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Effects of a gasoline spill on hibernating bats in the Sloans Valley cave system, Kentucky

By Julia Lankton, David Alvarez, Anne Ballmann, and Jo Ellen Hinck

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Effects of a gasoline spill on hibernating bats in the Sloans Valley cave system, Kentucky

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# Introduction

On January 30, 2014, a tanker truck rolled over and released approximately 7,750 gallons of unleaded gasoline in Sloans Valley, Kentucky, an area with extensive karst and caves. These caves are used as hibemacula by numerous species of bats, including the federally endangered Gray Bat (*Myotis grisescens*) and Indiana Bat (*M. sodalis*). One known hibemaculum in the area is located in Daniel Boone National Forect. The Sloans Valley karst system is believed to connect to the Cumberland River and Cumberland Lake (Simpson and Florea, 2009).

By February 28, 2014, approximately 450 gallons of gasoline were removed from the tanker, and approximately 1,377 gallons had been removed with sorbent pads and booms at two underflow dams set up in a dry creek bed. It was estimated that approximately 6,373 gallons were left in the environment. Daily monitoring of cave entrances and karst windows documented multiple locations with positive readings for volatile organics; some of these concentrations approached or exceeded the lower explosive limits, thus indicating that gasoline had traveled into the karst system. No additional removal or remedial efforts were undertaken, and soils and sediment remained saturated with gasoline.

The U.S. Fish and Wildlife Service, as a Natural Resource trustee and in cooperation with the responsible party, conducted bat surveys and tissue sampling of surrogate bats to address potential injury to federally-listed bats. Timing of the survey and tissue collection were critical because: 1) the bats were close to the period of emergence, when they come out of torpor (hibernation) and prepare for the maternity season; 2) bats that may have succumbed to potentially toxic effects of the gasoline could be rapidly consumed by cave invertebrates (crickets, spider, and beetles); and 3) the polycyclic aromatic hydrocarbons (PAHs) found in gasoline are quickly metabolized by many species, making a direct measurement of the parent compounds, and thus a link to potential injury difficult.

The U.S. Geological Survey (USGS) provided technical support to the Natural Resource Damage Assessment trustee to determine whether bats were exposed to gasoline from the tanker spill. The USGS National Wildlife Health Center (NWHC; Madison, Wisconsin) performed pathological and microbiological analyses on bats from reference and potentially impacted hibernacla within the spill area. The USGS Columbia Environmental Research Center (CERC; Columbia, Mssouri) analyzed gall bladders for PAH metabolites from all bats collected. Our findings are reported here.

# Methods

Sixty-three bats were collected from 10 sites from the Sloans Valley and Cave Creek reference cave systems in Kentucky in March 2014 (Figure 1). The presence and level of gasoline vapor (as volatile organic compounds in parts per million) were measured over the entrance of each cave (Shield Environmental Associates, 2014). Sites included nine Sloans Valley caves potentially contaminated by gasoline and one unexposed reference cave (South Goldson). Specimens included 31 Tri-colored Bats (*Perimyotis subflavus*), 31 Little Brown Bats (*Myotis lucifugus*), and 1 Rafinesque’s Big-Eared Bat (*Corynorhinus rafinesquii*). No specimens of either endangered bat species (gray bat, Indiana bat) were collected. Fifty-three bats (including four bats found dead) were collected from caves potentially exposed to gasoline, and 10 Little Brown Bats were collected from the reference site. Live bats were euthanized by cervical dislocation (Federal Collection Permit #TE070584-10; Stae Collection Permit #SC1411006) and carcasses (euthanized and those found dead) were shipped overnight on ice packs to NWHC. Carcasses were necropsied within 24 to 48 hours of receipt.

## Pathology and Microbiology

At necropsy, bats were weighed, sexed, and examined externally, including examination of the wing membrane and muzzle for evidence of white-nose syndrome (WNS; Appendix 1). Some bats were examined under ultraviolet light to better assess the presence of fungal lesions (see Turner and others 2014). Body condition and postmortem condition were also noted. A ventral midline incision from chin to pelvis was made and internal organs were grossly examined. Samples for histopathological analysis were excised and fixed in 10% neutral buffered formalin. Gall bladder with bile and portions of the liver, kidney, spleen, brain and lung were collected with acetone-rinsed instruments for possible contaminant analysis and saved frozen at -10 C degrees either in glass vials (similar tissues from same site pooled) or individually in foil. Gall bladders were shipped on ice packs for overnight delivery by FedEx to USGS Columbia Environmental Research Center (CERC) for analysis, and the remaining tissues were saved frozen at NWHC in the event that further testing was requested.

A wing swab from each bat was submitted to NWHC’s microbiology lab for PCR testing for *Pseudogymnoascus destructans*, the causative agent of WNS as described in Muller and others (2013). Histopathology was performed on 14 potentially exposed bats and 5 reference bats (Table 1). This subset of bats was selected to determine whether histopathological differences were present between bats with potential exposure to the spilled fuel and those from reference location. Bats were chosen for histopathology based on the presence and level of gasoline vapor measured over the entrance of each cave (Shield Environmental Associates, 2014) and whether they were found alive or dead. All bats found dead were histologically examined. Formalin-fixed tissue samples were routinely processed for histopathology, and were stained with hematoxylin and eosin to evaluate tissue architecture, cellular morphology, and the presence of lesions associated with petroleum vapor exposure ; skin sections were also stained with periodic acid-Schiff to assess fungal infection. Tissues examined histologically included skin from wing membrane, muzzle, flank and prepuce, a cross section of the head with nasal cavity, trachea, lung, brain, heart, liver, kidney, spleen, stomach, and intestinal tract. Due to the qualitative nature of histopathology and the relatively low numbers of animals examined microscopically in this case, statistical analysis of these data was not performed.

## Contaminant Analysis

Metabolism of many potentially toxic PAHs by vertebrates occurs in the liver in two phases. Phase I restuls in the formation of hydroxylated (OH-PAH) or epoxide derivatives of the parent compounds. In Phase II, these enzymatically oxidized products are converted to highly water soluble glurcoronide and sulfate conjugates, which facilitates excretion into the bile. The aim of analytical method is to free the water soluble PAH conjugates into an aqueous medium from the bile of the organism, form the free OH-PAHs through enzymatic hydrolysis, and recover the OH-PAH metabolites through extraction and enrichment procedures for quantification using high performance liquid chromatography (HPLC) with fluorescence detection (Gale and others, 2012).

Gall bladders from multiple individuals (up to 10) of the same species and collection site were combined into a single composite sample immediately following necropsy (Table 2). Combining samples was necessary to meet mass requirements of the analytical method because little to no bile was present in the gall bladders. A total of 13 composite samples were formed. Samples were frozen at -10 degrees and shipped to CERC as previously described. Upon arrival at CERC, all samples were stored frozen (-20 ºC) until analysis.

Small portions of liver were attached to all gall bladder samples. Individual gall bladders could not be discerned from the surrounding matrix; therefore, the entire sample was macerated and extracted to recover the PAH metabolites. Samples were extracted into an aqueous buffer solution, treated with β-glucuronidase (enzyme), and incubated at 37ºC for 3 hours. The OH-PAHs were then extracted from the aqueous buffer with ethyl acetate. Size exclusion chromatography was used to isolate the OH-PAHs from lipids and other biogenic compounds. A final enrichment/cleanup step was performed by passing the extracts through C18 solid-phase extraction cartridges to remove additional interfering chemicals from the samples. Analyses were performed using an Agilent 1100 HPLC with fluorescence detection. A series of quality control samples including procedural blanks, matrix blanks, matrix spikes, and a positive control (i.e., bile from environmentally exposed largemouth bass, *Micropterus salmoides*) were included with the analyses. Results for all quality control samples were within expected laboratory limits based on the method validation data (unpublished internal laboratory quality control data). Few detections of OH-PAHs were present in the procedural and matrix (black carp bile) blanks run concurrently with the gall bladder samples. The detections were very near the method detection limits ranging from 0.11 ng/mL for 2-hydroxychrysene to 0.71 ng/mL for 1-hydroxypyrene. Recoveries of the OH-PAHs spiked into the black carp bile ranged from 74 to 107% with an average of 96%. Recovery of naturally-incorporated conjugated PAHs, in the form of glucuronide and/or sulfate PAHs, in the bile of a largemouth bass used as a positive control ranged from 62 to 111% with an average of 94% (unpublished internal laboratory quality control data).

# Results and Discussion

## Pathology and Microbiology

Significant necropsy, histopathology and microbiology results are summarized in Table 2. Grossly, most of the bats in potentially exposed and reference groups had white particulate material on the face or wing membranes, evidence of injury to wing membranes, or both, which are suggestive of WNS. Eighteen of 53 (34%) potentially gasoline-exposed bats were considered to be in poor body condition with diminished fat stores, wheras 0 of 10 reference bats were in poor body condition. The postmortem condition of most bats was good to fair, with minimal to mild postmortem decomposition. There was moderate postmortem decomposition in 2 of 4 bats found dead.

In euthanized bats, blood within the mouth and respiratory tract, including nasal cavity and lungs, was a common finding due to the method of euthanasia. In some bats, hemorrhage associated with euthanasia may have interfered with the gross detection of other lesions within the respiratory tract. There were no other significant findings on necropsy.

Three bats from (one each of Tri-colored Bat, Little Brown Bat, and Rafinesque’s Big-Eared Bat) from Great Rock Sink West tested negative for *P. desctructans* through PCR analysis, whereas all remaining bats in both potentially exposed and reference groups were positive. All bats for which histopathology was performed had microscopic skin lesions characteristic of WNS. Because WNS can only be diagnosed by a combination of both *P. desctructans*-positive PCR and microscopic lesions, the bats for which histopathology was performed are designated as WNS-positive (19 bats), whereas those bats for which histopathology was not performed but which were *P. desctructans* PCR positive (that is, positive for the fungus that causes WNS) are designated as WNS-suspect (41 bats). Microscopic lesions of WNS are distinctive and are not considered confounding factors in the examination of bats for lesions caused by gasoline exposure.

Microscopically, all bats had variable presence of acute hemorrhage in the respiratory tract (euthanasia artifact). In some cases, severe hemorrhage obscured tissue architecture and interfered with full microscopic evaluation of the respiratory tract. Most potentially exposed bats examined histologically (12 of 14; 86%) had microscopic changes in the lungs characterized by increased numbers of pulmonary macrophages. Pulmonary macrophages, normally found in low numbers in the lung, are resident white blood cells that respond to the presence of edema fluid, congestion, hemorrhage, and/or tissue damage, and are involved in clearance of inhaled particulate material. Elevated numbers of pulmonary macrophages (designated as pulmonary histiocytosis) indicates a subacute to chronic inflammatory response. Pulmonary histiocytosis was not noted in the 5 reference bats examined.

Potentially exposed bats had an average of 33 macrophages/40x field (range 8-66/40x field), whereas reference bats averaged 11/40x field (range 4-17/40x field). Although the numbers of pulmonary macrophages in many potentially exposed bats were elevated compared to reference bats, a normal reference range for this measure has not been established for hibernating bats. Additionally, the part of lung (e.g. cranial vs caudal) sampled for histopathology was not standardized, so differnces due to the part of lung examined microscopically cannot be ruled out.

A few potentially exposed bats (3 of 14; 21%) had accumulated edema fluid within alveolar spaces of the lung. Several (8 of 14; 57%) had evidence of macrophages engulfing red blood cells, a common finding with chronic congestion. Because there were no other significant findings to account for the increased numbers of macrophages, edema and/or congestion are considered the most likely causes. Evidence of congestion was also noted in 1 of 5 (20%) reference bats. Low numbers of inflammatory cells surrounded pulmonary blood vessels in some potentially exposed bats (7 of 14; 50%); however, this change was also present in 4 of 5 (80%) reference bats and is considered a background lesion.

In general, the nasal mucosa was within normal limits in most specimens. A Little Brown Bat from Minton Hollow Cave had a mild acute rhinitis considered unlikely to be related to gasoline exposure, which would be expected to result in a more chronic lesion. Significant inflammation, necrosis, or fibrosis of the upper or lower respiratory tract was not seen in any bat. Other microscopic findings are considered incidental and include evidence of ectoparasite-associated damage to the skin (particularly the ears), superficial bacterial infections of the muzzle, mild gastroenteritis (likely endoparasite-related), intestinal trematodiasis, and splenic congestion. There were no significant changes in the brain, bone marrow, liver, or kidney tissues in any species examined.

In summary, abnormal findings that were greater in the potentially exposed bats when compared to reference bats, which includes poor body condition (34% potentially exposed bats, 0% reference bats) and increased number of pulmonary macrophages (86% of potentially exposed bats, 0% reference bats). The increased number of pulmonary macrophages is tentatively attributed to edema and/or congestion. Although edema and congestion are non-specific changes, both may be associated with irritation from gasoline vapor inhalation.

Although pulmonary edema can be clinically significant if severe or if occurring in a debilitated animal , the clinical significance of the pulmonary histiocytosis at the time of death is considered to be low. There was no notable difference in the degree of histiocytosis between bats found dead and euthanized bats. A cause of death for the bats found dead could not be determined, but complications associated with WNS are considered possible. Based on the absence of significant autolysis in the bats found dead, death likely occurred relatively recently (within the week preceding collection). Although gasoline exposure cannot be ruled out as a contributing factor to poor body condition in some animals, the significance of this finding is unclear, as many factors can contribute to the amount of body fat in an individual bat and bats with WNS often lose excessive body weight during hibernation.

## Contaminant Analysis

Ten of the 13 bile composite samples had no measureable levels of OH-PAHs above the method reporting limits (Table 3). Three of the samples (Tri-colored Bat from Goldson Cave 1; Tri-colored Bat from Goldson Cave 3; and Little Brown Bat from Minton Hollow Cave) had one detection each slightly greater than the reporting limit. The detections, all at very low concentrations, were limited to only 1 compound each, which prevented pattern analysis for source discrimination. In addition, the detected copounds were not the lower molecular weight OH-PAHs, such as hydroxylated naphthalenes, which would be indicative of the volatile PAH fraction found in fuels. Consequently, it is unlikely that these bats were systemically affected by the fuel spill. It is more likely that these low detections, consisting of the metabolites of higher molecular weight and less volatile PAHs, were the result of residues from a food source or background interferences (false positives) from the attached liver in the samples.

# Acknowledgments

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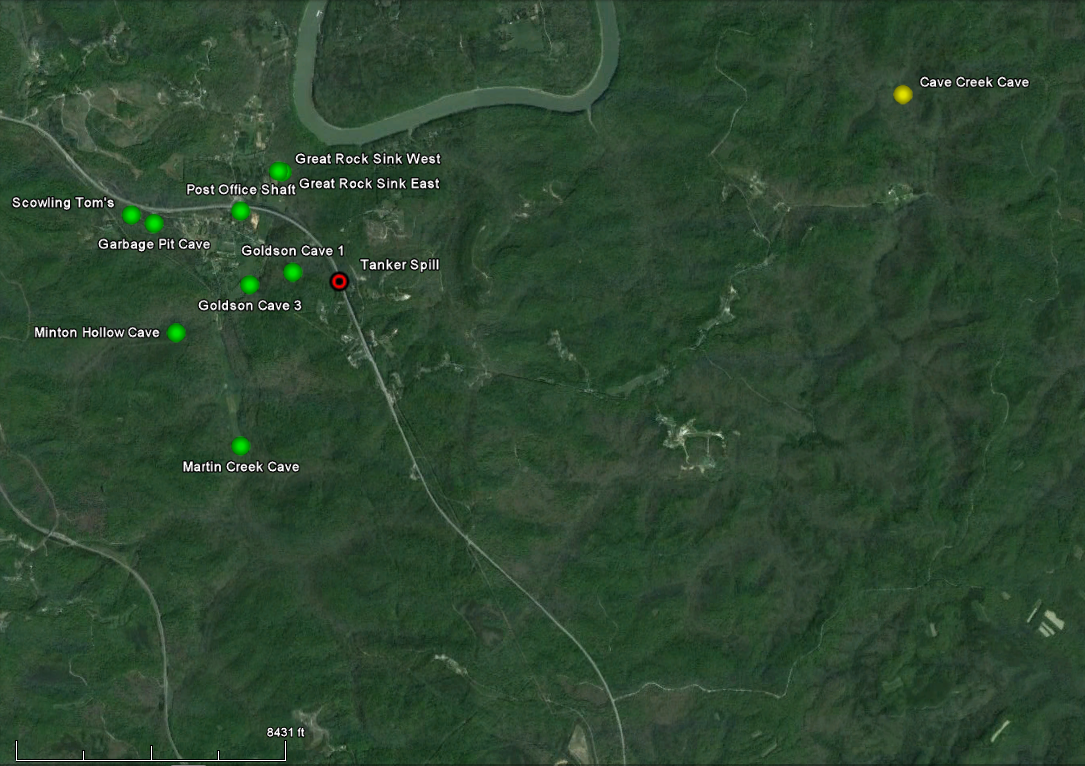
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1. Bat sample locations near Sloans Valley, Kentucky (Google Earth image, September 2014). Potentially exposed sites are green; the reference site is yellow.
2. Bats selected for histopathological analysis.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species, Location | Lat/Long  (Decimal degrees) | Exposed/Reference | n | Found Dead/  Euthanized |
| Tri-colored Bat | | | | |
| Goldson Cave 1 | 36.93273/-84.52921 | Exposed | 1 | Euthanized |
| Minton Hollow Cave | 36.92760/-84.54179 | Exposed | 3 | Dead |
| Garbage Pit Cave | 36.93696/-84.54409 | Exposed | 4 | Euthanized |
| Goldson Cave 3 | 36.93169/-84.53390 | Exposed | 1 | Dead |
| Little Brown Bat | | | | |
| Minton Hollow Cave | 36.92760/-84.54179 | Exposed | 5 | Euthanized |
| Cave Creek Cave | 36.94775/-84.463883 | Reference | 5 | Euthanized |

1. Summary of field necropsies. Individuals in bold were found dead; all others were euthanized.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Species and Site** | | **ID** | **Body Condition** | **Postmortem Condition** | **Histopathology Performed** | ***Pseudogymnoascus destructans* status a** | **Pulmonary Macrophages per 40x field** |
| Tri-colored Bat | | | | | | | |
|  | Goldson Cave 1 (collected 3/6/14) | | |  |  |  |  |
|  |  | 24731-001 | Emaciated | Fair | Yes | Positive (Hd, PCR) | 33 |
|  | Minton Hollow Cave (collected 3/6/14) | | |  |  |  |  |
|  |  | **24731-002** | Emaciated | Poor | Yes | Positive (H, PCR) | 14 |
|  |  | **24731-003** | Emaciated | Fair | Yes | Positive (H, PCR) | 24 |
|  |  | **24731-009** | Fair | Fair | Yes | Positive (H, PCR) | 57 |
|  | Garbage Pit Cave (collected 3/6/14) | | |  |  |  |  |
|  |  | 24731-015 | Fair | Fair | Yes | Positive (H, PCR) | 37 |
|  |  | 24731-016 | Emaciated | Fair | Yes | Positive (H, PCR) | 45 |
|  |  | 24731-017 | Fair | Not noted | Yes | Positive (H, PCR) | 29 |
|  |  | 24731-018 | Fair | Poor | Yes | Positive (H, PCR) | 21 |
|  |  | 24731-019 | Not noted | Not noted | No | Positive (PCR) | Not applicable |
|  |  | 24731-020 | Fair | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-021 | Not noted | Not noted | No | Positive (PCR) | Not applicable |
|  |  | 24731-022 | Fair | Good | No | Positive (PCR) | Not applicable |
|  | Great Rock Sink West (collected 3/7/14) | | | |  |  |  |
|  |  | 24731-023 | Good | Fair | No | Negative (PCR) | Not applicable |
|  | Great Rock Sink East (collected 3/7/14) | | |  |  |  |  |
|  |  | 24731-026 | Emaciated | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-027 | Fair | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-028 | Emaciated | Fair | No | Positive (PCR) | Not applicable |
|  |  | 24731-029 | Emaciated | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-030 | Emaciated | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-031 | Emaciated | Fair | No | Positive (PCR) | Not applicable |
|  |  | 24731-032 | Emaciated | Good | No | Positive (PCR) | Not applicable |
|  | Martin Creek Cave (collected 3/7/14) | | |  |  |  |  |
|  |  | 24731-033 | Emaciated | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-034 | Emaciated | Fair | No | Positive (PCR) | Not applicable |
|  |  | 24731-035 | Fair | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-036 | Emaciated | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-037 | Emaciated | Fair | No | Positive (PCR) | Not applicable |
|  |  | 24731-038 | Fair | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-039 | Fair | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-040 | Fair | Good | No | Positive (PCR) | Not applicable |
|  | Post Office Shaft (collected 3/7/14) | | | |  |  |  |
|  |  | 24731-041 | Emaciated | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-042 | Fair | Poor | No | Positive (PCR) | Not applicable |
|  | Goldson Cave 3 (collected 3/7/14) | | |  |  |  |  |
|  |  | **24731-043** | Emaciated | Fair | Yes | Positive (H, PCR) | 33 |
| Little Brown Bat | | | | | | | |
|  | Minton Hollow Cave (collected 3/6/14) | | |  |  |  |  |
|  |  | 24731-004 | Fair | Good | Yes | Positive (H, PCR) | 21 |
|  |  | 24731-005 | Good | Good | Yes | Positive (H, PCR) | 41 |
|  |  | 24731-006 | Fair | Not noted | Yes | Positive (H, PCR) | 21 |
|  |  | 24731-007 | Fair | Fair | Yes | Positive (H, PCR) | 8 |
|  |  | 24731-008 | Emaciated | Fair | Yes | Positive (H, PCR) | 66 |
|  |  | 24731-010 | Not noted | Not noted | No | Positive (PCR) | Not applicable |
|  |  | 24731-011 | Fair | Fair | No | Positive (PCR) | Not applicable |
|  |  | 24731-012 | Fair | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-013 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-014 | Fair | Good | No | Positive (PCR) | Not applicable |
|  | Great Rock Sink West (collected (3/7/14) | | | |  |  |  |
|  |  | 24731-024 | Good | Good | No | Negative (PCR) | Not applicable |
|  | Scowling Tom's (collected 3/14/14) | | |  |  |  |  |
|  |  | 24731-044 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-045 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-046 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-047 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-048 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-049 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-050 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-051 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-052 | Fair | No noted | No | Positive (PCR) | Not applicable |
|  |  | 24731-053 | Fair | Good | No | Positive (PCR) | Not applicable |
|  | Cave Creek Cave (collected 3/14/14) | | | |  |  |  |
|  |  | 24731-054 | Fair | Good | Yes | Positive (H, PCR) | 13 |
|  |  | 24731-055 | Good | Good | Yes | Positive (H, PCR) | 4 |
|  |  | 24731-056 | Fair | Good | Yes | Positive (H, PCR) | 8 |
|  |  | 24731-057 | Good | Good | Yes | Positive (H, PCR) | 15 |
|  |  | 24731-058 | Good | Fair | Yes | Positive (H, PCR) | 17 |
|  |  | 24731-059 | Good | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-060 | Fair | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-061 | Good | Fair | No | Positive (PCR) | Not applicable |
|  |  | 24731-062 | Fair | Good | No | Positive (PCR) | Not applicable |
|  |  | 24731-063 | Good | Good | No | Positive (PCR) | Not applicable |
| Rafinesques' Big Eared Bat | | | | | | | |
|  | Great Rock Sink West (collected 3/7/14) | | | |  |  |  |
|  |  | 24731-025 | Emaciated | Good | No | Negative (PCR) | Not applicable |

a *Pseudogymnoascus destructans* (White nose syndrome) status was determined by histopathology (H) and/or Polymerase Chain Reaction (PCR).

1. Analysis of PAH metabolites in bat bile collected in 2014.

[All concentrations are nanograms of analyte per milliter of bile]

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | 2-OH-naphthalene | 1-OH-napthalene | 9-OH-fluorene | 2-OH-fluorene | 9-OH-phenanthrene | 1-OH-pyrene | 2-OH-chrysene | 3-OH-benzo(a)pyrene |
| Tri-colored Bat | | | | | | | | |
| Goldson Cave 1 | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | 0.14 | <0.37 |
| Minton Hollow Cave | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Garbage Pit Cave | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Great Rock Sink West | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Great Rock Sink East | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Martin Creek Cave | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Post Office Shaft | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Goldson Cave 3 | <3.67 | <26 | <4.4 | 0.41 | <0.40 | <0.22 | <0.13 | <0.37 |
| Little Brown Bat | | | | | | | | |
| Minton Hollow Cave | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | 0.43 |
| Great Rock Sink West | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Scowling Tom’s | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Cave Creek Cave a | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |
| Rafinesques' Big Eared Bat | | | | | | | | |
| Great Rock Sink West | <3.67 | <26 | <4.4 | <0.31 | <0.40 | <0.22 | <0.13 | <0.37 |

a Reference site