PFAS levels in Michigan Deer from the Oscoda Area, Iosco County

Michigan Department of Health and Human Services

Division of Environmental Health

Michigan Fish Consumption Advisory Program

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Summary

In October of 2018, the Michigan Department of Health and Human Services (MDHHS) and Michigan Department of Natural Resources (MDNR) issued a ”Do Not Eat” advisory for deer harvested by hunting within five miles of Clark’s Marsh in Oscoda Township. The advisory was due to high levels of per- and polyfluoroalkyl substances (PFAS), specifically perfluorooctane sulfonate (PFOS), found in deer taken within five miles of the Marsh. The five-mile range was selected to be protective based on deer behavior and not based on specific PFAS results in the deer muscle and liver samples.

In 2020, an additional 44 deer were collected from around Clark’s Marsh and had muscle and liver tested for PFAS. Deer from the 2020 collection also had detectable levels of various PFAS, including PFOS. These deer samples, in addition to those collected in 2018, allowed analyses that were not possible with the limited 2018 data. The new data analysis identifies an apparent relationship between detections of PFOS in liver samples and the collection location distance from Clark’s Marsh. This relationship identifies an increasing likelihood of PFOS being detected in liver samples the closer the deer is to Clark’s Marsh. An additional 22 muscle samples from hunter-submitted deer during the regular hunting season were also tested for PFAS in 2019. No PFAS were detected in those muscle samples.

Based on this new information, MDHHS is issuing an update to the “Do Not Eat” advisory area to be for deer taken within three miles of Clark’s Marsh. The updated advisory area can be described as:

Oscoda Township East (T24N, R09E) Sections: 17, 18, 19, 20, 21, 27, 28, 29, 30, 31, 32, 33, and 34

AuSable Township (T23N, R09E) Sections: 4, 5, 6, 7, and 8

Wilbur Township (T23N, R08E) Sections: 1, 2, and 12

Oscoda Township West (T24N, R08E) Sections: 13, 14, 23, 24, 25, 26, 27, 34, 35, and 36

MDHHS continues to recommend not eating kidneys or liver from any deer statewide because many chemicals, including PFAS, can accumulate in these organs.

Purpose and Background

This report provides updates on the white-tailed deer (deer) tissue PFAS levels measured in the Oscoda area in Iosco County and the existing "Do Not Eat" advisory for the area. In October 2018, PFOS levels found in muscle and organ samples from one Oscoda area deer were highly elevated. Due to these levels, MDHHS, in collaboration with MDNR, issued a "Do Not Eat" advisory for deer harvested within five miles of Clark's Marsh. This report summarizes the additional deer tissue data collected, through voluntary hunter-submitted deer heads and a targeted deer collection, since the advisory was issued in 2018.

Iosco County surface water PFAS levels

Military activities at the former Wurtsmith Air Force Base (WAFB) began in 1923. The base is located in Oscoda, Iosco County, Michigan. In 1993, the base closed and portions have been turned over to the Oscoda Airport Authority for reuse as an industrial park and airfield. The 5,221-acre site is bounded by Van Etten Lake to the north and east, Oscoda and Au Sable Townships to the east and south, the Huron National Forest (including wetlands associated with the Au Sable River) to the south, and the Au Sable State Forest to the north and west. Lake Huron is less than one mile east of the site (Figure 1).

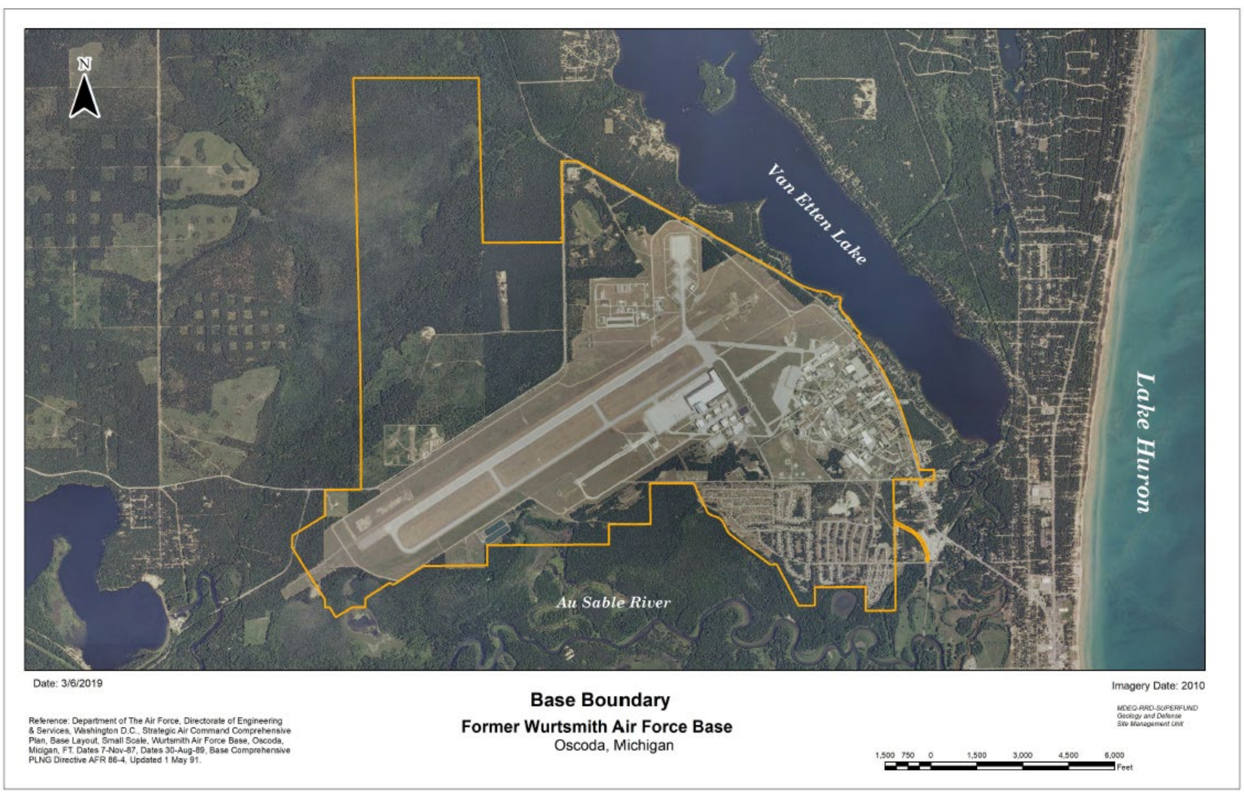

Figure 1: Map of the Former Wurtsmith Air Force Base and surrounding area (taken from https://www.michigan.gov/documents/pfasresponse/MAP_Wurtsmith_650201_7.pdf)

There are two former fire-training (FT) sites at WAFB. FT-01 is located on the northeast end of the runway and was used from 1951 to 1958. FT-02, located at the southwest end of the base near Clark's Marsh (which is north of the Au Sable River) was used from the 1950s to the early 1990s. PFOS-based aqueous film-forming foams (AFFF) were likely used in this area beginning in the 1970s. Data collected by the Michigan Department of Environment, Great Lakes, and Energy (EGLE) and others have shown that PFOS and other perfluorinated chemicals have contaminated this area, leached through the sandy soil into the groundwater, and migrated into the surface water and sediments in the ponds at Clark's Marsh. Thirteen PFAS were found at varying levels in the most recent surface water sampling results for select PFAS at Clark's Marsh (Table 1; EGLE 2020).[1] PFAS have also been found in other nearby water bodies, including Van Etten Lake, the Au Sable River, and Allen Lake (MDHHS 2017).

Table 1: Range of PFAS in Clark's Marsh surface water in nanograms per Liter (ng/L or parts per trillion [ppt]) in 2018 (EGLE 2020).

PFAS	Surface water PFAS levels (ng/L)*
Perfluoroheptanesulfonic acid (PFHpS)	3.89-19.20
Perfluorobutanesulfonic acid (PFBS)	2.31-8.43
Perfluorobutanoic acid (PFBA)	16.60-33.30
Perfluorodecanoic acid (PFDA)	ND
Perfluoroheptanoic acid (PFHpA)	10.80-48.80
Perfluorohexane sulfonate (PFHxS)	106.00-478.00
Perfluorohexanoic acid (PFHxA)	37.10-131.00
Perfluoropentane sulfonic acid (PFPeS)	2.24-12.60
Perfluorononanoic acid (PFNA)	1.60-8.86
Perfluorooctane sulfonamide (PFOSA)	ND – 5.31
Perfluorooctane sulfonate (PFOS)	83.00-1,410.00
Perfluorooctanoic acid (PFOA)	30.80-145.00
Perfluoropentanoic acid (PFPeA)	48.70-110.00
Perfluorotetradecanoic acid (PFTeDA)	ND
Perfluoroheptane sulfonic acid (PFHpS)	3.95-19.20

* = ND indicates that the PFAS was not detected.

Deer tissue sample collection and testing

In April 2018, 20 deer were collected by the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), in the Oscoda area. Samples of muscle tissue, liver, kidney, and fat were tested for PFAS (MDHHS 2019). Because elevated levels of PFOS were identified in multiple muscle and organ samples from one deer along with detections of PFOS in additional deer, additional deer collections were planned. In fall 2019, MDNR and MDHHS asked hunters who harvested deer near Clark's Marsh to submit deer heads for PFAS testing along with the standard disease testing. These individuals hunted on land within five miles of Clark's Marsh. Twenty-two deer heads were submitted to MDNR from this area. Only samples of muscle tissue were available for PFAS testing and specific

[1] 2018 data can be found at https://www.michigan.gov/pfasresponse/0,9038,7-365-86511_82704_83952-455897--,00.html. Sampling results from Clark's Marsh are also available upon request for the years 2011, 2012, 2014, 2016 and 2017.

geographic coordinates were not available for these samples. Because the location-specific information was not available, these deer tissue results could not be included in the spatially-based statistical analyses (described later) used for evaluating modification of the consumption guideline area. Nevertheless, the information was useful for understanding the overall potential for PFOS to be found muscle tissue in deer in this area.

These hunters submitted deer heads to the MDNR at the local deer check stations for CWD testing and for PFAS testing. MDNR Wildlife Disease Lab staff collected approximately 100 to 200 grams (g) of available muscle tissue from the submitted deer heads. Tissue samples were placed in sealable plastic bags and labeled. All samples were frozen until processed for analysis at the MDHHS Analytical Chemistry Laboratory.

In March 2020, APHIS sharpshooters harvested deer and collected tissue samples through an inter-agency agreement with MDNR and funding provided to MDNR through MDHHS. Protocols for tissue sampling were standardized in alignment with those used in the MDNR Wildlife Disease Laboratory.

APHIS collected 44 deer (age 0.5 years and older; 14 male, 30 female) within five miles of Clark's Marsh in 2020. Collection efforts were planned to collect deer as close as possible to the marsh due to the deer with elevated PFOS levels in 2018 that was collected in very close proximity to the marsh. Therefore, APHIS focused on the five-mile advisory area around the marsh; with some deer being taken within two miles of the marsh. It is possible that deer collected in the spring had stayed in the area throughout the winter and may have potentially been exposed to PFAS by drinking contaminated marsh surface water. The following tissue samples were collected:

- Muscle – 100-200 g, without connective tissue or tendons
- Liver – 100-200 g

Muscle was collected from the hamstring, the large muscle mass in the hind quarters of the deer. No specific area of the liver was targeted for sampling during field collection of the samples. Tissue samples were placed in sealable plastic bags and labeled. All samples were frozen until processed for analysis at the MDHHS Analytical Chemistry Laboratory.

Field assessments for bovine tuberculosis (bTB) were conducted by APHIS at the time of harvest, and laboratory assessments for bTB (O'Brien et al. 2001; O'Brien et al. 2002) and Chronic Wasting Disease (CWD) were conducted by MDNR's Wildlife Disease Laboratory to prevent exposure to MDHHS Analytical Chemistry Lab personnel. All deer, whether hunter-submitted or APHIS-collected, were tested and found negative for bTB and CWD. Samples were then processed and analyzed for PFAS.

At the time of field collection, individual deer were uniquely identified by a number, and all samples were labeled. (See Appendix A for a list of identifiers). Additional data collected included:

- the location (GPS preferred) of collection;
- results of TB field assessment (i.e., presence or absence of pleural lesions in the chest cavity suggestive of bTB[Error! Bookmark not defined.]);
- date and time of collection;
- position of the entry hole, exit wound, and bullet type;
- sex of the animal; and
- health description of the animal.

MDNR communicated the results of the bTB and CWD testing to the MDHHS Analytical Chemistry Laboratory prior to the lab starting their analysis for PFAS.

All deer were also aged by MDNR. Tooth eruption and wear is a widely used, accepted, and reliable method for estimating age in white-tailed deer, particularly in younger animals, which comprise most of the population. Ages of the deer are estimated based on eruption of the teeth through the gums and wear of the cheek teeth (premolars and molars) (Severinghous 1949).

PFAS analysis in deer tissue

The MDHHS Analytical Chemistry Laboratory homogenized, extracted, and analyzed all samples for PFAS. The laboratory's method for PFAS uses reversed-phase high-performance liquid chromatography with multiple reaction monitoring tandem mass spectrometry (HPLC-MRM-MS/MS) following their standard operating procedures. Using the same methods developed for fish tissue analysis, only 11 PFAS were quantified in tissue, even though additional PFAS are included in the methodology.

Table 2: PFAS analyzed in deer muscle and liver tissue harvested in Michigan by the MDHHS Analytical Chemistry Laboratory.

Abbreviation	Name
PFHxA*	Perfluorohexanoic acid
PFHpA*	Perfluoroheptanoic acid
PFOA	Perfluorooctanoic acid (branched and linear)
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnA	Perfluoroundecanoic acid
PFDoA	Perfluorododecanoic acid
PFTriA	Perfluorotridecanoic acid
PFTeA	Perfluorotetradecanoic acid
PFHxDA*	Perfluoro-n-hexadecanoic acid
PFODA*	Perfluoro-n-octadecanoic acid
PFBS*	Perfluorobutanesulfonic acid
PFHxS	Perfluorohexane sulfonate (branched and linear)
PFOS	Perfluorooctane sulfonate (branched and linear)
PFDS	Perfluorodecanesulfonic acid
PFOSA	Perfluorooctane sulfonamide

* These chemicals are not found in tissue samples although they are part of the methodological process.

Results for PFAS levels in deer muscle and liver

No PFAS were detected in the muscle tissue from deer collected by hunters in fall 2019.

Only two PFAS, PFOS and PFHxS, were detected in muscle samples collected in March 2020 (Table 3). Seven PFAS (PFDA, PFOS, PFDS, PFHxS, PFNA, PFUnA, and PFOSA) were detected in liver samples. Of the PFAS detected, PFOS had the maximum level in both the muscle (82.6 ppb) and liver (2,970 ppb) samples.

Table 3: Summary of PFAS levels (in parts per billion [ppb] or nanograms per gram [ng/g]) detected in deer collected in March 2020 from Iosco County, near Clark's Marsh.

PFAS	Deer tissue	Number of samples with detections	Range (minimum to maximum in parts per billion [ppb]) *
PFDA	Muscle	0/44	ND
	Liver	8/44	ND – 1.27
PFDoA	Muscle	0/44	ND
	Liver	0/44	ND
PFDS	Muscle	0/44	ND
	Liver	5/44	ND – 15.4
PFHxS	Muscle	3/44	ND – 0.88
	Liver	4/44	ND – 4.11
PFNA	Muscle	0/44	ND
	Liver	9/44	ND – 1.16
PFOA	Muscle	0/44	ND
	Liver	0/44	ND
PFOS	Muscle	7/44	ND – 82.6
	Liver	9/44	ND – 2,970
PFOSA	Muscle	0/44	ND
	Liver	2/44	ND – 1.93
PFTeA	Muscle	0/44	ND
	Liver	0/44	ND
PFTriA	Muscle	0/44	ND
	Liver	0/44	ND
PFUnA	Muscle	0/44	ND
	Liver	5/44	ND – 1.41

* ND stands for non-detect. The detection limit for all PFAS was 0.25 ppb.

Eighteen out of the 44 deer had detectable levels of PFAS in muscle and/or liver samples (Table 4). Thirteen deer were female and ranged from 0.5 to 12.5 years old. Five of the deer were male and ranged from 0.5 to 5.5 years old. The highest PFOS levels was in deer #2039 (as shown in Table 4), with 82.6 ppb detected in muscle and 2,970 ppb in liver.

Table 4: Listing of PFAS detected in muscle and liver samples (in parts per billion [ppb] or nanograms per gram [ng/g]) from individual deer collected in March 2020.

Deer Sample Number	Age (years) and Sex (Male [M] or Female [F])	Muscle (ppb)*	Liver (ppb)*
2003	5.5 F	ND	PFDA 0.38
2005	8.5 F	ND	PFOS 3.46
2006	4.5 F	PFOS 0.34	ND
2007	5.5 F	ND	PFDA 0.34
2008	4.5 F	ND	PFOS 26.3
2010	1.5 M	PFHxS 0.68 PFOS 41.9	PFDA 0.6 PFDS 4.44 PFHxS 2.3 PFNA 0.4 PFOS 1,090 PFUnA 0.77
2012	1.5 F	ND	PFNA 0.25
2018	1.5 F	ND	PFUnA 0.31
2021	12.5 F	ND	PFOS 3.87
2027	2.5 F	PFOS 27.1	PFDS 2.13 PFOS 454
2028	3.5 M	ND	PFNA 0.36
2034	0.5 M	ND	PFNA 0.26
2038	3.5 F	ND	PFDA 0.62 PFNA 0.38 PFUnA 0.32
2039	1.5 F	PFOS 82.6	PFDA 1.18 PFDS 15.4 PFOS 2,970 PFOSA 1.93 PFUnA 1.41
2041	5.5 M	PFOS 30.3	PFDA 1.19 PFDS 2.76 PFHxS 1.88 PFNA 1.16 PFOS 1,370
2043	0.5 M	PFHxS 0.37 PFOS 42.4	PFDA 1.27 PFDS 5.31 PFHxS 2.26 PFNA 0.97 PFOS 2,010 PFOSA 0.41 PFUnA 1.25

Deer Sample Number	Age (years) and Sex (Male [M] or Female [F])	Muscle (ppb)*	Liver (ppb)*
2044	0.5 F	PFHxS 0.88 PFOS 3.27	PFHxS 4.11 PFNA 0.47 PFOS 66.8
2045	8.5 F	ND	PFDA 0.49 PFNA 0.41

* ND stands for non-detect. The detection limit for all PFAS is 0.25 ppb.

Summary of PFAS results from 2018-2020 in the Oscoda area in Iosco County

Deer from the Oscoda area around Clark's Marsh have been tested for PFAS in 2018 (MDHHS 2019), 2019, and 2020. The range of PFAS from the 2018 collection are included in the tables below for comparison.

From all three collections, there is a total number of 86 muscle samples. No PFAS were detected in the 2019 muscle samples. PFDS, PFHxS, and PFOS were detected in at least one deer muscle sample from the 2018 and 2020 collections (Table 5). PFDS was only detected in one muscle sample collected in 2018 at 1.72 ppb. PFHxS was detected in four muscle samples, one from 2018 and three from 2020 with a maximum of 3.64 ppb. PFOS was detected in 10 muscle samples, three from 2018 and seven from 2020 with a maximum of 547.77 ppb.

Table 5: Comparison of deer muscle PFAS levels (in parts per billion [ppb] or nanograms per gram [ng/g]) from deer samples collected in 2018, 2019, and 2020 from Iosco County

PFAS	**Range: lowest to highest results measured in parts per billion (ppb) (number of samples found to have PFAS/total number of samples)**		
	Iosco County deer harvested in 2020 (44 samples) *	**2019 Hunter submitted deer heads** *	**Iosco County deer harvested in 2018 (20 samples)** *
PFDA	ND (0/44 samples)	ND (0/22 samples)	ND (0/20 samples)
PFDoA	ND (0/44 samples)	ND (0/22 samples)	ND (0/20 samples)
PFDS	ND (0/44 samples)	ND (0/22 samples)	ND – 1.72 (1/20 samples)
PFHxS	ND – 0.88 (3/44 samples)	ND (0/22 samples)	ND – 3.64 (1/20 samples)
PFNA	ND (0/44 samples)	ND (0/22 samples)	ND (0/20 samples)
PFOA	ND (0/44 samples)	ND (0/22 samples)	ND (0/20 samples)
PFOS	ND – 82.6 (7/44 samples)	ND (0/22 samples)	ND – 547.77 (3/20 samples)
PFOSA	ND (0/44 samples)	ND (0/22 samples)	ND (0/20 samples)
PFTeA	ND (0/44 samples)	ND (0/22 samples)	ND (0/20 samples)
PFTriA	ND (0/44 samples)	ND (0/22 samples)	ND (0/20 samples)
PFUnA	ND (0/44 samples)	ND (0/22 samples)	ND (0/20 samples)

* ND stands for non-detect. The detection limit for all PFAS is 0.25 ppb.

Samples from 64 livers were taken from the 2018 and 2020 deer collections. PFDA, PFDoA, PFDS, PFHxS, PFNA, PFOS, PFOSA, PFTriA, and PFUnA were detected in at least one deer liver sample (Table 6). It appears that the liver samples collected in 2018 have higher levels of detected PFAS than the 2020 liver samples.

Table 6: Comparison of deer liver PFAS levels (in parts per billion [ppb] or nanograms per gram [ng/g]) from deer samples collected in 2018 and 2020 from Iosco County

PFAS	**Range: lowest to highest results measured in parts per billion (ppb) (number of samples found to have PFAS/total number of samples)**	
	Iosco County deer harvested in 2020 (44 samples)*	**Iosco County deer harvested in 2018 (20 samples)***
PFDA	ND – 1.27 (8/44 samples)	ND – 4.92 (10/20 samples)
PFDoA	ND (0/44 samples)	ND – 4.31 (1/20 samples)
PFDS	ND – 15.4 (5/44 samples)	ND – 63.3 (2/20 samples)
PFHxS	ND – 4.11 (4/44 samples)	ND – 4.61 (1/20 samples)
PFNA	ND – 1.16 (9/44 samples)	ND – 5.78 (15/20 samples)
PFOA	ND (0/44 samples)	ND (0/20 samples)
PFOS	ND – 2,970 (9/44 samples)	ND – 6,080 (13/20 samples)
PFOSA	ND – 1.93 (2/44 samples)	ND – 3.05 (1/20 samples)
PFTeA	ND (0/44 samples)	ND (0/20 samples)
PFTriA	ND (0/44 samples)	ND – 0.7 (1/20 samples)
PFUnA	ND – 1.41 (5/44 samples)	ND – 8.08 (14/20 samples)

* ND stands for non-detect. The detection limit for all PFAS is 0.25 ppb.

Deer PFOS levels evaluated by distance from Clark's Marsh

Although PFAS exposure in deer is not fully understood, surface water in Clark's Marsh is a potential PFAS exposure source to area deer. Using the available GPS coordinates, distance from Clark's Marsh was used to analyze a potential association with the observed PFAS levels in deer tissue. For further details on the analysis see Appendix B. This analysis was limited to the APHIS-collected deer as those deer had both muscle and liver samples collected along with the exact GPS coordinates where the deer was collected.

The PFAS detected in any of the 64 liver samples collected in 2018 or 2020 were PFDA (18/64), PFDoA (1/64), PFDS (7/64), PFHxS (5/64), PFNA (24/64), PFOS (22/64), PFOSA (3/64), PFTriA (1/64), and PFUnA (16/64). The only PFAS detected in any of the 86 muscle samples collected in 2018, 2019, or 2020 were PFDS (1/86), PFHxS (4/86), and PFOS (10/86). PFOS was the most frequently detected PFAS in muscle samples (about 12%) and was the second most frequent PFAS detected in the liver samples (about 34%). Additionally, an apparent positive relationship was observed between PFOS levels in liver and muscle samples (Table 4[2]). While PFNA was detected in about 38% of liver samples, there were no detections of PFNA in muscle samples. Therefore, the PFOS detections in deer were evaluated further with a focus on the liver samples as those had more detections than the muscle samples.

As distance from Clark's Marsh increased, there was a decrease in the number of deer liver samples with PFOS detections (Figure 2). The greatest distance from Clark's Marsh that a deer had detectable PFAS in a liver sample was over four miles away (27.5 ppb in a liver sample collected in 2018[3]). Logistic regression was used to model the probability of having a detectable PFOS level in a deer liver sample. Given that muscle levels of PFOS tended to be lower than liver PFOS levels, if PFOS was not likely to be detected in the liver there would be an even lower chance that it was detected in the muscle. The further away the deer was collected from Clark's Marsh, the less likely they were to have detectable PFOS in their liver (Figure 3). That analysis revealed that for every one-mile increase in distance from Clark's Marsh, the odds of having a detectable liver PFOS concentration decreased by a factor of 0.413 (95% CI: 0.205, 0.767).[4] The percent detections are presented in three groups, less than one mile, one to two miles, and more than two miles from Clark's Marsh (Table 7), with the groupings based on the number of samples and detections. The only PFOS detection in muscle tissue from a deer located more than two miles from Clark's Marsh was in a deer collected in 2018 at 0.47 ppb.[5]

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[2] Appendix B, Figures 6 and 7 also present the relationship between PFOS liver and muscle levels. In general, liver PFOS levels were higher than muscle PFOS levels and a greater number of samples had detects in liver compared to muscle.

[3] This was deer #5 from the 2018 deer collection. There was no detectable PFOS in the muscle sample.

[4] Additional description of this analysis, including an analysis for each data collection are presented in Appendix B.

[5] This was deer #15, a 1-year-old female, from the 2018 deer collection with a PFOS detected in the muscle tissue at 0.47 ppb, no detect of PFOS in the liver sample, and 1.23 ppb of PFOS in the deer's kidney sample (MDHHS 2019).

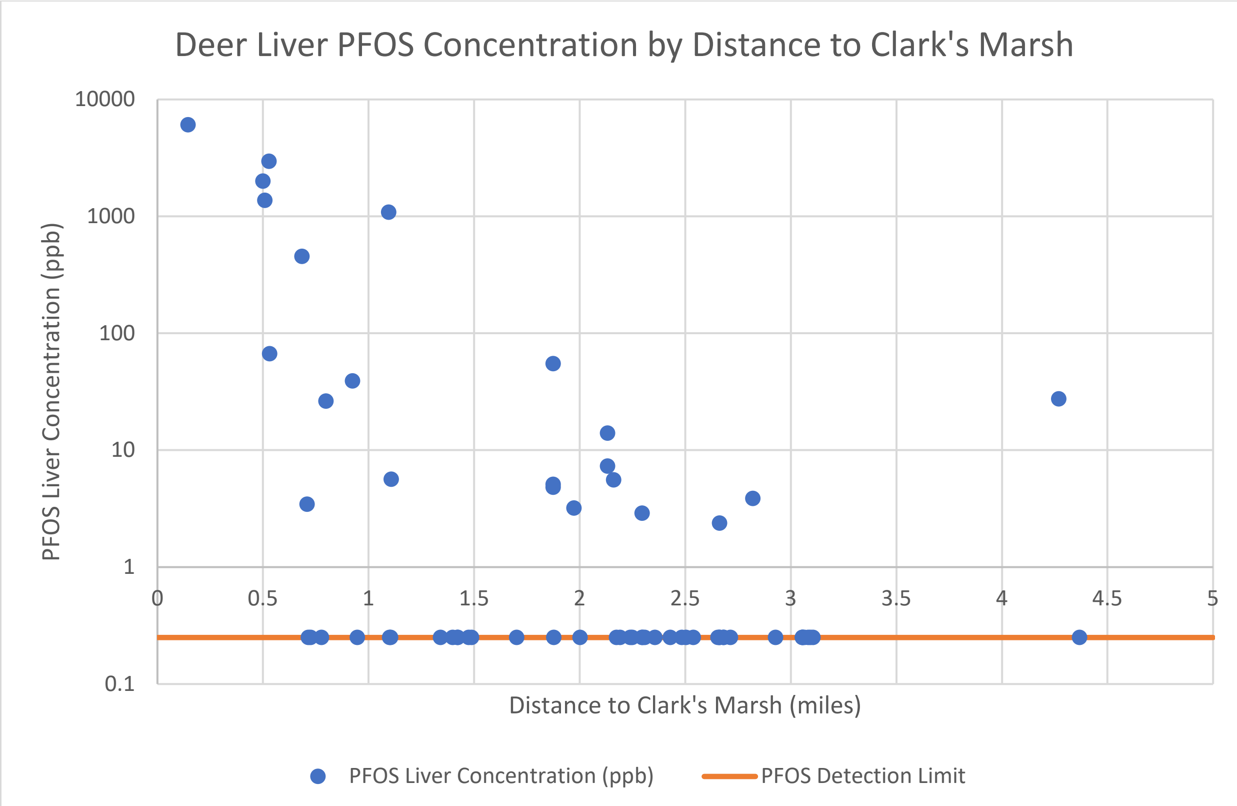

Figure 2: Individual deer liver PFOS levels (in parts per billion [ppb] or nanograms per gram [ng/g]) by distance of the deer collection locations from Clark's Marsh. This includes deer that were collected in 2018 and 2020. The orange line is set at 0.25 ppb, which is the detection limit. Points on the line are liver samples that did not have a detection of PFOS.

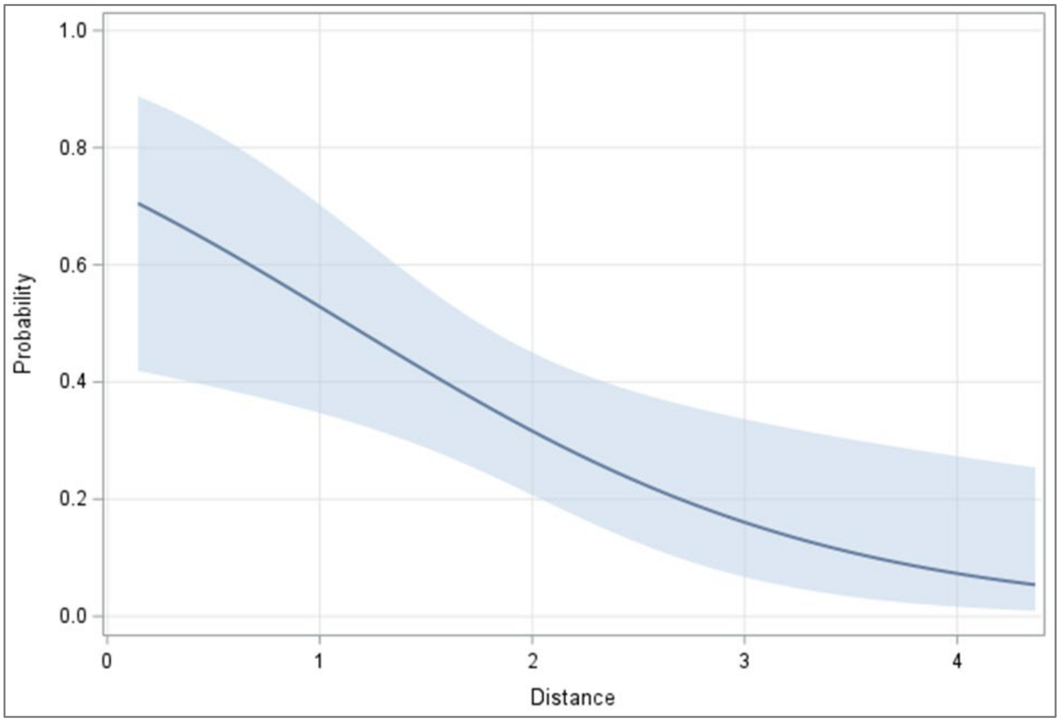

Figure 3: Modeled probability (shaded area is the 95% upper and lower confidence limits) for a detection of PFOS in liver and distance in miles from Clark's Marsh.

Table 7. Number and percent of PFOS Detections by Distance from Clark's Marsh in deer samples collected in 2018 and 2020 from Iosco County.

Distance from Clark's Marsh	Number of PFOS Detections in Liver	Percent with PFOS Detections in Liver	Number of PFOS Detections in Muscle	Percent with PFOS Detections in Muscle
<1 Mile	9/13	69%	7/13	54%
1-2 Miles	6/16	38%	2/16	13%
>2 Miles	7/35	20%	1/35*	3%

* This deer (deer #15, 2018) had 0.47 ng/g PFOS in the muscle sample and did not have detectable PFOS in the liver sample (MDHHS 2019).

Deer PFOS levels evaluated by sex and family group

To determine if any information could be provided to area hunters based on deer sex or family group, the PFOS levels in deer were evaluated by these factors. APHIS harvested deer from 2018 and 2020 included data regarding sex and family group. There was not a significant difference found in liver PFOS levels between male and female deer. Statistical analysis did reveal a significant relationship between family group status and liver PFOS detection, but due to the limited number of family groups, further analysis between groups was not warranted. Due to the small number of family groups (n=5) with PFOS detections in the samples collected in 2018 and 2020, future studies with larger samples sizes may be needed to explore this potential relationship. See Appendix B for additional discussion.

Table 8. Range of deer liver PFOS levels by family group (in parts per billion [ppb] or nanograms per gram [ng/g]) from deer samples collected in 2018 and 2020 from Iosco County

Group	Year	Number of Deer	Number of samples with PFOS detections	Range of Liver PFOS (ppb)*
A	2020	3	1/3	ND – 3.46
B	2020	2	0/2	ND
C	2020	3	0/3	ND
D	2020	4	0/4	ND
E	2020	3	0/3	ND
F	2020	2	0/2	ND
G	2020	4	0/4	ND
H	2020	2	0/2	ND
I	2018	3	1/3	ND – 2.38
J	2018	2	1/2	ND – 2.89
K	2018	2	2/2	7.33 – 14
L	2018	3	3/3	4.83 – 54.9
M	2018	3	0/3	ND

* ND indicates non-detect. The detection limit was 0.25 ppb for all PFAS.

Considerations on the available data

While the data show that not all deer in the area surrounding Clark's Marsh have detectable levels of PFOS or other PFAS in muscle, there was a wide range of PFOS detected. PFOS detections in muscle ranged from 0.34 to 547.77 ppb (ng/g). There are uncertainties with the way the deer may be exposed to PFAS. While we are assuming Clark's Marsh is the source of deer PFAS exposure, additional studies are needed to confirm or identify other sources of PFAS to area deer. Observational data on deer food sources and actual samples of food and water for PFAS testing would be informative, but this data is not currently available. Also, additional information on the absorption, retention, and half-life of PFAS in white-tailed deer are needed to fully understand how a deer's PFAS exposure relates to the levels measured in tissues.

Based on information from MDNR, the two collection months, March and April, are comparable in terms of deer behavior and can be grouped. Additionally, this may represent a more sedentary population than seen during hunting season in the fall (MDNR communications, January 2021). With a dataset of more than 60 deer, statistical analyses could be run. However, even with more than 60 deer, these analyses were limited by the high number of non-detects in the deer sampled from the Oscoda area. As a result, the statistical analysis only focused on PFOS[6] and utilized a categorical variable for detection. Statistical analyses did not include the quantitative measure of PFOS concentration.

A consideration in evaluating the data to determine a consumption guideline is how many meals people could have from a single deer. A single deer may result in enough venison for a family to eat for most of the year. This is very different from fish consumption where people eat two fish filets from one fish and multiple fish from a waterbody. Taken together, and including other factors described above, understanding the variation of PFAS levels in the population is more challenging with deer than with fish.

Additional analyses were done to examine whether any family group or sex differences could be identified. Family group analysis was limited by the small number of family groups with liver PFOS detections (n=5) and the possibility that the deer were genetically unrelated because the grouping was determined based on location at harvest and not DNA testing. Although we were able to see a relationship between group status and detection, the sample was not large enough to conduct additional analyses involving specific family groups.

Sex analysis was also limited due to the large proportion of female deer (n=48) harvested compared to male deer (n=16). Future analysis of PFAS levels by family group and sex may find associations that did not appear with this limited analysis.

Conclusions

Based on the currently available data, MDHHS concludes that deer in the Clark's Marsh area are still being exposed to PFAS.

- There appears to be a relationship between detections of PFOS in liver samples and how far the collection location was from Clark's Marsh, with PFOS more likely to be detected the closer the deer was to Clark's Marsh.
- Only a single deer more than two miles from Clark's Marsh had detectable PFOS in a muscle sample, indicating that deer more than two miles away are unlikely to have detectable PFOS in muscle.

Recommendations

MDHHS recommends that the "Do Not Eat" advisory be changed from within five miles of Clark's Marsh to within three miles of Clark's Marsh. Supporting information for this change are:

- PFOS was less likely to be detected in liver samples the further away the deer collection location was from Clark's Marsh.

[6] PFOS was detected in 10 muscle samples out of 86 total muscle samples from all three collections and detected in 22 liver samples out of 64 total liver samples from both collections.

    - This relationship between distance from the marsh and PFOS detection is more apparent in the 2020 dataset, as there were more detections of PFOS in deer liver lending confidence to the relationship. See Appendix B for more details.
- Out of the 64 deer collected in 2018 and 2020, 35 were collected from more than two miles away from Clark's Marsh. Only a single deer out of the 35 had a detection of PFOS, at 0.47 ppb (ng/g) in a muscle sample.

No muscle samples from hunter-submitted deer heads, from the 2019 hunting season, had detectable PFAS. As these were hunter-harvest deer taken further out from Clark's Marsh than the APHIS-collected deer, it may reflect difference in the time of year of the collection or just that the deer collected did not have sufficient PFAS exposure to have detectable levels in muscle tissue.

Public Health Action Plan

MDHHS will work with MDNR to develop a new map of the "Do Not Eat" advisory area.

MDHHS will communicate the updated "Do Not Eat" guideline with communities in the Oscoda area.

MDHHS will work with MDNR to continue to communicate the statewide "Do Not Eat" consumption advisory for deer organ meat such as liver and kidney because of the potential for elevated levels of PFAS and other contaminants.

MDHHS will be available to provide input to any work to investigate deer exposure to PFAS, including but not limited to how deer are exposed to PFAS through (e.g., surface water, diet), how quickly PFAS is taken up and present in tissues, and how long (half-life) PFAS remains in white-tailed deer. This type of research is beyond the work that MDHHS routinely does as part of issuing fish and wild game consumption guidelines, but MDHHS staff would be available to provide a public health perspective on any studies.

References

Michigan Department of Health and Human Services. (MDHHS) 2017. Health Consultation. Perfluorooctane Sulfonate (PFOS) in Fish. Former Wurtsmith Air Force Base Oscoda, Iosco County, Michigan EPA Facility ID: MI5570024278. February 14, 2017. Available at: https://www.michigan.gov/documents/mdhhs/WAFB_Fish_HC__Final__2-14-2017_552188_7.pdf.

Michigan Department of Health and Human Services. (MDHHS) 2019. PFAS levels in Michigan Deer and Eat Safe Wild Game Guidelines. January 11, 2019. Available at: https://www.michigan.gov/documents/pfasresponse/2019-01-11_MDHHS_deer_technical_report_644330_7.pdf.

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O'Brien DJ, Schmitt SM, Fierke JS, et al. 2002. Epidemiology of Mycobacterium bovis in free-ranging white-tailed deer, Michigan, USA, 1995–2000. Prev Vet Med. 54(1):47-63.

Severinghous, CW 1949. Tooth development and wear as criteria of age in white-tailed deer. J Wildl Manage 13:195–216.

Appendix A: Age and sex for deer harvested in Iosco County in March 2020

Deer ID	Date	County	Latitude	Longitude	Sex	Condition	TB Field Assessment	Family Group
2001	3/2/2020	Iosco	44.43482	-83.43549	M	Healthy	Negative	unknown
2002	3/2/2020	Iosco	44.43386	-83.43677	M	Healthy	Negative	unknown
2003	3/2/2020	Iosco	44.43090	-83.39627	F	Healthy	Negative	Group A
2004	3/2/2020	Iosco	44.42727	-83.42496	F	Healthy	Negative	unknown
2005	3/2/2020	Iosco	44.43076	-83.39574	F	Healthy	Negative	Group A
2006	3/2/2020	Iosco	44.44389	-83.37383	F	Healthy	Negative	unknown
2007	3/2/2020	Iosco	44.42689	-83.42191	F	Healthy	Negative	unknown
2008	3/2/2020	Iosco	44.44399	-83.37342	F	Healthy	Negative	unknown
2009	3/2/2020	Iosco	44.43047	-83.39575	F	Healthy	Negative	Group A
2010	3/2/2020	Iosco	44.43654	-83.41007	M	Healthy	Negative	unknown
2011	3/2/2020	Iosco	44.43316	-83.35959	F	Healthy	Negative	Group B
2012	3/2/2020	Iosco	44.43355	-83.35972	F	Healthy	Negative	Group B
2013	3/2/2020	Iosco	44.42853	-83.41203	F	Healthy	Negative	Group C
2014	3/2/2020	Iosco	44.42820	-83.41228	F	Healthy	Negative	Group C
2015	3/2/2020	Iosco	44.42853	-83.41262	F	Healthy	Negative	Group C
2016	3/4/2020	Iosco	44.39975	-83.41657	F	Healthy	Negative	Group D
2017	3/4/2020	Iosco	44.39963	-83.41672	F	Healthy	Negative	Group D
2018	3/4/2020	Iosco	44.39950	-83.41688	F	Healthy	Negative	Group D
2019	3/4/2020	Iosco	44.39995	-83.41631	F	Healthy	Negative	Group D
2020	3/4/2020	Iosco	44.43378	-83.43788	M	Healthy	Negative	Group E
2021	3/4/2020	Iosco	44.40392	-83.36040	F	Healthy	Negative	unknown
2022	3/4/2020	Iosco	44.43367	-83.43825	F	Healthy	Negative	Group E
2023	3/4/2020	Iosco	44.42522	-83.41630	M	Healthy	Negative	unknown
2024	3/4/2020	Iosco	44.43346	-83.43890	F	Healthy	Negative	Group E
2025	3/4/2020	Iosco	44.43641	-83.41022	F	*see note	Negative	Group F
2026	3/4/2020	Iosco	44.43627	-83.41008	F	Healthy	Negative	Group F
2027	3/4/2020	Iosco	44.43886	-83.40213	F	Healthy	Negative	unknown
2028	3/26/2020	Iosco	44.42610	-83.36874	M	Healthy	Negative	unknown
2029	3/26/2020	Iosco	44.41165	-83.35137	F	Healthy	Negative	Group G
2030	3/26/2020	Iosco	44.42851	-83.39968	F	Healthy	Negative	unknown
2031	3/26/2020	Iosco	44.41136	-83.35106	M	Healthy	Negative	Group G
2032	3/26/2020	Iosco	44.41117	-83.35131	M	Healthy	Negative	Group G
2033	3/26/2020	Iosco	44.41066	-83.35111	F	Healthy	Negative	Group G
2034	3/26/2020	Iosco	44.41630	-83.35868	M	Healthy	Negative	unknown

Deer ID	Date	County	Latitude	Longitude	Sex	Condition	TB Field Assessment	Family Group
2035	3/26/2020	Iosco	44.41594	-83.35873	M	Healthy	Negative	unknown
2037	3/26/2020	Iosco	44.41491	-83.35886	M	Healthy	Negative	unknown
2038	3/26/2020	Iosco	44.41204	-83.36217	F	Healthy	Negative	Group H
2039	3/26/2020	Iosco	44.43942	-83.39899	F	Healthy	Negative	unknown
2040	3/26/2020	Iosco	44.42317	-83.44289	F	Healthy	Negative	unknown
2041	3/26/2020	Iosco	44.43875	-83.39855	M	Healthy	Negative	unknown
2042	3/26/2020	Iosco	44.41464	-83.35903	M	Healthy	Negative	unknown
2043	3/26/2020	Iosco	44.43930	-83.39840	M	Healthy	Negative	unknown
2044	3/26/2020	Iosco	44.43897	-83.39903	F	Healthy	Negative	unknown
2045	3/26/2020	Iosco	44.41184	-83.36223	F	Healthy	Negative	Group H
*Deer was emaciated, hair loss, hips protruding.								

Appendix B: Data Analyses

Study Design and Statistical Power

The PFAS data supporting statistical evaluations in this report are the result of an observational study design, where data are generated opportunistically as opposed to through a randomized sampling procedure. As such there are no predefined treatment and control groups for comparison; however, the sample data are suitable for evaluating the strength of association between deer PFAS concentrations and distance from Clark's Marsh. While a randomized design would be preferred, wildlife sampling studies are rarely based on true randomized sampling designs because wildlife, particularly large game species, are generally captured opportunistically and true randomization precluded. As a result, we did not perform a formal power analysis to determine sample size, but rather planned for numbers of deer liver and muscle tissue samples adequate to generate meaningful statistical summaries conditioned on success of the APHIS deer collection. Generally, sample sizes on the order of 20 are adequate to understand data distributions and to generate useful summary statistics. The statistical modeling requires more than 20 samples, but if relationships are strong, meaningful two-variable models can be developed with 40 to 60 observations provided that relationships are not overly complex. If future collection of tissue samples from deer are performed, it is recommended that the currently available data be used to develop formal data quality objectives and to develop an understanding of the number of samples needed to achieve them at reasonable levels of confidence, precision, and accuracy.

Methods

Statistical analyses were done using R version 3.6.1 and SAS version 9.4 with figures and tables created with Microsoft Excel (Microsoft 365). Data used in statistical analyses were the 2018 and 2020 APHIS harvested deer samples that were tested for 11 quantified PFAS (Table 2). Due to the higher proportion of samples testing positive for PFOS in the liver, this compound was used in all statistical analyses.

Percentage of PFAS detected in Oscoda area, Iosco County, deer liver

For liver samples that had detectable PFAS levels, the percent contribution of each individual PFAS was calculated ((detected level of individual PFAS/sum of all detected PFAS) x100). For the 2018 deer liver samples, 19 had detectable PFAS, with 13 having PFOS as the primary PFAS (Figure 4). For the 2020 deer liver samples, 17 had detectable PFAS, with 9 having PFOS as the primary PFAS (Figure 5).

Figure 4: Contribution of individual PFAS in deer with liver detections for ≤1 PFAS from deer samples collected in 2018 from Iosco County

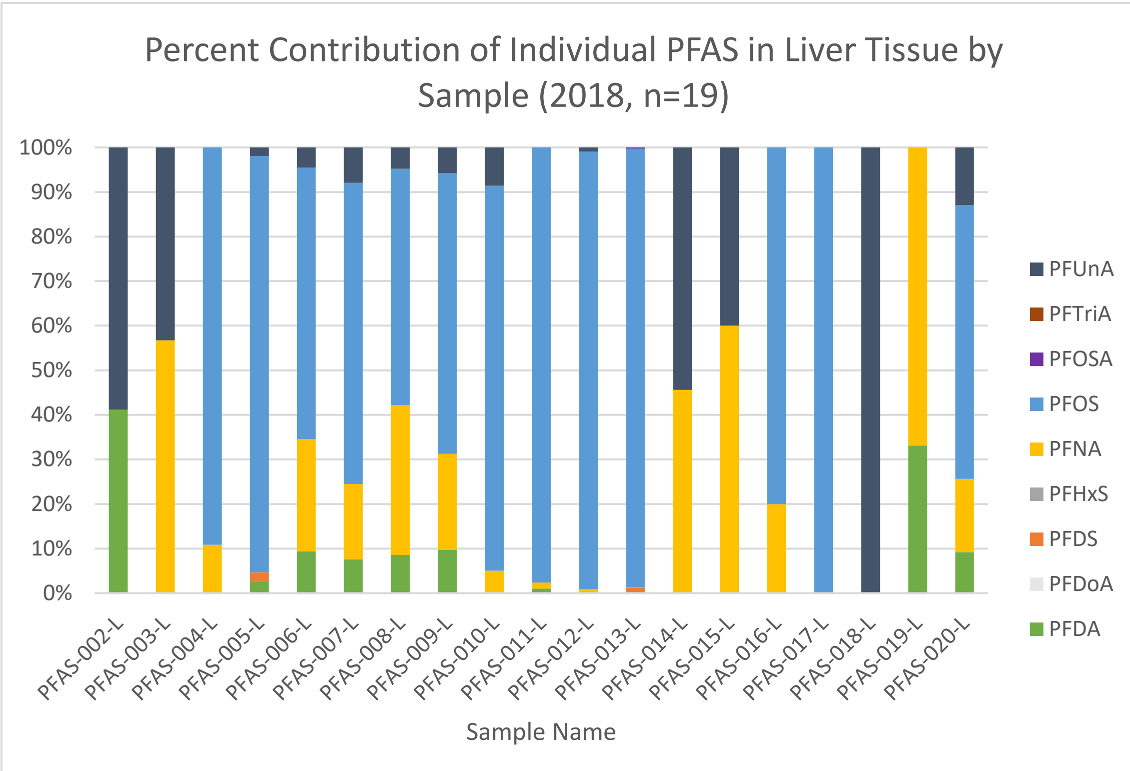
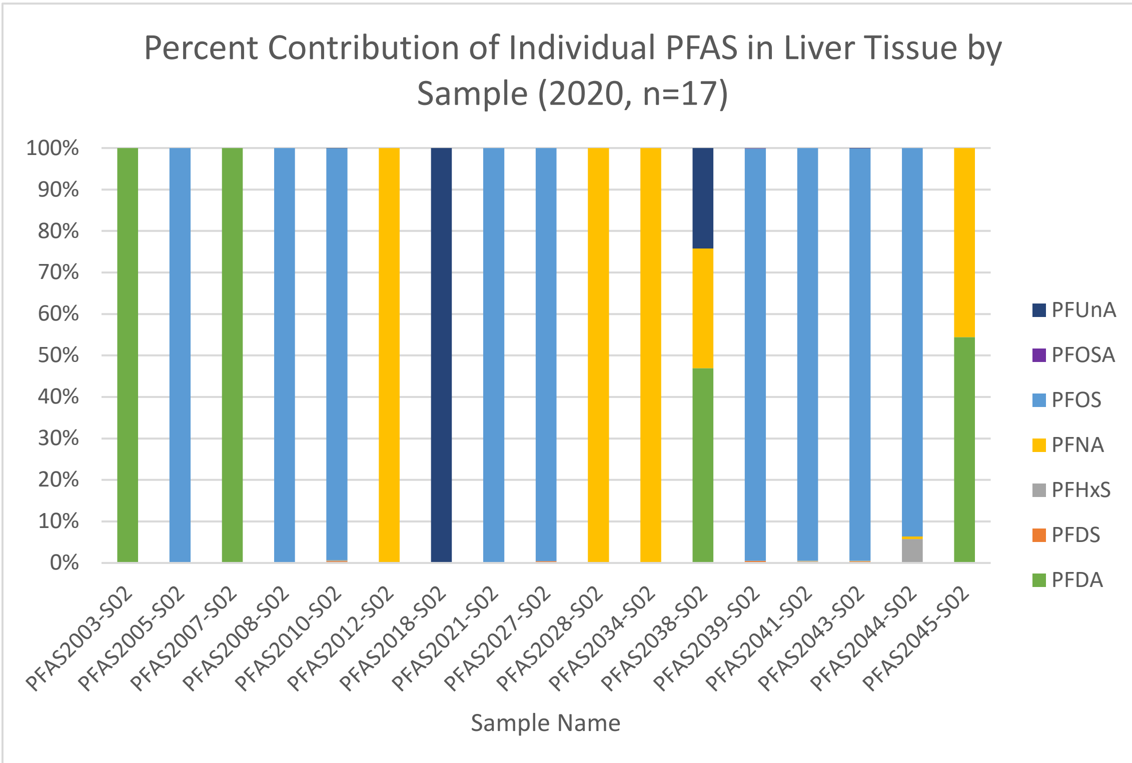


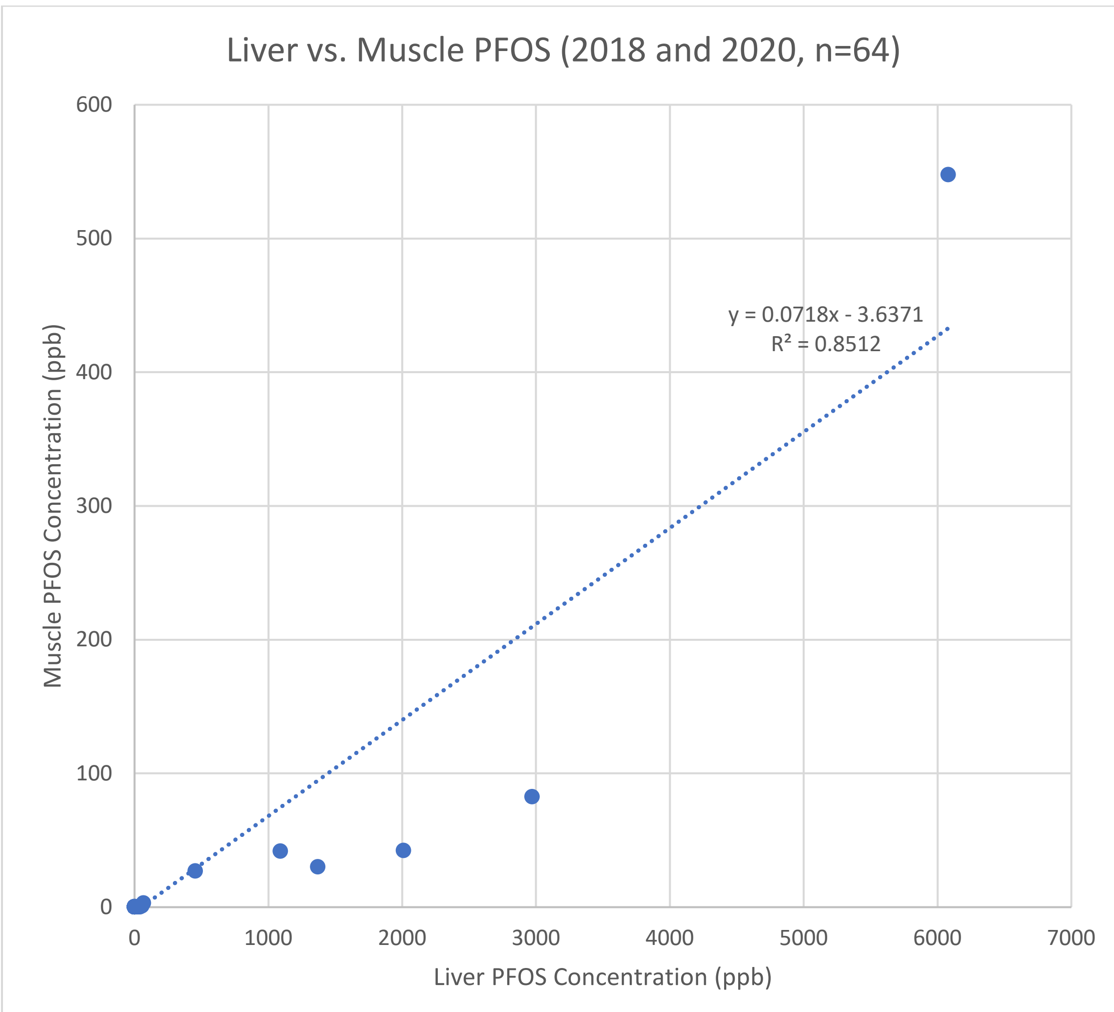
Figure 5: Contribution of individual PFAS in deer with liver detections for <u><</u>1 PFAS from deer samples collected in 2020 from Iosco County



Liver and Muscle PFOS Comparison

Data were available for samples collected from both liver and muscle tissue in Oscoda area deer (n=64). In the current sample, there was a higher proportion of samples that had detectable amounts of PFOS in the liver (n= 22) compared to muscle tissue (n= 10). MDHHS currently advises individuals to not consume wild game organs because the liver and kidneys filter chemicals and metals from the blood which often leads to high concentrations of chemicals such as PFOS in the organs.[7] In order to assess the relationship between liver and muscle PFOS concentrations, a linear regression equation was calculated along with a coefficient of determination (R^2) for the full sample (n=64; Figure 6), a sample excluding an extreme outlier (n=63; Figure 7), and a sample excluding non-detect values (n=24; Figure 8).

Figure 6. Comparison of liver and muscle PFOS (in parts per billion [ppb] or nanograms per gram [ng/g]) from deer samples collected in 2018 and 2020 from Iosco County



[7] https://www.michigan.gov/documents/mdhhs/2018-11-13_WG_Organs_web_638623_7.pdf

Figure 7. Comparison of liver and muscle PFOS excluding an extreme outlier (in parts per billion [ppb] or nanograms per gram [ng/g]) from deer samples collected in 2018 and 2020 from Iosco County

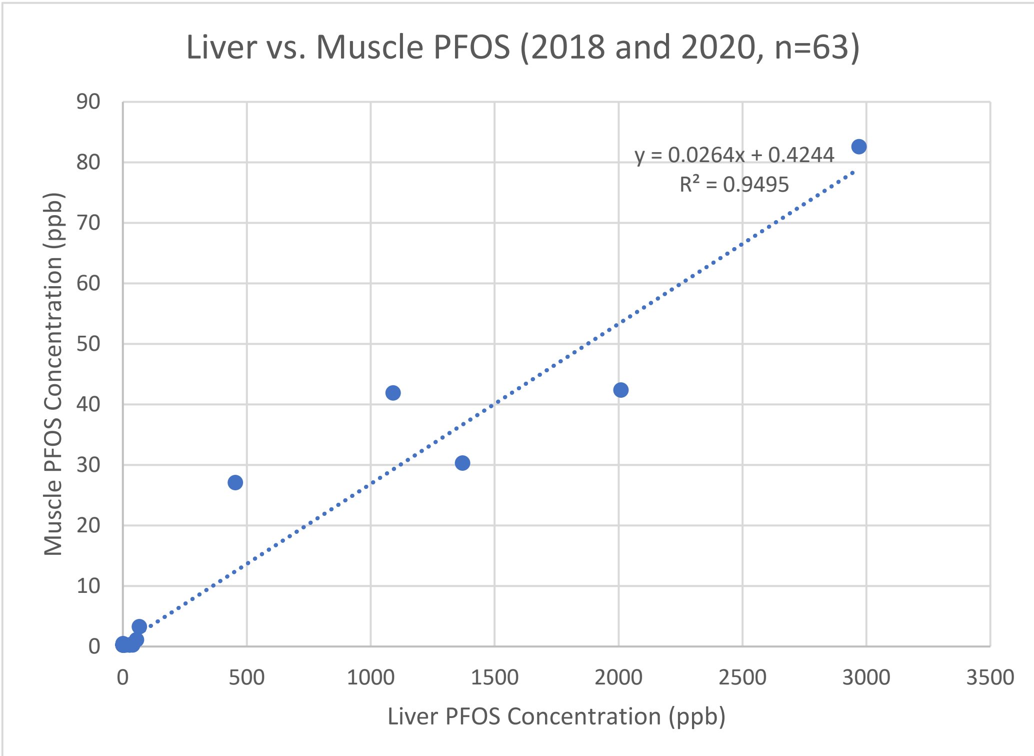
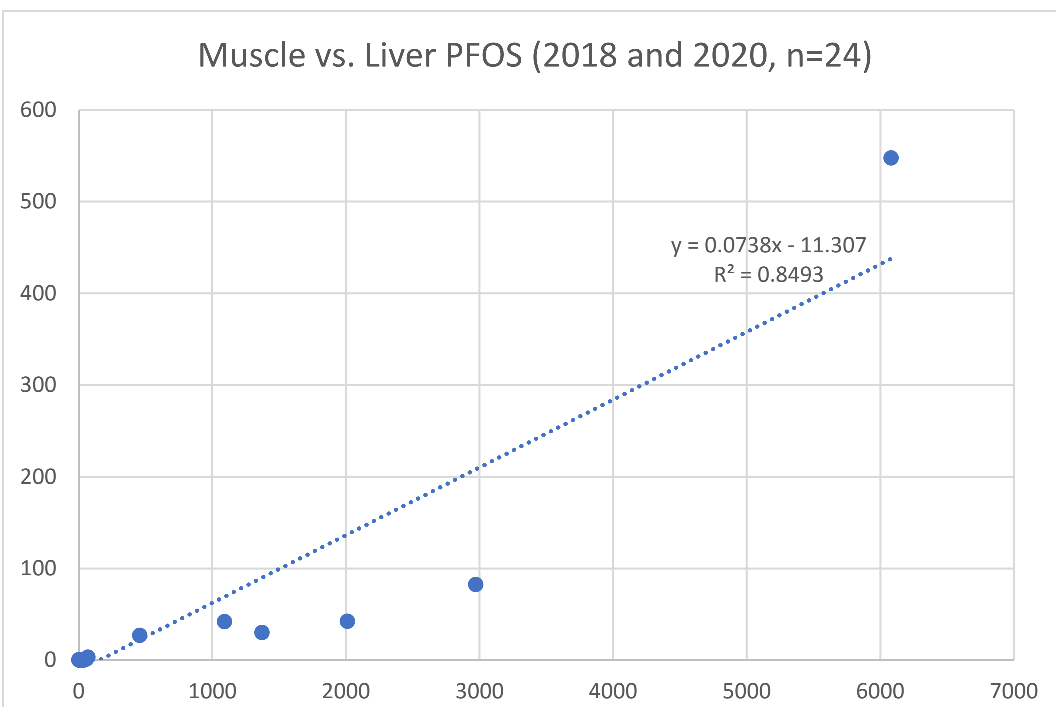


Figure 8. Comparison of liver and muscle PFOS excluding non-detects (in parts per billion [ppb] or nanograms per gram [ng/g]) from deer samples collected in 2018 and 2020 from Iosco County



Distance Analysis

Precise GPS coordinates were available for each APHIS harvested deer (Figure 9). Clark's Marsh, a known source of PFAS contamination in the Oscoda area, has GPS coordinates of 44.439535, -83.388318. Using this location, distance in miles from Clark's Marsh was calculated for each of the APHIS-harvested deer (in 2018 and 2020) using the National Oceanic and Atmospheric Administration (NOAA) Latitude/Longitude Distance Calculator, adapted from Ed Williams with permissions.[8] A graph of PFOS concentrations by distance from Clark's Marsh showed an initial trend of decreasing liver PFOS levels with increasing distance from Clark's Marsh (Figure 10). PFOS detection levels were then transformed to a dichotomous variable, with 0 indicating a non-detect for PFOS and 1 indicating a detection of PFOS at or above 0.25 ppb in liver and muscle samples. Data were then uploaded to R and SAS for analyses. The following packages were installed in R to complete the analyses: 'aod' and 'ggplot2'. Data were then imported into R and descriptive statistics were calculated for the categorical outcome (detect or non-detect) and predictor (distance from Clark's Marsh in miles). Logistic regression coefficients were calculated with 95% confidence intervals using the profiled log-likelihood. Additionally, odds ratios with 95% confidence intervals and predicted probabilities were obtained for both liver (Figure 11) and muscle (Figure 12) tissue. The exact code and output details are listed below.

Figure 9. Map of APHIS Harvested Deer, 2018 and 2020 with a 2-mile buffer from Clark's Marsh

**Note: Multiple deer may be represented by a single point if they were part of a family group harvested from the same GPS coordinates.*

Blue markers: 2018 Targeted Deer (n= 20)

Red markers: 2020 Targeted Deer (n= 44)

[8] https://www.nhc.noaa.gov/gccalc.shtml

Figure 10. Log scale of deer liver PFOS concentration (in parts per billion [ppb] or nanograms per gram [ng/g]) by distance from Clark's Marsh (in miles) from deer samples collected in 2018 and 2020 from the Oscoda area

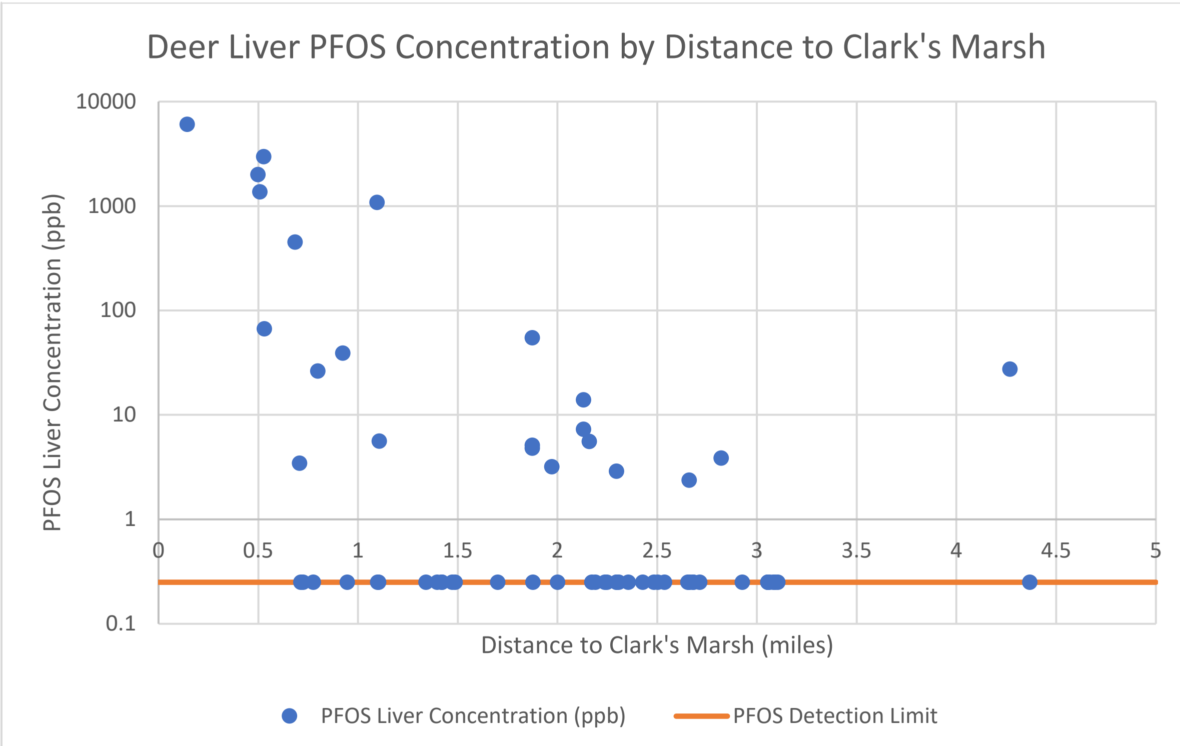


Figure 11. Liver PFOS Logistic Regression Model (Predicted Probabilities) for Oscoda area deer sampled in 2018 and 2020 (Detect Status 1 = PFOS detected in liver sample)

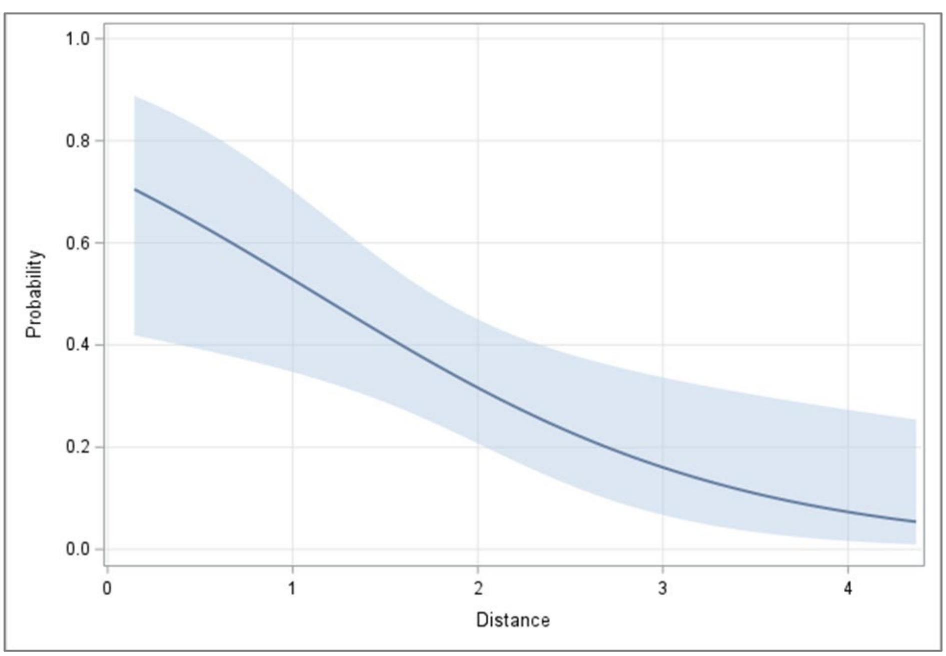
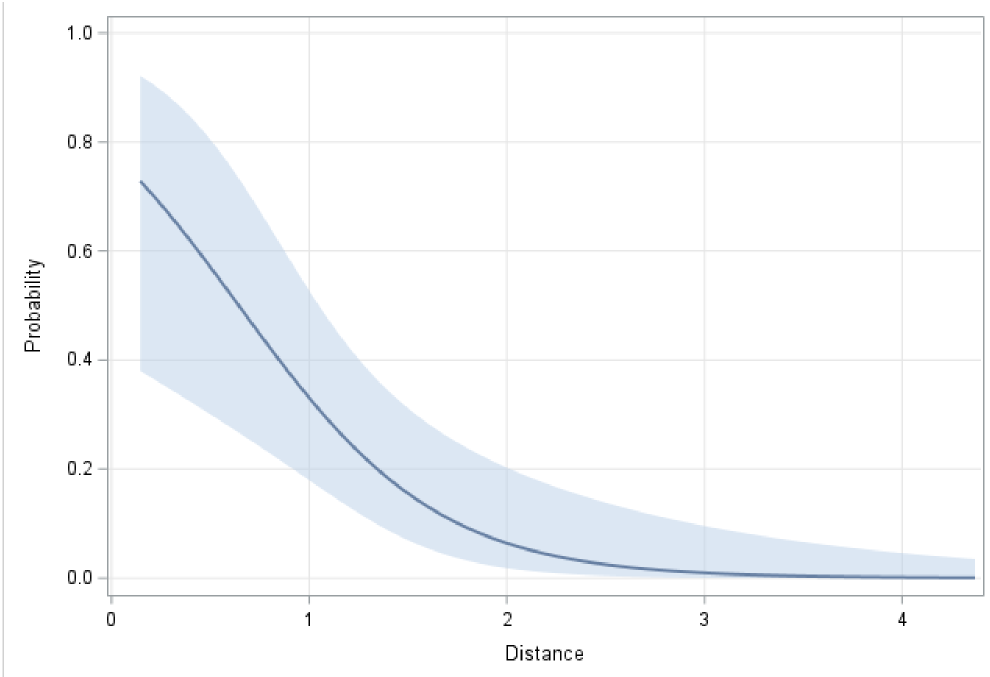


Figure 12. Muscle Logistic Regression Model for Oscoda area deer (sampled in 2018 and 2020) (Group 1= PFOS detected in liver sample)



Note: This analysis was completed with a high proportion of non-detect samples (84%), which weakens the strength of the model and limits the conclusions that can be made. However, this initial analysis found a similar trend of decreasing PFOS concentrations with increasing distance from Clark's Marsh for both liver and muscle tissue samples.

Superimposed Liver PFOS Model and Muscle PFOS Concentration

To better visualize the liver and muscle PFOS concentrations, charts were superimposed to show both the liver PFOS regression model and muscle PFOS concentrations in linear (Figure 13) and logarithmic (Figure 14) scales. Distance is displayed on the horizontal axis, with the left vertical axis displaying the predicted probability of a PFOS detection and the right vertical axis displaying the muscle PFOS concentration of deer from the Oscoda area.

Figure 13. Liver PFOS Logistic Regression Model with Muscle PFOS Concentrations (ppb) for Oscoda area deer sampled in 2018 and 2020

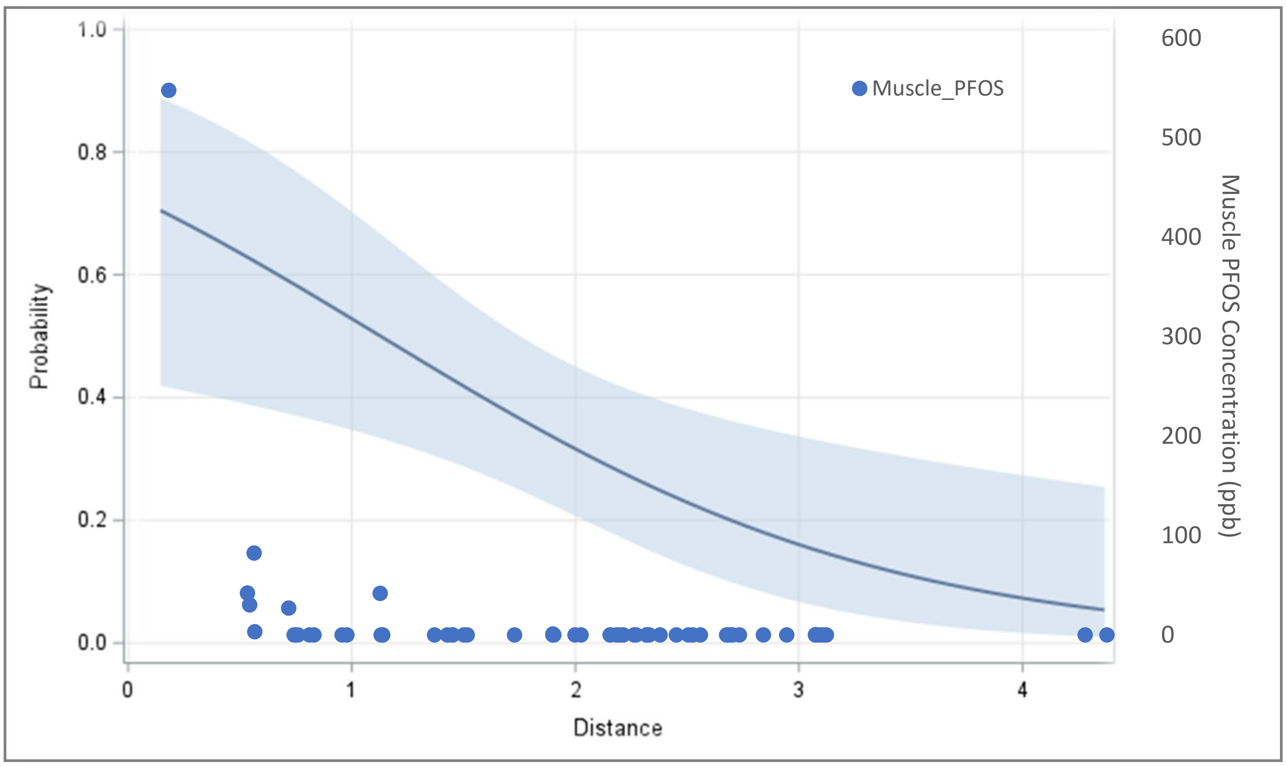
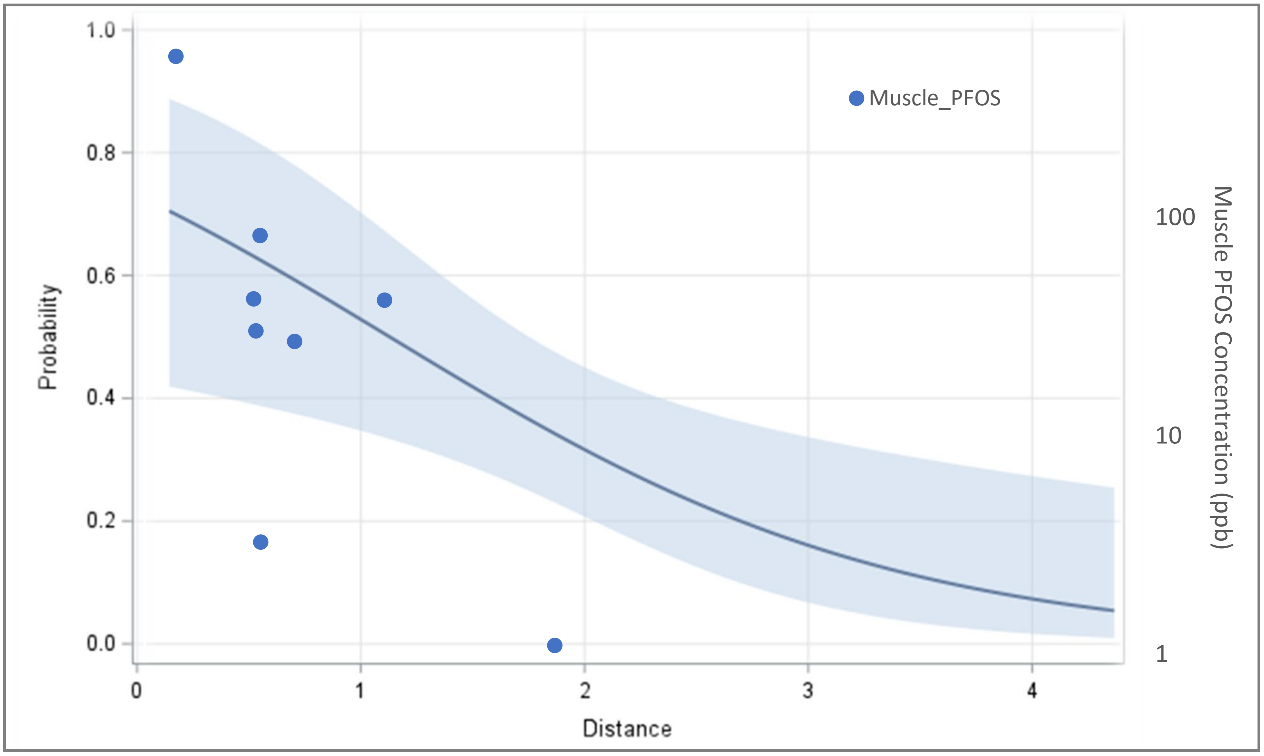


Figure 14. Liver PFOS Logistic Regression Model with Muscle PFOS Concentrations (ppb – logarithmic scale) for Oscoda area deer sampled in 2018 and 2020



Distance by Year

To assess for differences in PFOS concentrations between deer harvested in 2018 and 2020, the logistic regression described previously was completed independently for each year. Although there was variation between years, it was not statistically significant (2018 95% CI: 0.194, 0.891; 2020 95% CI: 0.010, 0.296) (Figure 15). The exact code and output details are listed below. If future sample collection is completed, further investigation of potential variation in deer PFOS concentrations over time is warranted.

Figure 15. Odds Ratios with 95% Confidence Intervals for Liver PFOS Detection by Miles from Clark's Marsh, by year – 2018 and 2020

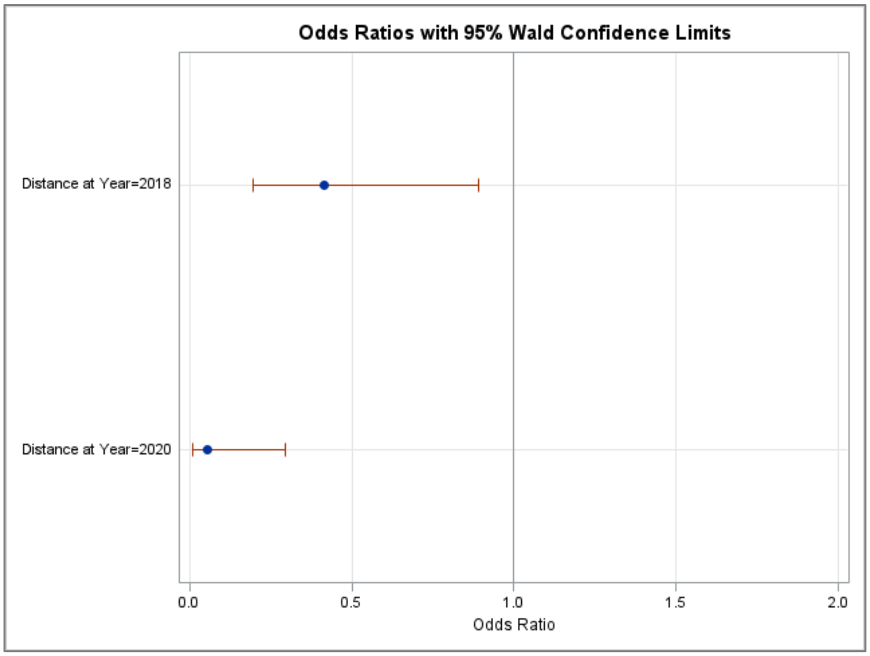
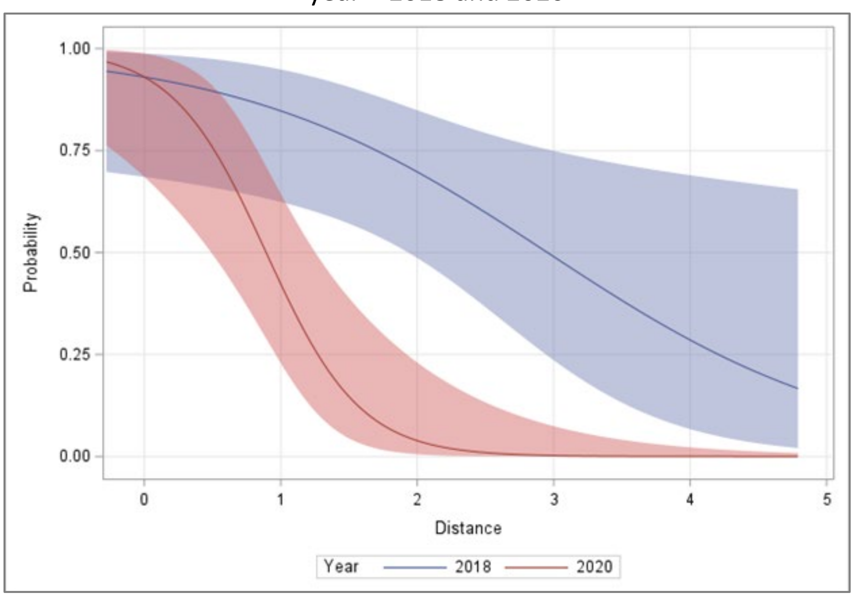


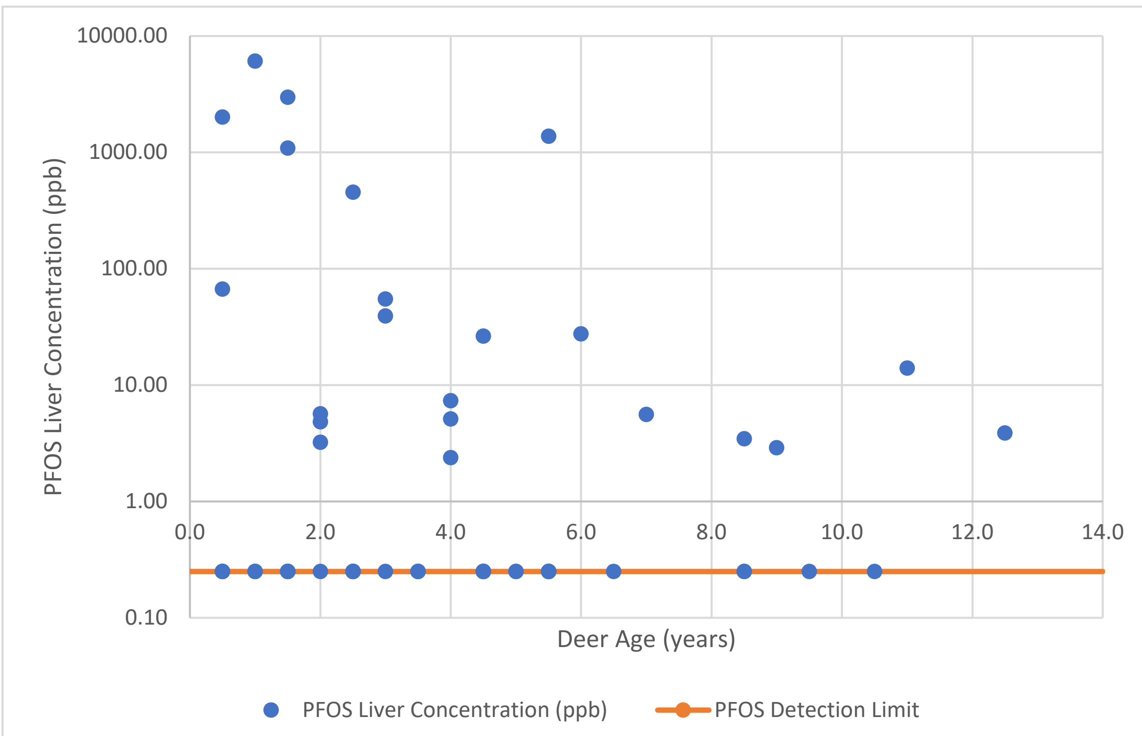
Figure 16. Logistic Regression for Liver PFOS Detection by Miles from Clark's Marsh, by year – 2018 and 2020



Age Summary

For all APHIS harvested samples in 2018 and 2020, data regarding age, family group, and sex were also available. Most deer were young, with an average age of about 4 years at the time of collection. There was an apparent slight decrease in liver PFOS concentration with increasing age in the samples from the Oscoda area in 2018 and 2020 (Figure 17). Although PFOS concentrations were higher among younger deer, this may have been in part due to the high proportion of samples that were collected from deer less than 5 years old (44/64 samples). Future research beyond the scope of this analysis might further investigate trends in deer PFAS concentrations using age-adjustment methods and advanced statistical methods.

Figure 17. Oscoda area deer liver PFOS concentration (ppb) by deer age, 2018 and 2020



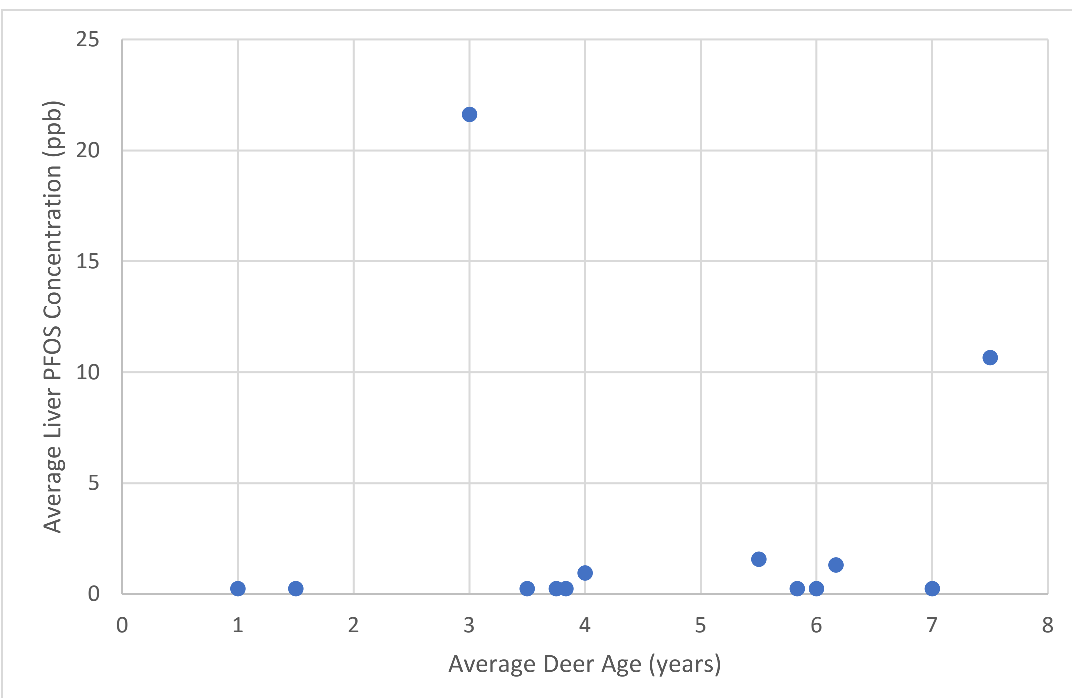
Family Group Analysis

Data regarding family group of deer was provided by APHIS and determined by location at time of harvest. Due to the small and non-parametric sample of deer that were included as part of a group, a Fisher's exact test of indepedence was used to test for an association between PFOS liver concentration detection and family group status. A contingency table of observed frequencies was created to report the number of samples in each group and detection status (Table 9). Expected frequencies were also calculated using the chi.square test in R prior to running the Fisher's exact test. The exact code and output details are listed below. A scatter plot of family average liver PFOS concentration by average age was to control for distance (Figure 18). A visual inspection of this data did not reveal any distinct patterns in liver PFOS concentration by deer age. Due to the limited sample size and scope of the current analysis, further research is warranted regarding deer family groups and PFAS concentrations. While this research is needed, it is outside of the mission of MDHHS.

Table 9. Contingency table of liver PFOS detection status by deer family group status

	Non-detect (0)	**Detect (1)**
Non-group (0)	14	14
Group (1)	28	8

Figure 18. Family group average liver PFOS concentrations (in parts per billion [ppb] or nanograms per gram [ng/g]) by average age from samples collected in 2018 and 2020



Sex Analysis

Male deer were found to have a slightly higher concentration of PFOS in the liver (279.71 ppb) compared with females (203.79 ppb; Figure 19). With a high proportion of the deer being female (75%), a nonparametric Fisher's exact test of independence was used to test for differences in liver PFOS concentration by sex. There was not a significant difference found in liver PFOS levels between male and female deer. A contingency table of observed frequencies was created to report the number of samples in each group and detection status (Table 10). Expected frequencies were also calculated using a chi-square test of independence prior to running the Fisher's exact test. The exact code and output details are listed below.

Figure 19. Average Liver PFOS Concentration (parts per billion [ppb] or nanograms per gram [ng/g]) of Oscoda Area Deer in 2018 and 2020

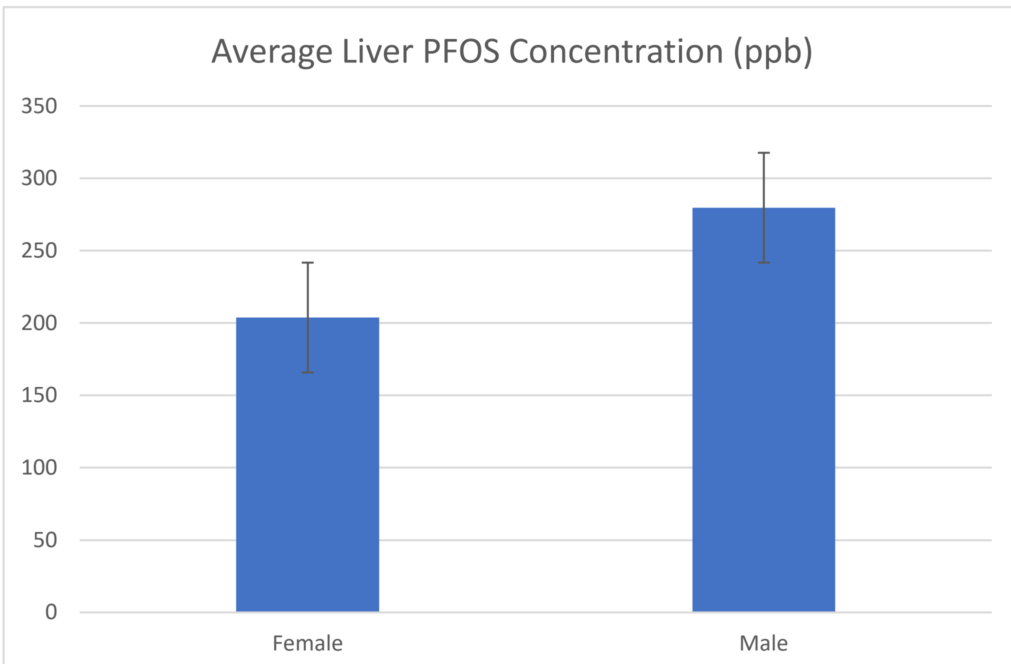


Table 10. Contingency table of liver PFOS detection status by deer sex

	Non-detect (0)	**Detect (1)**
Male (0)	12	4
Female (1)	30	18

Limitations

These analyses were limited by the low prevalence of PFAS detections and the high number of non-detects in the deer sampled from the Oscoda area. As a result, the statistical analysis only focused on PFOS and utilized a categorical variable for detection and did not include the quantitative measure of PFOS concentration (ppb). Other limitations impacted specific analyses, with the distance analysis being limited by a lack of knowledge surrounding deer behavior prior to harvest. Although water sampling has identified Clark's Marsh as a source of PFAS contamination in the Oscoda area, deer migration and travel patterns make it difficult to draw conclusions regarding whether the contamination is the only source of PFAS to area deer. Family group analysis was further limited by the small number of family groups with liver PFOS detections (n=5) and the possibility that the deer were genetically unrelated because the grouping was determined based on location at harvest and not DNA testing. Although we were able to see a relationship between group status and detection, the sample was not large enough to conduct additional analyses involving specific family groups. Sex analysis was also limited due to the large proportion of female deer (n=48) harvested compared to male deer (n=16). Future analysis of PFAS levels by family group and sex may find associations that did not appear with this limited analysis.

Statistical Code

R Code and Output: Distance Analysis

```
> install.packages("aod")
Installing package into ‘C:/Users/koole/Documents/R/win-library/3.6’
(as ‘lib’ is unspecified)
--- Please select a CRAN mirror for use in this session ---
trying URL 'https://repo.miserver.it.umich.edu/cran/bin/windows/contrib/3.6/aod_1.3.1.zip'
Content type 'application/octet-stream' length 322896 bytes (315 KB)
downloaded 315 KB

package ‘aod’ successfully unpacked and MD5 sums checked

The downloaded binary packages are in
    C:\Users\koole\AppData\Local\Temp\1\RtmpamnyYM\downloaded_packages
> install.packages("ggplot2")
Installing package into ‘C:/Users/koole/Documents/R/win-library/3.6’
(as ‘lib’ is unspecified)
trying URL 'https://repo.miserver.it.umich.edu/cran/bin/windows/contrib/3.6/ggplot2_3.3.2.zip'
Content type 'application/octet-stream' length 4069762 bytes (3.9 MB)
downloaded 3.9 MB

package ‘ggplot2’ successfully unpacked and MD5 sums checked

The downloaded binary packages are in
    C:\Users\koole\AppData\Local\Temp\1\RtmpamnyYM\downloaded_packages
> library(aod)
Warning message:
package ‘aod’ was built under R version 3.6.3
> library(ggplot2)
```

```
Warning message:
package 'ggplot2' was built under R version 3.6.3
> ## import data
> setwd("C:\\Users\\Koole\\desktop")
> deerdata <- read.csv(file='OscodaDeerData.csv')
> head(deerdata)
      Name Year Distance Liver_PFOS Detect_Status
1 PFAS-001 2018 4.367898       0.25             0
2 PFAS-002 2018 2.661627       0.25             0
3 PFAS-003 2018 2.661627       0.25             0
4 PFAS-004 2018 2.661627       2.38             1
5 PFAS-005 2018 4.269191      27.50             1
6 PFAS-006 2018 2.131958      14.00             1
> ##descriptive data
> summary(deerdata)
       Name          Year         Distance         Liver_PFOS
 PFAS-001: 1   Min.   :2018   Min.   :0.1444   Min.   :   0.250
 PFAS-002: 1   1st Qu.:2018   1st Qu.:1.1065   1st Qu.:   0.250
 PFAS-003: 1   Median :2020   Median :2.1459   Median :   0.250
 PFAS-004: 1   Mean   :2019   Mean   :1.9689   Mean   : 222.773
 PFAS-005: 1   3rd Qu.:2020   3rd Qu.:2.6616   3rd Qu.:   4.902
 PFAS-006: 1   Max.   :2020   Max.   :4.3679   Max.   :6080.000
 (Other) :58
 Detect_Status
 Min.   :0.0000
 1st Qu.:0.0000
 Median :0.0000
 Mean   :0.3438
 3rd Qu.:1.0000
```

```
 Max.   :1.0000

> sapply(deerdata, sd)
Error in var(if (is.vector(x) || is.factor(x)) x else as.double(x), na.rm = na.rm) :
  Calling var(x) on a factor x is defunct.
  Use something like 'all(duplicated(x)[-1L])' to test for a constant vector.
> ##logistic regression
> mylogit <- glm(detect_status ~ distance, data = deerdata, family = "binomial")
Error in eval(predvars, data, env) : object 'detect_status' not found
> mylogit <- glm(Detect_Status ~ distance, data = deerdata, family = "binomial")
Error in eval(predvars, data, env) : object 'distance' not found
> mylogit <- glm(Detect_Status ~ Distance, data = deerdata, family = "binomial")
> summary(mylogit)

Call:
glm(formula = Detect_Status ~ Distance, family = "binomial",
    data = deerdata)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.3365  -0.8137  -0.6676   1.0179   2.3826

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   0.9985     0.6540   1.527  0.12681
Distance     -0.8846     0.3340  -2.648  0.00809 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 82.367  on 63  degrees of freedom
Residual deviance: 74.234  on 62  degrees of freedom
AIC: 78.234

Number of Fisher Scoring iterations: 3

> ##CIs using profiled log-likelihood
> confint(mylogit)
Waiting for profiling to be done...
                2.5 %     97.5 %
(Intercept) -0.252093  2.3437796
Distance    -1.585908 -0.2649365
> ##CIs using standard errors
> confint.default(mylogit)
                 2.5 %     97.5 %
(Intercept) -0.2832567  2.2801868
Distance    -1.5392151 -0.2299678
> ##odds ratios and 95% CI
> exp(cbind(OR=coef(mylogit), confint(mylogit)))
Waiting for profiling to be done...
                   OR     2.5 %     97.5 %
(Intercept) 2.7141127  0.7771725  10.4205479
Distance    0.4128828  0.2047618   0.7672547
> save.image("C:\\Users\\koole\\Desktop\\LogRegression")
```

Logistic Regression Output - CLEAN: Distance Anlysis

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.3365	-0.8137	-0.6676	1.0179	2.3826

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	0.9985	0.6540	1.527	0.12681	
Distance	-0.8846	0.3340	-2.648	0.00809	**

Signif. codes: 0 '***' 0.001 **'**' 0.01** '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 82.367 on 63 degrees of freedom

Residual deviance: 74.234 on 62 degrees of freedom

AIC: 78.234

Number of Fisher Scoring iterations: 3

> ##CIs using <u>profiled log-likelihood</u>

	2.5 %	**97.5 %**
(Intercept)	-0.252093	2.3437796
Distance	-1.585908	-0.2649365

> ##CIs using <u>standard errors</u>

	2.5 %	**97.5 %**
(Intercept)	-0.2832567	2.2801868
Distance	-1.5392151	-0.2299678

> ##odds ratios and 95% CI

	OR	2.5 %	97.5 %
(Intercept)	2.7141127	0.7771725	10.4205479
Distance	0.4128828	0.2047618	0.7672547

Muscle PFOS Regression Output (SAS)

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Distance	0.138	0.040	0.475

Liver PFOS Regression Output (SAS) – by year

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Distance at Year=2018	0.416	0.194	0.891
Distance at Year=2020	0.055	0.010	0.296

R Code and Output: Group Analysis

```
###import data
> setwd("C:\\Users\\Koole\\desktop")
> groupdata <- read.csv(file='GroupAnalysis.csv')
> head(groupdata)
> ###calculate expected frequencies
> test <- chisq.test(table(groupdata$Group, groupdata$Detect))
> test
	Pearson's Chi-squared test with Yates' continuity correction
data:  table(groupdata$Group, groupdata$Detect)
X-squared = 4.2262, df = 1, p-value = 0.0398
> test$expected
        0      1
  0 18.375  9.625
  1 23.625 12.375
> ###Fisher's exact test of independence
> test <- fisher.test(table(groupdata$Group, groupdata$Detect))
> test
	Fisher's Exact Test for Count Data
data:  table(groupdata$Group, groupdata$Detect)
p-value = 0.03306
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.08360509 0.95087480
sample estimates:
odds ratio
 0.2917815
```

R Code and Output: Sex Analysis

```
###import data
> setwd("C:\\Users\\Koole\\desktop")
> sexdata <- read.csv(file='SexAnalysis.csv')
> ###calculate expected frequencies
> test <- chisq.test(table(sexdata$Sex, sexdata$Detect))
> test
       Pearson's Chi-squared test with Yates' continuity correction
data:  table(sexdata$Sex, sexdata$Detect)
X-squared = 0.36941, df = 1, p-value = 0.5433
> test$expected
      0    1
  0 10.5  5.5
  1 31.5 16.5
> ###Fisher's exact test of independence
> test <- fisher.test(table(sexdata$Sex, sexdata$Detect))
> test
       Fisher's Exact Test for Count Data
data:  table(sexdata$Sex, sexdata$Detect)
p-value = 0.5445
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.4485106 8.7569646
sample estimates:
odds ratio
   1.78429
```