

**DEPARTMENT OF THE INTERIOR  
U.S. FISH AND WILDLIFE SERVICE  
REGION 5**



**ENVIRONMENTAL CONTAMINANTS PROGRAM  
OFF-REFUGE INVESTIGATIONS SUB-ACTIVITY**

**NY, MA, VT, NH, CT, PA, IN – Evaluation of the Potential Role of Environmental  
Contaminants in Significant Bat Mortality in Conjunction with White-Nose  
Syndrome (WNS) in the Northeastern United States**

Project ID: FFS#: 5F44 and DEC ID: 200950001.1(Final Report)  
(filename:5F44BatWNSECinvestigation-finalFY14.docx)

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August 2015

Congressional Districts:

NY: 19 – 23

NH: 1

MA: 1

VT: 1

CT: 6

PA: 9 - 11

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## EXECUTIVE SUMMARY

This study was originally developed in 2008 to investigate whether there may be a link between environmental contaminants and the newly discovered syndrome affecting bats known as White-Nose Syndrome (WNS). It quickly became clear as WNS research progressed that WNS is an emerging infectious disease caused by a newly described psychrophilic (cold-loving) fungus, currently known as *Pseudogymnoascus destructans*. The goal of this study shifted from the evaluation of environmental contaminants as a proximate cause of WNS to an evaluation of the role contaminants may play in contributing additional stresses to bats that are already under significant threats from WNS.

A review of pesticide use in primarily New York and California found that the most commonly used class of pesticides in recent use are the dinitroanilines, neonicotinoids, pyrethroids, chlorophenoxy compounds, organophosphates, carbamates, substituted benzenes, triazines and glyphosate. Many of these chemicals, depending on exposure, may pose chronic or acute toxicity to bats, as predicted by mammalian median lethal dose (LD<sub>50</sub>) data and/or evidence indicating endocrine disruptive or other sublethal effects.

We analyzed bat carcasses (see Table 1 for sample information) collected in New York State for the above classes of pesticides, organochlorine pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). This analysis found relatively low concentrations of pesticides such as dichlorodiphenyltrichloroethane (DDT) and metabolites, oxychlorodane, dieldrin, thiamethoxam (neonicotinoid insecticide), chlorpyralid (chlorophenoxy herbicide), and dalapon. We found a single bat with concentrations of several organophosphate compounds, likely indicative of poisoning. Two bats displayed brain cholinesterase (ChE) activity consistent with exposure to ChE inhibiting pesticides. We recommend that attention be paid in future studies to the effects on bats of the organophosphates, carbamates, neonicotinoids and

pyrethroids. These pesticides are widely used, can bioaccumulate and may have high toxicity to non-target organisms.

Concentrations of mercury (Hg) in bat fur differed between states, with general increases in bat fur Hg concentrations as follows: MA>VT>NH>NY>PA. Some bat fur concentrations exceeded fur Hg concentrations (10.8 mg/kg) that are associated with adverse behavioral effects in deer mice. Three bats had fur Hg concentrations greater than 20 mg/kg, suggesting Hg enrichment in the environment.

The mean concentration of PBDE detected in bats for this study was 0.61 mg/kg, with concentrations in individual bats as high as 8.8 mg/kg in a bat from Massachusetts. The effect of PBDEs on bats is unknown but is explored further in Secord et al. (2015).

We analyzed 26 additional bat carcasses (or carcass composites) from the Northeastern United States for emerging contaminants such as pharmaceuticals, personal care products and hormones. Most compounds were not detected or detected in only a few bats. Caffeine, salicylic acid and thiabendazole were the most frequently detected emerging contaminants, detected in about 23% (caffeine) to 80% (salicylic acid) of bat samples. Other compounds detected in at least 15% of bat samples were digoxigenin, ibuprofen, warfarin, penicillin V, testosterone and N,N-diethyl-meta-toluamide (DEET). These data are further discussed in Secord et al. (2015).

# I. INTRODUCTION

## IA. BACKGROUND

This study proposal was originally developed in 2008 to investigate whether there may be a link between environmental contaminants and the newly discovered syndrome affecting bats known as White-Nose Syndrome (WNS). WNS was first reported during the winter of 2006-2007 in a small number of bat hibernacula in New York, possibly contributing to the death of about 8,000 bats. Findings, in addition to the white fungus appearing on the noses, wings, and membranes of bats, were low fat reserves, emaciation, clustering near the colder hibernacula mouths, unresponsive behavior within the hibernacula, and premature emergence during the daytime and/or when temperatures were too low to support an insect prey base.

At the time this proposal was developed, pathologists and other researchers from a number of agencies and universities had failed to confirm pathogens or other agents of likely concern. Health evaluations had included assessments of gross pathology, histopathology, virology, bacteriology, parasites, immune suppression (e. coli test, t-cell subset capacity), blood chemistry (white blood cell, hematocrit, etc.), and a characterization of external fungi.

Scientists have since determined that WNS is an emerging infectious disease caused by a newly described psychrophilic (cold-loving) fungus, currently known as *Pseudogymnoascus destructans*. Since its first documented appearance in New York in 2006, WNS has spread rapidly throughout the Northeast and is expanding through the Midwest. As of August 2013, WNS was confirmed in 23 States (Alabama, Connecticut, Delaware, Georgia, Illinois, Indiana, Kentucky, Maine, Maryland, Massachusetts, Minnesota, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, and West Virginia) and 5 Canadian provinces (New Brunswick, Nova Scotia, Ontario, Prince Edward Island, and Quebec). Three additional states (Arkansas, Iowa, and Oklahoma) are considered suspect for WNS based on the detection of the causative fungus on bats within those states, but with no associated disease to date. The U.S. Fish and Wildlife Service (USFWS) has estimated that over 5.5 million to 6.7 million bats of several species have now died from WNS (USFWS 2012).

The diagnostic feature of WNS is the white fungal growth on muzzles, ears, or wing membranes of affected bats, along with epidermal erosions that are filled with fungal hyphae (Blehert *et al.* 2009; Meteyer *et al.* 2009). In addition to the presence of the white fungus, initial observations showed that bats affected by WNS were characterized by some or all of the following: 1) depleted fat reserves by mid-winter; 2) a general unresponsiveness to human disturbance; 3) an apparent lack of immune response during hibernation; 4) ulcerated, necrotic, and scarred wing membranes; and 5) aberrant behaviors including: shifts of large numbers of bats in hibernacula to roosts near the entrances or unusually cold areas; large numbers of bats dispersing during the day from hibernacula during mid-winter; and large numbers of fatalities, either inside the hibernacula, near the entrance, or in the immediate vicinity of the entrance (USFWS 2011). Although the exact process by which WNS leads to death remains undetermined, it is likely that

the reduced immune function during torpor compromises the ability of hibernating bats to combat the infection (Bouma *et al.* 2010; Moore *et al.* 2011).

The spread of WNS over the past seven years has generally progressed as would be expected of a transmissible agent spreading along known migratory pathways and overlapping summer ranges of hibernating bat species. It is hypothesized to spread via bat-to bat contact, as well as contact with contaminated cave/mine substrate (Kunz and Reichard 2010; USGS 2011).

It has been hypothesized that *P. destructans* may have recently arrived in North America from Europe (Puechmaile *et al.* 2011). Although *P. destructans* has been isolated from five bat species in Europe, research suggests that bat species in Europe may be immunologically or behaviorally resistant, having co-evolved with the fungus (Wibbelt *et al.* 2010). Pikula *et al.* (2012), however, confirmed that bats found dead in the Czech Republic exhibited lesions consistent with WNS infection.

## IB. EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON BATS

The goal of this study has shifted from the evaluation of environmental contaminants as a proximate cause of WNS to an evaluation of the role contaminants may play in contributing additional stresses to bats that are already under significant threats from WNS. We also offer a hypothesis in Secord *et al.* (2015) that certain contaminants may increase the susceptibility of bats to WNS and/or limit the ability of bats to marshal defenses to WNS.

Environmental contaminants, particularly organochlorine pesticides, have been implicated historically in bat mortality (Clark and Shore 2001). It is also possible that more recently used classes of pesticides (e.g., organophosphates, pyrethroids, neonicotinoids) may be adversely affecting bat populations by interfering with metabolic, neurologic, or immune functions. Emerging contaminants, such as detergents and surfactants (alkylphenol ethoxylates, perfluorosulfonates), antibacterials (triclosan), pharmaceuticals and plasticizers (phthalates, bisphenol A) are also of increasing concern in the environment due to their widespread use, and properties such as estrogenicity and bioaccumulation (Kolpin *et al.* 2002). Bats may be more susceptible to the effects of contaminants than other mammals due to their high longevity, high metabolic rates, low reproductive rates, and annual hibernation cycles requiring significant fat deposition followed by extreme fat depletion during hibernation (Clark and Shore 2001).

## IC. PROJECT OBJECTIVES

The specific original objectives of this project were to:

**Objective #1** – Assess pesticide use practices in areas affected and unaffected by WNS to identify any pesticides of concern.

**Objective #2** – Evaluate land use practices in the vicinity of bat hibernacula and summer roosts to elucidate any relationship with adjacent land use and WNS.

**Objective #3** – Monitor bats at summer roosts and fall swarm areas near hibernacula to collect any dead or incapacitated bats. Archive bat tissues for subsequent contaminant and biomarker analysis.

**Objective#4** – Determine whether bats collected near areas of pesticide use or other known or suspected contaminants have concentrations of contaminants or brain cholinesterase (ChE) inhibition in tissues that may adversely affect health and survival of bats and potentially contribute to WNS.

**Objective #5** – Determine insect abundance and chemical quality at summer roosts and fall swarm sites at WNS and non-WNS affected areas to evaluate whether prey availability is affecting the health of bats or bats are consuming insects with potentially high concentrations of contaminants.

As our knowledge of WNS has improved, specifically our understanding that the symptoms were related to a fungus called *P. destructans* and that this fungal disease was rapidly advancing into new states, suggesting a transmissible disease, our study objectives changed. With respect to Objectives 1 and 2, although we evaluated pesticide use as part of this study, it does not appear that WNS discriminates between states, rural vs. urban areas or areas with potentially low vs. potentially high chemical/pesticide use. Additional information on pesticide use and implications for bat health will be presented in the Results.

Pursuant to Objectives 3 and 4, we analyzed contaminant and brain ChE concentrations in a large number of bats collected dead or incapacitated at hibernacula, as well as bats from rabies monitoring programs and bats otherwise found dead or moribund. No bats were sacrificed for this study.

With respect to Objective 5, reports from early in the evaluation of WNS did not suggest that bats were entering hibernation underweight. Reichard *et al.* (2010) determined that bats swarming at WNS affected sites appeared to deposit sufficient fat reserves during the fall, but these reserves appeared to be depleted relative to unaffected bats during both the final stages of pre-hibernation and the early stages of hibernation. Blehert *et al.* (2011) reported that fat depletion is not consistently observed among all bats with WNS. We determined that it remained appropriate to analyze insects for contaminants of emerging concern, but measures of insect abundance were not warranted.

Our focus shifted to evaluating the exposure of bats to pesticides, Hg and emerging contaminants in order to better understand the role that hazardous substances may play in impacting bat physiology and behavior independent of WNS. Also, we have considered whether the widespread and pervasive nature of some of these chemicals may reduce the ability of bats to combat and survive WNS.

## II. METHODS

### IIA. PESTICIDE USE PRACTICES

We initially evaluated pesticide use practices in New York in order to identify any potential relationship between pesticide use patterns and WNS. We evaluated pesticide use data from New York for the years 2003 – 2005 (and later added data from 2009 that was posted in ~ 2013) to evaluate whether there may have been patterns of use by county that corresponded with WNS

in New York bats or any increased pesticide usage in the years preceding the onset of WNS in New York. After we found no apparent relationship in New York between pesticide use practices and WNS, we expanded this objective to document pesticide use and pesticide use temporal changes in other parts of the country in order to evaluate the overall threats that current use pesticides may present to bats.

## IIB. COLLECTION OF BAT CARCASSES, INSECTS, AND GUANO

We collected over 135 bat carcasses from sites across New York, Pennsylvania, Connecticut, Vermont, New Hampshire, and Massachusetts. These included dead or dying bats collected at hibernacula, as well as bats sent to us from the New York State Health Department from counties of interest. No bats were sacrificed for this study, in an effort to minimize mortality among bat populations that are already severely distressed. Due to the manner of bat carcass collection, the length of time between mortality and collection varied.

Bat carcasses (with brains removed for rabies testing) collected in the field by the New York State Health Department were frozen soon after collection and shipped frozen to the New York Field Office. Other bat carcasses were sent to us frozen by State or USFWS personnel in New York, Pennsylvania, Vermont, Massachusetts and New Hampshire. After receipt, we maintained all carcasses at -80 degrees F until processing and shipment to the laboratory. All carcasses were weighed prior to shipping and shipped whole (except for carcasses analyzed for emerging contaminants) on dry ice using an overnight carrier. For emerging contaminants analysis, in order to reduce analytical interference, we removed the wings from bats prior to shipping them to the laboratory. Some bat samples shipped to the laboratory consisted of multiple bats from the same location and date of collection (see Secord et al. 2015).

For Hg analysis, a small amount (~ 1-10 mg) of hair was clipped from the back of a bat carcass and shipped frozen to the laboratory, as described above.

As much brain tissue was removed as feasible, weighed, transferred to acid-washed jars and shipped frozen to the laboratory for analysis of ChE. Brains that were desiccated or emulsified were not analyzed.

Ten insect samples were collected in 2009/2010 in New York, Pennsylvania, Vermont, New Hampshire and Massachusetts using both malaise and light traps. They consisted predominantly of moths. An additional nine mosquito samples were collected in Pennsylvania and New York by local health departments. Additional insect samples were collected by the TestAmerica laboratory in Texas, California and Nevada. Some of these samples were submitted for analysis of emerging contaminants. Additional analysis is pending.

We collected guano from a number of locations throughout the northeast and have archived them. Most samples were of too small a mass to allow for chemical analysis.

## IIC. CHEMICAL ANALYSIS OF BAT AND INSECT TISSUE

A summary of chemical analyses performed on various samples is presented in Table 1. Twelve bats were submitted for analysis of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated terphenyls (PCTs), carbamates, organophosphates (OPs), and

organochlorine pesticides (OCs), including total dichlorodiphenyltrichloroethane (tDDT), chlordane, dieldrin, endrin, benzene hexachloride (BHC), mirex, and toxaphene. These 12 bats were collected from Onondaga or Ulster Counties, New York in the period of August 2008 through March 2009. This suite of chemicals was selected because they have been associated with mortality/toxicity in fish and wildlife. All bats analyzed were *Myotis lucifugus* (MYLU).

An additional 16 bats were analyzed for neonicotinoids, chlorophenoxy herbicides, OPs, pyrethroids, dinitroanilines, and triazines. The sixteen bats were collected from Ulster, Essex and Onondaga Counties from February 2009 through July 2009. These pesticides were selected for analysis because they were determined to be applied in New York in the greatest quantities in the time period 2003 – 2005 (Figure 2). All bats analyzed were *Myotis lucifugus* or *M. sodalis* (MYSO).

In late 2010, based on the earlier chemical analytical results showing detections of PBDEs and evaluation of pesticide use indicating significant use in New York State of neonicotinoids and substituted benzenes, we submitted 10 bat carcass samples for analysis of neonicotinoids, substituted benzenes and PBDEs. All bats analyzed were *Myotis lucifugus*, *M. sodalis* or *Eptesicus fuscus* (EPFU). One additional *M. lucifugus* that was suspected of being poisoned was analyzed for organophosphates and carbamates.

Thirty bat fur samples were analyzed for Hg and 19 brain tissue samples were analyzed for ChE.

Twenty six bat samples (8 – PA; 3 – MA; 2 – NH; 2 – VT; 11 – NY) were sent to the laboratory in January, 2012 for analysis of emerging contaminants. Data are reported and discussed in Secord et al. (2015). Insects were collected as described in the Methods Section. Three insect samples were analyzed for emerging contaminants.

<b>Table 1. Bat Analytical Sample Summary</b>			
Type Analysis	Matrix	# Samples	Date of Analytical Report
PCBs, PBDEs, PCTs, carbamates, OPs, OC pesticides	Bat carcass with brain removed (NY)	12	11/17/2009
Neonicotinoids, chlorphenoxy, OPs, pyrethroids, dinitroanilines, triazines	Bat carcass with brain removed (NY)	16	7/6/2009
Neonicotinoids, substituted benzenes, PBDEs	Bat carcass (NY & New England)	10	3/16/2011
OPs/carbamates	Bat carcass (NY)	1	3/16/2011
Hg	Bat hair (NY,PA,MA,VT,NH)	30	3/16/2011
Cholinesterase (ChE)	Bat brain (NY,PA,MA,VT,NH)	19	3/16/2011
Emerging Contaminants (pharmaceuticals, antibacterials, hormones etc)	Bat carcass with wings removed (NY,PA,MA,VT,NH)	26	April, 2014
Emerging Contaminants	Insects	3	July, 2013

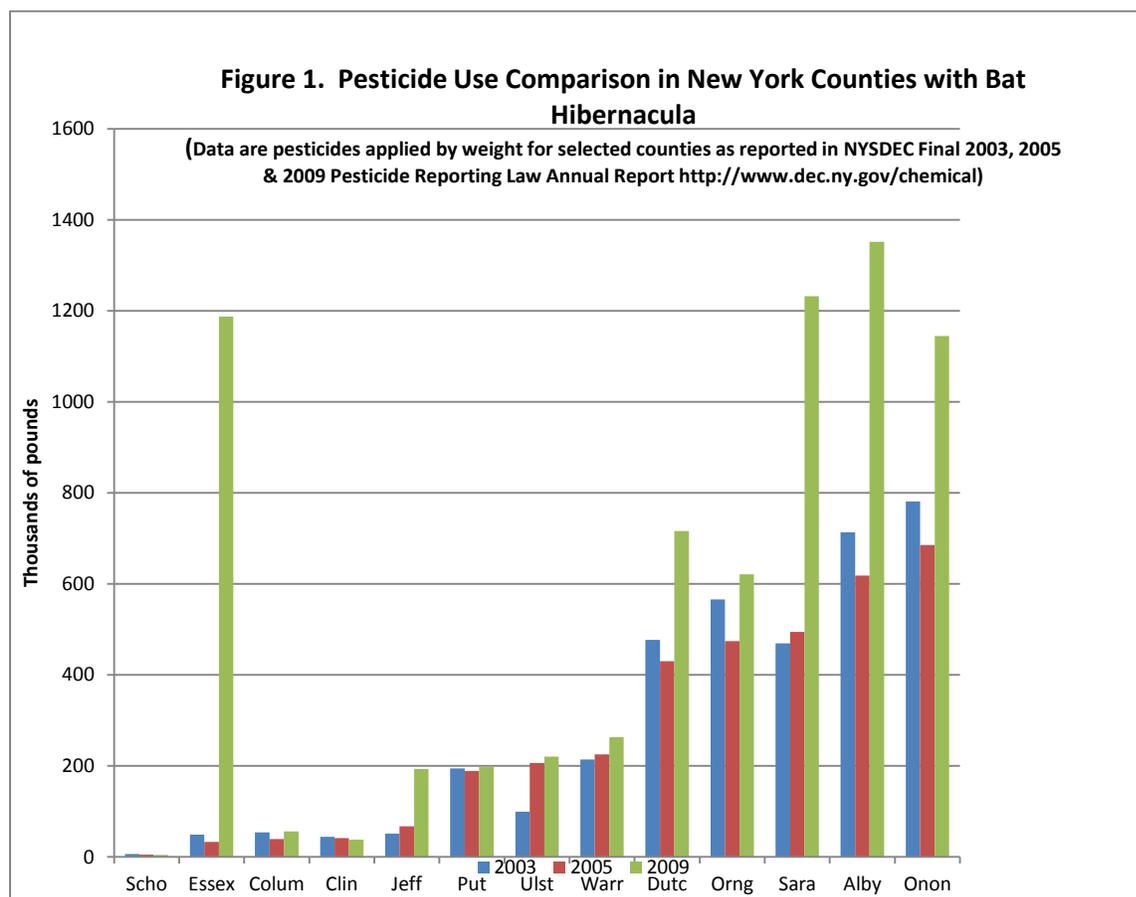
#### IID. DATA AND STATISTICAL ANALYSIS

Means calculated in this report do not include data reported as non-detect (ND). All statistical analyses were performed in EXCEL. For the evaluation of fur Hg data, a single factor analysis of variance was performed on Hg concentrations by state, followed by a post hoc two tailed t-test (equal variance) with a Bonferoni correction to determine whether differences were significant. Fur Hg concentrations between adult and juvenile bats were compared using a two-tailed t-test, assuming unequal variances.

### III. RESULTS

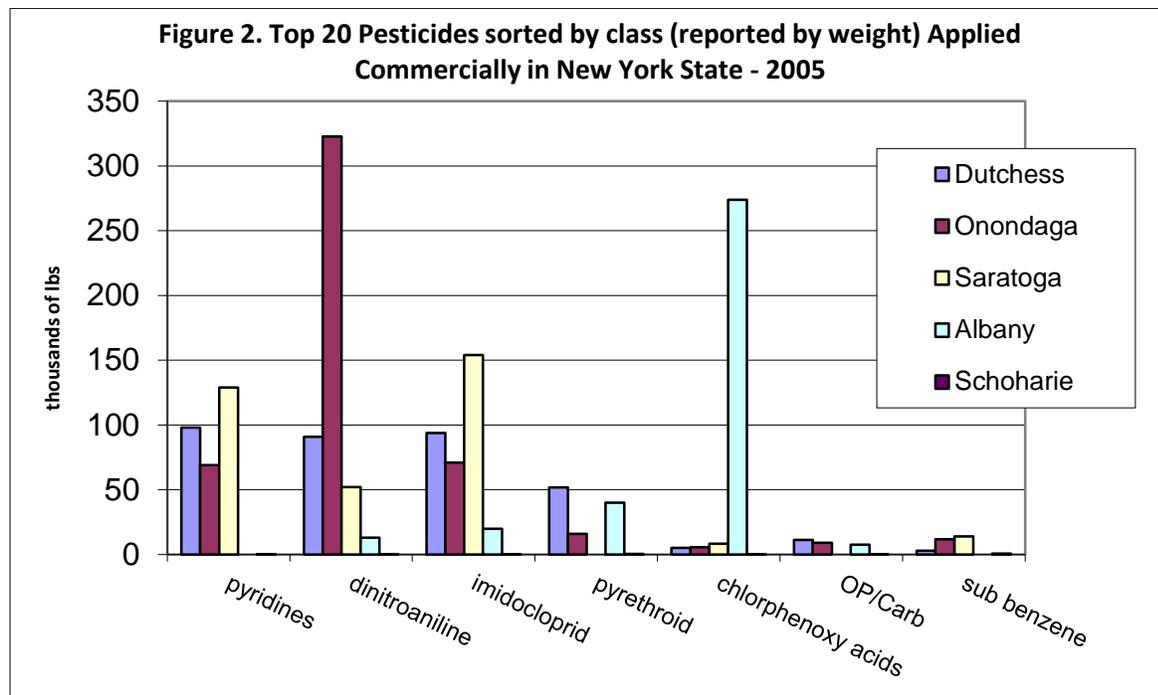
#### IIIA. PESTICIDE USE PRACTICES

New York State maintains a pesticide use database that was developed in 1998 (<http://www.dec.ny.gov/chemical/27506.html>). At the start of this project in 2009, based on pesticide use data from 2003 through 2005, we evaluated pesticide use in a number of New York counties and determined that New York counties with low overall pesticide use (e.g. Schoharie) had bats with symptoms of WNS, as did counties with high pesticide use (e.g. Onondaga, Saratoga). We later added pesticide use data from 2009. The 2009 data indicated a large increase in pesticide use in some counties between 2005 and 2009, but still no obvious relationship between the pounds of pesticides used and WNS in bats. See Figure 1.



By evaluating the top ten pesticides used in New York from 2003 – 2005 (<http://www.dec.ny.gov/chemical/37855.html>) and also data compiled from <http://pmep.cce.cornell.edu/psur/> on the top 20 products by weight used by commercial applicators in New York, we determined that the most commonly used classes of pesticides in New York during those years were the substituted benzenes, pyridine/carboxylics, chlorphenoxy acids, triazines, dinitroanilines, pyrethroids, neonicotinoids, OPs, carbamates, and glyphosate (Figure 2). The New York database also reported on the use of other pesticides that are typically sold and reported in gallons rather than pounds. These pesticides were dominated by

disinfectants (e.g., calcium hypochlorite), kerosene and oil based compounds, metal-based compounds (e.g. copper azole), herbicides such as atrazine, metalochlor, glyphosate and fungicides such as mancozeb (<http://www.dec.ny.gov/chemical/27506.html>).



The State of California also maintains a pesticide use reporting database (<http://www.cdpr.ca.gov/docs/pur/purmain.htm>). Among the top 100 pesticides used (acres treated state-wide) in 2011 were many of the same pesticides used in New York State in 2005, including glyphosate, imidacloprid, chlorpyrifos, a number of pyrethroids, pendimethalin, and mancozeb (<http://www.cdpr.ca.gov/docs/pur/pur11rep/11sum.htm>). The 2011 “Summary of Pesticide Use Data for California” reported an increase in the use of carcinogenic compounds from 2009 – 2011, particularly the fungicides, mancozeb and idoprione. During that same time period, they reported an increase in the use of OPs and carbamates, particularly chlorpyrifos and malathion (<http://www.cdpr.ca.gov/docs/pur/pur11rep/11sum.htm#pestuse>).

The CropLife Foundation reported information from the National Pesticide Use Database 2002 (Gianessi and Reigner 2006). The most used herbicides and fungicides included glyphosate, atrazine, 2,4-D, metalochlor, pendimethalin, triflurin, chlorothalinil, and mancozeb.

Fishel (2009) reported on home and garden pesticide use trends in the U.S. Among their top ten most commonly used conventional home and garden pesticide active ingredients were 2,4-D, glyphosate, pendimethalin, diazinon, mecoprop (MCP), carbaryl, dicamba, malathion, chlorthal-dimethyl (DCPA) and benefin.

Many of the pesticides mentioned above can also be found on the U.S. Environmental Protection Agency’s (USEPA) “Final List of Chemicals for Initial Tier 1 Screening” for endocrine

disruption (<http://www.epa.gov/scipoly/oscpendo/pubs/prioritysetting/finallist.html>). This list of 67 pesticide active ingredients includes glyphosate, many of the pyrethroids, OPs, imidacloprid (neonicotinoid), atrazine and simazine, many of the carbamates, and substituted benzenes, such as chlorothalnil and dichlobenil. This USEPA list was developed based on exposure potential and not likely endocrine disruptor properties.

A summary of this pesticide use information is presented in Table 2. Our analysis found that, for the most part, the same classes of pesticides have been used in New York State as in California. Some of these classes of pesticides are also reported as commonly used by households and are considered to pose an exposure risk by USEPA, warranting their evaluation for endocrine disruptor properties. We note that pesticide use practices are continually evolving as new products are developed or existing products are used more or less widely. The information provided here is just a snapshot of general pesticide use, as reported from several information sources.

Pesticide Class	Examples	NY Top 20 from 2005*	CA Top 50 from 2007 **	USEPA endocrine disruption***	Home & Garden Top 10 ****
Dinitroanilines	Pendimethalin, proflumicarb	X	X		X
Substituted Benzenes	Chlorobenzene, dichlorobenzene	X	X	X	
Neonicotinoids	Imidacloprid, thiamethoxam	X	X	X	
OPs/Carbamates	Chlorpyrifos, malathion (OP); carbaryl, mancozeb (carbamate)	X	X	X	X
Pyrethroids	Bifenthrin, cypermethrin	X	X	X	
Chlorophenoxy	2,4-D, chlorpyralid	X	X		X
Triazines	Atrazine, simazine	X	X	X	
Glyphosate	Glyphosate	X	X	X	X
Other	Petroleum based, inorganic, etc		X	X	X
Note: This table is intended to present general information only on pesticides commonly used, as reported by various sources					
* <a href="http://pmep.cce.cornell.edu/psur">http://pmep.cce.cornell.edu/psur</a> (top 20 products noted and sorted into classes)					
** <a href="http://www.pesticideinfo.org/DS.jsp?sk=30">http://www.pesticideinfo.org/DS.jsp?sk=30</a>					
*** <a href="http://www.epa.gov/scipoly/oscpendo/pubs/prioritysetting/finallist.html">http://www.epa.gov/scipoly/oscpendo/pubs/prioritysetting/finallist.html</a>					
**** Fishel (2009)					

## IIIB. BACKGROUND INFORMATION ON COMMONLY USED CLASSES OF PESTICIDES

### *ORGANOPHOSPHATES AND CARBAMATES*

Organophosphates and carbamates are two classes of highly toxic insecticides that act by inhibiting the enzyme acetylcholinesterase and causing neurotoxic and other effects (Grue *et al.* 1997). They are generally quickly hydrolyzed in the blood and eliminated from the body, although some OP compounds (such as chlorpyrifos) may be stored in fat tissue (USEPA 2013; <http://www.inchem.org/documents/pims/chemical/pimg001.htm>).

### Chlorphenoxy-Type Compounds & Dalapon

The chlorphenoxy herbicides are generally used to control broadleaf weeds and include 2,4-D, 2,4,5-T and MCPP. They are not significantly stored in fat (USEPA 2013). The chemical 2,4,5-T has been banned for all uses for over 25 years due its carcinogenic properties and adverse impacts on mammalian reproduction (<http://pmep.cce.cornell.edu/profiles/herb-growthreg/fatty-alcohol-monuron/fenoprop/silvex-2-79-canc.html>).

Dalapon is an organochlorine herbicide in no specific chemical class. It is used to kill vegetation such as grasses and cattails (<http://extoxnet.orst.edu/pips/dalapon.htm>). Dalapon is a component of the commercial formulation, “Dimension”, an herbicide and fertilizer product commonly used in New York State in 2004 and 2005. Dalapon has relatively low toxicity to mammals, with rat oral LD<sub>50</sub>s ranging from 7,570 – 9,330 mg/kg body weight, although it is regarded as moderately toxic to humans (<http://extoxnet.orst.edu/pips/dalapon.htm>). The octanol-water partition coefficient (Log K<sub>ow</sub>) for dalapon is 0.78, indicating a low propensity to bioaccumulate. Bioconcentration factors (BCFs) of less than 1 have been measured for a number of mammals ([www.epa.gov/ogwdw/pdfs/factsheets/soc/tech/dalapon.pdf](http://www.epa.gov/ogwdw/pdfs/factsheets/soc/tech/dalapon.pdf)).

### *PYRETHROIDS*

Pyrethroid insecticides are neurotoxins that include a diverse group of broad spectrum products with agricultural, horticultural, veterinary, and residential uses. They may be increasingly used as a substitute for insecticides such as OPs (Gan 2006). The pyrethroid insecticide, bifenthrin, was among the top ten pesticides (by weight) used by certified commercial applicators in 2003 – 2005 in New York (<http://www.dec.ny.gov/chemical/27506.html>). A few of the pyrethroids have high mammalian toxicity (as represented by the rat LD<sub>50</sub>) and a high bioaccumulation potential (See Table 5). In particular, the “third generation” of pyrethroids have acute oral toxicities rivaling the toxicity of OP, carbamate and organochlorine pesticides. These pyrethroids include esfenvalerate, deltamethrin, bifenthrin, tefluthrin, flucythrinate, cyhalothrin and fenprothrin (Mueller-Beilschmidt 1990).

A number of pyrethroid compounds are on the USEPA “Final List of Chemicals for Initial Tier 1 Screening” as part of the Endocrine Disruptor Screening Program<sup>1</sup> (<http://www.epa.gov/endo/pubs/prioritysetting/finallist.html>).

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<sup>1</sup> The Final List of Chemicals for Initial Tier 1 Screening was developed based on exposure potential and not likely endocrine disruptor properties.

### *SUBSTITUTED BENZENES*

Substituted benzenes are typically used as fungicides on a variety of agricultural crops, including grapes, tomatoes, apples and potatoes. They are in the top ten classes of pesticides used in New York (see Figure 2). The substituted benzenes currently registered for use are considered to have low toxicity in mammals, with LD<sub>50</sub>s for rats generally in excess of 5,000 mg/kg body weight (Fishel 2012). The substituted benzenes, chlorothalnil and dichlobenil, are on the USEPA “Final List of Chemicals for Initial Tier 1 Screening” as part of the Endocrine Disruptor Screening Program (<http://www.epa.gov/endo/pubs/prioritysetting/finallist.html>).

### *TRIAZINES (SIMAZINE, ATRAZINE) & DINITROANILINE (PENDIMETHALIN)*

The herbicides, atrazine and simazine, have a low bioaccumulative potential and are not expected to accumulate in bat tissues. Atrazine has been demonstrated to adversely affect endocrine and reproductive systems in mammals ([www.epa.gov/teach/chem\\_summ/Atrazine\\_summary.pdf](http://www.epa.gov/teach/chem_summ/Atrazine_summary.pdf)). Pendimethalin has a comparatively higher bioaccumulative potential<sup>2</sup>. We included these three compounds in our analysis of bats from New York State because of their high usage in New York. Pendimethalin is a dinitroaniline herbicide that is one of the most commonly used herbicides in New York. The 2005 New York State pesticide use data reported pendimethalin as the number one pesticide used by weight in the state, although it was noted that pendimethalin was a minor component (generally ~1%) of a commercial product that combines small amounts of herbicide with much larger amounts of fertilizer. Pendimethalin is also commonly used as a home and garden herbicide and was among the top ten pesticides in the National Pesticide Use Database 2002 (Fishel 2009; Gianessi and Reigner 2006). Pendimethalin is considered highly toxic to fish and slightly toxic to mammals (<http://pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/pendimethalin-ext.html#1>). A study with mice indicated an acute LD<sub>50</sub> of 3,189 mg/kg body weight (<http://sitem.herts.ac.uk/aeru/footprint/en/Reports/511.htm>).

### *NEONICOTINOIDS*

The neonicotinoid insecticides are becoming more widely used and comprised 24% of the global insecticide market in 2009 (Jeschke *et al.* 2011). The number 1 and 2 selling neonicotinoids in 2009 were imidacloprid and thiomethoxam (Jeschke *et al.* 2011). The neonicotinoids have been found to cause oxidative stress and neurological damage in rats and immune suppression in mice (Badgular *et al.* 2013; Duzguner and Edogaan 2010; Kimura-Kuroda *et al.* 2012). Due to information indicating that there is a link between neonicotinoids used in agriculture and a decline in bee numbers, the European Union proposed a two year ban on the use of thiamethoxam, imidacloprid and clothianidin on crops attractive to honeybees, beginning in

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<sup>2</sup> Atrazine in humans has a biological half-life of 10.8 – 11.2 hours (ATSDR 2003), a Log K<sub>ow</sub> of 2.75 and has a low bioaccumulation potential ([www.epa.gov/safewater/pdfs/factsheets/soc/tech/altrazine.pdf](http://www.epa.gov/safewater/pdfs/factsheets/soc/tech/altrazine.pdf)). Simazine has a Log K<sub>ow</sub> of 2.18 and a low bioaccumulation potential ([www.epa.gov/safewater/pdfs/factsheets/soc/tech/simazine.pdf](http://www.epa.gov/safewater/pdfs/factsheets/soc/tech/simazine.pdf)). Pendimethalin was considered by the USEPA to be persistent, bioaccumulative and toxic under Emergency Planning and Community Right to Know Act of 1986 [EPCRA] (U.S. EPA, 1997). It has a Log K<sub>ow</sub> of 5.18. ([oehha.ca.gov/multimedia/biomon/pdf/020910Pendimethalin.pdf](http://oehha.ca.gov/multimedia/biomon/pdf/020910Pendimethalin.pdf)).

December of 2013. <http://www.lawbc.com/regulatory-developments/entry/proposal-for-restriction-of-neonicotinoid-products-in-the-eu/>

A summary of acute mammalian toxicity (as represented by rat LD<sub>50</sub>s) and lipid solubility (as represented by the Log K<sub>ow</sub>) for many commonly used pesticides is presented in Table 3.

**Table 3. Rat Acute Toxicity (LD<sub>50</sub>) Values and Octanol-Water Partition Coefficients (Log K<sub>ow</sub>) for Selected Chemicals of Concern**

Compound	Chemical Class	Rat Oral LD <sub>50</sub> * (mg/kg body weight)	Log K <sub>ow</sub> @
Parathion	Organophosphate	2 – 30	3.8
Methyl Hg	Organo-metallic	29.9	1.7 – 2.5
Dieldrin	Organochlorine	37	5.4
Bifenthrin	Pyrethroid	50 – 70	> 6
Dichlorvos	Organophosphate	70.4 – 250	1.47
Chlorpyrifos	Organophosphate	95 – 270	4.92
DDT	Organochlorine	113 – 800	5.7
Cypermethrin	Pyrethroid	150 – 500	6.6
2,4-D	Chlorphenoxy	375 – 666	2.01
Imidacloprid	Neonicotinoid	424	0.57
Penta-PBDE	Polybrominated diether	500 – 5,000	6.57
Pendimethalin	Dinitroaniline	1,050 – 5,000	5.18
Thiamethoxam	Neonicotinoid	1,563	-0.13
Atrazine	Triazine	3,090	2.3
Dichlobenil	Substituted Benzene	3,160	2.74
HCB	Organochlorine	3,500	5.89
Pentachloronitrobenzene	Substituted Benzene	5,000	4.22
Dalapon	Unclassified Herbicide	7,570 – 9,330	0.78

Red – highly toxic; orange – moderately toxic; yellow – slightly toxic (USEPA Toxicity Classes)

\* LD<sub>50</sub> data from the following sources:

- [Exttoxnet pesticide profiles \(extoxnet.orst.edu/pips/\);](http://extoxnet.orst.edu/pips/)
- [edis.ifas.ufl.edu/pdf/PI/PI09800.pdf;](http://edis.ifas.ufl.edu/pdf/PI/PI09800.pdf)
- [http://www.esd.worldbank.org/popstoolkit/POPsToolkit/POPSTOOLKIT\\_COM/ABOUT/CHEMICAL/DIELDRIN.HTM](http://www.esd.worldbank.org/popstoolkit/POPsToolkit/POPSTOOLKIT_COM/ABOUT/CHEMICAL/DIELDRIN.HTM)
- [www.ncbi.nlm.nih.gov/pubmed/15369322](http://www.ncbi.nlm.nih.gov/pubmed/15369322)
- [www.vce.org/Hg/methyl\\_Hg.pdf](http://www.vce.org/Hg/methyl_Hg.pdf)

@ Log K<sub>ow</sub> data from the following sources:

- [www.cerij.or.jp/ceri\\_en/hazard\\_assessment\\_report/pdf/en\\_62\\_73\\_7.pdf](http://www.cerij.or.jp/ceri_en/hazard_assessment_report/pdf/en_62_73_7.pdf)
- [pmep.cce.cornell.edu/profiles/extoxnet/](http://pmep.cce.cornell.edu/profiles/extoxnet/)
- [water.epa.gov/.../Archived-Technical-Fact-Sheet-on-Hexachlorobenzene.pdf](http://water.epa.gov/.../Archived-Technical-Fact-Sheet-on-Hexachlorobenzene.pdf)
- [foehha.ca.gov/multimedia/biomon/pdf/020910Pendimethalin.pdf](http://foehha.ca.gov/multimedia/biomon/pdf/020910Pendimethalin.pdf)
- [circa.europa.eu/Public/irc/env/bio.../library?l=.../thiamethoxam](http://circa.europa.eu/Public/irc/env/bio.../library?l=.../thiamethoxam)
- [www.epa.gov/oppsrrd1/REDS/pcnb\\_red.pdf](http://www.epa.gov/oppsrrd1/REDS/pcnb_red.pdf)
- [www.efsa.europa.eu/en/efsajournal/doc/654.pdf](http://www.efsa.europa.eu/en/efsajournal/doc/654.pdf)
- [www.rivm.nl/bibliotheek/rapporten/679102050.pdf](http://www.rivm.nl/bibliotheek/rapporten/679102050.pdf)
- <http://www.atsdr.cdc.gov/toxprofiles/tp155-c4.pdf>
- <http://www.cdpr.ca.gov/docs/emon/pubs/ehapreps/eh9403.pdf>
- <http://publications.gc.ca/site/archivee-archived.html?url=http://publications.gc.ca/collections/Collection/En1-34-2-2002E.pdf>

@ The K<sub>ow</sub> represents the lipophilicity and hydrophobicity of a chemical and how it thermodynamically distributes between aqueous and organic phases. It is considered a reasonable surrogate for lipids in biological organisms and therefore is a measure of bioaccumulation potential (Mackay 1982).

### IIIC. CHEMICAL ANALYTICAL RESULTS

#### *ORGANOCHLORINE PESTICIDES/PCBS*

Twelve bat carcasses from New York State were analyzed for OC pesticides, PCBs and PCTs (Table 4; Figure 3). For the most part, these compounds were not detected in bat carcasses in excess of the reporting limit. Metabolites of DDT (particularly dichlorodiphenyldichloroethylene (p,p'-DDE) were detected at generally low concentrations, with a bat from Ulster County, New York having a p,p'-DDE concentration of 2.4 parts per million (mg/kg) wet weight (ww). Dieldrin was detected at low concentrations in most bats sampled. The maximum dieldrin concentration of 0.18 mg/kg (ww) was detected in a bat from Onondaga County, New York.

Analyte	Reporting Limit (RL) (mg/kg wet weight, ww)	Mean Conc (mg/kg, ww)	Max Conc (mg/kg ww)
HCB	0.002	0.003 (6 detects)	0.004
PCB Total	0.01	ND	
PCT Total	0.01	ND	
Beta BHC	0.002	0.009 (2 detects)	0.011
Diazinon	0.02	ND	
Dieldrin	0.002	0.027 (11 detects)	0.18
Heptachlor epoxide	0.002	0.006 (1 detect)	0.006
Oxychlordane	0.002	0.016 (11 detects)	0.061
p,p'-DDD	0.002	0.014 (6 detects)	0.049
p,p'-DDE	0.002	0.333 (12 detects)	2.4
p,p'-DDT	0.002	0.024 (6 detects)	0.12
Alpha BHC	0.002	ND	
Alpha chlordane	0.002	ND	
Cis-nonachlor	0.002	ND	
Delta-BHC	0.002	ND	
Endrin	0.002	ND	
Gamma-BHC	0.002	ND	
Gamma chlordane	0.002	ND	
Mirex	0.002	ND	
o,p'-DDD	0.002	ND	
o,p'-DDE	0.002	ND	
o,p'-DDT	0.002	0.003 (1 detect)	0.003
Toxaphene	0.05	ND	
Trans-nonachlor	0.002	ND	

## PBDEs

Polybrominated biphenyl ethers were analyzed in 12 bat carcasses from New York and an additional ten bat carcasses from New York, Massachusetts, Pennsylvania, New Hampshire and Vermont (Table 5). Total PBDE concentrations ranged from the 0.001 – 0.002 mg/kg detection limit to 8.85 mg/kg (ww) in a bat from Massachusetts. The overall mean concentration of PBDEs was 0.59 mg/kg (ww). Further discussion of the PBDE data is found in Secord et al. (2015).

**Table 5. Polybrominated Diphenyl Ether (PBDE) Concentrations in Bat Carcasses from New York and other Northeastern States.**

Bat ID	PBDE Congener Concentration (mg/kg wet weight) (Reporting Limit of 0.001 – 0.002 mg/kg ww)						
	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154	Total PBDE
ON101	0.005	0.039	0.024	0.006	0.005	0.002	0.081
ON102	0.003	0.22	0.18	0.024	0.018	0.006	0.451
ON103	ND	0.015	0.15	0.004	0.005	0.001	0.175
ON104	0.002	0.01	0.005	0.003	0.001	ND	0.021
ON6710	ND	0.004	0.005	ND	0.001	ND	0.010
ON7103	ND	0.048	0.38	0.004	0.006	0.002	0.440
ON7435	ND	0.006	0.004	0.001	ND	ND	0.011
ON7552	ND	0.007	0.006	ND	0.001	ND	0.014
ON7565	0.002	0.007	0.004	0.001	ND	ND	0.014
UL758	0.002	0.017	0.011	0.002	0.002	ND	0.034
UL759	ND	0.028	0.01	0.003	0.001	ND	0.042
UL829	0.002	0.077	0.61	0.15	0.004	0.002	0.845
NH04B	ND	0.267	0.207	0.07	0.67	0.027	1.241
NH03B	ND	0.003	ND	0.002	ND	ND	0.005
MA01B	0.231	6.03	1.4	0.624	0.314	0.251	8.850
MA02B	0.005	0.05	0.039	0.009	0.029	ND	0.132
NY17B	0.003	0.086	0.062	0.004	0.03	0.005	0.190
NY20B	ND	0.014	0.01	ND	0.026	ND	0.05
PA05B	ND	0.028	0.029	0.003	0.024	0.009	0.093
PA07B	ND	0.018	0.014	0.006	ND	ND	0.038
PA08B	0.003	0.036	0.024	0.007	ND	ND	0.07
VT02B	ND	0.038	0.03	0.005	ND	ND	0.073

“ON” (Onondaga County) and “UL” (Ulster County ) bats are from New York State.

## ORGANOPHOSPHATES AND CARBAMATES

For this study, we evaluated 10 carbamate compounds in 13 bats from Onondaga and Ulster Counties, New York. No carbamate compounds were detected at a detection limit of 0.01 mg/kg. Organophosphates were evaluated in 30 bats from Onondaga, Ulster, Essex and Warren Counties, New York. No OPs were detected in 29 of these bats at detection limits of 0.02 to 0.1 mg/kg. A bat that was collected dead at a warehouse in the Syracuse, New York area did have concentrations of ethyl p-nitrophenyl thionobenzenephosphonate (EPN), chlorpyrifos, dichlorvos, parathion and mevinphos in its tissues at concentrations ranging from 0.04 – 0.458

mg/kg ww (Table 6). The brain tissue from this bat was too desiccated to allow evaluation for cholinesterase.

<b>Table 6. Organophosphate compounds in One Bat from Syracuse, NY (March 2011)</b>	
Compound	Concentration (mg/kg ww) (DL = 0.01 mg/kg)
EPN	0.12
Chlorpyrifos	0.196
Coumaphos	<0.01
Diazinon	<0.01
Dichlorvos	0.458
Dimethoate	<0.01
Malathion	<0.01
Methyl Parathion	<0.01
Mevinphos	0.04
Parathion	0.277
Phorate	<0.01
Terbufos	<0.01

#### *BRAIN CHOLINESTERASE (ChE)*

Brain ChE was measured in 19 bats (Table 7). The mean ChE concentration was 9.1  $\mu\text{mol/g/min}$ , with a standard deviation of 5.1  $\mu\text{mol/g/min}$ . The brain ChE value for bat MA01A was rejected as it is outside of the range that would be considered possible for bat brain (Sparks, personal communication, 2014). We are assuming that this result may have been a laboratory computational error. The laboratory control tissue was bovine brain tissue, with control ChE concentrations measured between 2.23 and 3.24  $\mu\text{mol/g/min}$ . We do not consider bovine brain tissue to be a suitable control tissue for evaluating bat brain ChE since ChE concentrations from bat brains typically exceed the ChE concentrations in bovine brain. See Discussion Section of this report.

**Table 7. Brain Cholinesterase in Bats Collected at Sites in the Northeastern United States (2008 – 2010)**

Bat ID	Species	Mass of Bat (gms)	Cholinesterase (conc in $\mu\text{mol/g/min}$ )	Collection Location	Collection Date
PA04A (juvenile)	EPFU	13.1	11.3	Crawford County, PA	July 2009
PA06A	EPFU	10.8	7.86	Lewisburg, PA	3/25/09
PA09A	MYLU	7.72	11.2	Schuykill, PA	2/09/09
PA12A (juvenile)	EPFU	12.6	0.44	Bucks County, PA	6/27/09
MA01A*	MYLU	1.82	68.4	Chester, MA	4/02/08
MA02A	MYLU	3.83	13.9	Sheffield, PA	2/09/08
MA03A	EPFU	4.66	8.88	Chester, MA	7/16/09
NH02A	MYLU	2.31	21.7	Unity, NH	4/03/09
NH03A	EPFU	5.78	7.02	Effingham, NH	9/11/08
NH04A (juvenile)	EPFU	4.3	7.37	Dunbarton, NH	6/25/09
NH05A	EPFU	7.02	2.79	Plymouth, NH	4/23/08
VT01A	MYLU	5.11	9.66	Plymouth, VT	2/02/10
VT02A	MYLU	2.77	11.6	Plymouth, VT	1/20/10
VT03A	MYLU	3.12	15.3	Johnson, VT	Jan-Feb/10
NY03A	MYLU	11.9	1.17	Arnot Forest, NY	8/30/08
NY16A	MYLU	8.83	10.3	Jamesville, NY	3/19/09
NY17A (juvenile)	EPFU	6.7	4.84	Pine City, NY	7/10/09
NY18A	MYLU	7.23	9.84	Barton, NY	2/10/09
NY20A	MYLU	3.61	8.5	Hooper, NY	4/1/10

All bats were adults unless otherwise noted

\* Data rejected as outside typical limits for brain cholinesterase in bats

MYLU = *Myotis lucifugus*; EPFU = *Eptesicus fuscus*

*CHLORPHENOXY HERBICIDES (INCLUDES CHLORPHENOXY ACIDS/ESTERS, PYRIDINE CARBOXYLIC HERBICIDES AND DALAPON)*

The compounds chlorpyralid and dalapon were the only chlorphenoxy-type compounds detected in bat tissue (see Table 8). Picloram, 2,4-D, DCPA and MCPP were not detected at a detection limit of

0.01 mg/kg ww. Chlorpyralid is a pyridine carboxylic herbicide commonly used as a residential lawn weed killer. Dalapon is an organochlorine in no specific chemical class. It is used to kill vegetation such as grasses and cattails (<http://extoxnet.orst.edu/pips/dalapon.htm>).

Bat ID	County/ Date Collected	Chlorpyralid Concentration (mg/kg ww)	Dalapon Concentration (mg/kg ww)
BR100	Warren/Feb 2009	ND	0.098
BR101*	Warren/Feb 2009	0.012	0.01
BR102	Warren/Feb 2009	0.01	ND
BR103*	Warren/Feb 2009	0.031	ND
BR104	Warren/Feb 2009	ND	ND
AR100	Tompkins/July 2008	0.23	0.027
ON5257	Onondaga/July 2008	0.011	0.011
ON6706	Onondaga/August 2008	0.021	0.034
ON7432	Onondaga/August 2008	0.013	ND
ON7433	Onondaga/August 2008	ND	ND
ON7436	Onondaga/Sept 2008	ND	ND
ES761	Essex/2009	ND	ND
ES1053	Essex/2009	0.11	0.08
ES1300	Essex/2009	ND	ND
UL740	Ulster/2009	ND	ND
UL1103	Ulster/2009	ND	ND
* <i>Myotis sodalis</i> ; all other bats <i>M. lucifugus</i>			

#### *PYRETHROIDS*

No pyrethroids were detected in sixteen bats from New York (detection limit of 0.02 mg/kg).

#### *SUBSTITUTED BENZENES*

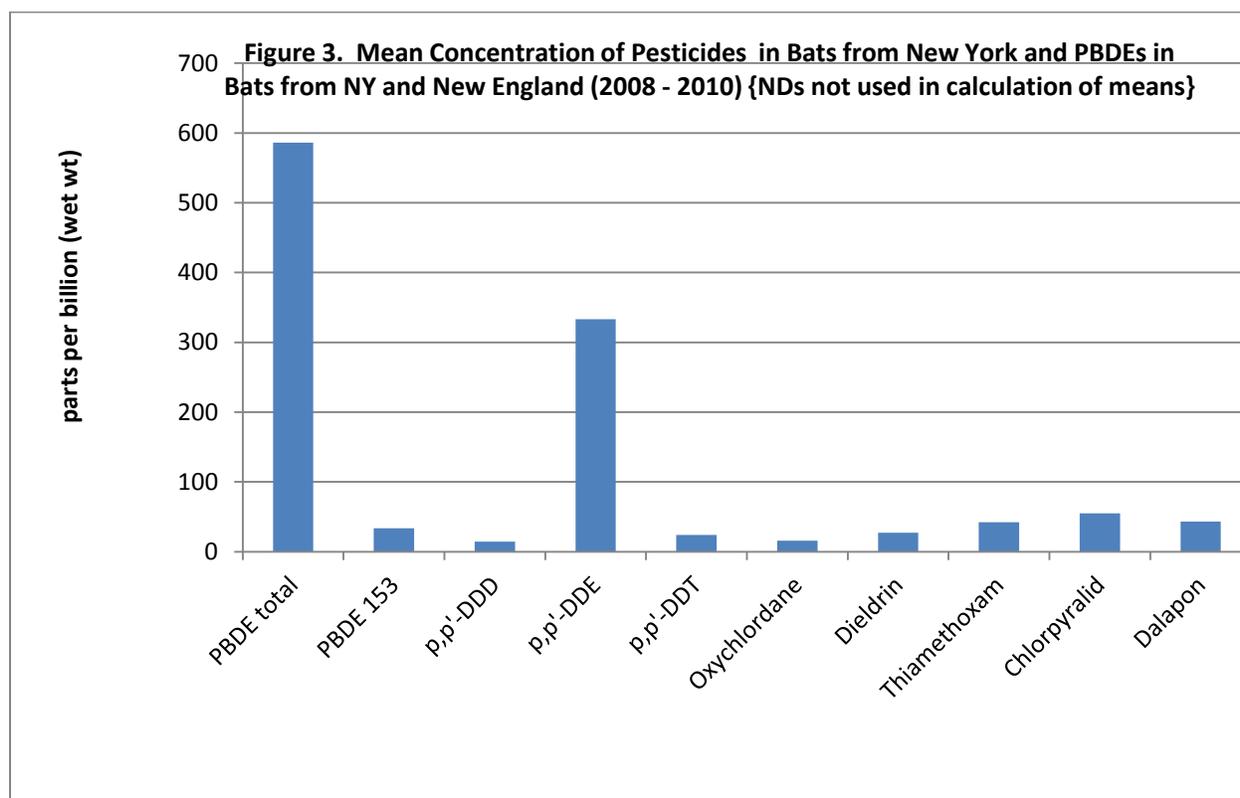
Ten bats from New York, Massachusetts, Pennsylvania, Vermont and New Hampshire were tested for four substituted benzene compounds: chloroneb, dichlorobenil, dicloran, and pentachloronitrobenzene. No substituted benzenes were detected at a detection limit of 0.002 mg/kg.

### TRIAZINES (SIMAZINE, ATRAZINE) & PENDIMETHALIN

None of these three chemicals was detected at detection limits of 0.02 and 0.05 mg/kg in any of the sixteen bat samples.

### NEONICOTINOIDS

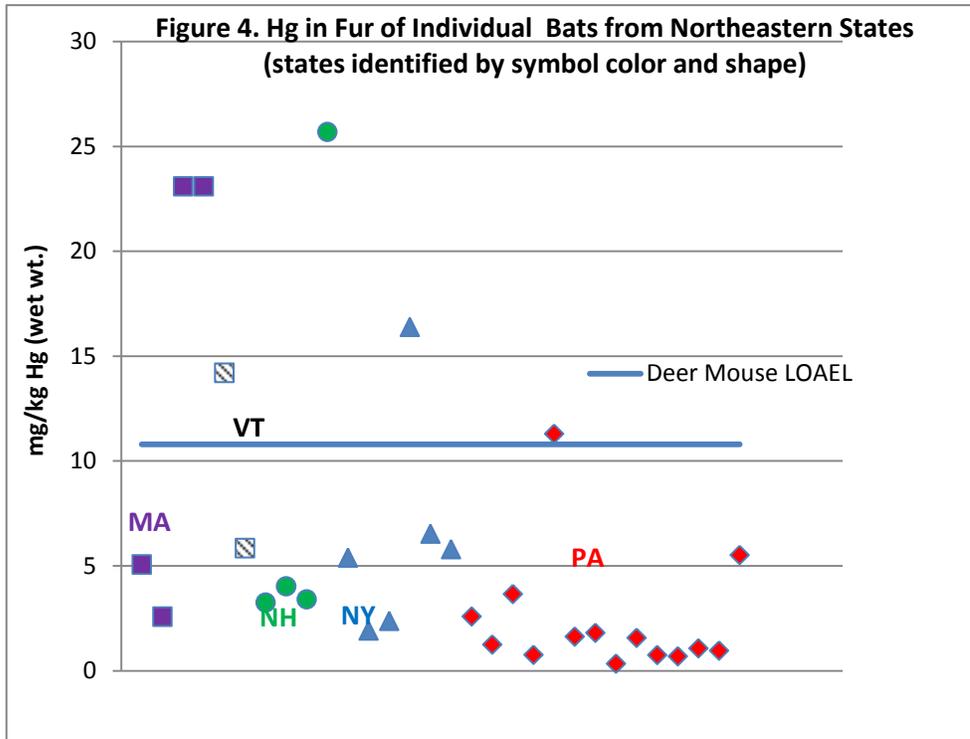
We tested 16 bats from five New York Counties for the neonicotinoid insecticides: imidacloprid, thiamethoxam, clothianidin, acetamiprid, imazapic and dithiopyr<sup>3</sup>. Thiamethoxam was detected in two bats from a hibernaculum in New York State at concentrations of 51 and 33 parts per billion (ng/g) ww. Both of these bats were collected alive, with obvious signs of WNS, in February of 2009. A summary of Pesticides and PBDEs detected in bat carcasses from this study is shown in Figure 3.



<sup>3</sup> An additional 10 bats from several states were analyzed for imidacloprid, thiamethoxam, clothianidin, acetamiprid, as well as thiacloprid. None of these compounds was detected, but the detection limits were very high at 2 and 6 mg/kg.

### Hg IN BAT FUR

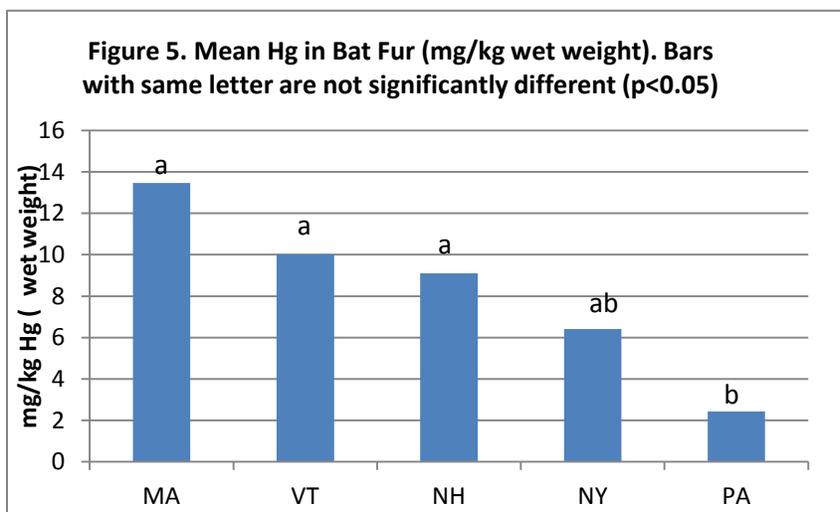
The Hg concentration in individual bat fur sampled as part of this study ranged from 0.34 to 25.7 mg/kg ww, with an overall mean concentration of 6.1 mg/kg ww (Figure 4; Table 9). There were statistically significant differences in bat fur Hg concentrations between states, with bats from PA having significantly less Hg in fur than bats from VT, NH or MA ( $p < 0.05$ ) (Figure 5). The mean Hg concentration in adults across all states (8.9 mg/kg ww) was significantly greater than the mean Hg concentration in juveniles (2.3 mg/kg ww) ( $p = 0.004$ ).



**Table 9. Mean Hg Concentration in Fur of Bats Collected from Northeastern States**

State	Mean Hg Concentration (mg/kg ww)	Sample Size	Species
Pennsylvania	2.4	14	3 MYLU, 11 EPFU
New York	6.4	6	5 MYLU, 1 EPFU
New Hampshire	9.1	4	2 MYLU, 2 EPFU
Vermont	10.0	2	2 MYLU
Massachusetts	13.5	4	2 MYLU, 2 EPFU

MYLU = *Myotis lucifugus*; EPFU = *Eptesicus fuscus*



#### *EMERGING CONTAMINANTS IN INSECT SAMPLES*

Three samples of insects were analyzed for emerging contaminants. A much smaller number of analytes was detected at greater than the reporting limit in insects than in bat samples (Table 10).

**Table 10. Concentration of Emerging Contaminants in Insects (2010/2011) from Three Locations in the United States (all concentrations ng/g wet weight)**

Compound > LOD (Limit of Detection)	Texas Mosquitoes	Las Vegas, NV & Sacramento, CA Mosquitoes	NE-Bug 1 (VT- mostly moths)
Cotinine	19.7		
Caffeine		29.9	42.5
Sulfamerazine		71.7	51.2
Trimethoprim		137	20.4
Pentoxifylline		5.5	
Sulfamethoxazole	16.9		
Flumequine	2		
Salicylic acid	250	245	689
Bisphenol A			128
DEET	24.8		632
Warfarin			54.2

## *DATA QUALITY*

Since bat carcasses were collected opportunistically, the time between time of death and collection and preservation via freezing is not always known. There may have been degradation of contaminants or moisture/lipid loss that may have influenced concentrations of analytes.

## IV. DISCUSSION

### IVA. PESTICIDE THREATS TO BATS

The U.S. has changed its pesticide use patterns over the last 50 years, eliminating the notoriously toxic and persistent organochlorine pesticides, such as DDT and chlordane. However, as discussed below and summarized in Table 3, newer classes of pesticides may be equally hazardous to mammals and persistent in biological tissue. Our evaluation of recent pesticide use in New York and California, as well as other information sources, reveals that pesticide usage across the United States is recently dominated by classes of pesticides such as the dinitroanilines, neonicotinoids, pyrethroids, chlorophenoxy compounds, OPs, carbamates, substituted benzenes, triazines and glyphosate.

These pesticides vary in their toxicity and persistence. One relative measure of mammalian toxicity can be expressed as the rat oral LD<sub>50</sub><sup>4</sup>. As can be seen from Table 3, some OP and pyrethroid insecticides that are currently registered for use in the U.S. have rat oral LD<sub>50</sub>s that are in the USEPA “moderate toxicity” class. Some of the pyrethroid insecticides and even the herbicide, pendimethalin, also have Log K<sub>ow</sub>s (measure of bioaccumulation potential) that are greater than 3, indicating that they may bioaccumulate in organisms.

Pesticides may also pose chronic toxicity or indirect lethality by impacting reproduction, behavior, and immune function. Pesticides such as such as atrazine, glyphosate, pyrethroids, carbamates, OPs, and dinitroanilines are believed to cause endocrine disruption (TEDX 2011).

### IVB. CHEMICAL ANALYSIS OF BATS

#### *ORGANOCHLORINE PESTICIDES/PCBS*

The only notable detections of organochlorine pesticides/PCBs in bat carcasses were fairly low concentrations of DDT isomers, with a single bat from Ulster County, New York having a p,p'-DDE concentration of 2.4 mg/kg ww (Figure 3). This concentration is at the lower end of DDE concentrations in bats from around the world reported in Table 5.2 of Clark and Shore (2001)<sup>5</sup>. Dieldrin was detected at low concentrations in most bats sampled. The maximum dieldrin concentration of 0.18 mg/kg ww was detected in a bat from Onondaga County, New York. This is an order of magnitude less than dieldrin concentrations reported in Clark and Shore (2001).

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<sup>4</sup> LD50s may not appropriately estimate toxicity of lipophilic compounds in that LD50s will be low when fat reserves are low and high when fat reserves are high (Clark and Shore 2001)

<sup>5</sup> Clark and Shore (2001) summarize DDE data from multiple bat species from studies published from the mid-1970s through the early 1990s. Mean DDE concentrations in bat carcasses ranged from 8.33 to 150 mg/kg ww, as reported in Table 5.2.

## ORGANOPHOSPHATES

Organophosphates have been reported in bat tissues from other studies. Sparks (2006) found OPs in three of nine bats collected by the Indiana Health Department (methyl parathion at 0.015 mg/kg; chlorpyrifos at 0.18 mg/kg; diazinon at 0.034 mg/kg). Sparks (2006) also reported chlorpyrifos at concentrations ranging from 0.0008 to 0.0042 mg/kg (ww) in six Indiana bats from Rays Cave and Wyandote Cave, Indiana ; dichlorvos was detected in bat guano from Coon and Grotto Caves (Indiana) at concentrations of 0.0086 and 0.011 mg/kg (ww).

Whereas the data from Sparks (2006) may indicate a broad environmental exposure to OPs and a persistence of low concentrations of these compounds, our data (Table 6) likely indicate acute exposure to OPs, potentially used as an insecticide at the warehouse where the bat was found dead. The concentrations we report (with the exception of chlorpyrifos) are about an order of magnitude greater than those reported by Sparks (2006).

It is interesting that the OP parathion was detected in the single bat from Syracuse. Parathion is no longer registered by USEPA for any use (USEPA 2013). It is one of the most toxic OP compounds, with a rat oral LD<sub>50</sub> of 2 - 30 mg/kg body weight (compared to 95 – 270 mg/kg body wt for chlorpyrifos and 70 - 250 mg/kg body wt for dichlorvos ) (<http://edis.ifas.ufl.edu/pi087>; <http://extoxnet.orst.edu/pips/parathio.htm>; [extoxnet.orst.edu/pips/dichlorv.htm](http://extoxnet.orst.edu/pips/dichlorv.htm)).

The bats from this study in which no OPs were detected may indicate no significant exposure to these compounds or no significant retention in bat tissues. Most of these bats had likely died from WNS and may have had characteristically low fat reserves – the primary reservoir of persistent OPs.

A larger issue concerning the potential health of bats is that these chemicals not only continue to be used, but at least in California, there was an increased use from 2009 – 2011, particularly of the pesticides chlorpyrifos and malathion (<http://www.cdpr.ca.gov/docs/pur/pur11rep/11sum.htm#pestuse>). Chlorpyrifos has a fairly low rat oral LD<sub>50</sub> and is reasonably lipid soluble, based on the Log K<sub>ow</sub> of 4.92 (Table 3), suggesting that it has the potential to be acutely toxic to mammals and may be persistent in fat tissue. The OPs cause acute effects such as respiratory failure and asphyxia (Wilkinson 1976), and may also induce hypothermia in birds and mammals and impact endocrine, immune and reproductive function (Grue *et al.* 1997). Disruption of these physiological functions in bats could have serious consequences, given the unique physiology of a hibernating mammal with a low reproductive rate. Animal studies have shown that there may be additive effects when OPs are combined with other pesticides, including herbicides, carbamates and pyrethroids (Costa and Murphy 1983; Ahmad 2007; Trimble and Lydy 2006).

## CHOLINESTERASE INHIBITION

A discussion of the brain ChE data must be prefaced with an understanding that the time of death in relation to the collection and freezing of these bat carcasses is largely unknown. There is the potential that ChE re-activation or degradation may have impacted the detected concentrations of brain ChE. The range of brain ChE concentrations in these bats was 0.44 – 21.7 µmol/g/min, with a mean brain ChE concentration of 9.1 µmol/g/min (Table 7). The mean ChE concentration

was 11.3  $\mu\text{mol/g/min}$  for MYLU and 6.3  $\mu\text{mol/g/min}$  for EPFU. Eidels *et al.* (2014-in prep; Sparks, personal communication, 2014) found that brain ChE in wild-caught reference EPFU was 10.6  $\mu\text{mol/g/min}$  (n=11). In that same Eidels *et al.* study, EPFU surviving the highest single dose of chlorpyrifos (60  $\mu\text{g/g}$ ) had a brain ChE of 6.2  $\mu\text{mol/g/min}$ .

Clark and Rattner (1987) reported brain ChE in little brown bats (MYLU) exposed to various concentrations of the OP orthene in the range of 3.0 – 10.1  $\mu\text{mol/g/min}$ . These authors used 50% of the mean or one standard deviation less than the mean as an indication of cholinesterase inhibition (as has been done in other studies). Due to the variability in the carcass collection for this study, we opt for a more conservative approach to suggest ChE inhibition. We found that two bats in particular, NY03A (MYLU) and PA12A (EPFU), had brain ChE concentrations that were over 90% lower than other conspecific bats sampled (1.17 and 0.44 micromol/g/min, respectively – see Table 7). These bats were of reasonable body mass (suggesting they were not desiccated specimens) and were collected during the summer months, when there is the potential for active use of ChE-inhibiting compounds. These bats were not analyzed for organophosphates or carbamates.

#### *PYRETHROIDS*

No pyrethroids were detected in bats as part of this study. Although pyrethroids were not detected in this analysis, we consider this class of pesticides a possible threat to bats due to their high usage, as well as the high mammalian acute toxicity and persistence of the newer pyrethroids such as esfenvalerate, deltamethrin, bifenthrin, tefluthrin, flucythrinate, cyhalothrin and fenpropathrin. Pyrethroids have been detected in bat carcasses and guano in other studies (Clark and Shore 2001).

#### *CHLORPHENOXY-TYPE COMPOUNDS AND DALAPON*

Chlorpyralid and dalapon were detected in bat carcasses. Little is known of their toxicity to bats, but dalapon acute toxicity in rats and bioaccumulative potential are low (Table 3).

#### *TRIAZINES (SIMAZINE, ATRAZINE) & PENDIMETHALIN*

None of these commonly used herbicides and fungicides was detected in bat carcasses. The USEPA is evaluating the environmental fate and ecological risk associated with atrazine as part of their registration review for this very commonly used herbicide that has been shown to affect endocrine and reproduction systems ([http://www.epa.gov/oppsrrd1/reregistration/atrazine/atrazine\\_update.htm](http://www.epa.gov/oppsrrd1/reregistration/atrazine/atrazine_update.htm)).

It may be prudent to consider monitoring biological tissues for dinitroaniline herbicides (specifically pendimethalin). Pendimethalin is a commonly used herbicide with a high Log  $K_{ow}$  (5.18), suggesting it may be persistent in biological tissues. It has also been classified as a possible human carcinogen by the USEPA based on increases in liver tumors in rats (USEPA 1997), and was found to have estrogenic and anti-androgenic activity in *in vitro* reporter gene assays in Chinese hamster ovary cells (Kojima *et al.* 2004).

#### *NEONICOTINOIDS*

The neonicotinoid, thiomethoxam, was detected in two bat carcasses. Both bats were *Myotis sodalis* collected in February, 2009 from a hibernaculum in New York . Thiomethoxam is used for seed treatment or as a foliar or ground spray insecticide for a variety of crops, including tomatoes, strawberries, corn and stone fruits. Like other neonicotinoid compounds, it translocates throughout the plant structure and is found in all plant parts, including nectar and pollen (Stoner and Eitzer 2012). The neonicotinoid insecticides are becoming more widely used and comprised 24% of the global insecticide market in 2009 (Jeschke *et al.* 2011). Thiomethoxam was the second largest selling neonicotinoid globally in 2009 (Jeschke *et al.* 2011).

The neonicotinoids have been found to cause oxidative stress and neurological damage in rats and immune suppression in mice (Badgular *et al.* 2013; Duzguner and Edogaan 2010; Kimura-Kuroda *et al.* 2012). Due to information indicating that there is a link between neonicotinoids used in agriculture and a decline in bee numbers, the European Union proposed a two year ban on the use of thiamethoxam, imidacloprid and clothianidin on crops attractive to honeybees, beginning in December of 2013. <http://www.lawbc.com/regulatory-developments/entry/proposal-for-restriction-of-neonicotinoid-products-in-the-eu/>

We were unable to find information in the literature on concentrations of neonicotinoids in tissues of wild mammals exposed to these chemicals. The presence of thiamethoxam in bat tissues in mid-winter, months after likely dietary exposure, suggests that this chemical can be persistent in bat tissues, despite its low Log  $K_{ow}$  (Table 3).

The LD<sub>50</sub> in rats varies from 140 mg/kg body weight (bw) for acetamiprid to 5,000 mg/kg bw for clothianidin, indicating the potential for variable acute toxicity among neonicotinoid pesticides (Legocki and Polec 2008, as cited in Goulson 2013).

Mason *et al.* (2013) have noted that WNS is one of a number of wildlife diseases that have appeared since the advent of neonicotinoid use in the 1990s. They have hypothesized that neonicotinoids may have contributed to abnormal behavior that has been described in bats with WNS. This behavior includes flying outside during the day in temperatures at or below freezing and clustering near the entrance to hibernacula ([www.fws.gov/northeast/pdf/white-nosefaqs.pdf](http://www.fws.gov/northeast/pdf/white-nosefaqs.pdf) ). Our data do not appear to point to neonicotinoids as a significant contributor to WNS.

## *Hg*

The Hg concentrations in bat fur sampled as part of this study ranged from 0.34 to 25.7 mg/kg ww, with an overall mean concentration of 6.1 mg/kg ww (Figures 4 & 5).

There have been a number of investigations of Hg exposure in bats. Osborne *et al.* (2011) summarized data on Hg concentrations in bats sampled at 44 sites across New England and the mid-Atlantic states (including New York). Adult fur Hg concentrations ranged from 0.69 mg/kg ww in a red bat from the Monongahela National Forest in West Virginia to 120.31 mg/kg ww in a big brown bat from along the Little River in New Hampshire. The mean fur Hg concentration for all bats sampled as part of the Osborne *et al.* (2011) study was approximately 7 mg/kg ww for females (n=389) and 10 mg/kg ww (n=213) for males (see Figure 44 of Osborne *et al.* 2011).

Massa and Grippo (2000) examined various Chiroptera species from rivers in Arkansas that were under fish consumption advisories for Hg and found fur Hg levels ranging from 1 to 30 µg/g (fresh wet weight - fw). They also found Hg was elevated in bat muscle, kidney, liver, and brain when compared to a reference site.

Adult bats sampled in the vicinity of Onondaga Lake, a Hg contaminated lake in central New York, had mean Hg concentrations in fur of 15.4 mg/kg ww, with Oneida Lake reference area adult bats having 8.7 mg/kg ww Hg in fur (Yates *et al.* 2012). Miura *et al.* (1978) examined various species of Chiroptera from areas in Japan sprayed with Hg fungicides. In 1965 and 1966, they measured total fur Hg in these bats and found mean Hg concentrations of 33.0 mg/kg (fw) and 33.7 mg/kg (fw), respectively. Wada *et al.* (2010) found that big brown bats at a Hg contaminated site in Virginia contained an average of 28 mg/kg ww Hg in fur.

Lowest observed adverse effect levels (LOAELs) have not been developed for bats; however some bats sampled for this study have concentrations of Hg in fur that exceed a Hg fur concentration (10.8 mg/kg) associated with adverse effects in deer mice (Burton *et al.* 1977). Six bat fur samples (2 MA, 1 NH, 1 NY, 1 PA 1 VT) had Hg concentrations in excess of a deer mouse fur LOAEL of 10.8 mg/kg ww. Three bats had fur Hg concentrations exceeding 20 mg/kg ww, typical of Hg-enriched areas. They were an adult male *Eptesicus fuscus* from Pittsfield, MA an adult female *Myotis lucifugus* from Hinsdale, MA and an adult female from Plymouth, NH. Nam *et al.* (2012) reported Hg in fur of bats from a Hg contaminated ecosystem at 132 mg/kg (much higher than detected in this study), compared with 3.09 mg/kg (fresh weight) in reference area bats. They documented evidence of Hg-associated neurochemical changes in bats with increasing Hg concentrations.

#### IVC. EMERGING CONTAMINANTS IN INSECTS

Our sample size for insects is too limited to allow for a detailed analysis. Of the compounds detected in bats, caffeine, salicylic acid, bisphenol A, DEET, warfarin and pentoxifylline were detected in at least one insect sample, with salicylic acid detected in all samples.

## V. CONCLUSIONS

Bats, like all wildlife, are exposed to a myriad of pesticides, many of which have the potential to adversely affect reproduction, immune and endocrine function, behavior and survival. Our study showed that few of these pesticides accumulated in the bats we tested. Our data may be limited because most of the bats had died as a result of WNS and consequently had low fat reserves, the reservoir of many of the contaminants we evaluated. We recommend that any future investigations evaluate chemical concentrations in bats with more typical fat reserves. Based on the chemical and toxicological characteristics of current-use pesticides, we suggest that particular attention be paid in future studies to the effects of OPs, carbamates, neonicotinoids and pyrethroids on bats. Our study also revealed that two herbicides, chlorpyralid and dalapon, accumulated in bat tissue, a reminder that herbicides should not be disregarded as potential toxicants to wildlife.

Hg in bat fur was greatest in New England bats and least in Pennsylvania bats. Three bats had Hg concentrations in fur ( $> 20$  mg/kg), similar to bat fur Hg concentrations detected in Hg enriched environments. Two out of 19 bats had brain ChE concentrations indicative of exposure to ChE-inhibiting substances, such as organophosphate or carbamate pesticides.

The possible implications of CECs, including pharmaceuticals, personal care products and PBDEs in bats, is discussed in Secord et al (2015).

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