Untargeted Poisonings of Southern African Wildlife

Judith Rodriguez Salas, MSFS[1]*; Meaghan Drumm, MSFS[1]; Stephen Donovan, PhD[1]; Alana Balogh, BFA[1]; Amanda Mohr, MSFS[1], Barry Logan, PhD[1]

[1]The Center for Forensic Science Research and Education, Willow Grove, PA

INTRODUCTION

Throughout history, pesticides have been widely used against wildlife on the continent of Africa. Pesticides are used to protect crops and farmland against insects, pest, and wild animals that attack livestock. Poachers are also known to use pesticides, while hunting big game. The use of pesticides as a means to kill animals, however, causes inadvertent poisonings of other species such as scavengers who feed from the dead carcass, or if an unintended animal ingests the poisoned bait (e.g. fruit, crops) that are left behind.

The goal of this study was to determine a viable in-field extraction technique with limited supplies and/or resources that could be performed in the field and sent to the laboratory for the identification of selected pesticides following a suspected poisoning event.

METHODS

In-Field Extraction

Performed using minimal materials that are inexpensive and easy to find:
-Acetone, glass jars, paper towels and a funnel

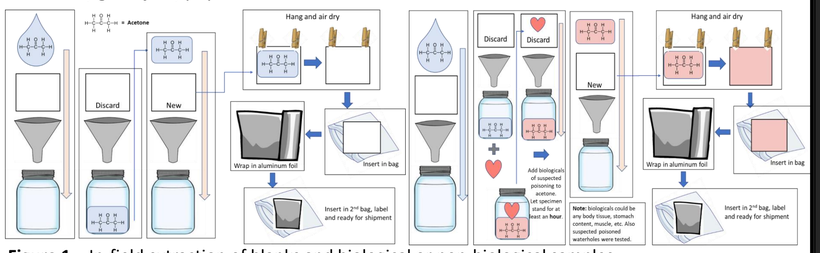

Figure 1 – In-field extraction of blanks and biological or non-biological samples.

Qualitative Analysis:

- Waters™ Acquity UPLC®, Waters™ Xevo® TQD MS (LC/MS-MS)
- Analytical column Acquity UPLC® BEH C18 (1.7 µm, 2.1 x 100 mm)
- MPA: Ammonium Formate pH 4
- MPB: 0.1% Formic Acid in Methanol
- Injection volume 10 µL
- MS at ESI+
- Data processing Waters MassLynx™ v4.1 software

Time (min)	Flow (mL/min)	% A	% B
Initial	0.45	90	10
0.5	0.45	90	10
4	0.45	20	80
4.1	0.45	90	10
5	0.45	90	10

Pesticide Scope (Spiking Mix):

Strychnine, Monocrotophos, 3-Hydroxycarbofuran, Carbofuran, Aldicarb, Aldicarb Sulfoxide, Aldicarb Sulfone, Carbaryl and Methomyl
ISTD: Carbaryl – D7

Lab extraction:

1. Paper towels were cut out in 2 x 2 in pieces
2. Positive Control: 100 µL of 10 ng/µL spiking mix applied to paper towel cutout
3. Add 5 mL of ethyl acetate, cap and shake
4. Allow for passive extraction (10 min)
5. Pour over to new test tube and add 20 µL of 10 ng/µL of ISTD
6. Evaporate to dryness at 40°C
7. Reconstitute in 200 µL of 90:10 MP
8. Vortex and centrifuge @3800 rpm for 10 min
9. Transfer to autosampler vials for analysis

Method Validation

Fit-for-purpose validation adapted from the American Standards Board (ASB) validation standard including:

- Limit of detection (LOD), recovery, carryover, interferences with other pesticides, stability at 23°C, 33.9°C and -80°C.

RESULTS

A total of 105 samples were analyzed during a 4-year period (2016 to 2020). Samples analyzed were collected by local agencies following the in-field extraction and sent via courier mail. Positive samples were distributed from 3 different countries: Zambia, Zimbabwe and Mozambique. (Figure 2)

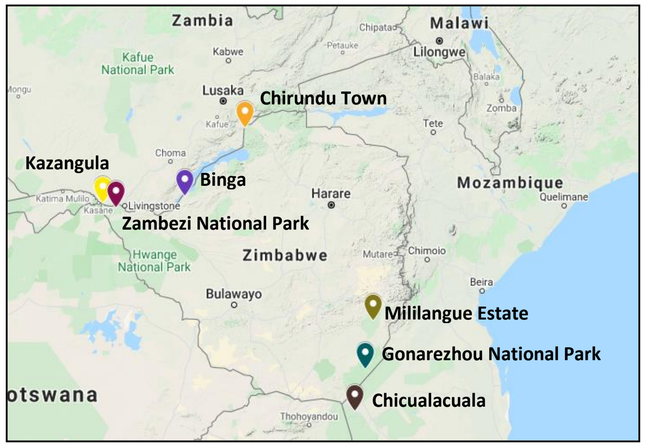

Figure 2. Location of collected positive samples.

BACKGROUND AND RESULTS FOR SELECT CASES

Case 1 and 2 – A total of twelve hippopotami from the same bloat were found dead within five days of the first identification. Local wildlife conservationist groups identified that a new irrigation scheme that used pesticides was located near the pool where this bloat lived. The pesticides that were identified were ***aldicarb, carbofuran and traces of aldicarb sulfoxide***. Days later another two hippopotami were found dead near the same location, and it was thought that the prior pesticide dump on the water pool was the responsible.
Location: Binga, Zimbabwe

Case 3 – Carcasses belonging to two adult buffalos were identified 20 meters apart. The carcasses were covered by branches and portions of the meat were missing. A nearby adult male lion was found in distress and required attendance. Local wildlife agencies believe that the buffalo was poisoned, and the lion ate from the remains. Stomach contents from the buffalo were analyzed and a wide variety of pesticides were identified: ***aldicarb, aldicarb sulfoxide, aldicarb sulfone and carbofuran***.
Location: Kazangula, Zambia

Case 5 – In this case, three (3) samples tested positive for pesticides. Liver and stomach contents from vultures were analyzed along with the stomach contents of a deceased elephant. The carcass of the dead elephant was suspected to be the source for the death of 104 vultures identified in the nearby area by second-hand poisoning. The *elephant stomach* contents contained ***aldicarb, aldicarb sulfoxide, carbofuran, 3-hydroxycarbofuran, traces of methomyl and traces of carbaryl***. The *vulture liver* contained ***aldicarb, aldicarb sulfoxide, aldicarb sulfone and carbofuran*** and the *vulture stomach* contents contained ***aldicarb, aldicarb sulfoxide, aldicarb sulfone, carbofuran, 3-hydroxycarbofuran and traces of carbaryl***. These drug findings line up with the possible line of events that the local wildlife agencies suspected: the elephant was poisoned, and the vultures were an indirect casualty after consuming the poisoned meat.
Location: Chicualacuala, Mozambique

RESULTS CON'T

Case #	Species	Results	Location
4	Watermelon	Carbofuran	
6	Warthog	Carbaryl, Carbofuran	
7	Vulture	Carbofuran	
	Elephant	Methomyl, Carbofuran, Carbaryl	
8	Knob-billed geese	Carbaryl	
9	Marula Fruit	Monocrotophos, Carbofuran, Aldicarb	
10	Elephant	Monocrotophos, Carbofuran, Aldicarb	
11	Bateleur Eagle	Monocrotophos	
12	Kudu	Methomyl, Aldicarb, Carbofuran, Aldicarb Sulfoxide	

From the 105 samples analyzed for pesticides, 24 were positive for one or more pesticides. None of the positive samples were positive for **strychnine. Carbofuran** was the identified in 67% of the samples, most of the time identified with **aldicarb** and **monocrotophos. Aldicarb,** was identified in 46% of the samples and **monocrotophos** was identified on 38% of the samples. (Figure 3)

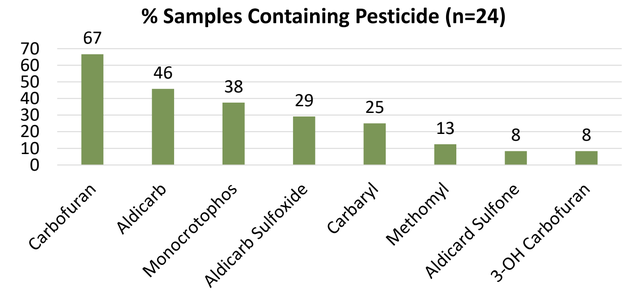

Figure 3. Percentage of samples containing pesticides

DISCUSSION AND CONCLUSION

Pesticides have been used for decades, but some animals end up as unwanted targets of these poisonings. A sample collection, extraction procedure and analytical method were developed successfully for the analysis of some common pesticides.

The case histories show how secondhand poisoning and habitat contamination are a huge issue that kill animals indiscriminately and can create a chain reaction that could unknowingly harm many additional animals.

Being able to detect and report the findings in these cases to the African wildlife agencies can help them in their prosecution of offenders.

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