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2,4-D Presence in Animal Fur

Ghennya E Shain

Cal Poly Humboldt, ges46@humboldt.edu

Keith Druen

Cal Poly Humboldt, kpd24@humboldt.edu

Alondra Salazar

Cal Poly Humboldt, as517@humboldt.edu

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Keith Druen, Alondra Salazar, Ghennya Shain, Dr. Jeffrey Schineller¹, Dr. Jenny A. Cappuccio¹

¹ CHEM 435L - Biochemistry II Lab - Department of Chemistry, Cal Poly Humboldt, Arcata, CA, USA

Abstract

2,4-dichlorophenoxyacetic acid, commonly known as 2,4-D, is a widely utilized herbicide in weed control. 2,4-D is considered toxic, and may pose detrimental effects to wildlife if used recklessly. Here we sought to quantitatively analyze for the presence of 2,4-D on the fur of local fauna found in Humboldt county, namely Gray fox (*Urocyon cinereoargenteus*), Brush rabbit (*Sylvilagus bachmani*), and Deer mice (*Peromyscus maniculatus*). We used purification techniques such as washes, as well as extraction techniques including liquid-liquid extraction, and analysis through gas chromatography (GC) in order to visualize 2,4-D. Positive results could spell trouble for the local wildlife, and have severe consequences.

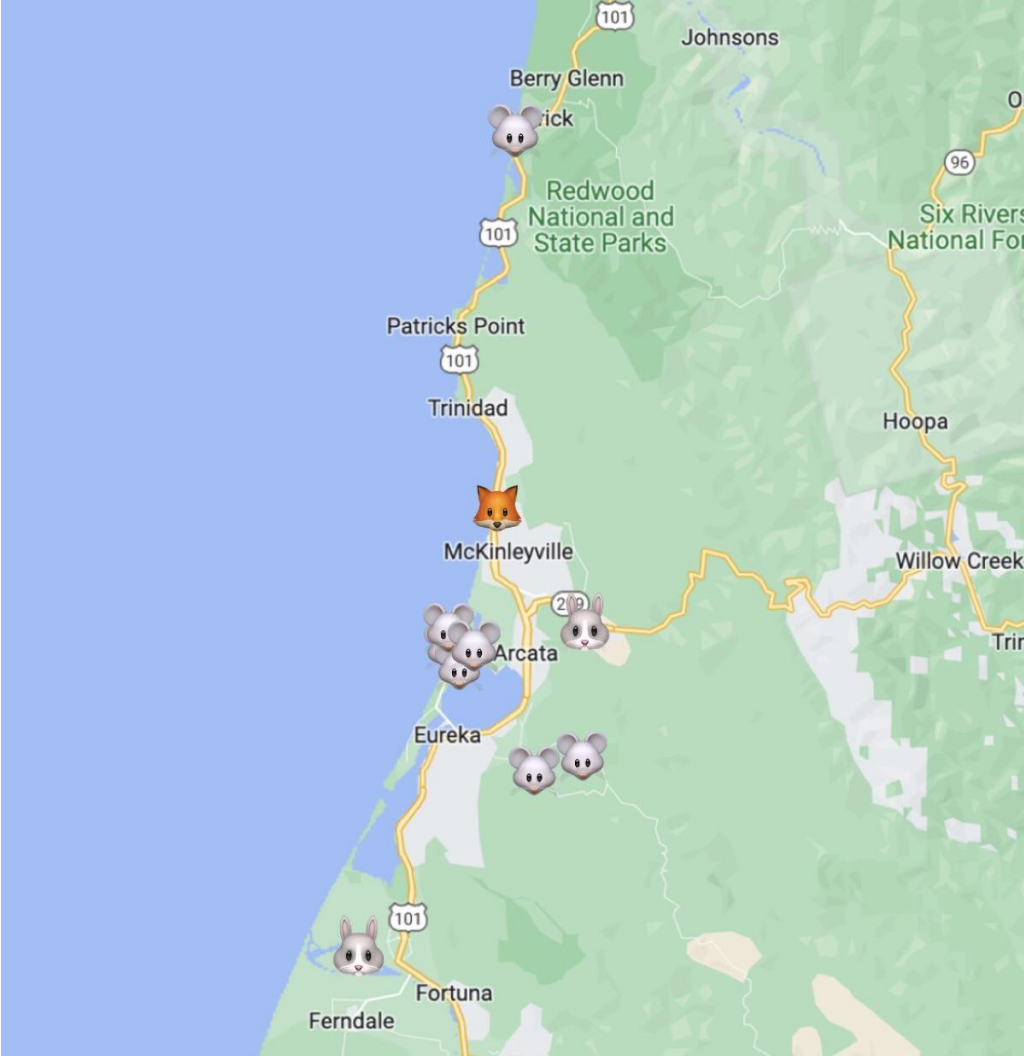


Figure 1. Localities of specimen

Introduction

Persistent organic pollutants (POPs) are chemicals that are resistant to environmental degradation, and depending on the chemical, can pose health risks to local flora and fauna. In Humboldt county one of the most common sprays used in local farms is 2,4-dichlorophenoxyacetic acid (2,4-D), a toxic auxin-type herbicide, which, when ingested, may be manifested in the animal's fur.



Figure 2. Grey fox, Deer mouse, and Brush rabbit

Negative effects of 2,4-D have been documented in lab animals as researchers noted brain size changes, as well as effects on neurotransmitters and neural connections development. Further, litter size changes and presence of 2,4-D in breast milk was noted for animals that were known to be exposed to the compound.

Grey fox (*Urocyon cinereoargenteus*), Brush rabbit (*Sylvilagus bachmani*), and Deer mice (*Peromyscus maniculatus*) are species that are found in the Humboldt county area, and were the specimens chosen for fur analysis.

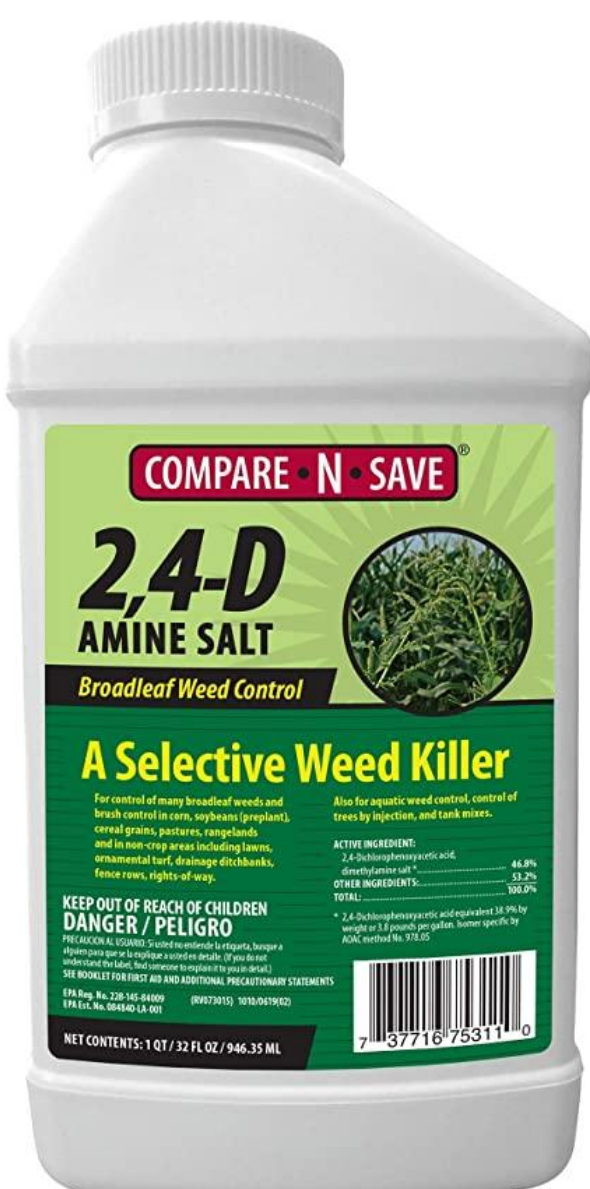
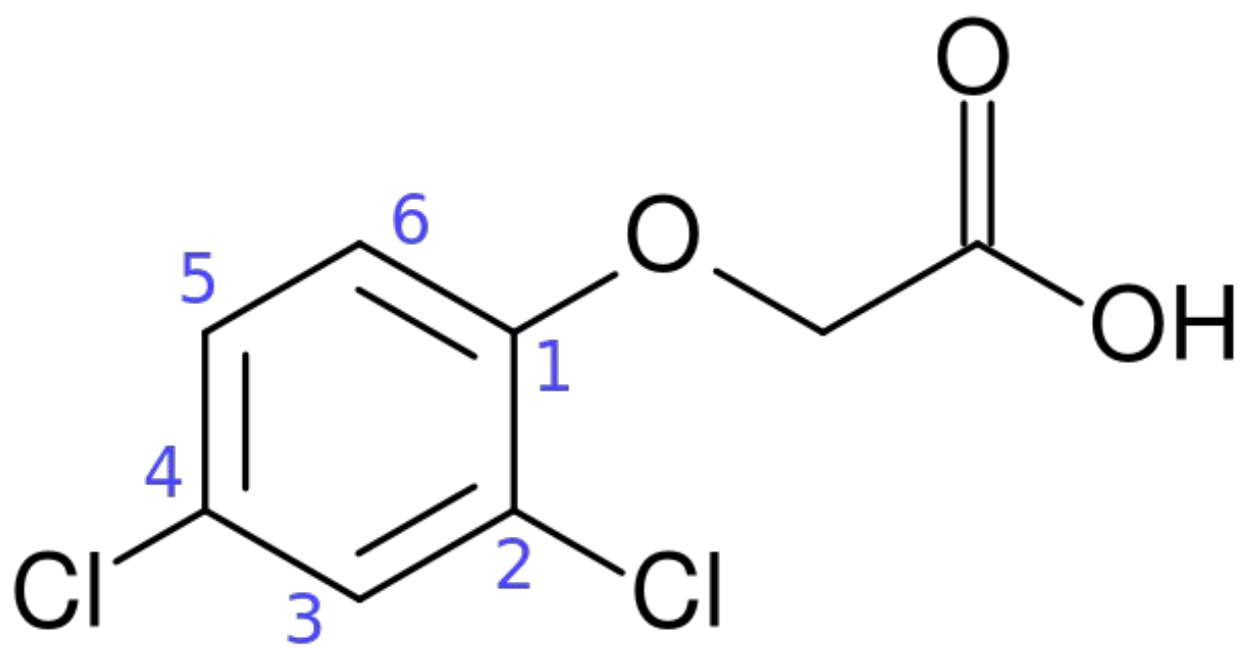


Figure 3. 2,4-dichlorophenoxyacetic acid (2,4-D) structure and commonly sold form

Methods

1. Collection of animal fur from a housecat, as well as three types of wildlife: fox, mice, and rabbits from the vertebrate museum at Cal Poly Humboldt.
2. Purification of samples by washing with DI water, shampoo (0.3–1% poly- ethylene lauryl ether), and DI water once again in order to get rid of impurities such as dust particles.
3. Incubation of the samples in 3 M HCl in a rocker.
4. Liquid-liquid extraction with 4:1 Hexane:DCM, and removal of the top aqueous layer for analysis.
5. Esterification of samples by AMES synthesis.
6. Analysis via GC by set temperature sequence with loaded samples of spiked cat hair as positive control, cat hair as negative control, wildlife fur samples of interest, and standards for calculations.



Figure 4. Peromyscus maniculatus (Deer Mice) Specimen.

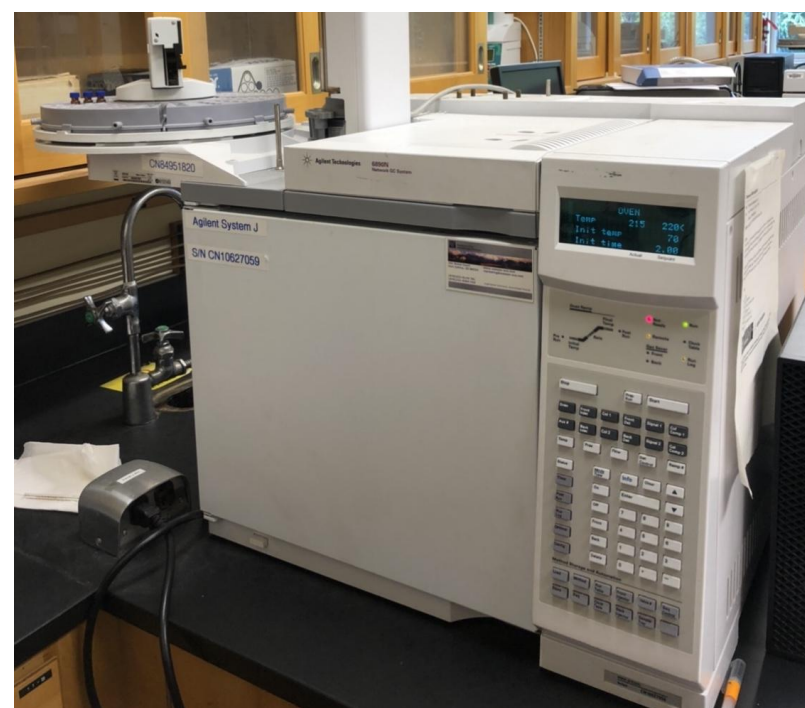


Figure 5. GC instrument

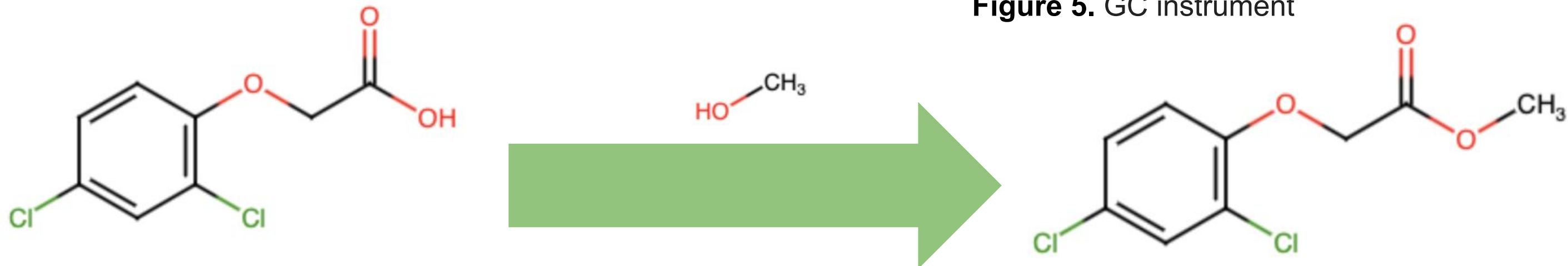


Figure 6. Esterification of 2,4-D with methanol and BF3.

Results

A series of tests were performed, where an esterification procedure to lower the boiling point of 2,4-D was found necessary in order to achieve results via GC analysis.

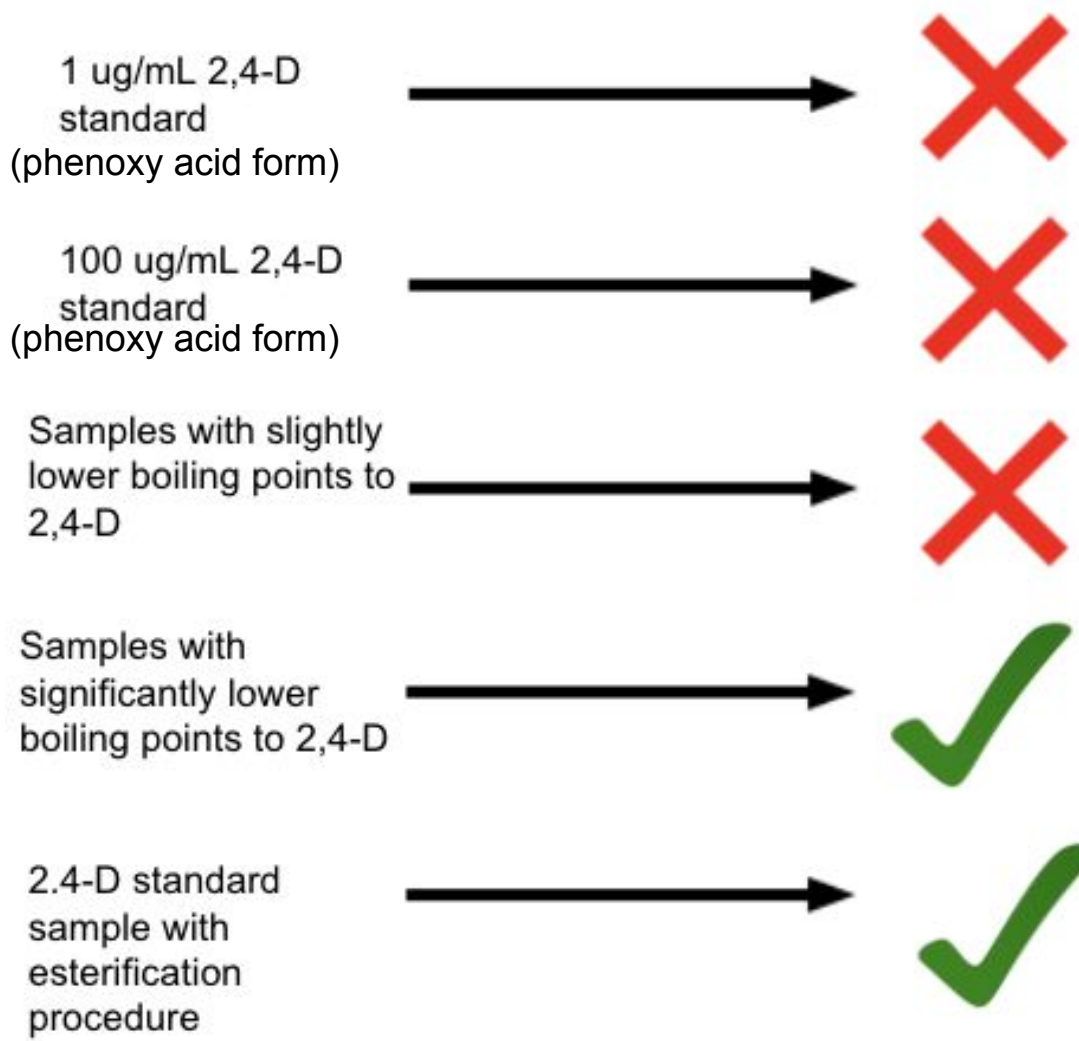


Table 1. Concentration of 2,4-D in samples with positive results.

Sample name	Area Under Curve	Concentration of 2,4-D (ug/mL)
Spiked Cat 1	4.568	2.110
Spiked Cat 2	10.653	4.921
Brush Rabbit (7782)	1.634	0.755
Brush Rabbit (8548)	1.466	0.677

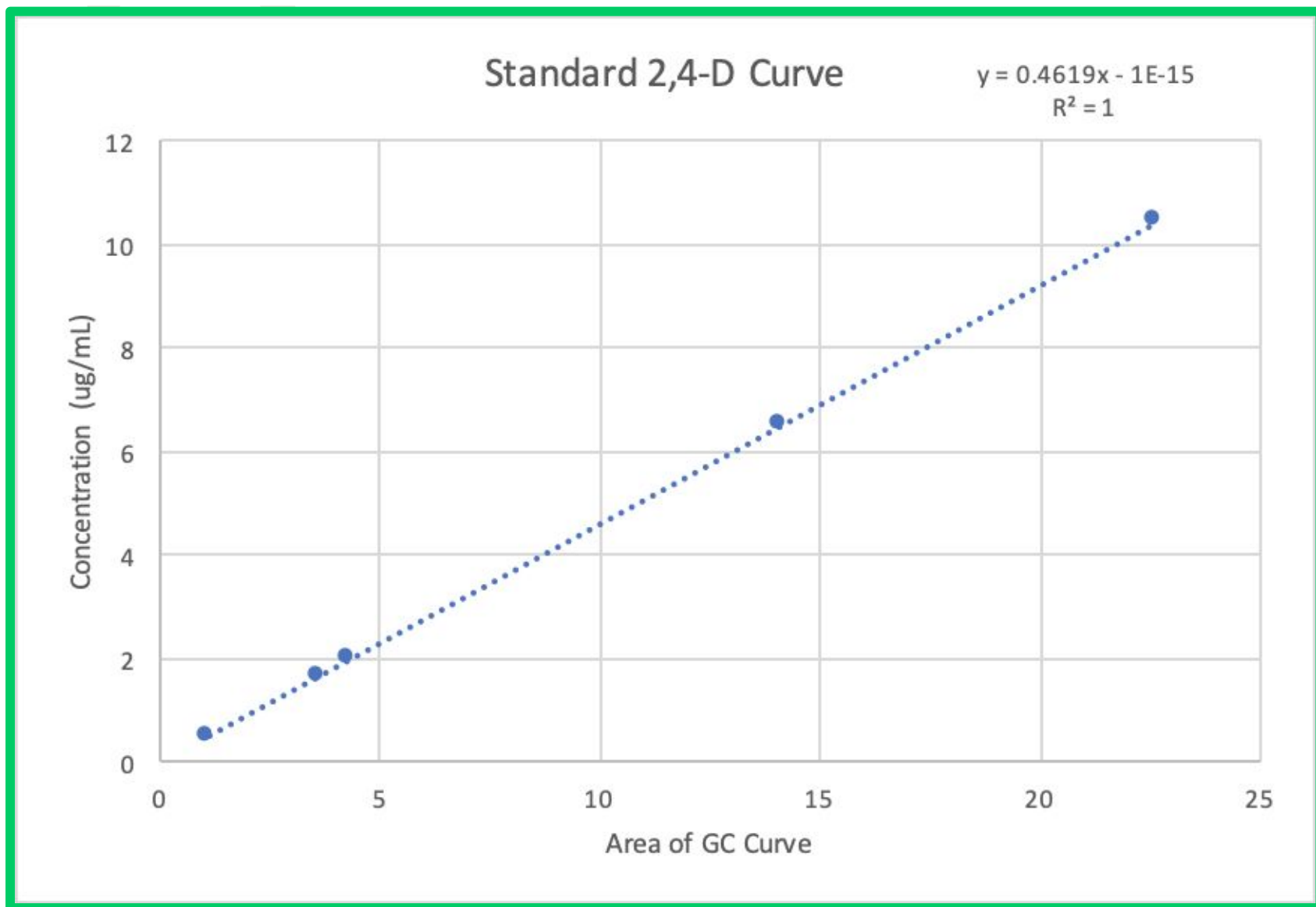


Figure 7. Standard curve of 2,4-D.

Results

Results of animal fur samples indicate a presence of 2,4-D in both Brush rabbit samples, and no presence of 2,4-D in the Grey fox or Deer mice.

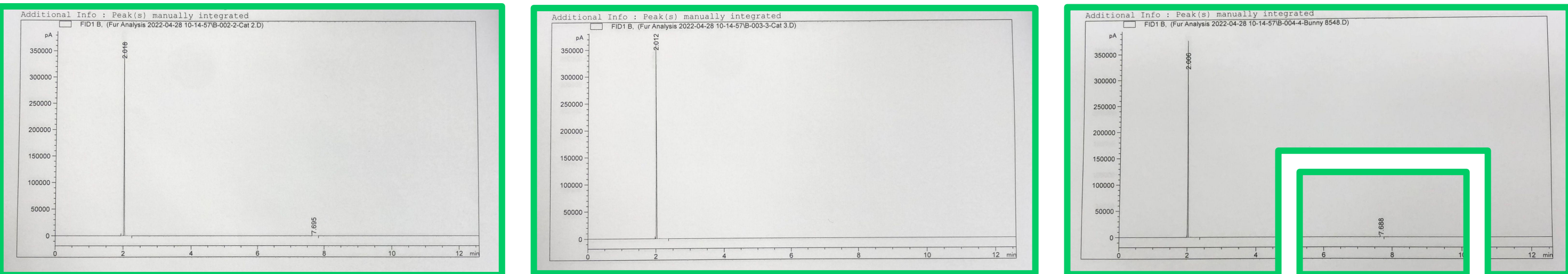
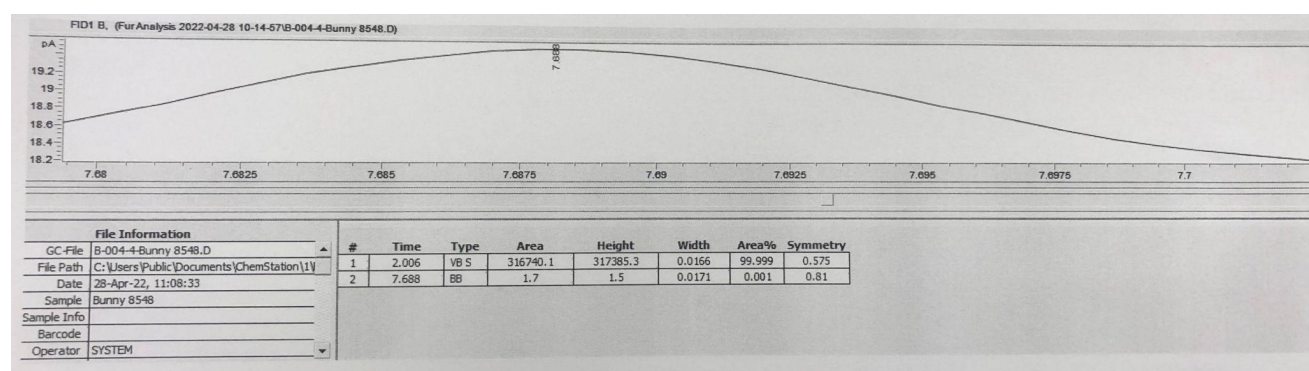


Figure 8. GC graph results. From left to right: positive control cat sample that was spiked with 2,4-D analyte, negative control cat sample that was not spiked, Brush rabbit sample with peak indicating positive results for 2,4-D.



Zoomed in 2,4-D peak

Please scan the QR code for an update on the current progression of our data analysis. Due to the continual growth and improvement of our work we wanted to provide you with the most up to date content. Thank you.

<https://kpd241.wixsite.com/fur-analysis-gc>

Conclusions

GC analysis of the standard samples provided no data at the expected region where 2,4-D presence would be indicated when analyzing samples in the phenoxy acid form. However, peaks were present in the data indicative of the solvent as well as the analyte (2,4-D) in esterified samples.

Results indicate that Rabbit samples obtained from Loleta and in Arcata near Glendale were positive for 2,4-D at concentrations of 0.677 and 0.755 ug/mL respectively.

Further testing with a wider range of samples, including other species, would be beneficial to yield diagnosable results.

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Dr. Jeffrey Schineller

References

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