Analysis of Carbamate and Organophosphate Pesticides in Animal Poisoning Cases by Gas Chromatography/Mass Spectrometry

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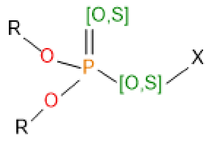
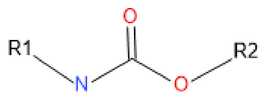
Introduction

In underdeveloped countries, pesticides are widely available especially as agriculture is a large economic area in the region.[1] While pesticides do find a legitimate use in the agricultural sector, the ease of access to these pesticides has led to their use in illegal activities, such as the poisoning of wildlife. Pesticides are most commonly introduced to wildlife via poisoned bait meat, meat that is laced with pesticides meant to kill animals that are predating on cattle, especially in regards to lions. These incidences affect many species besides the lions being targeted including vultures, hyenas and can even make their way into the human food chain. Additionally, vultures often tend to be targeted by poachers to avoid detection by officials during their illegal activities that are often used to fund terrorism. Vultures are facing declines in population and possible extinction, in part due to these poisonings. Avian species are poor metabolizers of these two classes of pesticides, leaving them even more vulnerable to poisonings.[1,2]

Carbamate and organophosphate (OP) pesticides are both acetylcholinesterase inhibitors. Acetylcholine is a neurotransmitter involved in all physiological response mechanisms in animals and acetylcholinesterase is necessary to break it down. When these pesticides inhibit acetylcholinesterase, acetylcholine builds up in the nerve junctions leading to toxic central nervous system effects and ultimately leading to convulsions followed by paralysis and ultimately respiratory failure due to the diaphragm muscles ceasing to work. OPs bind irreversibly to the enzyme and require new enzymes to be synthesized for function to be restored while carbamates bind reversibly to the enzyme, making them somewhat less dangerous.[1,3,4]



Organophosphate Structure



Carbamate Structure

Although methods have been previously developed to analyze these two classes of pesticides, many analyses are long and no method for this application exists.[4-6] For this method to be applicable to these situations, a simple extraction and collection method that would work on multiple matrices needed to be developed along with a shorter GC/MS method that was capable of separating and identifying the most commonly used pesticides.

Methods

In-field Acetone Extraction

1. Stomach contents, bait meat, urine or blood are placed into a glass container
2. Commercial grade acetone is added to the container
3. Mixing occurs for at least one minute
4. Mixture is filtered through a paper towel placed in a funnel into a glass container
5. Filtrate is applied to a paper towel and allowed to dry

In-lab Reconstitution and Liquid/Liquid Extraction

1. A cutting of the paper towel is put into a vial and ethyl acetate is added
2. After one hour, the ethyl acetate is decanted off and this is repeated once more
3. 2 mL of water is added to the tube containing the ethyl acetate that was decanted to wash the organic layer
4. Aqueous layer is removed and 2 mL of 5% sodium bicarbonate is added to wash the organic layer
5. Aqueous layer is removed and 2 mL of saturated sodium chloride solution are added to wash the organic layer and remove water
6. Aqueous layer is removed and 0.5 g of sodium sulfate is added to further remove water
7. Organic layer is removed and dried down under a stream of nitrogen gas
8. Residue is reconstituted in 50-500 µL ethyl acetate and analyzed by the developed GC/MS method

Results

Table 1: GC/MS Method Parameters

GC	Agilent 7890A
Column	HP-4MS Ultra Inert
Column Length	30 m
Carrier Gas	Hydrogen
Injection Type/Volume	Splitless/1 µL
Inlet Temperature	250 ºC
Oven Program	40 ºC for 1 minute 15 ºC/min to 170 ºC, hold for 5 minutes 25 ºC/min to 350 ºC, hold for 3 minutes
Flow	1.1 mL/min
MS	Agilent 5975C
MS Scan Range	35-500 amu

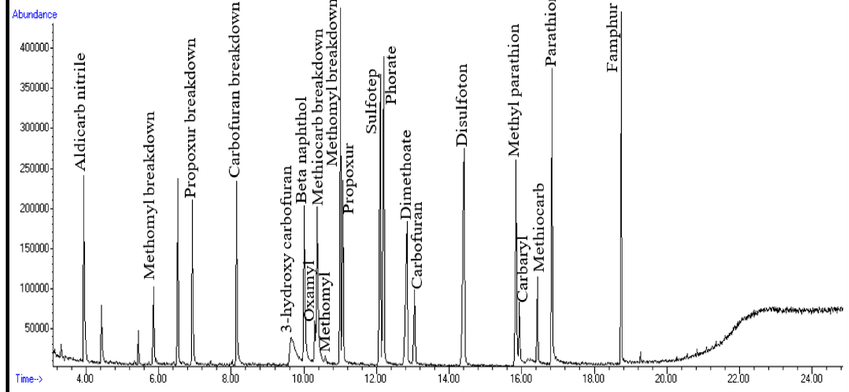
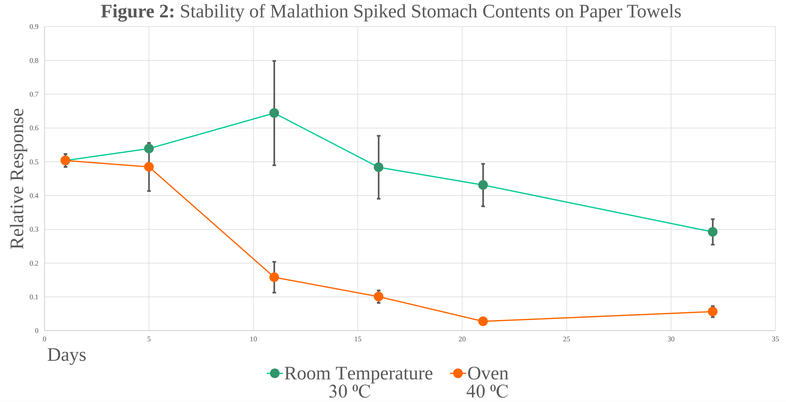


Figure 1: Standard Mix of Carbamate and OP Pesticides on Developed Method

Table 2: Recovery Data from Extractions

	Beta-Naphthol	Carbaryl
Recovery from In-Field Extraction	8.85%	8.48%
Recovery from Lab Extraction	73.57%	70.64%
Recovery from Both Extractions	6.51%	5.99%

Figure 2: Stability of Malathion Spiked Stomach Contents on Paper Towels



Results

Table 3: Limit of Detection for Neat Standards

LOD (ng on column)	Compounds
1	Aldicarb nitrile, propoxur
10	Methomyl, 3-hydroxycarbofuran, thionazin, sulfotep, phorate, dimethoate, carbofuran, disulfoton, methyl parathion, carbaryl, methiocarb, parathion, famphur
25	Triethyl thiophosphate, oxamyl

Conclusion

A method has been developed that is capable of separating and identifying the most commonly used pesticides in wildlife poisoning cases. While this research aims to target poisonings occurring in Africa, the method can be applied globally. This method has decreased the run time of previous methods and was able to identify carbamate pesticides that generally require liquid chromatography, specifically aldicarb in the form of aldicarb nitrile. A simple, in-field extraction method was developed for this analysis and has proven efficient. An extraction video detailing the in-field procedure and including safety precautions was made and is being sent to collaborators in Africa who will in turn be sending samples back to the United States for analysis.

A recovery study of the in-field and liquid-liquid lab extraction demonstrated the efficiency of the developed extraction methods and revealed that most of the product is lost in the stomach contents, but the developed extraction is recovering around 70%. This is not of large concern because it is expected that during these poisonings large quantities of pesticide will be applied to the bait meat. Additionally, since this method is qualitative, the recovery is sufficient because the pesticides can be detected. Malathion seemed least stable over the 32 day period when stored in an oven at 40 ºC, which is slightly concerning as these samples will be exposed to the heat in Africa and possibly during shipping. Overall, this is not a concern for the testing of authentic samples as they are expected to have a high quantity present and should still be able to be detected.

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Acknowledgments

The authors would like to thank Mandi Mohr, Melissa Friscia and Alex Krotulski for their aid and guidance during this research; colleagues Stephanie Reichardt, Amanda D'Orazio, Brianna Robbins, Margret Potoczniak, Courtney Mower and Meaghan Drumm for their input and aid in filming for the extraction video; the Center for Forensic Science Research and Education and Arcadia University for this research opportunity and for providing the resources to make this research possible.