A PRELIMINARY EVALUATION OF DIOXINS (POLYCHLORODIBENZODIOXINS AND POLYCHLORODIBENZOFURANS)

IN WILD GAME TAKEN FROM THE FLOODPLAIN ALONG THE TITTABAWASSEE RIVER

The Dow Chemical Company
Midland, Michigan

July 2004

Definitions and Acronyms

ND	Non-detect
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDF	Polychlorinated dibenzofuran
SC	Smith's Crossing (study site)
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF	Toxic Equivalency Factor
TEQ	Toxicity Equivalency Quotient
TEQ_{DF}	TCDD equivalent concentration due to PCDDs and PCDFs
TEQ_{MIN}	TCDD equivalent concentration in which congeners below the method detection limit are assigned a proxy value of 0.0.
TEQ_{MID}	TCDD equivalent concentration in which congeners below the method detection limit are assigned a proxy value of ½ the method detection limit.
TEQ_{MAX}	TCDD equivalent concentration in which congeners below the method detection limit are assigned a proxy value equal to the method detection limit.
USDA	United States Department of Agriculture
WHO	World Health Organization

1.0 EXECUTIVE SUMMARY

In the Fall of 2003, in response to community questions, Dow funded a study to obtain information relevant to the consumption of wild game taken along the Tittabawassee River. The concentration of specific types, or congeners of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), were measured in wild deer, turkeys, and squirrels taken from a reference location north of Midland and two locations south of Midland (at Smith's Crossing Road and near Imerman Park) along the Tittabawassee River. The concentrations of PCDDs and PCDFs were converted to, and reported as, the Toxic Equivalency Quotients (TEQ). Some PCDDs and PCDFs congeners were not detected in some samples. In these cases, the non-detected congener concentration was reported as ½ the detection limit, rather than as zero, using standard EPA methodology.

As anticipated, the TEQ concentrations of wild game samples taken south of Midland were greater than TEQ concentrations from wild game north of Midland. This increase in TEQ concentrations was likely related to the increased concentrations of PCDDs and PCDFs reported in soil south of Midland along the Tittabwassee River. Deer meat (venison) was lower in TEQ than was squirrel meat, which was lower than Turkey muscle, which was lower than deer liver.

Since the most significant source of background dioxin exposure is via the diet, the TEQ concentrations detected in the wild game sampled were compared to TEQ concentrations measured and reported in the national, commercial food supply by the U.S. Department of Agriculture (USDA). The TEQ concentrations for all venison, all squirrel, and turkey with the skin off from both north and south of Midland were within the range of TEQ concentrations reported in beef, fish, pork and poultry by the USDA, as part of nationwide market basket surveys. Figure 1 compares the venison TEQ concentrations from all three locations on the Tittabawassee River to the TEQ concentrations reported by the USDA in beef, fish, poultry and pork. The "Y" axis is depicted in "TEQ_{MID} (ng/kg)" which is the same as parts per trillion (ppt). The results from the other wild game samples are compared to these and other data in section 4.1 of this report. Average TEQ concentrations for turkey with the skin on taken south of Midland were at the level of the Michigan fish advisory of 10 ppt. Average TEQ concentrations for liver from deer taken at Smith's Crossing Road were 11 ppt and at Imerman Park were 64 ppt.

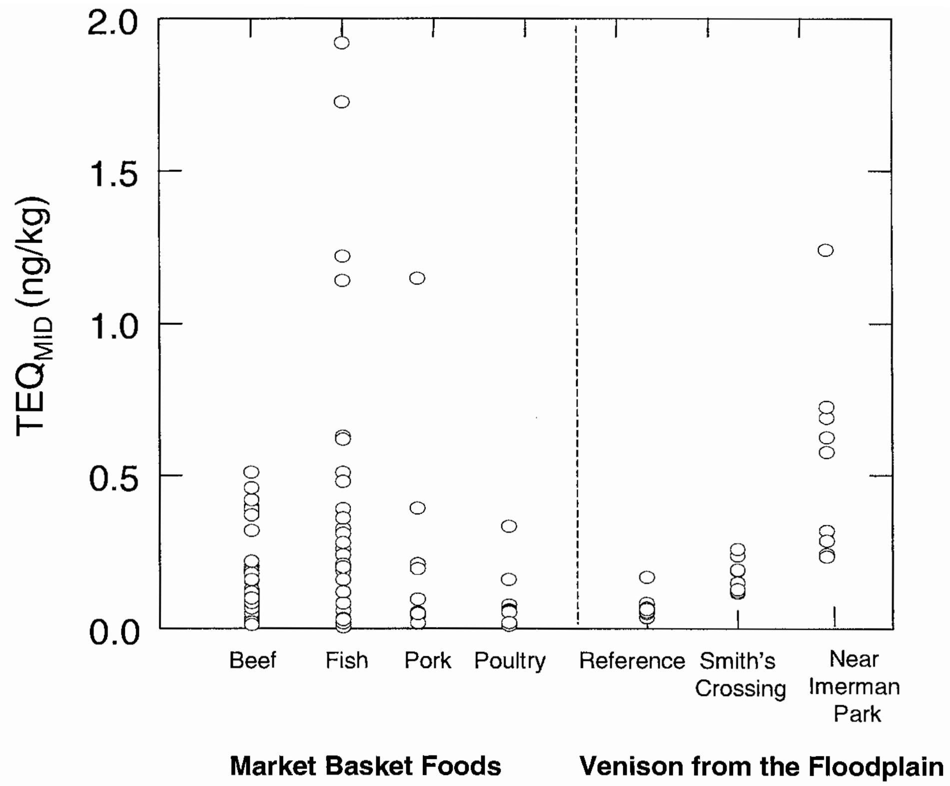

Figure 1. TEQ Concentrations In Commercially Available Meats Versus Venison Obtained north and south of Midland Along the Tittabawassee River. [From The USDA Continuing Survey of Food Intake by Individuals. Survey years 1994-1996.]

Venison (on average of 123 pounds per deer which provides approximately 60 pounds of venison) is far more significant in terms of potential exposure than the small volume of squirrel meat (approximately 5 ounces per squirrel) and the maximum legal limit of turkeys (approximately 15 pounds total). It can be reasonably assumed that a hunter or individual consuming wild game would substitute venison, turkey, and/or squirrel in place of other dietary protein sources, such as beef, fish, pork, and poultry, obtained from the national food supply. Therefore, based on the Market Basket survey data shown in Figure 1, substitution of venison taken from the floodplain area would not increase dietary background exposure to PCDDs and PCDFs. Similarly, turkey and squirrel taken from all three locations would not be appreciably different than these protein sources from commercial sources, provided that turkey skin is removed, which is a common practice for removing feathers. While liver TEQ concentrations from deer taken from the locations south of Midland were higher than TEQ concentrations reported in the commercial food supply, liver from wild deer is not typically eaten and if eaten, would only represent a few meals. Thus, a limited intake of deer liver should not significantly increase the overall average intake of PCDDs and PCDFs, assuming only a few meals of deer liver per year were consumed.

It should be noted that neither the USDA nor the U.S. Food and Drug Administration (FDA) have regulatory limits for food nor do they provide directly relevant guidance that we are aware of other than to eat a varied diet. These agencies are aware that it is possible to purchase protein (e.g. fish) that may have on average higher TEQ concentrations than the deer meat or turkey with skin off reported in this study, and yet there is no warning from these federal agencies regarding fish consumption related to dioxin.

The Michigan Department of Community Health (MDCH) has set a 10 ppt limit for TEQ concentrations in fish. When TEQ concentrations in fish exceed this limit, MDCH advises reduced fish consumption. TEQ concentrations of venison, squirrel, and turkey with the skin off taken from all three locations were below this 10 ppt limit.

TEQ concentrations in venison averaged 0.07 parts per trillion (ppt) from deer taken north of Midland at the reference location. Venison concentrations from deer taken at the Smith's Crossing Road and Imerman Park south of Midland averaged 0.17 and 0.52 ppt, respectively. The highest TEQ concentrations were measured in deer liver, which can be explained by the liver's ability to concentrate or sequester dioxins. TEQ concentrations in deer liver averaged 0.57 ppt from the reference location, while concentrations at the two locations south of Midland averaged 11 ppt and 64 ppt.

The meat from wild turkeys was analyzed in two ways: skin on and skin off. TEQ concentrations for turkey samples with the skin on averaged 0.18 ppt at the reference location and 10 ppt at each of the locations south of Midland. Removal of the turkey skin and fat, which is a common practice to avoid plucking the feathers, resulted in TEQ concentrations approximately 50% lower, or an average of 6.5 ppt at both locations south of Midland. The higher TEQ concentrations in turkey compared to venison was likely due to greater soil ingestion by turkeys and a difference in their diet.

TEQ concentrations in squirrel samples averaged 0.07 ppt from the reference location, compared to 0.40 ppt and 1.3 ppt at the two locations south of Midland.

To understand potential exposure better, Dow has provided a grant to the University of Michigan to further evaluate wild game and other possible dioxin exposure pathways. This includes an evaluation of TEQ concentrations in the blood of residents living along the Tittabawassee River south of Midland compared to other residents in Midland and Saginaw Counties as well as compared to other resident living in Michigan outside of Midland and Saginaw Counties. This study will provide a much clearer understanding of whether consuming wild game taken from the Tittabawassee River area has any impact on dioxin exposure.

Additional details about this study, including its design, methods, detailed results and statistical analysis, can be found in the ENTRIX report that will be provided later. Currently, this report is considered preliminary until a technical audit of the ENTRIX report can be finalized.

2.0 INTRODUCTION AND METHODS

2.1 Wild Game Harvesting Locations

Figure 2 shows the upstream and downstream collection sites for the study. The reference site was located on privately owned land approximately 5.5 miles upstream of Midland, Michigan. The reference site is far enough away to prevent game animals from exposure south and east of Midland. Smith's Crossing is the first downstream collection site and it is located approximately 3.8 miles downstream of Midland. The second downstream site is located near Imerman Park (IP), approximately 13 miles downstream of the Dow dam. All of these locations are comprised of agricultural land and forest.

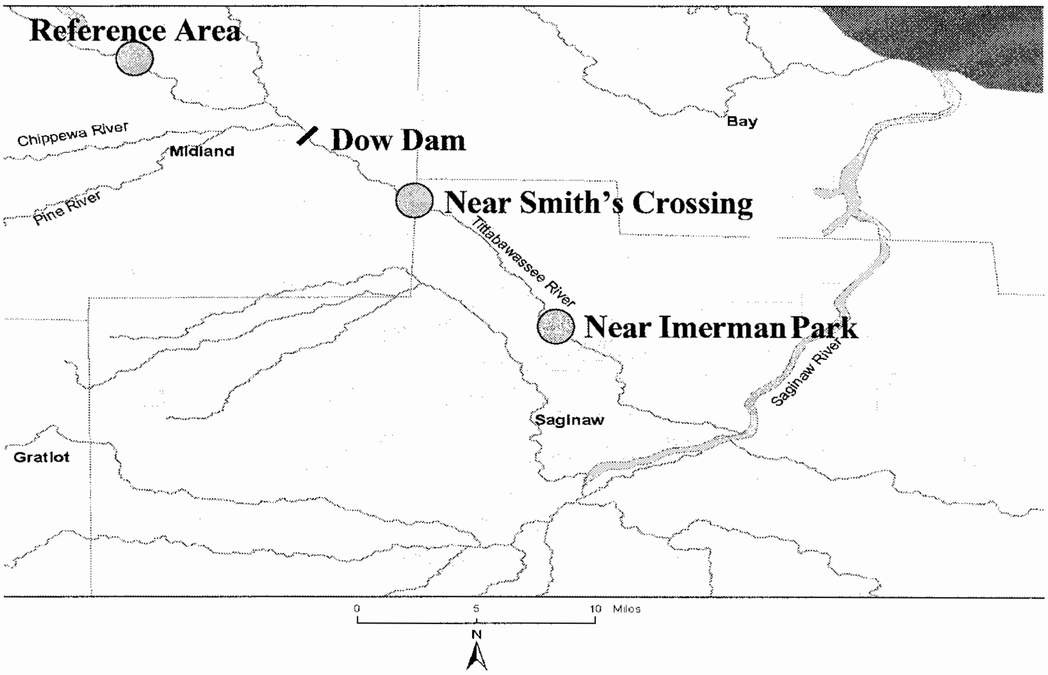

Figure 2. Wild game sampling locations along the Tittabawassee River

Deer and Turkey were harvested between November of 2003 and January of 2004 by United States Department of Agriculture (USDA) Wildlife Service personnel. Squirrels were harvested by ENTRIX personnel, the contractor hired to conduct this study. The number of each species harvested according to location is shown in Table 1.

Table 1. Number of wild game animals collected from the three sampling locations

Study Site	# White-tailed deer collected	# Wild turkeys collected	# Fox squirrels collected
Reference (private property)	14	12	12
Smith's Crossing (Dow Property)	14	11	10
Near Imerman Park (private property)	10	1	10
Total	**38**	**24**	**32**

Standard hunting practices were used to dress all wild game and care was taken not to contaminate the samples with soil from the floodplain. Both liver and muscle tissues were collected from deer, while only muscle tissues were collected from wild turkeys and squirrels. All of the collected meat samples were sent to Agriquality Limited laboratories for analysis by USEPA-approved high resolution GC/MS methods (EPA method 8290). A few duplicate meat samples were sent to Alta laboratories in order to check the reproducibility and reliability of the results between Agriquality Limited and Alta.

2.2 Calculation of Toxic Equivalency Concentrations (TEQ) and Statistics

Since there are 17 different compounds which comprise the PCDDs and PCDFs of interest, the conventional approach is to convert all of the concentrations of these 17 compound (or congeners) into a single concentration (a sum) proportionate to the most toxic dioxin congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD. Each congener's concentration is multiplied by its own toxic equivalency factor (TEF). The TEF is an approximate estimate of the congener's potency with respect to TCDD. All of the TEF-derived concentrations for the 17 congeners are added together to obtain a sum known as the Toxic Equivalency Quotient, or TEQ. The TEQ is what is referred to in this study. An example of this approach is shown in the following table where the concentration for each of the 17 PCDD/PCDF congener is shown in the 2nd column. The third column shows the WHO TEF value for the congener. The fourth column is the TEF-adjusted concentration of the congener.

Table 2. Example TEQ calculation [1]

Chemical	Concentration (ng/kg, wet weight)	WHO TEF [2]	TEF-Adjusted Concentration (ng/kg, wet weight)[3]
2,3,7,8-TCDD	0.015	1	0.015
1,2,3,7,8-PeCDD	0.028	1	0.028
1,2,3,4,7,8-HxCDD	0.025	0.1	0.002
1,2,3,6,7,8-HxCDD	0.020	0.1	0.002
1,2,3,7,8,9-HxCDD	0.022	0.1	0.002
1,2,3,4,6,7,8-HpCDD	0.098	0.01	0.001
OCDD	0.427	0.0001	0.000
2,3,7,8-TCDF	0.519	0.1	0.052
1,2,3,7,8-PeCDF	0.206	0.05	0.010
2,3,4,7,8-PeCDF	0.833	0.5	0.417
1,2,3,7,8,9-HxCDF	0.349	0.1	0.035
2,3,4,6,7,8-HxCDF	0.071	0.1	0.007
1,2,3,4,7,8-HxCDF	0.026	0.1	0.003
1,2,3,6,7,8-HxCDF	0.028	0.1	0.003
1,2,3,4,6,7,8-HpCDF	0.039	0.01	0.000
1,2,3,4,7,8,9-HpCDF	0.048	0.01	0.000
OCDF	0.097	0.0001	0.000
		Total TEQ =	**0.577** [4]

[1] Congeners below the method detection limit were assigned a proxy value equal to ½ the method detection limit.

[2] Toxic equivalency factor is relative to 2,3,7,8-TCDD. TEFs are based on Van den Berg et al., 1998.

[3] Product of the concentration of each congener and its TEF value.

[4] Sum of the TEQ for each individual congener

The final row of this table shows the cumulative TEQ (0.577 ng/kg or parts per trillion) for this particular sample containing 17 different PCDDs and PCDFs.

In the event the detection limit for a particular congener was not exceeded, the congener's presence (or non-presence known as non-detects) was treated in the following manner: 1) Assuming the non-detect was really zero, 2) Assuming the non-detect was really present at half the detection limit, and 3) Assuming the non-detect was present at the detection limit of the assay. The designation of TEQ_{MIN} concentrations represents the TEQ sum assuming the non-detects were "0". TEQ_{MID} concentrations represent the TEQ sum assuming the non-detect congeners were present at one-half (½) the detection limit. TEQ_{MAX} concentrations represent the TEQ sum by assuming the non-detects were present at the assay's detection limit. Consequently, the TEQ_{MIN} is the lowest possible estimate while the TEQ_{MAX} is the highest possible TEQ measurement for a particular sample. A variety of statistical tests were utilized for comparison purposes. The statistical tests were set so that statistically significant differences in TEQ between the three collection sites would be accurately identified with only a 5% chance of being wrong.

3.0 RESULTS

3.1 Concentrations of PCDDs and PCDFs in Wild Game Animals, Statistical Analysis and Congener Profile.

Table 3.0 shows the TEQ results for deer muscle (venison), deer liver, turkey (skin on) and squirrel harvested from the three locations. The values are all in parts per trillion (ng/kg) on a whole wet (wet weight) basis.

Table 3. Mean concentrations of TEQ_{MID}[1] (ng/kg, wet weight) ± standard deviation in wild game animals collected from the reference sampling location and two sampling locations downstream of Midland, MI

	Reference	Smith's Crossing	Near Imerman Park
Deer Muscle	^{a}0.0665 ± 0.0345	^{b}0.169 ± 0.0490	^{c}0.523 ± 0.318
	(n= 13)	(n=10)	(n=10)
Deer Liver	^{a}0.572 ± 0.253	^{b}11.1 ± 5.49	^{c}64.0 ± 56.8
	(n=13)	(n=10)	(n=10)
Turkey Muscle [2]	^{a}0.181 ± 0.0819	^{b}10.2 ± 6.98	
	(n=12)	(n=12)	
Squirrel Muscle	^{a}0.0711 ± 0.0206	^{b}0.402 ± 0.418	^{b}1.32 ± 1.58
	(n=12)	(n=10)	(n=10)

[1] $TEQ_{MID:}$ Congeners below the method detection limit were assigned a proxy value equal to ½ the method detection limit. Concentrations reported as mean ± standard deviation (ng/kg, wet weight)

[2] Turkey muscle data were combined for Smith's Crossing and near Imerman Park since there was only a single sample harvested near Imerman Park.

Different superscript letters indicate a significant difference at $p < 0.05$.

These data demonstrate that the TEQ concentrations in wild game down stream of Midland are statistically higher than the upstream reference site. The Imerman Park deer concentrations were statistically greater than concentrations measured in deer taken at Smith's Crossing. The same statistical differences were observed for deer liver. Concentrations of TEQs in muscle and liver tissues were not statistically different among male and female deer. Therefore, male and female deer data were combined as shown in the preceding table.

Average (mean) concentrations of TEQs were greater in turkeys collected at downstream locations compared to the reference sampling location. The one Turkey muscle value shown for Smith Crossing and Imerman represents an average (mean) for both sites since only one Turkey was taken near Imerman park, which is speculated as being due to poor Turkey habitat at this location. Table 4 shows the difference in Turkey when the analysis is done just on the meat versus meat attached to skin. The majority of the PCDDs and PCDFs are located in the skin which is consistent with the fatty nature of skin; PCDDs and PCDFs preferentially distribute to fat tissue in the body.

Table 4. Average concentrations of TEQ_{MID} (ng/kg, wet weight) in muscle and skin from turkeys collected from the reference and Smith's Crossing sampling locations.

	Reference	Smith's Crossing
Skin on meat (ng/kg, wet weight) [1]	0.26 ± 0.12 (n= 3)	12.0 ± 7.93 (n=3)
Skin off meat (ng/kg, wet weight) [1]	0.12 ± 0.03 (n=3)	6.49 ± 5.90 (n=3)
Skin (ng/kg, wet weight) [1]	0.2.3 ± 0.71 (n=3)	120 ± 125 (n=3)
Ratio of TEQ concentrations in skin off meat to skin on meat (unitless) [2]	0.52 ± 0.24 (n=3)	0.49 ± 0.12 (n=3)

[1] TEQ_{MID}: Congeners below the method detection limit were assigned a proxy value equal to ½ the method detection limit. Concentrations reported as mean ± standard deviation (ng/kg, wet weight). Note also that the analyses were conducted on the same sample of wild turkey (3 per location) that were split and analyzed as skin on meat, skin off meat, and skin.

[2] Ratio of TEQ concentrations in skin off meat to skin on meat was calculated for each individual sample before conducting statistics.

TEQ concentrations in squirrel muscle were found to be significantly different between reference and downstream sampling locations. However, the squirrel TEQ concentrations from Smith's Crossing and Imerman Park were not statistically different.

3.2 Congener Profile

Figure 3 shows the pattern of the PCDD and PCDF congeners measured in the wild game samples. There are notable differences between the upstream and downstream samples. For the upstream samples, a greater overall proportion of the TEQ is comprised of two dioxin congeners, 2,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzo-p-dioxin. In contrast, the downstream samples are comprised primarily of furans: 2,3,4,7,8-pentachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzofuran. The greater proportion of 2,3,4,7,8-pentachlorodibenzofuran found in the downstream samples is consistent with the presence of this one congener in flood plain soils. In addition, this one congener demonstrates a greater affinity for the liver since it is highly taken up by the liver and bound to a protein known as cytochrome P-450 1A2 in the liver.

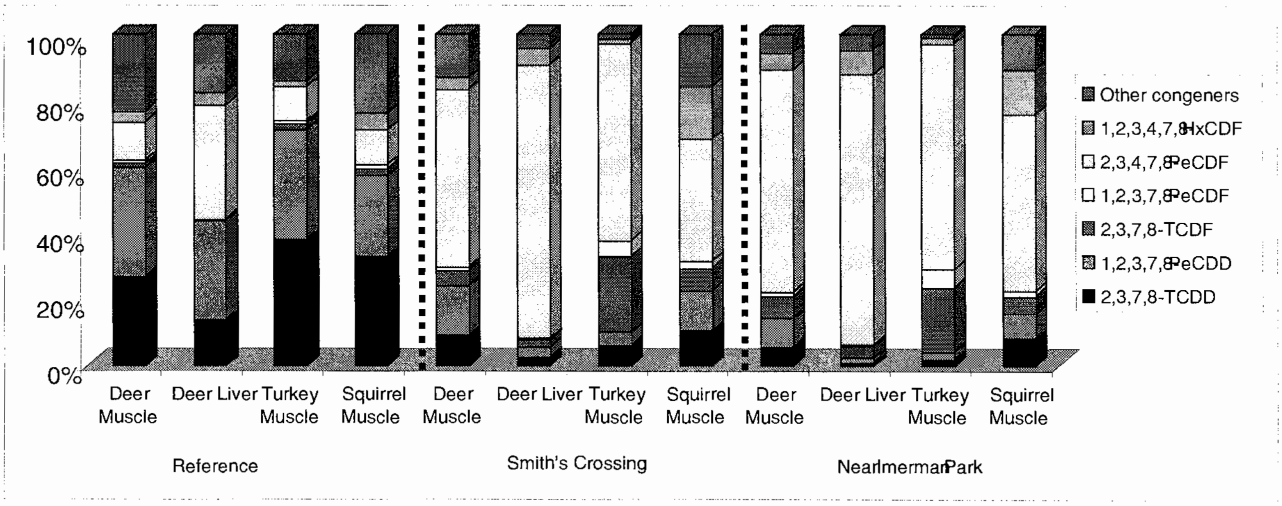

Figure 3. Percent contribution of PCDD and PCDF congeners to the total TEQ concentration in wild game animals (Other congeners included 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 1,2,3,6,7,8-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, and OCDF, which individually constituted < 5% of the total TEQ.)

4.0 DISCUSSION

Wild game samples taken south of Midland were higher in PCDD/PCDFs than wild game samples taken north of Midland. This increase in TEQ is probably due to incidental ingestion of floodplain soils. For example soil ingestion by wild game occurs during grooming dirt off of their fur, through accidental ingestion during browsing on vegetation on the ground, or actively seeking to increase the amount of trace elements in their diet or in the case of turkeys, ingestion of grit (small rocks and gravel) as an aid in digestion. The likely reason for the lower concentrations of TEQ in deer muscle relative to wild turkeys is probably related to soil ingestion. For example, incidental soil ingestion by deer has been estimated to be less than 2% of their diet whereas wild turkeys consume up to 9% soil in their diet (Beyer et al., 1994). While the amount of incidental soil ingestion is unknown for squirrels, it is likely to be intermediate to that of deer and wild turkey. The greater concentrations of TEQs in deer liver compared to deer muscle can be explained by the ability of the liver to sequester PCDDs and PCDFs through mechanisms such as induction of the cytochrome P450 enzyme, CYP1A2, and subsequent binding to this same enzyme (Santostefano et al., 1996).

The majority of dioxin exposure comes from food, primarily meat, poultry, fish, and dairy products in the national food supply (Hays and Aylward, 2003; Liem et al., 2000). There are a number of studies that have characterized the amounts of PCDDS/PCDFs present in these food items. These commercial food studies are generally referred to as "Market Basket" surveys. Thus, it is a useful and relevant comparison to view the wild game sampling results against the Market Basket Survey data. As shown in Figure 1 above, the Market Basket Survey TEQ concentrations in beef, pork, poultry, and fish were in general greater than TEQ concentrations in deer from the floodplain, including deer collected near Imerman Park (See Figure 1 in the Executive Summary of this report). TEQ concentrations of venison taken south of Midland along the Tittabawassee River were comparable to TEQ concentrations of meats in the national food supply. Of the game evaluated in this study, venison is the primary meat that would be consumed by local hunters. Sample results from this study in comparison to results from foods sampled by USDS, depicted in Figure 1 are summarized below (Table 5).

Table 5. Summary of Wild Game TEQ data (wet wet in ng/kg or ppt) versus the overall U.S. Food Supply Average TEQ

National Food Supply Average	Deer North of Midland	Deer South of Midland	Squirrel North of Midland	Squirrel South of Midland	Turkey North of Midland	Turkey South of Midland	Michigan Fish Advisory
0.04 to 2 ppt	0.07 ppt Livers – 0.57 ppt	0.17 and 0.52 ppt Livers – 11 ppt and 64 ppt	0.07 ppt	0.40 and 1.4 ppt	0.18 ppt	6.5 ppt (skin off) 10 ppt (skin on)	10 ppt

Table 5 demonstrates that venison and squirrel are within the range of dioxin TEQ existing in the National food supply. This table also demonstrates that turkey, once the skin is removed, is lower in concentration than the fish advisory set for dioxins by the State of Michigan.

5.0 CONCLUSIONS

Wild game harvested south of Midland showed higher TEQ concentrations than wild game taken north of Midland. For venison, which is the most common and abundant wild game meat, the concentrations were no different than TEQ concentrations present in beef, pork, poultry and fish tested by the USDA and have entered the national food supply. Concentrations of TEQs in venison at the two locations south of Midland averaged 0.17 and 0.52 ng TEQ/kg (parts per trillion). This was within the range of concentrations of TEQs from nationwide market basket surveys for meats, dairy, and eggs (0.004 – 1.1 ppt). Deer liver was found to have the highest TEQ concentrations and these concentrations were higher than TEQ concentrations found in commercial food supply. Much of this TEQ is attributable to one furan congener, 2,3,4,7,8-pentachlorodibenzofuran for which recent scientific studies have shown to be much less potent than 2,3,7,8-tetrachlorodibenzo-p-dioxin, or TCDD (NTP, 2003a and 2003b). In addition, deer liver does not provide a substantive number of meals nor is it typically eaten. For turkey, the PCDD/PCDF concentrations were primarily localized in the skin, presumably in the fatty portion of the skin. The usual practice of processing wild turkey involves skin removal in order to avoid the tedious job of manually plucking the feathers which will reduce potential exposure. TEQ concentrations in turkey with the skin removed were slightly higher than reported by the USDA in the national food supply; however, these results were below the fish advisory level set by the Michigan Department of Community Health when the skin is removed. Also, the limited amount of wild turkey meat available to the hunters (legal limit of two, one in the spring and on in the fall) should not significantly increase their background dioxin exposure from food. TEQ concentrations from squirrels taken south of Midland were 0.4 ppt and 1.32 ppt, and were within the range of meats sampled by USDA from the national food supply, although, squirrel meat would not be expected to contribute substantively to a typical diet.

In conclusion, these data demonstrate that individuals who consume venison, squirrels, or turkeys with the skin off taken south of Midland would incur no greater exposure than by eating meat, fish, or poultry from the national food supply. While deer liver showed higher TEQ concentrations than commercial meat products, an occasional meal of deer liver taken downstream of Midland in the Tittabawassee River floodplain should have no significant impact on the amount of background dioxin concentrations.

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