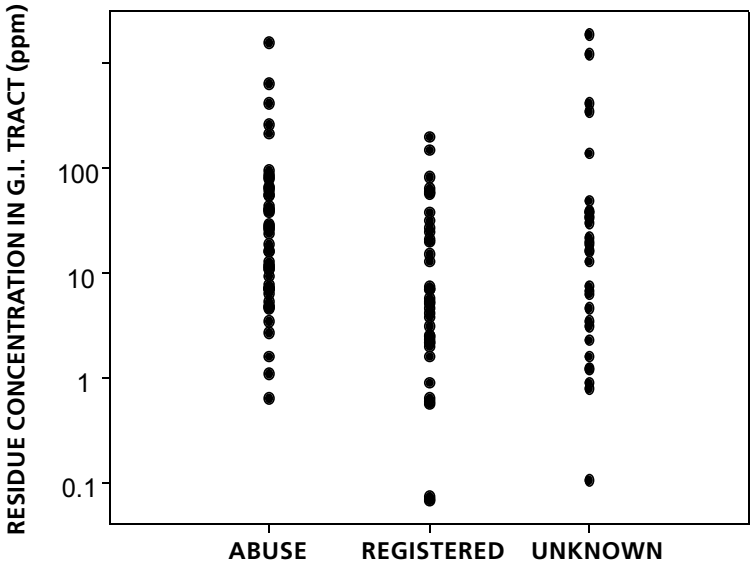
recommended to also rinse the feet and feathers of birds suspected of having been dermally exposed (e.g., Hooper et al. 1989), although rehabilitators are reminded that this may enhance pesticide absorption in a live bird. Residue analysis is a costly proposition, so sending a sample to be analyzed cannot be done lightly. It has been customary in several diagnostic centers to run a cholinesterase assay first and then a residue analysis if the former is positive. As the pesticide arsenal moves away from cholinesterase inhibitors to embrace new chemistry, this strategy will cease to be sufficient, although it will still be useful to direct the residue analysis. Chemical analyses are most expensive and least likely to succeed when the chemist has no idea of the nature of the pesticide being sought.

The level of a pesticide measured in a carcass is extremely variable. For example, Figure 3 shows levels of diazinon residues recovered in grass samples taken from the upper gastrointestinal tracts of poisoned ducks or geese.

Occasionally, criminal abuse cases are characterized by very high residue levels in gut contents because concentrated baits are used. In practice, however, the extensive overlap in residue levels between abuse cases and poisonings resulting from labeled pesticide use means that residue levels cannot reliably be used to separate those two situations. Figure 4 shows the extensive overlap in the concentration of carbofuran in the gut contents of birds of prey killed through abuse, through labeled pesticide use, or under unknown circumstances.

For reasons outlined earlier, the quantity of pesticide found in poisoned wildlife is often poorly correlated with measured levels of cholinesterase depression. Therefore, when the presence of a cholinesterase inhibitor is confirmed through a cholinesterase assay, any finding of a cholinesterase-inhibiting

FIGURE 4. CARBOFURAN CONTENT OF GASTROINTESTINAL TRACTS OF POISONED BIRDS OF PREY SEPARATED BETWEEN KNOWN ABUSE CASES, NORMAL USES, OR WHERE CIRCUMSTANCES WERE UNKNOWN.



Data from U.S., Canada, and U.K. cases reviewed by Mineau et al. 1999.

pesticide, regardless of its concentration, should be considered significant. Also, failure to find a pesticide agent through chemical analysis does not disprove that intoxication occurred.

Anticoagulants

Wildlife rehabilitators should be aware of at least one other class of pesticides: the anticoagulants. This is because the frequency of poisonings has increased dramatically in recent years, coinciding with the increased use of a new generation of single-dose products that are much more toxic and much more likely to lead to secondary poisoning in nontarget species. Anticoagulants in this group include difenacoum, brodifacoum, bromadialone, flocoumafen, and difethialone. Birds admitted to rehabilitation centers and diagnosed as nonpesticide cases often carry anticoagulant residues (Canadian Wildlife Service, unpublished), but the significance of these residues is not known. The second generation anticoagulants have a high affinity for the liver, where they bind to specific receptors. Once bound, residues can be extremely persistent —e.g., several years for brodifacoum, at least in rat livers (http://www.epa.gov/REDs/2100red.pdf). A pressing question is whether the presence of residues makes an animal more sensitive to this class of chemicals if re-exposed. In two recent surveys of cases in New York and California (Stone et al. 1999 and Hosea 2000, respectively) it has been established that birds of prey in proximity to urban centers have a high risk of exposure. This is in keeping with regulations and labeling that restrict use of these products to Norway and black (roof) rats or house mice in and around habitations or in sewers.

As with any anticoagulant intoxication, vitamin K is antidotal. Frazier (2000) recommends 2.5–5.0 mg/kg intramuscularly or orally every 24 hours (or 0.2–2.2 mg/kg every 4–8 hours), to be continued for at least 10–14 days in the case of warfarin or at least for 1 month in the case of the newer, single-dose products.

New pesticides

A few recent introductions are worth mentioning. Possibly the most interesting from the point of view of birds is chlorfenapyr, the first of a new class of pyrrole insecticides. In response to advocacy by the American Bird Conservancy and subsequent scientific criticism, this insecticide was the first pesticide to be denied a registration in the U.S. based solely on its toxicity to birds. It has very high acute toxicity (on par with some of the more toxic OPs and CBs), although mortality is typically delayed for a few days after exposure. It also causes notable effects on reproduction at extremely low levels of exposure (http://www.epa.gov/opprd001/chlorfenapyr/toc.htm). The high persistence and high aquatic toxicity of chlorfenapyr were undoubtedly factors that contributed to registration denial for cotton applications in the United States. Concerns continue because chlorfenapyr is registered in a number of countries, including areas comprising the wintering ranges of many North American bird species. New registration proposals are also being considered in the U.S.

Kills of pigeons and game birds have been reported in France with two other new insecticides used as seed dressings: imidacloprid and fipronil (Annual reports from l'Office National de la Chasse). It is not clear how extensive and/or important these problems will be, and whether kills may also result from other formulations of these same products.

A number of other insecticides from totally new chemical classes are gaining in popularity in the U.S. and elsewhere. On the positive side, some of these products may represent improvements over the OP and CB pesticides they are replacing. For example, some require a lesser amount of a more selective, or target-specific substance that can be applied with equal efficacy. On the negative side, we are ill prepared to ascertain their presence in wildlife tissue and there are no biochemical diagnostic techniques to aid in the investigation of a mortality incident. In the authors' opinion, development of the latter should be a requirement of any new pesticide registration; unfortunately, this is not currently the case.

Note

Part II of this paper will appear in the next issue of the *Journal of Wildlife Rehabilitation*.

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