DEPARTMENT OF NATURAL RESOURCES

Assessing Neonicotinoids Exposure in Wild Deer in Minnesota: Pilot Study Update, 24-January, 2021

Recent research conducted in South Dakota has raised concerns about potential adverse effects of neonicotinoid exposure in captive white-tailed deer including reduced survival and productivity. To assess whether free-ranging deer in Minnesota are exposed to neonicotinoids (neonics), we began a pilot study with the following 3 objectives:

1) Assess the feasibility of working with deer hunters to obtain biological samples from free-ranging white-tailed deer for disease screening and collecting relevant metadata for those samples.
2) Estimate exposure of neonics in white-tailed deer across different regions of Minnesota.
3) If high levels (>0.33 ng/g) of neonics are discovered in white-tailed deer, provide a basis to direct future research on potential impacts of neonic exposure on fawn survival and recruitment.

Recap of fall 2019 spleen collection efforts

- We initiated a "Citizen-Science" based approach to collect spleens from hunter-harvested deer across the state during the fall 2019 hunting season.
    - Deer permit areas (DPAs) were categorized based on estimated use intensity of agricultural products commonly treated with neonics, specifically corn, soybeans and wheat. Our goal was to collect 800 spleens total, with half our samples originating from DPAs with heavy crop coverage (≥66%), and the remainder equally split between moderate (33-65%) and little/no use (<33%) crop coverage.
    - Interested hunters (n=1,836) volunteered to receive a sampling kit in the mail and reported on the sex, age, date of harvest, and kill location (deer permit area).
    - 770 spleens and 517 tooth samples were submitted by hunters throughout the state and an additional 29 spleens were collected by DNR staff from opportunistic deer (agency culling and sick deer). Total spleens collected = 799.

- Submitted spleen samples did not reflect our original sampling design and was heavily weighted toward the little/no use crop coverage DPAs (n=497, 62%), followed by the moderate use (n=220, 28%) and heavy use (n=81, 10%) DPAs (Fig 1). The number of spleens collected from each DPA is displayed in Figure 1. This was likely due to the greater proportion of our deer harvest coming from our forest and transition zones.

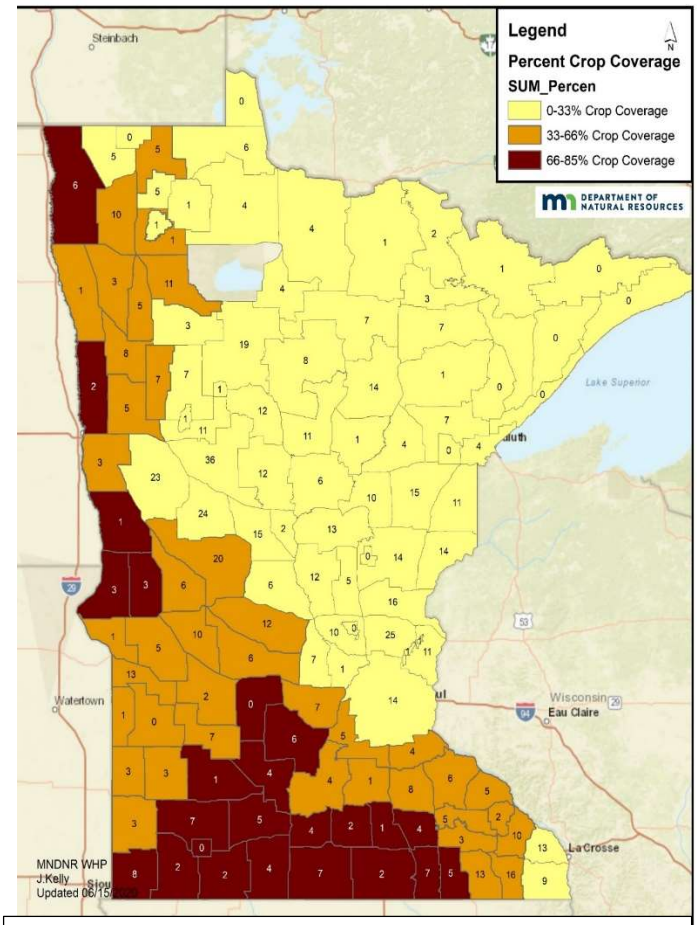

Figure 1. Deer spleens collected by DPA, fall 2019.

Results of the ELISA Screening Test

In spring 2020, spleen samples were sent to Ecdysis Foundation Laboratory (Estelline, SD) to test for the presence of imidacloprid, one of the most commonly used neonics, using enzyme-linked immunosorbent assay (ELISA). This screening test is inexpensive ($25/sample) and generated results directly comparable to the SD captive deer study, which used the same test from the same lab. However, concern was raised by the agricultural industry that the ELISA was not a valid test for neonics, and that our findings may be not usable. Thus, we planned to send a subset of spleen samples (up to 100) from the ELISA screening to Michigan State University (MSU) Veterinary Diagnostic Laboratory (Lansing, MI) to test for neonics using mass spectrometry, which can differentiate between multiple neonic compounds, but is more expensive ($100/sample).

ELISA test results had been expected by late summer 2020; however, due to the Covid-19 pandemic, the lab was closed over the summer and had reduced capacity through the fall. The preliminary results for MN's samples were received in mid-December 2020.

Primary Findings

ELISA results indicate that **≥61% of deer spleens had exposure to neonics**. The ELISA test kit is specific to imidacloprid, but it may cross-react with other neonics (such as clothianidin, which has very similar chemical composition) and we are not sure how this may influence the signal. Thus, while the initial ELISA results demonstrate exposure in most spleen samples, the exact magnitude of those values may differ if cross-reaction were happening. The reason we sent the subset of samples to MSU for mass spectrometry is to gain confidence as what compounds are being picked up by the ELISA, and to ensure our interpretation is accurate.

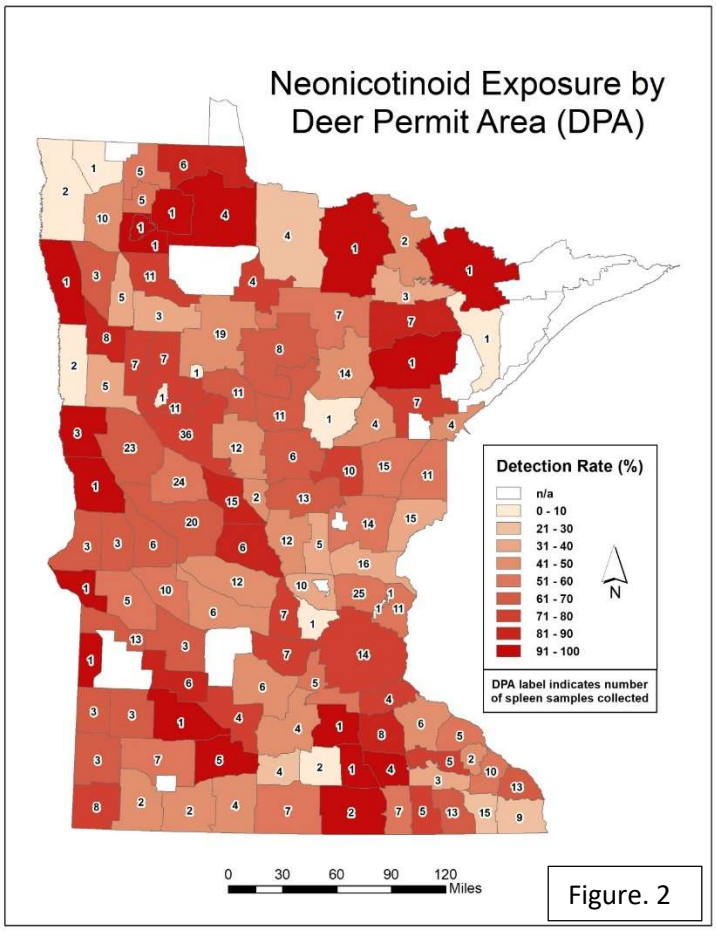

Figure. 2

- Of the 61% of sampled deer (n=482) that were exposed to neonics, **29% (n=140) had concentrations high enough (>0.33 ng/gm) to adversely affect fawn survival, according to SD study**; 26% (n=125) were classified as medium (0.165-0.33 ng/gm) and 45% (n=217) were classified as low (>0-0.165 ng/gm) exposures.
- **Deer harvested from all agricultural use categories, including the Forest Zone, were exposed to neonics** (Fig. 2)
- 39% of deer sampled were not exposed to neonics.
- Our sample included representation of both sexes (65% males, 35% female) and all age classes (70% adults, 24% yearlings, and 6% fawns); likelihood of exposure to neonic was similar among all age/sex cohorts

Preliminary Results from Mass Spectrometry at MSU

In late-December, we submitted at subset of 57 spleen samples to MSU to test for exposure to neonics with mass spectrometry. This included a mix of exposure levels from the ELISA results; these data were blinded from MSU staff. On 19-January, I was contacted by Dr. Justin Zyskowski from MSU, whom had begun processing the first 8 samples from our submission and was concerned because he

was finding evidence of neonics in each sample, and wanted to ensure we provided samples with zero exposure from the ELISA analysis. I provided him two sample IDs from our ELISA dataset with zero exposure and two high exposure (>3.0 ng/gm) for him to analyze; again he found evidence of neonics in all 4 samples. To further discuss how to proceed with the testing, we set up a conference call for 19-January between MSU staff and myself, Eric Michel, and Patrick Hagen.

During this call, Dr. John Buchweitz, Section Chief of Toxicology at MSU, explained that their mass spectrometry test can pick up the chemical signature for 9 neonic compounds and their metabolites. Since the ELISA test was focused solely on imidicloprid, it is likely that deer in our dataset were exposed to >1 neonic compound. Further, Dr. Buchweitz explained that it would be extremely unlikely that the parent neonic compound, such as imidicloprid, would be present in the spleen of a deer, as the body would break down the parent compound (primarily in the liver, but the breakdown would start in the gut), leaving only breakdown products or metabolites that would accumulate in the spleen. However, they are able to identify and differentiate the metabolites to build the panel backwards and identify the parent compound; this process will take more time and required the development of known standards using spleen tissue. Together, we identified the following next steps:

1) MSU would run all 57 spleen samples to test for existence of the parent compounds for imidacloprid, clothianidin, and thiamethoxam (the most commonly used neonics in MN). This would provide us a quantitative result for the presence of each compound. **COMPLETED**
2) MSU would run all 57 spleen samples for evidence of 19 metabolites for these 3 parent compounds and provide a qualitative result (presence or absence) for each. **COMPLETED**
3) If metabolites are detected, MSU will create a panel for these compounds against known standards to be able to quantify the amount of neonic present in each sample. These data will then offer a direct comparison to the exposure levels indicated by the ELISA results. **In Process (will take ~ 2 months)**

On 21-January, we received the following statement from Dr. John Buchweitz from MSU:

- "Liquid chromatography tandem quadrupole mass spectrometry analysis of 57 deer spleen samples submitted to the Michigan State University Veterinary Diagnostic Laboratory did not reveal spectral confirmation of the neonicotinoid pesticides clothianidin, imidacloprid, or thiamethoxam in their native form to a limit of quantitation of 0.1 ppb (ng/g). Spectral analysis did, however, reveal **preliminary evidence to suggest the presence of several neonicotinoid breakdown products and possible metabolites in <u>every sample</u>**. These findings are currently under investigation by the laboratory and awaiting confirmatory analysis."

It is encouraging that the preliminary MSU results <u>support the ELISA findings</u> that neonics are present in the deer spleen samples and further suggests that the 61% estimate may be a minimum exposure level, and it could be quite higher.