

INSECTICIDE RESIDUES IN BATS AND GUANO FROM INDIANA

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ABSTRACT. Over recent decades, numerous species of bats have been declining, but the causes are not well understood. One of the causes often mentioned is that of environmental toxicants. Feeding on insects makes insectivorous bats more likely to be exposed to insecticides. We sent nine bats (5 Indiana myotis, *Myotis sodalis*, and 4 northern myotis, *Myotis septentrionalis*) through U.S. Fish & Wildlife Service (USF&W) sources to be tested for toxicants. Three of these proved to have organophosphate (OP) insecticide residues: chlorpyrifos (0.18 µg/g), diazinon (0.034 µg/g), and methyl parathion (0.015 µg/g). Chlorpyrifos was also detected in all six dead Indiana myotis found during the USF&W Service biennial mid-winter hibernacula surveys in Ray's and Wyandotte Caves, both important Indiana myotis hibernacula. Chlorpyrifos or dichlorvos (another OP) was found in Indiana myotis guano from all four caves sampled (Wyandotte, Coon, Grotto and Ray's). These data are particularly surprising since OP insecticides are thought to have little bioaccumulation in living tissues or in food chains. Their presence in a tissue sample is indicative of exposure shortly before death (O'Shea & Clark 2002; Hill 1989, 1995). Even though exposure to low doses might not be the primary cause of mortality, such exposure could impair echolocation, coordination and response time which, in turn, could lead to significant injuries and death of bats in the field (O'Shea & Clark 2002). Presence of OPs in bat guano from caves may suggest insecticide application near these hibernacula and constitutes clear evidence of oral exposure through persistence of toxicants in water or through the food chain.

Keywords: Cholinesterase inhibition, organophosphate insecticides, bats, residues

Apart from being an important part of the ecosystem, insectivorous bats are the main predators on nocturnal insects; and they consume numerous agricultural pest insects (Whitaker 1995; Lee & McCracken 2005). Nevertheless, many North American bats, including the federally-endangered Indiana myotis (*Myotis sodalis*), are declining. The total population of the Indiana myotis was estimated to be approximately 380,000 bats in 2001, down from an estimated 880,000 bats in 1960 (Clawson 2002). Whitaker et al. (2002) indicated that the evening bat (*Nycticeius humeralis*), the little brown myotis (*Myotis lucifugus*), the eastern red bat (*Lasiurus borealis*) and the hoary bat (*Lasiurus cinereus*) are declining in Indiana. Habitat decrease and vandalism in hibernacula are some of the reasons bats decline. Another reason may be environmental contaminants such as pesticides. Several organochlorine pesticides have been im-

plicated in free-ranging bat mortality (Clark et al. 1978, 1980; Clark 1981). However, it is unknown how widespread these types of incidents may have been or what relation they may have had to range-wide bat populations. It is even less clear what effects present-day insecticides might have on bats. The decline of insectivorous bats could be partially related to pesticides. In addition to direct toxicity, there could be a decrease in food availability, sub-lethal toxic effects, or perhaps both. Regardless, the decline of insectivorous bats is not beneficial to agricultural pest managers.

The cholinesterase-inhibiting insecticides (organophosphates and carbamates) are widely used in Indiana, according to the National Pesticide Information Retrieval System (NPIRS) List of Pesticides Registered in Indiana for 2006. NPIRS contains federal pesticide registration data from the U.S. Environmental Protection Agency (EPA) and from state pesticide registration data. These insect-

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ticides are highly toxic but not very fat soluble, and were not previously reported to accumulate in living tissues (O'Shea & Clark 2002; Hill 1995) or in food chains (Schmidt et al. 2002). Hoffman et al. (2001) describe organophosphates (OPs) and carbamates as inhibitors of the enzyme cholinesterase (ChE). ChE hydrolyzes the neurotransmitter acetylcholine in nervous tissue. Clinical signs of toxicity include salivation, lacrimation, urination, defecation, tremor, convulsions and eventually paralysis (Grue et al. 1997). Death occurs due to respiratory failure and asphyxia (Wilkinson 1976). In addition to neurotoxic effects, anti-ChE compounds have been shown to exert effects on endocrine and immune function in higher vertebrates (Hoffman et al. 2001). Grue et al. (1997) reviewed the sub-lethal effects of ChE inhibitors on captive small mammals and birds. These include impaired thermoregulation (i.e., pronounced hypothermia, with normal body temperature usually resumed within 24 hours), reduced food consumption, and reproductive problems caused by impaired sexual behavior and changes in levels of reproductive hormones (i.e., reduced luteinizing hormone levels).

Alterations of thermoregulatory ability have potentially serious consequences to bats due to their metabolic complexities. Gordon (1993) suggested that hypothermia is the natural response of mammals in order to minimize the effects of toxicants. Bats often enter into torpor to conserve energy when demands are high and energy resources are low (Kurta et al. 1989). How anti-ChE compounds might affect this poorly-understood physiological response is unknown. Reduction in food consumption might also reduce reproductive success, pup or nestling weight, and survival of offspring in mammals and birds (Grue et al. 1997; Hill 1995). Reduced reproductive success, leading to fewer young, would be particularly bad for bats because of their low reproductive rate. Most species of bats in Indiana produce only one or two offspring per year (Whitaker & Hamilton 1998). Low reproductive rate dictates slow recovery of affected populations (Clark & Shore 2001).

McFarland (1998) analyzed ChE activity in brains of little brown myotis and northern myotis (*Myotis septentrionalis*) from agricultural sites in northern Missouri during summer and found brain ChE inhibition in some of the

bats. He suggested that these bats may be exposed to sub-lethal levels of OP and/or carbamate insecticides. Chlorpyrifos was detected at low levels in nearly every bat collected and analyzed on Fort Leonard Wood, Missouri during 2002–2003 (BHE 2004, 2005). Land (2001) was the first to report detections of OP residues in bat guano.

Given mounting evidence that bats in other locations are being exposed to OPs, the present work is a preliminary study to determine whether or not Indiana myotis as well as other species of bats are being exposed to OPs in Indiana.

METHODS

All bats submitted to the Indiana Department of Health Rabies laboratory since 1966 have been forwarded to one of the authors (JOW) at Indiana State University for identification and research purposes. Many of these bats exhibited behavior that brought them to the attention of humans, i.e., many were on the ground sick, dying or dead. From 1966 to 2003, about 200 to 400 bats were submitted annually, but only 5.4% of these proved rabid (Whitaker & Douglas in press).

Nine bats that had been submitted to the Indiana Department of Health, Rabies laboratory from 1998 to 2000 were submitted through the U.S. Fish and Wildlife Service to the Geochemical & Environmental Research Group, Texas A&M laboratory for chemical analysis in August 2001. Five Indiana myotis and four northern myotis, all non-rabid, were analyzed (whole body, minus the brain which had been removed for rabies testing). Analyses included: OPs, organochlorine insecticides, polychlorinated biphenyls (PCBs), and pyrethroids. Due to the large sample mass required for carbamate insecticide analysis (35 g) those contaminants could not be investigated. The bats were kept frozen prior to chemical analysis. No separate stomach content examinations were conducted on these bats.

Recent guano samples were collected from priority hibernacula by placing plastic sheets under roosting/hibernating Indiana myotis. These sheets were retrieved at various intervals, and guano pellets were transferred to chemically-clean glass jars and kept frozen until analysis. In addition, any fresh dead Indiana myotis found during biennial mid-win-

Table 1.—Organophosphate insecticides ($\mu\text{g/g}$ wet weight) in the Indiana myotis (*Myotis sodalis*) and northern myotis (*Myotis septentrionalis*) found by members of the public and submitted to Indiana Department of Health for rabies testing. U, not determined.

	<i>Myotis sodalis</i>					<i>Myotis septentrionalis</i>			
ID #	16122	16204	16615	16638	16639	16729	16730	16784	16785
Date found	8/26	10/21	4/15	8/22	8/23	8/29	8/29	10/6	10/18
	1998	1998	2000	2000	2000	2000	2000	2000	2000
County	Marion	Vander- burgh	Vander- burgh	Vander- burgh	Marion	Vander- burgh	Decatur	Davies	Vander- burgh
Body mass (g)	5.44	7.4	4.73	5.8	6.08	4.94	4.03	6.39	6.12
Sex	M	F	F	U	U	U	U	M	M
Lipids (%)	12.9	33.7	3.46	9.1	3.03	15	3.52	22.8	40.3
Moisture (%)	60.7	40.2	56.5	62.2	61.5	49.8	51.4	51.6	28.8
Dieldrin	0.06	0.13	0.09	0.09	0.08	0.10	0.04	0.16	0.23
Heptachlor ep- oxide	0.02	0.14	0.02	0.02	0.01	0.02	0.01	0.11	0.05
Oxychlordane	0.1	0.19	0.14	0.21	0.09	0.21	0.04	0.12	0.28
p', p'-DDE	0.12	0.14	0.3	0.14	0.14	0.16	0.03	0.07	0.06
PCB total	0.62	0.53	1.3	0.92	—	4.2	0.89	0.39	0.8
Organophosphates:									
Chlorpyrifos	—	—	—	—	—	0.18	—	—	—
Diazinon	—	—	0.03	—	—	—	—	—	—
Methyl para- thion	—	0.02	—	—	—	—	—	—	—

ter hibernacula surveys ($n = 6$) were collected for chemical analysis.

Gas chromatography (GC)/mass spectrophotometer (MS) method in single ion monitoring (SIM) mode was used to perform chemical analysis on the carcasses. The tissue samples were extracted by the National Oceanic and Atmospheric Administration (NOAA) Status and Trends Method (MacLeod et al. 1985) with minor revisions (Brooks et al. 1989; Wade et al. 1988). The tissue samples were homogenized with a Teckmar Tisumizer. A 1–10 g sample (wet weight) was extracted with the Teckmar Tisumizer by adding surrogate standards, Na_2SO_4 , and methylene chloride in a centrifuge tube. The tissue extracts were purified by silica/alumina column chromatography to isolate the aliphatic and polycyclic aromatic hydrocarbon (PAH)/pesticide/PCB fractions. The PAH/pesticide/PCB fraction was further purified by high performance liquid chromatography (HPLC) in order to remove interfering lipids. The quantitative analyses were performed by capillary gas chromatography (CGC) with a flame ionization detector for aliphatic hydrocarbons, CGC with electron capture detector for pesticides and PCBs, and a mass spec-

trometer detector in the SIM mode for aromatic hydrocarbons (Wade et al. 1988).

RESULTS

Organophosphate insecticides were detected in three of the nine bats examined from the Indiana Department of Health, Rabies laboratory (Table 1). All the samples contained organochlorine pesticides. PCBs were detected in eight of nine bats (0.39–4.2 $\mu\text{g/g}$ wet weight). No pyrethroid insecticides were detected (Table 1). Due to the small size of the sample, no statistical analysis could be performed.

Chlorpyrifos (OP) was also detected in every Indiana myotis carcass and guano sample from Ray's Cave and Wyandotte Cave, both important Indiana myotis hibernacula (Table 2). No other OPs were detected in the Indiana myotis carcasses that were found dead in the caves.

Dichlorvos was detected in Indiana myotis guano from three of the four caves at concentrations ranging from 11–86 ppb, wet weight (Table 2).

DISCUSSION

The three bats with detectable levels of OP insecticides in their carcasses were found in

Table 2.—Organophosphorous insecticide residues (ppb, wet weight) in Indiana myotis guano and carasses from several priority Indiana myotis hibernacula in Indiana. U, not determined. OPs not detected include: dimethoate, disulfoton, ethoprop, fensulfthion, EPN, fenthion, malathion, merphos, methyl parathion, azinphos-methyl, phorate, demeton, diazinon, trichloronate, coumaphos, tetraethylpyrophosphate, mevinphos, monocrotophos, naled, parathion, ronnel, sulfo-tepp, sulprofos, tetrachlorvinphos, and protothiofos.

Cave	Guano from caves						Whole <i>Myotis sodalis</i>					
	Coon	Grotto	Ray's	Ray's	Wyandotte	Ray's	Wyandotte	Wyandotte	Wyandotte	Wyandotte	Wyandotte	Wyandotte
	April 04	April 04	April 04	Sept 03	April 04	Jan 03	April 04	Feb 02	Feb 03	Feb 03	Feb 03	Feb 03
Date												
Body mass (g)	N/A	N/A	N/A	N/A	N/A	7.9	8.0	6.2	4.6	5.1	5.9	
Lipid (%)	0.4	0.1	0.3	0.7	1.1	U	U	U	U	U	U	
Sex	N/A	N/A	N/A	N/A	N/A	F	U	F	M	M	M	
Moisture (%)	51.8	69.7	67.2	76.2	13.5	U	U	U	U	U	U	
Chlorpyrifos	<0.1	<0.08	0.7	0.2	1.0	0.8	1.1	1.6	3.2	4.2	1.1	
Dichlorvos	86	11	—	—	20	—	—	—	—	—	—	

Vanderburgh County. According to Indiana Agricultural Statistics Service census from February 1999, 55% of the total land area of Vanderburgh County is farmland (38% corn, 48% soybeans, and 12% wheat).

Chlorpyrifos is registered in Indiana for agricultural use on corn, soybeans and wheat to control agricultural pests. Up until 2002 it was also used in buildings to control cockroaches, fleas and termites. Today it has been phased out for home use (limiting the use for certified professional applicators) by EPA under a June 2000 agreement with the registrant (Dow AgroSciences which manufactures chlorpyrifos) (EPA 2000). Nevertheless, existing home use stocks purchased before 2002 may be used indefinitely for any purpose specified on the label.

Diazinon was registered in Indiana at 2000 for use on turf grass as well as for a variety of other homeowner uses, but residential uses have now been phased out.

In 1998, methyl parathion was registered for a variety of agricultural products such as corn, soybeans and wheat. Methyl parathion should only be used in open fields to control insects and is used as a spray (insecticides data courtesy of G.N. Saxton, Compliance Officer, Office of Indiana State Chemist, 2005 and ATSDR, Agency for Toxic Substances and Disease Registry).

Dichlorvos is registered for indoor, terrestrial non-food, greenhouse (food and non-food) and domestic outdoor use including turf and ornamental uses. There are no agricultural crop uses for this chemical. The majority of dichlorvos uses are indoors, including greenhouses, commercial, residential, industrial and farm buildings, food handling establishments and more (EPA 2005).

Several authors have suggested that since OPs do not accumulate in living tissue their presence is indicative of recent exposure prior to death (O'Shea & Clark 2002; Hill 1995, 1989). Nevertheless, the presence of OP residues in the absence of demonstrated ChE inhibition does not confirm clinical effects nor suggest causation in a specific mortality. It is important to state that in the present study the cause of death was not determined nor was it determined how long the OP-infected bats lived after they were exposed. It is also important that the samples were frozen for 10 months to three years prior to the chemical

analysis. Given that OP residues are supposedly rapidly eliminated from the living body (Hill 1989) and do not persist in the environment for long, it is likely that the residue levels detected are lower than they were in the body at the time of exposure.

Freed et al. (1979) studied the degradation of OPs in water. They found that the half life of OPs in aqueous systems ($\text{pH} = 7.4$) at 20°C ranged from seven hours to 130 days, according to the specific OP compound. OPs degradation rates at 37°C were 5–7 times greater. Ferrando et al. (1992) obtained similar results. They tested persistence of various insecticides (organochlorines, OPs and carbamate) in tap water ($\text{pH} = 7.5 \pm 0.5$) and unfiltered lake water ($\text{pH} = 9.0 \pm 0.5$) kept at 22°C . The most persistent insecticides in their study belonged to the OP (diazinon, half-life of 70.54 h in natural water and 79.19 h in tap water) and carbamate (thiobencarb, half-life 74.27 h in natural water and 247.66 h in tap water) groups. This implies that ChE inhibitors can build up in surface water after application to crop fields, especially since each field will be treated at a different time and sometimes more than once. They will persist in water for even longer during the colder spring months. The result can be an additional exposure media (water) to bats and other wildlife on top of the other exposure media (air, soil and food).

Even though ChE-inhibiting insecticides are labile in mammals; recovery of inhibited ChE activity from a single exposure may vary from a few hours for most carbamates to 1–3 weeks for OP compounds (Hill 1995). Repeated application of even short-lived chemicals may cause cumulative physiological effects without a corresponding accumulation of chemical residues (Hill 1995). Although it is possible that the bats were exposed to OPs by dermal or respiratory routes while flying in areas where an OP had been sprayed, it is most likely that these OP residues come from consuming contaminated insects or drinking contaminated water. If that is the case, bats are apt to be re-exposed when feeding on the same insects or drinking the same water night after night until contamination ceased.

Clark (1986) and Clark & Rattner (1987) conducted OPs acute toxicity laboratory studies using bats. The bats were dosed once with high (near lethal) doses of OP and were kept

(if they survived) for only 24 h before being sacrificed and tested to determine brain ChE activity. The relevance of these studies to field conditions in which chronic exposure to low doses is more likely to occur is questionable. Clark (1986) dosed little brown myotis and big brown bats (*Eptesicus fuscus*) by stomach intubation with methyl parathion. He found that bats lost coordination within 1 h and were still not able to right themselves at 24 h after exposure. Although it appears that bats are about eight times less sensitive than mice in terms of acute lethality, the oral dose estimated to cause loss of coordination in 50% of big brown bats was one-third or less than the LD_{50} of this species. Similar results were obtained when Clark & Rattner (1987) dosed little brown myotis with acephate. These data imply that exposure to non-lethal doses could result in death in the field when bats that have become debilitated are exposed to high risk situations such as predators, harsh sun, inclement weather, or drowning (Clark 1986). Another question was whether the lack of lethality during the 24 h studies might be related to bats entering torpor when subjected to stress—in this particular case, the stress of the toxicity study (Gordon 1993). This could have delayed acute effects therefore making it appear that bats are not as “sensitive” to OPs as mice. However, elongated torpor might cause energetic deficiencies when torpid bats do not feed for relatively long periods.

Sub-lethal inhibition of brain ChE activity, after OP applications to agricultural fields, were documented on several occasions. Maul & Farris (2005) documented significant levels of cholinesterase (ChE) inhibition in 8.7% of northern cardinals (*Cardinalis cardinalis*) sampled from agricultural field edges in north-east Arkansas 3–21 days post treatment. Guillén et al. (1991) documented whole body residues of fenitrothion in *Pipistrellus pipistrellus* the day after (0.54 ppm) and 21 days after (0.84 ppm) rice fields were treated. In addition, mean brain ChE levels in bats collected 21 days after treatment were significantly reduced. If similar sub-lethal reductions are occurring in the ChE enzymes of Indiana bats as a result of these documented OP exposures, and if ChE reductions are cumulative, persisting for many days, both the risk of trauma due to navigational impairment increases and foraging ability might be impaired

such that their high energy requirements could not be met. Even if the bats themselves manage to survive and recover, pregnant and nursing females, too low on energy, could lose their pups.

Sub-lethal doses of an organophosphate might reduce bats foraging capability for a few hours or days, and that could be sufficient to cause starvation-related mortality that would be nearly untraceable using current analytical chemistry techniques. Indeed, bats necropsied at the U.S. Geological Service Wildlife Health Laboratory in Madison, Wisconsin are often found to be emaciated (Grace McLaughlin, USGS Wildlife Health Laboratory pers. commun).

Eighteen different OPs were detected in guano from maternity roosts of cave bats (*Myotis velifer*) in Texas (Land 2001). The presence of OPs in guano from Texas and from caves in Indiana not only confirms bat exposure to OPs, it also demonstrates oral exposure (food, water or probably both). Demonstration of oral exposure through contaminated insects would be clear evidence that OPs are entering the food chain. If we assume low OP bioaccumulation in bats, their presence in guano may result from OP insecticides being applied near those hibernacula.

A specimen of Indiana myotis (16615), submitted in April 2000 contained detectable levels of diazinon (Table 1). It is likely that this exposure resulted from feeding on insects associated with a spring-time agricultural or turf grass application. Just emerging from hibernation therefore having low energetic reserves could have made that bat more sensitive to the toxic effect of OPs. A northern myotis (16729) submitted in August 2000 was found with detectable levels of chlorpyrifos (Table 1). It appeared to be in good condition, based on 15% lipid concentration in its body. It is possible that this bat was exposed to chlorpyrifos due to agricultural application or other use. An Indiana myotis (16204) submitted in October 1998 had detectable levels of methyl parathion and a whole body lipid concentration of 33.7% (Table 1). Speakman & Thomas (2003) indicate 30% fat reserves as the normal level prior to hibernation. It is more difficult to suggest a reason for using methyl parathion at the end of October. Although the cause of death was not determined in any of the nine bats sampled, it is possible that OP insecticide

exposure was a contributing cause of death in these three bats, especially since all three were in normal to good nutritional condition considering the season.

Of the 8262 bats submitted to the Indiana department of health rabies laboratory during the years 1966–2003, only 5.4% (445 bats) were rabid (Whitaker & Douglas in press). This means that nearly 95% of the bats died from other causes. Considering that many of these bats were found under suspicious circumstances (i.e., often ending up on the ground around human habitations), and that OPs can adversely influence behavior, we suggest that OPs may have directly or indirectly led to the deaths of the three bats. The fact that three of nine bats were found to be exposed to OPs might suggest frequent exposure to those toxicants in the overall population. Because of this, and also because of the lack of information regarding sub-lethal effects of these ubiquitous neurotoxins to living bats, more research is needed.

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