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Memorandum

Date:	4 October 2014
То:	National Parks Trust, British Virgin Islands Dr. Gad Perry Dr. Skip Lazell Dr. Lianna Jarecki
From:	Dr. Adam Duerr, West Virginia University
Subject:	Update on lead analysis in blood of Stout Iguanas and other lizards from the BVI

Background. Long-term use and local disposal of lead-acid batteries has created a possible sources of this toxic heavy metal to fauna of the BVI. In preliminary work conducted in 2012, I found what appeared to be elevated levels of lead in blood of *Anolis* lizards on Guana Island. These findings raised concerns about potential lead exposure in other species, including the endangered Stout Iguana (*Cyclura pinguis*).

In 2013, I began investigating lead exposure in Stout Iguanas on Guana Island. Availability of portable and reliable instruments (Lead Care II, Magellan Diagnostics, North Billerica, MA, USA) allowed for on-site testing of small blood samples (50 μ L). The instrument was designed to analyze human blood and has not been verified for herpetological use. Results from the Lead Care II unit tend to be biased low in birds (Boschetto and Pierce 2012). Results from reptiles have to be considered preliminary until supported by testing by an accredited lab. The unit has a detection range of $3.3 - 65 \mu g/dL$. Levels > $5 \mu g/dL$ are a concern in humans.

There are no established benchmarks for herpetofauna. Camus et al., (1998) interpreted blood lead levels of 0.2 mg/L ($20 \mu g/dL$) as suspicion of lead poisoning and levels of 0.5 mg/L ($50 \mu g/dL$) as diagnostic of lead poisoning. Although I am using these values in this preliminary analysis, it is important to note that there are no data to support levels of acute or chronic toxicity in iguanas. However, lead is not a biologically useful element and lead exposure at all levels is considered poisoning with potential chronic effects, which include decreased reproductive and survival rates.

Work in 2013. I analyzed 39 blood samples from iguanas captured on Guana Island and released following sampling and marking. These included 21 hatchlings, 8 subadults (ages 1-4), and 10 adults. One hatchling, three subadults, and four adults had elevated lead levels, ranging from $3.3 - 40 \mu g/dL$. The iguana with blood lead levels near the diagnostic category for lead poisoning (40 $\mu g/dL$) was a subadult.

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I also tested for lead exposure in 45 Anoles (*Anolis spp.*) on Guana Island (2012-2013). Results show 30 anoles had detectable lead levels in blood with four that fall in the suspicious category $\sim 20 \ \mu g/dL$ and three that fall in the diagnostic category (>50 $\mu g/dL$). Such high incidence of lead exposure is a concern, although these numbers are not representative of all anoles on Guana Island.

Lead exposure appears to be clustered around the hotel, reverse osmosis plant, and dump areas (Figure 1, red dots). This clustering suggests that lead contamination comes from multiple point sources on the island.

Lead exposure in lizards is not limited to Guana Island. In 2013, I also expanded the study of lead exposure to include other islands and found elevated lead in *Anolis* blood samples from locations on Little Camanoe, Marina Cay, Tortolla, and Virgin Gorda. The only island where samples were obtained that did not show elevated lead levels was Last Resort. Given this broader distribution of lead exposure, it is possible that lizards (and animals, including humans) from other islands are also exposed to lead.

Management implications. If lead exposure does indeed come from localized sources, it should be possible to remove those sources and mitigate negative effects from this toxic element.

Work planned for 2014. For 2014, I will continue analysis of lead exposure in BVI lizards to better define the distribution of exposure and the proportion of the population that may be affected. In addition to iguanas, I will also look at lead exposure in snakes



Figure 1. Capture locations of lizards that were samples for lead exposure. Elevated lead levels are shown in red, levels below detection limits are shown in black. Circles are Circles: Crested Anoles; Triangles: Saddle Anoles; Squares: Stout Iguanas

(*Alsophis pororicensis*) and other anoles, to explore how lead moves up the food chain. Finally, I will continue looking at lead exposure in anoles on other islands of the BVI. *To allow for a reference site that is lacking human population, I am asking permission to sample on Great Dog.*

Future work. The Lead Care II unit has a limited range for quantifying blood lead levels. To verify results obtained by the Lead Care II unit, I archived samples from 33 iguanas from Guana Island in 2013. These archived samples include 33 blood spots of ~50 microliters stored on treated filter paper. These samples were exported to the US using a CITES permit obtained by

Dr. Perry and a CITES import permit to the IUCN Iguana Specialist Group. Lab-based lead analyses have a lower detection limit of $0.5 \ \mu g/dL$ and such results will better show where exposure to lead occurs. I am collaborating with the Diagnostic Center for Population and Animal Health, Michigan State University, to conduct lab-grade analyses. These will allow us to 1) detect much lower levels of lead exposure, 2) develop a correction factor for the Lead Care II unit for lizard blood, and 3) identify stable isotope signatures to distinguish sources of lead, such as leaded gasoline, lead paint, and car batteries (Komárek et al. 2008). This collaboration will also allow for analysis of a greater number of samples (from iguanas, anoles, and snakes) than current funding permits. For all of the field analyses conducted in 2014, I will archive samples for lab-based analyses. *To allow this lab analysis to proceed with the largest possible sample, I have asked Dr. Perry to request a CITES export permit again for blood samples, and he has informed me that he intends to request such a permit toward the end of the month, when field work is completed.*

Many hatchling iguanas sampled on Guana Island were too young to have fed much, so lead exposure must primarily have been from a maternal source, transferred via the egg. If funding allows, we would also like to conduct genetic analyses to determine relatedness among iguanas. Genetic analyses may be conducted by collaborators at West Virginia University, Purdue University, or University of Rhode Island. In addition to genetics, we can also assess maternal transfer of lead by analyzing lead content in iguana eggshells because lead replaces calcium at the metabolic level. *I have also asked Dr. Perry to request a CITES export permit for iguana eggshells*.

References

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