SPCB REPORT

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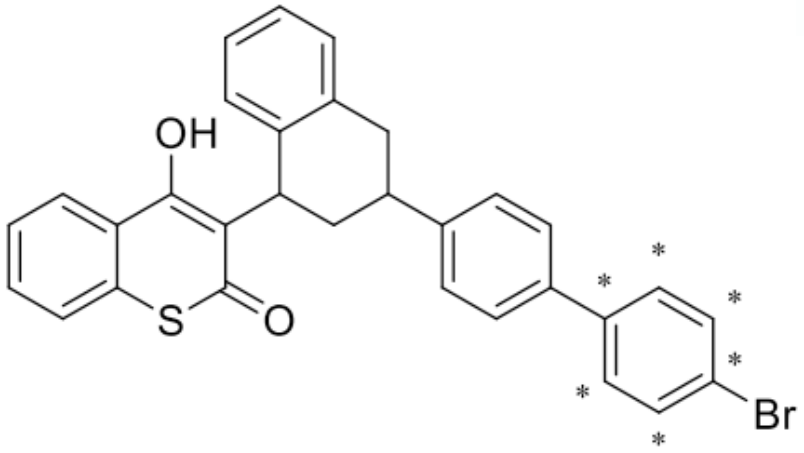
FINAL REPORT

Investigation of Rodenticide Pathways in an Urban System Through the Use of Isotopically Labelled Bait

01



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ABOUT OUR PROJECT

This study investigated developing an isotopically labelled bait that can be detectable through multiple trophic levels. Such a bait will allow us to trace exposure through the food chain. It will also help with the development of mitigation measures that are often proposed to limit exposure to rodenticides. The creation of such a product is critical for the development of data-driven mitigation measures and will allow for the testing of those already in place to assess whether they are effective at reducing exposure.

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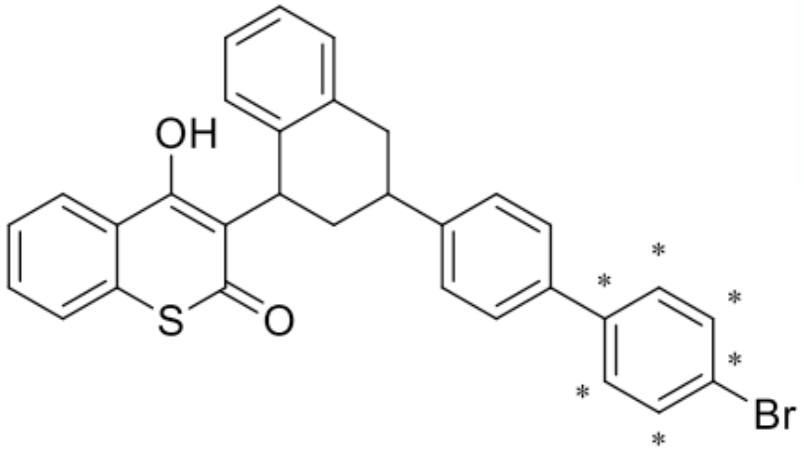
PHASES

The project involved multiple phases:

- Phase 1- Isotopically labelled bait design
- Phase 2- Labelling of rats
- Phase 3- Administering rats to captive coyotes
- Phase 4- Pilot field study and analyses of samples

03



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MAKING C13DFN

Our molecule was custom designed and manufactured
This technical was made into Difethialone (DFN) concentrate which was then made into the C13DFN rodenticide bait.

350mg of C13DFN

7500g rodenticide bait (25 ppm of C13DFN)

METHODS

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Rats labelled with C13DFN

The manufacturing of an isotopically labeled rodenticide had never been done before. We were very successful in the manufacture of this compound and the development of the bait by our research partners.
Rats were successful administered the isotopically labelled rodenticide (48 sublethally and 32 lethally). Lethally exposed rats were offered 40g of C13 DFN rodenticide bait and sublethally exposed rats were offered 4g of C13DFN rodenticide bait.

METHODS

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Captive coyote trial

Captive coyotes at USDA NWRC's Utah Field Station were divided into three separate groups based on feeding regimes that would replicate potential exposure scenarios in the field.

- Single
  - Coyotes fed one sublethally exposed rat
- Intermittent
  - Coyotes fed sublethally exposed rat on day 1, 3, 5, 7 and 10.
- Daily
  - Coyotes fed one sublethally exposed rat on day 1, 2, 3, 4, 5

Coyot ID	Sex	Rat	Exposure Schedule
1843	M	Whole	Single
1530	F	Ground	Single
1403	M	Ground	Intermittent
1410	F	Ground	Intermitent
1521	M	Ground	Daily
1440	F	Whole	Daily

METHODS

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Captive coyote trial

Post-exposure feces collection schedule

Coyotes in the **single-exposure group** had fecal samples collected for the 7 days post feeding.

For the **intermittent-exposure group**, feces collection commenced within 24-hr of the first feeding and continued every day during exposure period and for seven days after the last rat is consumed. Thus, scat was collected for 26 days.

For the **daily sequential-exposure group**, feces collection commenced within 24-hours of the first feeding and continued for all five days of the exposure period and for seven days after the last rat is consumed. Thus, scat was collected daily for up to 13 days.

Only coyotes 1843 and 1440 consumed whole rats. All other coyotes offered whole rats refused to consume any part of them. Therefore, rats were homogenized and mixed and offered to the remaining coyotes.

METHODS

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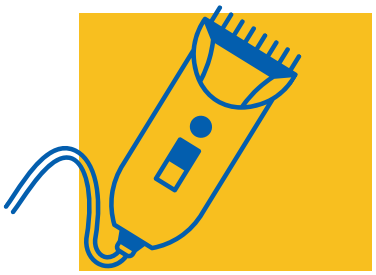

Captive coyote trial

Post-exposure hair collection schedule

Single exposure: Day 4, 7, 10, 13 and then every 2-4 weeks (pending tolerance of the animal to repeated capture and handling) for up to 9 months post-consumption or until the marker was no longer detected in at least two consecutive sampling periods, whichever occured first (and based on when lab results wer received, so that we recognized a lack of detection).

Intermittent exposure: Day 4, 7, 10, 13, 16, 19 and then every 2-4 weeks (pending tolerance of the animal to repeated capture and handling) for up to 9 months post-consumption or until the marker was no longer detected in at least two consecutive sampling periods, whichever occurred first (and based on when lab results were received, so that we recognized a lack of detection).

Daily sequential exposure: Day 4, 7, 10, 13 and then every 2-4 weeks (pending tolerance of the animal to repeated capture and handling) for up to 9 months post-consumption or until the marker was no longer detected in at least two consecutive sampling periods, whichever occurred first (and based on when lab results are received, so that we recognized a lack of detection).

METHODS

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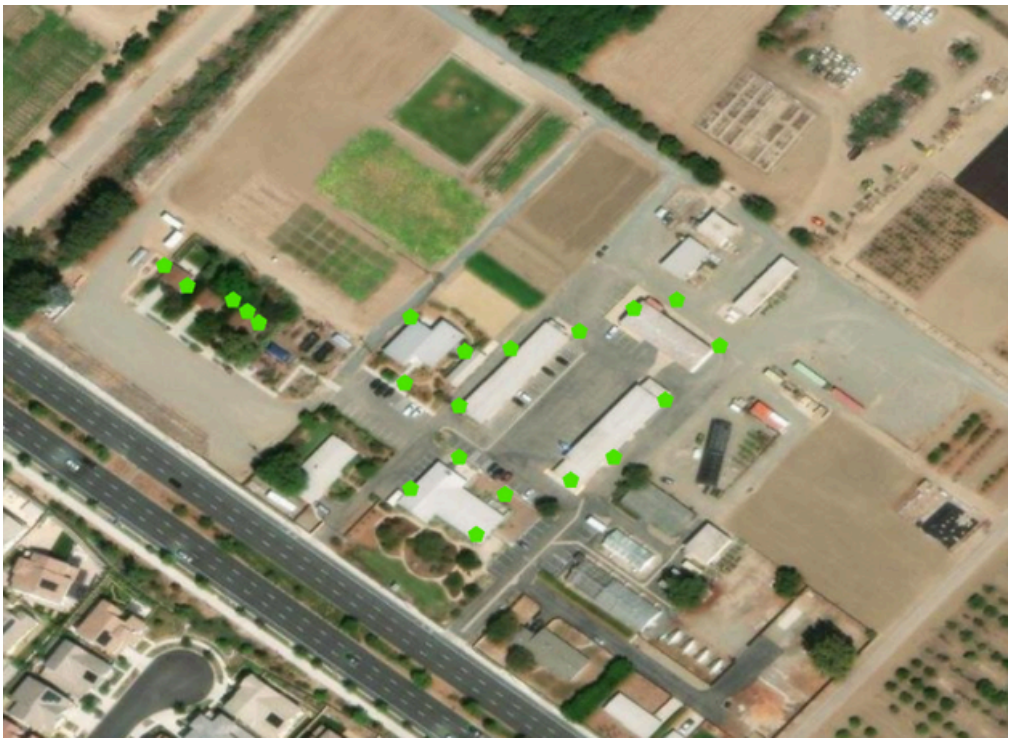
Field Trial

C13DFN rodenticide bait was placed in 21 bait stations that were spaced around buildings at the test site. C13DFN rodenticide bait was applied on 8/30/2022 and the bait stations were checked approximately every 30 days. C13DFN rodenticide bait consumption was monitored and any bait that was consumed was replaced until 6/28/23 when we stopped applying C13DFN. Scats were collected from the 100 acre property at least twice a week but sometimes up to three times a week from the time the bait was first applied until December 2023.



RESULTS

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Rats labelled with C13DFN

Lethal Exposure

In this group rats ate between 8.8 g-23.8 g of C13DFN rodenticide bait. The average amount of bait consumed by rats was 16.6g. All rats died by Day 10.

Sublethal Exposure

Rats in this group consumed between 0g and 4.1 g of C13DFN rodenticide bait with an average consumption of 3.81 g

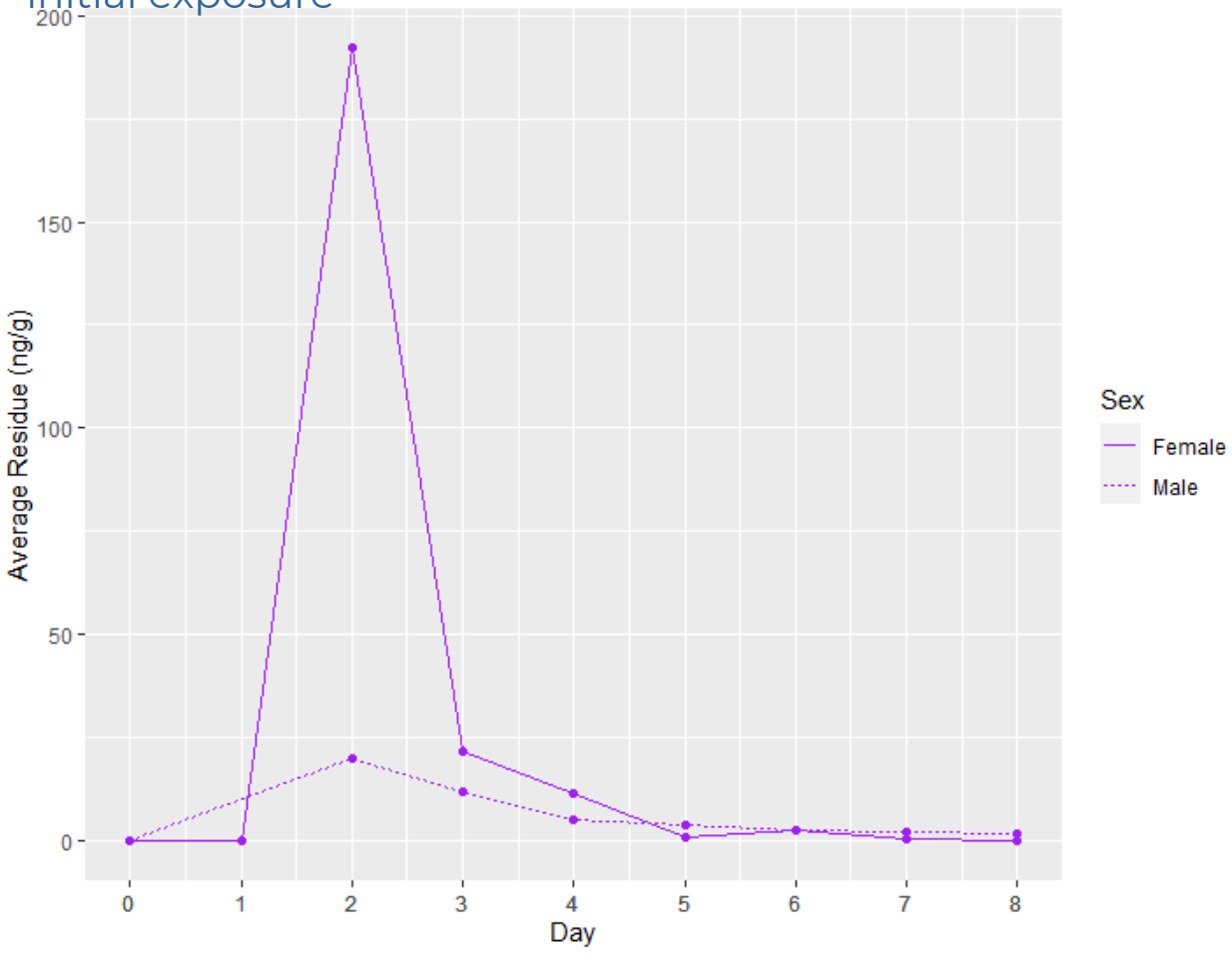
Six percent of our sublethally dosed rats succumbed to their exposure to the C13DFN rodenticide bait. All of the remaining rats were humanely euthanized by day 10.

RESULTS

Captive coyote trial

Single Exposure Group

C13DFN was detected in the female coyote's feces at day 2. It peaked on this day at 192 ng/g followed by a sharp drop off to 21.8 ng/g. By Day 8, C13DFN was no longer detected in the female coyote's feces. II
n the male coyote's feced, C13DFN was detected at Day 2 where it peaked at 20.2 ng/g. This was also followed by a decline. By day 8 the C13 DFN was still detected in the feces of the male coyote at 1.94 ng/g. It appears that the female and male excretion profiles differ significantly here, however, no feces were collected after the initial exposure

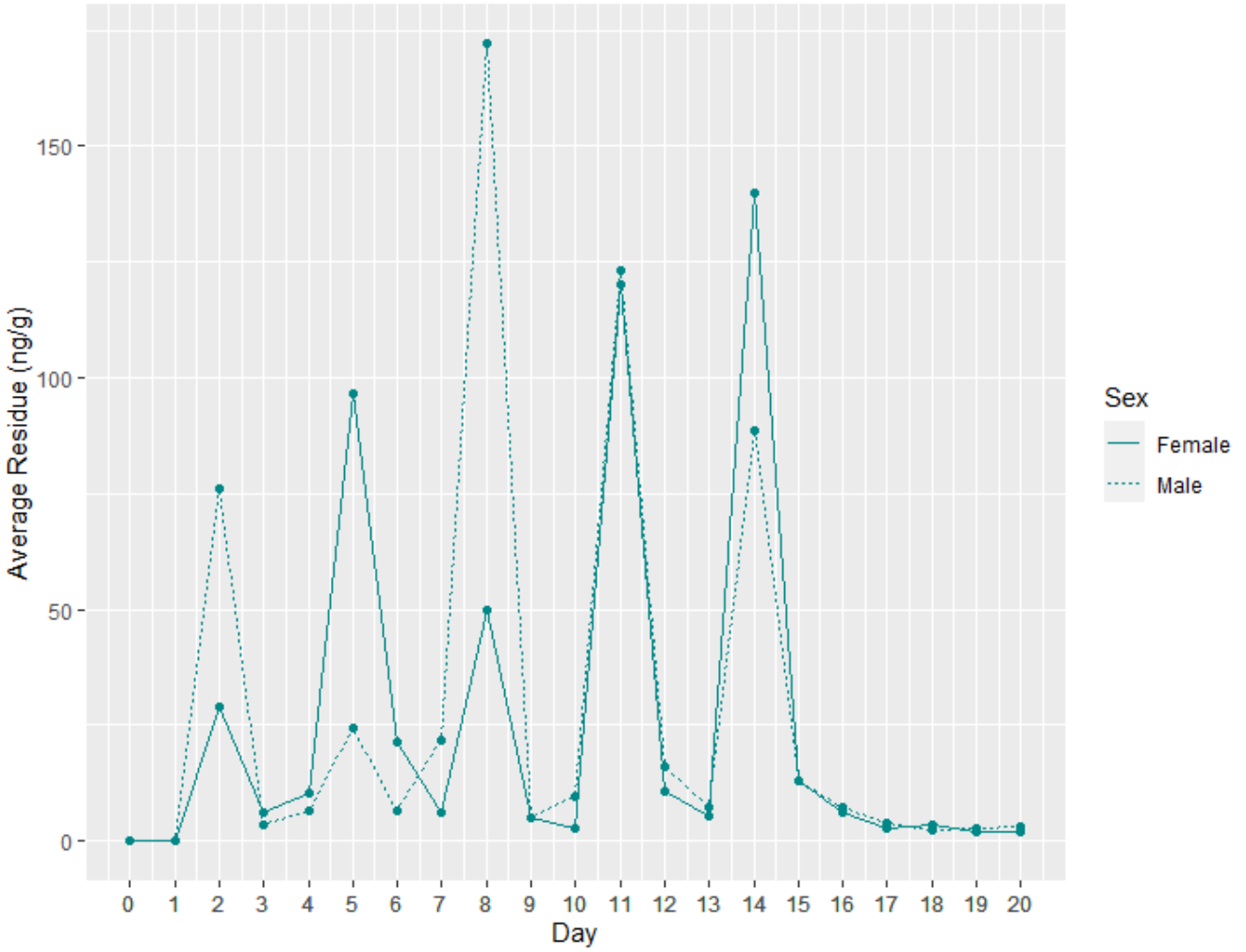


RESULTS

Captive coyote trial

Intermittent Exposure Group

C13DFN was detected in both the female and male coyote's feces at day 2 and peaked after every subsequent exposure. The excretion peaks for males and females occurred at the same time post exposure, however, their magnitude differed. After the final exposure, the presence of C13DFN declined, and was as low as 2.2 ng/g for the male coyote and 3.33 ng/g for the female coyote.

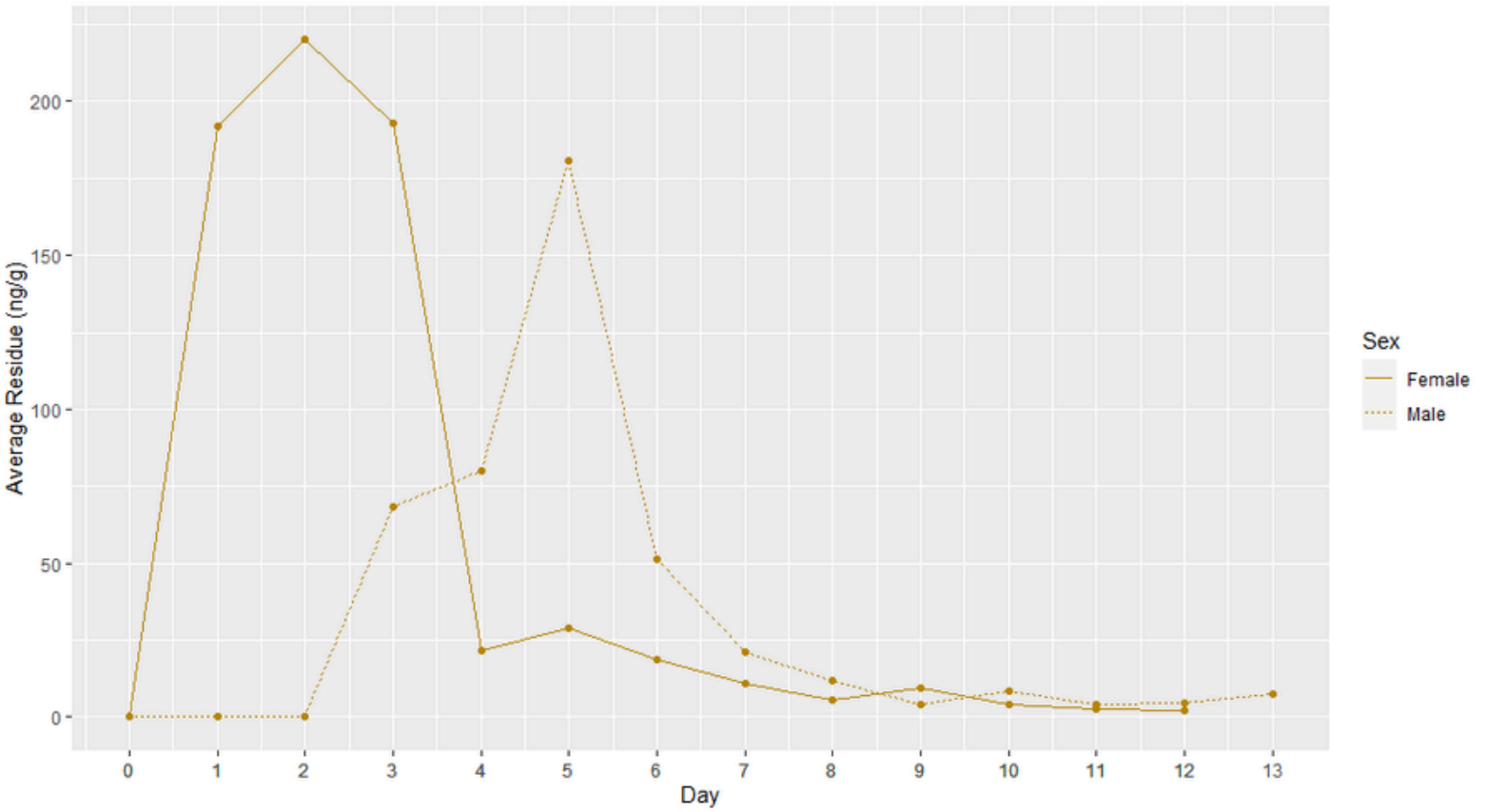


RESULTS

Captive coyote trial

Daily Exposure Group

C13DFN was detected in the female coyote's feces at day 1, peaked at day 2 and began to decline there after. By day 12, the residue was at its lowest at 2.31 ng/g. C13DFN was not detected in the male coyote's feces until day 3, peaked on day 5 and then continued to decline. By day 13, the residue level detected was 7.47 ng/g



RESULTS

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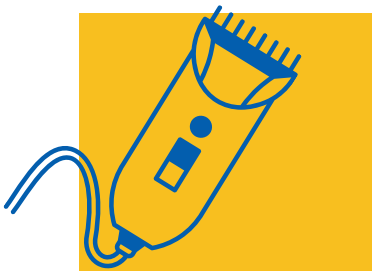

Captive coyote trial

Post-exposure hair collection

Two coyotes were discovered to have been exposed to warfarin and diphacinone rodenticide when hair samples were collected on Day 0. Since there was a delay in receiving the sample analyses, we did not know this until the animals had already been exposed to the C13DFN-exposed rats. Analyses of hair, yeilded only one positive detection of C13DFN from the female in the single exposure group. The detection was 71 days after first exposure.

RESULTS

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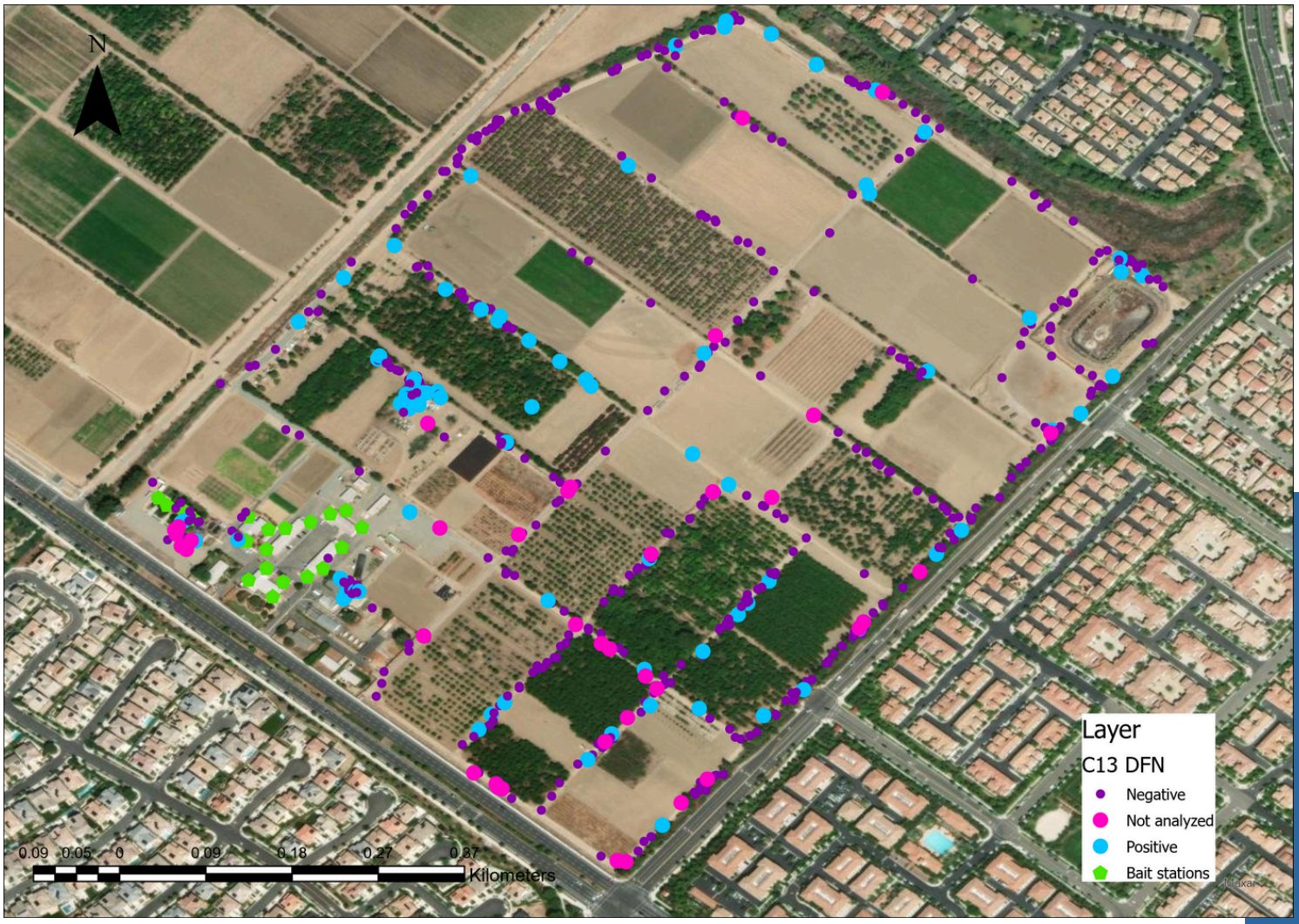
Field study

623 scats were collected and analyzed for C13DFN. We have detected C13DFN in 99 scats. The first detection was 14 days after the C13DFN was applied.

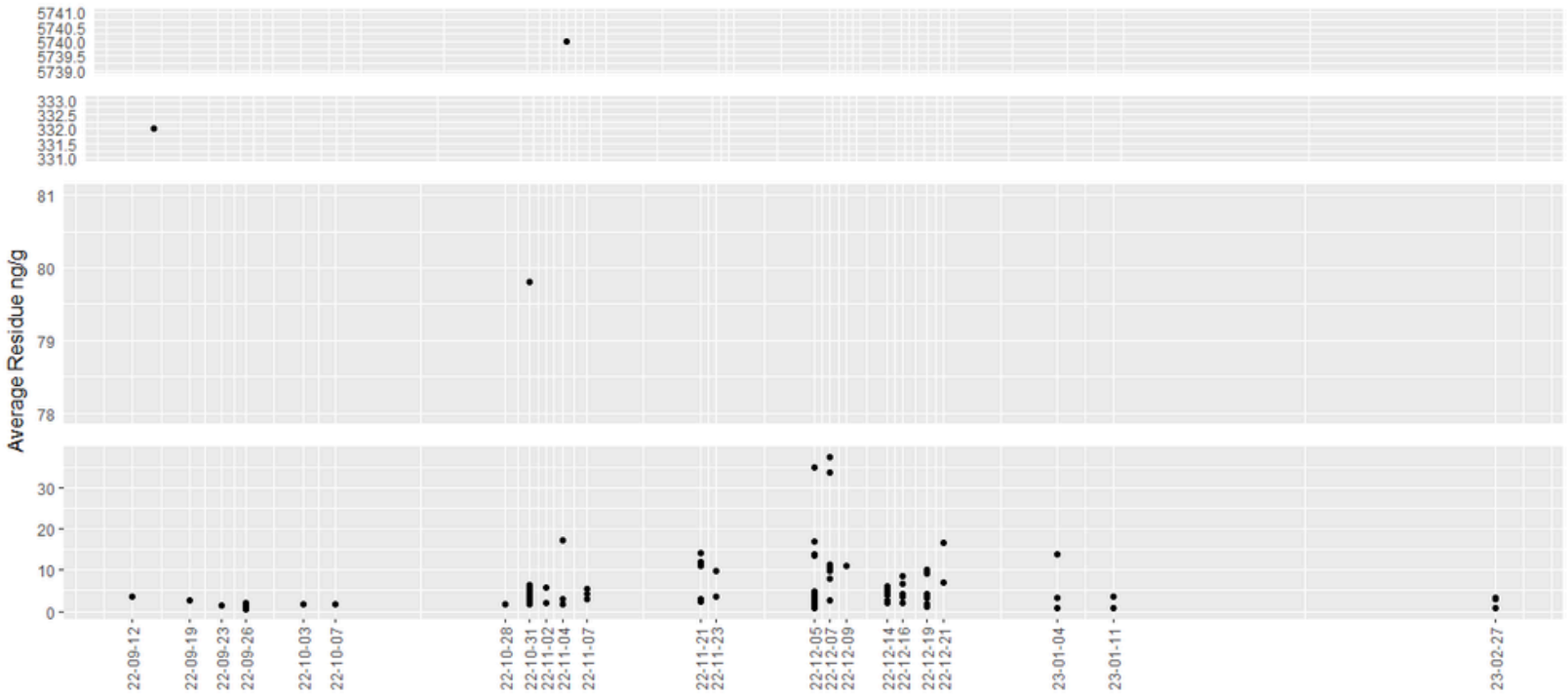


RESULTS

Field study

Residues of C13DFN were detected in coyote feces in September, October, November, December 2022 and January and February 2023 There were no detections between March and July or November and December 2023.

Residue levels were between 0-5740 ng/g. The majority of positive scats recovered had very low levels of residues with 93% of residues below 17.2 ng/g.



RESULTS

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Field study

Bait consumption from each station varied from 0 packets removed to 6 packets removed. The week had a significant effect on the consumption of bait (Kruskal-Wallis chi-squared = 232.54, df = 20, p-value < 2.2e-16). Twice as much bait was consumed between weeks 31 and 38 compared with weeks 1-16. The location of the bait station also had a significant impact on the amount of bait consumed (Kruskal-Wallis chi-squared = 122.21, df = 5, p-value < 2.2e-16).

Location	Mean no. of packets consumed
Barn	1.97
Annex	1.59
EFNEP	1.38
Landscape	1.15
Breezeway	0.769
Main Office	0.25

RESULTS

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Field study

Location of bait stations at study site

RESULTS

Field study

Bait station with consumed C13DFN rodenticide bait in it

DISCUSSION

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Studies on rodenticide exposure have focused on the recovery of information from dead, dying or sick individuals. This is likely an inaccurate picture of what is occurring on the landscape. However, tools to detect exposure in live individuals were unavailable unless animals were live captured and blood was drawn. This process requires extensive skill, often requires state and federal permits and often sample sizes are small. Without tools to accurately assess the pathways of rodenticide from the source of application, it is difficult to identify effective mitigation measures. These tools also very importantly allow for the assessment of mitigation measures, either prior to their implementation, or post implementation to monitor their success. This is the first time that a rodenticide has been isotopically labelled with the aim of trying to track it up the food chain. The process was very successful and achieved the marking of the primary consumer and then the secondary consumer.

DISCUSSION

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The detection of C13DFN in coyotes after it was legally applied in the field provides a lot of information. Firstly, it provides evidence that legally applied anticoagulant rodenticide is being moved up the food chain by the roof rat, proving that rats are a major part of the pathway of rodenticide exposure in urban environments. This is a really important piece of knowledge that should inform all future mitigation measures. Secondly, it also shows that secondary consumers will be exposed to legally applied anticoagulant rodenticides.

The C13DFN was detected 14 days after the application of the product. This is particularly important information disproving the myth that reduction in the time that a product is applied reduces the likelihood of exposure. It is even possible that reducing the time that a rodenticide is applied could lead to incomplete rodent management and in fact lead to more rats that could be exposed and increase the amount of exposure up the food chain.

DISCUSSION

The residues detected in the scats from the animals in the field study were much lower than those that were exposed in captivity. There could be several reasons why these values are different:

1. Detections were many days after the exposure event
2. Rats consumed very small amount of bait
3. Rats comprise a small percentage of the diet of coyotes at this site
4. The pharmacokinetics of how wild coyotes in southern California absorb, distribute, metabolize, and/or excrete anticoagulant rodenticides are different from those of the captive pack in Utah

DISCUSSION

As an educator of applicators, I would be considered to be a highly educated pesticide applicator. The fact that rodenticide that I applied legally was detected at all, demonstrates that it is likely that education will not reduce the risk of rodenticide exposure for the majority of applicators.

C13DFN was also detected at the furthest extent of the range of scat collection. This shows that even though the bait was applied directly adjacent to buildings, that the exposure is detected much further afield. Reducing the distance that bait can be applied from a building may have no impact on rodenticide moving through the food chain. The furthest detection was approximately 900 m from the point of application. This also demonstrates that the imposition of a 2500 ft (762 m)buffer from wildlife habitat areas to prevent exposure is unlikely to be successful

DISCUSSION

The conception and processes involved in achieving the success of this project was complicated and plagued with problems. Initially, we had significant delays with getting ethical approval from the university which is a requirement. However, once that was acquired, both phases 1 and 2 were swiftly completed. There were some brief issues with flooding and getting some of our samples analyzed at the NWRC Research Facility in Colorado. Phase 3 also faced some issues. Covid 19 delayed the start. The coyotes were extremely uncooperative and did not feed on the rats. This required a lot of trouble shooting and also a trip to the NWRC Research Facility in Utah in order to make the rats into meatballs which were successful accepted by the coyotes. However, a freezer failure caused the loss of all the lethally exposed rats. Phase 4 was successfully completed. We collected and analyzed 623 scats for the presence of C13DFN.

In future studies, the development of an isotopically-labelled anticoagulant rodenticide should would not encounter the same challenges, ethical approvals or equipment malfunctions.

MEET THE TEAM

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