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Abstract

2,4-dichlorophenoxyacetic acid, commonly known as 2,4-D, is a widely utilized pesticide 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.

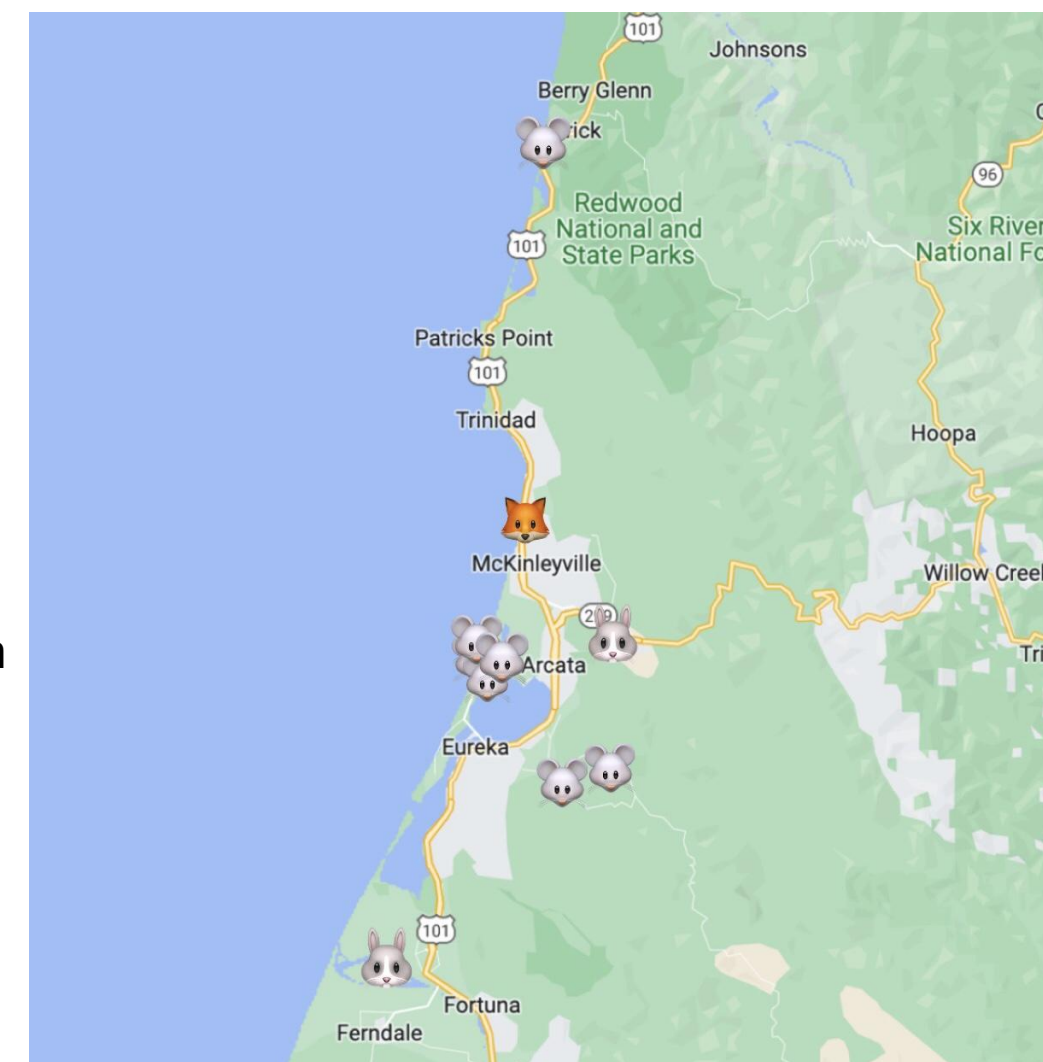


Figure 1. Localities of specimen

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.

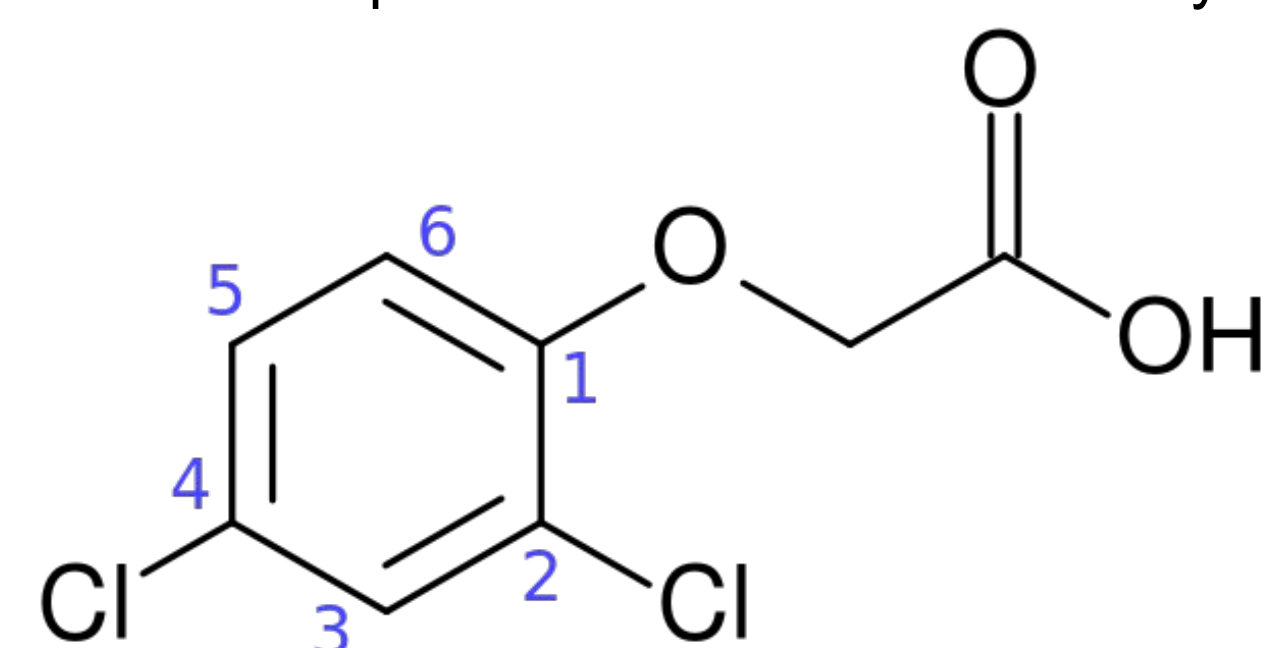


Figure 3. 2,4-dichlorophenoxyacetic acid (2,4-D) structure and commonly sold form

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. 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.

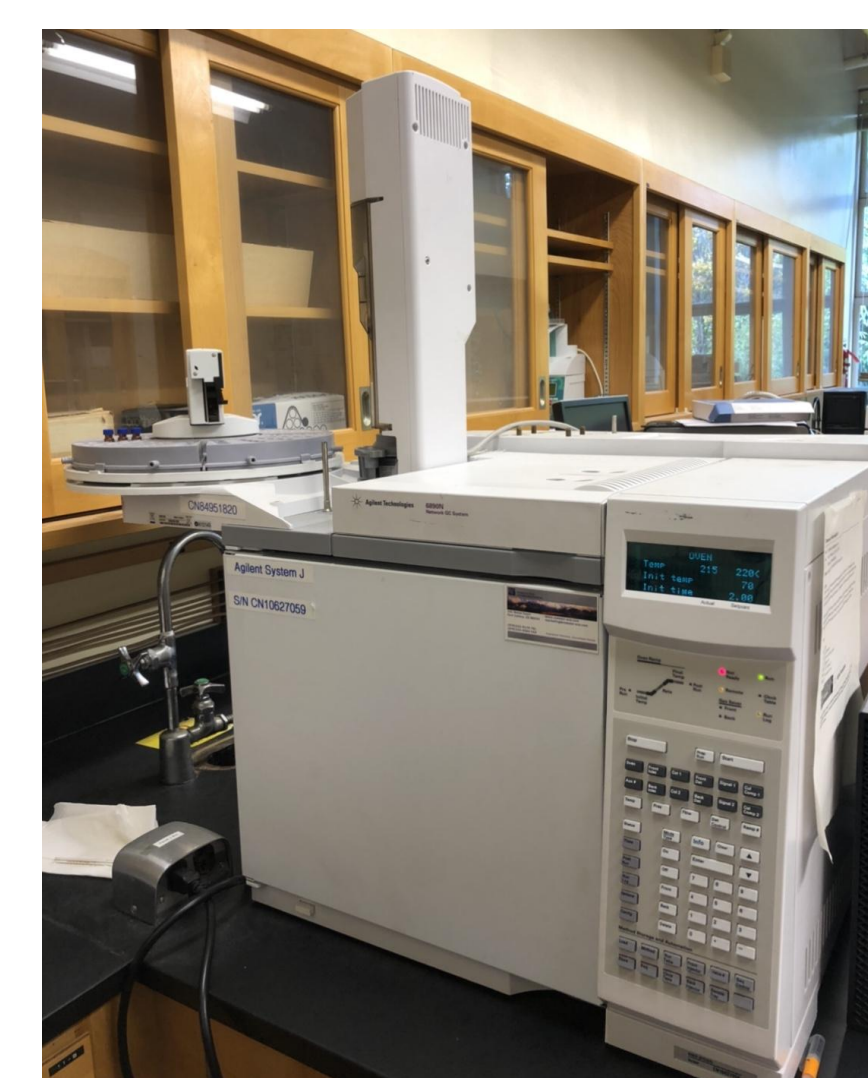


Figure 5. GC instrument

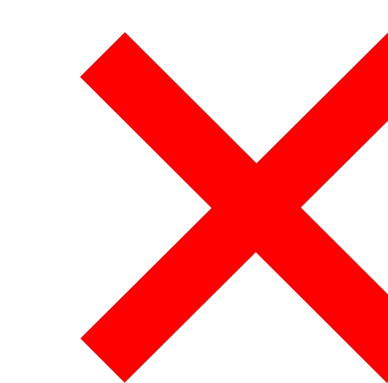
Results



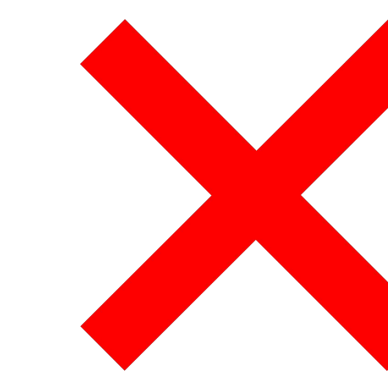
Please scan the QR code for an update on the current progression of our data analysis. Due to the continual growth and improvement of our work we wanted to provide you with the most up to date content. Thank you. <https://kpd241.wixsite.com/fur-analysis-gc>

Results

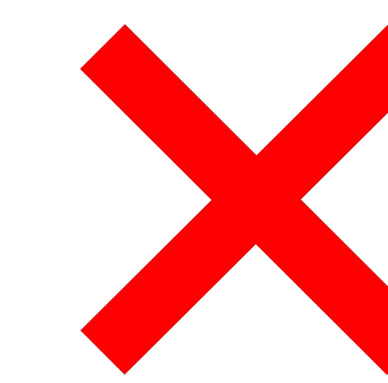
1 ug/mL 2,4-D standard



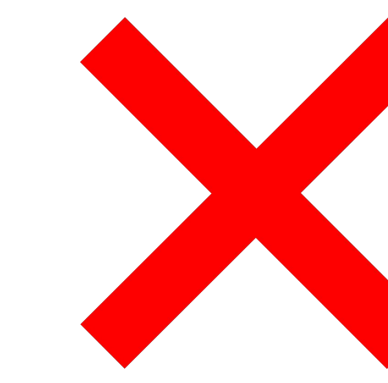
100 ug/mL 2,4-D standard



2,4-D standard sample with esterification procedure



Samples with slightly lower boiling points to 2,4-D



Samples with significantly lower boiling points to 2,4-D



Conclusions

GC analysis of the standard samples provided no data at the expected region where 2,4-D presence would be indicated. Peaks were only present in the data indicative of the solvent, and not the analyte (2,4-D). Further, changing temperature runs, retention times, and amount of sample injected into the machine, as well as running the sample after an esterification procedure, seemed to have no effect on the peaks.

Further testing would be beneficial to yield diagnosable results.

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References

1. 2,4-D. <https://www.atsdr.cdc.gov/toxsubstances/toxsubstance.cfm?id=104> (accessed Apr 24, 2022).
2. State of California. "2,4-Dichlorophenoxyacetic Acid (2,4-D)." <https://www.cdpr.ca.gov/Programs/OPA/Pages/NR2018-0001.aspx>
3. 2,4-D. <https://pubchem.ncbi.nlm.nih.gov/compound/24-Dichlorophenoxyacetic-Acid>
4. 2,4-D. <https://pubchem.ncbi.nlm.nih.gov/compound/24-Dichlorophenoxyacetic-Acid>
5. <https://pubchem.ncbi.nlm.nih.gov/compound/24-Dichlorophenoxyacetic-Acid>
6. <https://pubchem.ncbi.nlm.nih.gov/compound/24-Dichlorophenoxyacetic-Acid>
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