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DDT poisoning of big brown bats, *Eptesicus fuscus*, in Hamilton, Montana

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HIGHLIGHTS

- Misuse of DDT on a rabies-negative bat colony.
- First reported DDT-related bat mortality in 40 years.
- Highest reported concentrations of DDT and metabolites for a bat mortality event.

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ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) is an insecticidal organochlorine pesticide with; known potential for neurotoxic effects in wildlife. The United States Environmental Protection Agency (US EPA) registration for this pesticide has been cancelled and there are currently no federally active products that contain this ingredient in the U.S. We present a case of a colony of big brown bats (*E. Fuscus*) found dead in the attic roost of an administrative building; in the city of Hamilton, Montana from unknown cause. DDT and its metabolites; dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) were detected in bat tissues by gas chromatography/mass spectrometry (GC-MS) and quantified by gas chromatography tandem quadrupole mass spectrometry (GC-MS/MS). Concentrations of 4081 ppm DDT and 890 ppm DDE wet weight were found in the brain of one bat and are the highest reported concentrations in such a mortality event to date. This case emphasizes the importance of testing wildlife mortalities against a comprehensive panel of toxicologic agents including persistent organic pollutants in the absence of other more common disease threats.

1. Introduction

In its role as an insecticide, DDT is readily absorbed through the exoskeleton of insects in which it acts to disrupt nerve impulse conduction in a two-fold manner: by preventing the closure of sodium channels and by disturbing the opening of potassium channels (Cheremisinoff and Rosenfeld, 2010). Its persistent interference with chemical neurotransmission is lethal. In mammals, DDT is not readily absorbed across the dermis (Smith, 2010); therefore, the primary routes of exposure are ingestion and inhalation. This compound is highly lipophilic and accordingly accumulates in adipose tissues of exposed animals (Smith, 2010). As with insects, DDT and its metabolites exert a neurotoxic effect in humans and other mammals. Symptoms include paresthesia of the mouth and tongue, hypersensitivity to stimuli, vertigo, tremor, and convulsions (Cheremisinoff and Rosenfeld, 2010).

Populations of bats across the United States have previously tested positive for residual environmental contaminants like DDT and other persistent organochlorine pollutants (POPs) (Clark, 1981; Henny et al., 1982; Hernández et al., 2006; Kannan et al., 2010; O'Shea et al., 2001; Reidinger Jr, 1976). However, with the exception of one study, most of these surveys were conducted over 3 decades ago and do not reflect the current state of the environment. Given the finding of trace levels of legacy POPs in bats, it is unsurprising that they have been speculated to render bats more

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susceptible to the acquisition of newly prevalent diseases such as white-nose syndrome, caused by the fungal pathogen *Pseudogymnoascus destructans*. (Kannan et al., 2010; Secord et al., 2015). No association between chemical burden and disease has been proven, though.

From a historical and regulatory perspective, it is important to note that the U.S. Department of Agriculture (USDA) cancelled registrations for many of the agricultural uses of DDT in the late 1960's. In response to increasing scientific evidence for its persistence in the environment, adverse effects on wildlife health, and potential for impact on human health, all remaining crop uses were cancelled by the U.S. Environmental Protection Agency (EPA) in 1972. Although cancelled, the EPA maintained authority to grant exemptions for its use by Federal or State agencies for emergency conditions concerning human health; exemption types are described in Table 1 (EPA, 2017). Interestingly, within the realm of disease management, bats roosting in buildings were a frequent target of exempted DDT use because of potential for human exposure to rabies (Table 2)("Library of Congress," no date). However, no exemptions for its use have been issued since 1979. As such, there have been no cases documenting lethal exposures in bats to DDT in the United States since its 1976 exempted use in New Hampshire (Clark et al., 1978).

Forty years later on July 15, 2016, approximately one dozen big brown bats were found dead in a Ravalli County Administration Building (Hamilton, MT) stairwell and adjacent attic roost. This was the second occurrence of multiple bat deaths in the vicinity of the same building that prompted an investigation into the root-cause. Two bat carcasses were submitted to the Montana Department of Livestock Diagnostic Laboratory in Bozeman, MT. The brains of both bats tested negative for rabies. In the absence of any infectious disease or notable pathology, the carcasses of three bats were submitted to the Michigan State University Veterinary Diagnostic Laboratory for toxicologic analysis by GC-MS screen.

2. Materials and methods

2.1. GC-MS identification

Livers were harvested from two bats by a board-certified pathologist for the purpose of chemical extraction and identification of a toxin of unknown origin.

The GC-MS method made use of a pooled tissue extract prepared in accordance with the following procedure. In duplicate, 3 g samples of pooled liver were combined with 2 mL of Milli-Q water in separate Precellys homogenizer tubes (Bertin Technologies, Redondo Beach, CA) and homogenized using a Precellys Evolution Homogenizer (Bertin Technologies, Redondo Beach, CA). The resulting homogenates were transferred to separate glass tubes with an additional 4 mL of Milli-Q water and 1 mL diphenylamine (Sigma Aldrich, St. Louis, MO) internal standard (2 ppm diphenylamine final concentration). To one tube, 6 mL acidic (pH = 3.0) glycine buffer (glycine, Sigma Aldrich, St. Louis, MO; sulfuric acid, JT Baker, Phillipsburg, NJ) was added and vortexed, and to the other tube 6 mL basic (pH = 10.0) glycine buffer (Sigma Aldrich, St. Louis, MO) was added and vortexed. To each tube, 12 mL acetonitrile (Honeywell, Burdick and Jackson, Muskegon, MI) and 5 g sodium chloride (VWR, Radnor, PA) were sequentially added and vortexed. Each tube was centrifuged in a Beckman GS-6 centrifuge (Beckman Coulter, Brea, CA) to separate the organic solvent phase (acetonitrile, top layer). The organic phase was transferred to a QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, **S**afe) clean up tube (2.0 mg magnesium sulfate, JT Baker, Phillipsburg, NJ; 300 mg octadecyl, Sigma Aldrich, St. Louis, MO; 300 mg bondesil, Agilent, Santa Clara, CA). The original extract tubes were washed with an additional 12 mL acetonitrile, processed in the same manner described, and combined with the first extracts in the QuEChERS tube. An aliquot of 20 μL dimethylformamide (Sigma Aldrich, St. Louis, MO) was added to each of the acidic and basic preparations. The solvent volumes were reduced under nitrogen gas to approximately 10 mL and placed in a freezer to precipitate excess fat. After 1 h, the samples were centrifuged and the solvent layers were filtered through Acrodisc filters (Pall Corporation, Port Washington, NY) into new tubes. Solvents were evaporated completely under nitrogen and the extracts were reconstituted in 120 μL acetonitrile. These extracts were transferred to autosampler vials for GC-MS analysis. For each of the acidic and basic extracts, 50 μL of the final extract were also combined with 25 μL BSTFA (N,O-Bis(-trimethylsilyl)trifluoroacetamide) +1% TMCS (Trimethylchlorosilane)(Sigma Aldrich, St. Louis, MO) for derivatization in separate autosampler vials. All four samples (acidic, acidic + derivative, basic, basic + derivative) were qualitatively analyzed on an Agilent Technologies 7890A GC system coupled with an Agilent 5975 Mass Spectrometry detector (Agilent, Santa Clara, CA) configured with a 30 m × 0.25 mm X 0.25 μm film thickness DB 5MS (Agilent, Santa Clara, CA) column in full-scan mode (m/z 40–700). Acquisition and data processing were performed with ChemStation software. The pooled sample was screened against an extensive mass spectral library and was confirmed positive for DDT, under both acidic and basic extraction conditions, based on this initial screen.

Table 2
Specific and crisis exemptions to use DDT for the control of rabid bats.

State	Year	Type	Federal Register
Massachusetts	1974	Specific	39 FR 24530
Massachusetts	1975	Crisis	40 FR 43758
New Jersey	1975	Crisis	40 FR 43759
New Hampshire	1976	Crisis	41 FR 816
Pennsylvania	1976	Crisis	41 FR 6122
Delaware	1976	Crisis	41 FR 7809
Wyoming	1976	Crisis	41 FR 50855
Ohio	1976	Crisis	41 FR 50053
Massachusetts	1977	Crisis	42 FR 2526
Texas	1977	Crisis	42 FR 2527
Texas	1979	Crisis	45 FR 3875

Source: Library of Congress.

Table 1
State and U.S. federal pesticide exemptions under 40 CFR Part 166.

Exemption Type	Description
Specific	Used to avert a significant economic loss or significant risk to an endangered species, threatened species, beneficial organisms, or the environment.
Quarantine	Used to control the introduction or spread of any pest new to or not previously known to be widely prevalent within the U.S.
Public Health	Used to control a pest that will cause significant risk to human health.
Crisis	When the time from discovery of an emergency to the time needed to allow for the authorizations of specific, quarantine, or public health exemptions by the EPA is insufficient.

Source: U.S. EPA. Pesticide Registration Manual, 2017.