FINAL PROGRESS REPORT

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t(14;18) translocations in Dioxin-Exposed Workers
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ABSTRACT

Occupational exposure to dioxins has been associated with increased risk of Non-Hodgkin's lymphoma in previous epidemiologic studies. Environmental exposure to 2,3,7,8-TCDD following the Seveso accident has been associated with increased frequency of t(14;18) translocations, which is a critical step in the carcinogenesis pathway for follicular lymphoma. We investigated the association between dioxin exposure and the prevalence and frequency of t(14;18) translocations in 218 former workers of a chemical plant that produced chlorinated phenols and chlorphenoxy acids and 150 general population controls from an unexposed city, who were frequency matched to the exposed on age, gender, and ethnicity. The exposed had a mean blood 2,3,7,8 - TCDD level of 51.02 lipid-adjusted ppt (median 22.9) vs 2.75 (median 2.30) for controls, and a mean TEQ of 109.71 (median 62.38) vs 20.83 (median 17.20) for the unexposed. We did not observe an increase in the prevalence or frequency of t(14;18) translocations between the exposed and unexposed. We did, however, observe a significant increase in the frequency of t(14;18) translocations with increasing blood dioxin levels in the subset of workers who had current or past chloracne. We also investigated gene expression in a random sample of 60 exposed and 30 unexposed. Dioxin exposure, as measured by blood levels, was associated with statistically significant effects on gene expression in peripheral blood mononuclear cells in multiple candidate genes and pathways. In the AhR pathway, dioxin exposure was associated with significant up-regulation of AHR, ARNT, NRIP1, TIPARP, and TRIP11 while HSP90AA1 was significantly down-regulated. In the Anti-Apoptosis pathway, BAX, BCL2AI and BCL2L1 were significantly down-regulated while PTGS2 was significantly up-regulated. Dioxin exposure affected multiple genes in the inflammation pathway. IL8, PARP1, SEPINB2, NFKB1, and STAT3 were significantly up-regulated while IL17RB was significantly down-regulated. In addition, TNF and BACH2 were borderline up-regulated while CCL2 was borderline down-regulated. In the lipid metabolism pathway, MTMR7, GRN, and ALOX15B were significantly up-regulated, while ST8SIAI was borderline down-regulated. In the cell cycle and translation pathways, RB1 and CTBP2 were significantly up-regulated while PTN and EIF2S1 were significantly down-regulated. In the WNT signaling pathway, CTNNB1 was significantly up-regulated while WNT5A was borderline down-regulated. Among the other candidate genes, dioxin exposure was associated with significant up-regulation of TP53, PRDM1, NFIL3, and BTN1A1, significant down-regulation of HIST1H2BE and CDEBPD, and borderline up-regulation of ALDH3A2. This study provides important data on the molecular effects of dioxin on candidate genes and pathways. This study also suggests that there are important differences in susceptibility to the effects of dioxin in humans. This susceptibility is likely mediated through genetic or epigenetic mechanisms. Susceptibility to chloracne appears to provide a promising avenue for further investigation into the role of gene-environmental interactions in modulating dioxin toxicity.

LIST OF TERMS AND ABBREVIATIONS

AHR	Aryl Hydrocarbon Receptor
NHL	Non-Hodgkin's Lymphoma
PCB	Polychlorinated Biphenyl
PBMNs	Peripheral Blood Mononuclear Cells
PCDD	Polychlorinated Dibenzodioxins
PCDF	Polychlorinated Dibenzofurans
TCDD	2,3,7,8 - Tetrachlorodibenzodioxin
TEQ	Toxic Equivalence

SECTION 1

Significant Key Findings

This occupational cohort was highly exposed to a mixture of PCDDs and PCDFs as evidenced by their blood dioxin levels. PCDD and PCDF levels among the unexposed were in the range of background levels that we would expect from general population controls. The exposed had a mean 2,3,7,8 TCDD of 51.02 lipid-adjusted ppt (median 22.9) vs 2.75 (median 2.30) for controls and mean TEQ of 109.71 (median 62.38) vs 20.83 (median 17.20) for the controls. Both the exposed and unexposed had similar serum levels of coplanar PCBs, though these PCB levels are higher than what has been observed in the U.S. general population. The demographics of the exposed and unexposed were similar.

The prevalence of t(14;18) positive cells was similar between the unexposed group and population controls from Germany. The mean and median frequencies among the unexposed group were lower than German population controls.

Contrary to our primary hypothesis, we did not observe an increase in prevalence or frequency of t(14;18) translocations between the exposed an unexposed groups. We also did not observe an increase in the frequency of translocations with increasing serum TCDD or TEQ in the entire group of exposed and unexposed. We did, however, observe a significant increase in the frequency of translocations among the subset of occupationally exposed who had current or past chloracne.

Dioxin exposure was associated with significant effects on gene expression in peripheral blood mononuclear cells in multiple candidate genes and pathways. In the AhR pathway, dioxin exposure was associated with significant up-regulation of AHR, ARNT, NRIP1, TIPARP, and TRIP11 while HSP90AA1 was significantly down-regulated.

Except for a borderline up-regulation of ALDH3A2, none of the candidate genes in the drug metabolism pathway were significantly up-regulated which may reflect the type of tissue studied (PBMNCs and not liver cells).

In the Anti-Apoptosis pathway, BAX, BCL2AI and BCL2L1 were significantly down-regulated while PTGS2 was significantly up-regulated.

Dioxin exposure affected multiple genes in the inflammation pathway. IL8, PARP1, SEPINB2, NFKB1, and STAT3 were significantly up-regulated while IL17RB was significantly down-regulated. In addition, TNF and BACH2 were borderline up-regulated while CCL2 was borderline down-regulated. In the lipid metabolism pathway, MTMR7, GRN, and ALOX15B were significantly up-regulated, while ST8SIAI was borderline down-regulated.

In the cell cycle and translation pathways, RB1 and CTBP2 were significantly up-regulated while PTN and EIF2S1 were significantly down-regulated.

In the WNT signaling pathway, CTNNB1 was significantly up-regulated while WNT5A was borderline down-regulated.

Among the other candidate genes, dioxin exposure was associated with significant up-regulation of TP53, PRDM1, NFIL3, and BTN1A1 and significant down-regulation of HIST1H2BE and CDEBPD.

Individuals with and without chloracne also had significant differences in expression of multiple candidate genes. Interestingly AHRR was inversely associated with chloracne status.

Translation of Findings

The t(14;18) translocation is considered a critical step in the causal pathway for follicular lymphomas. Previous studies of dioxin-exposed workers have shown an increase in the risk of mortality from Non-Hodkin's lymphoma. A previous study of the Seveso population that was environmentally exposed to 2,3,7,8-TCDD showed an increase in the frequency of t(14;18) translocations with increasing blood TCDD level among those who had one or more translocations. This finding suggested that dioxin may increase the frequency of translocations, and therefore the risk of NHL, by preventing apoptosis, or death of these abnormal clones.

While we did not observe an increase in the overall frequency of t(14;18) translocations between our exposed and unexposed groups, we did observe a significant increase in the frequency of translocations among the subset of exposed who had current or past chloracne. When workers are exposed to high levels of dioxins, some develop chloracne while others do not. This suggests that there are important differences in susceptibility between workers who do and do not develop chloracne. Our study on t(14;18) translocations also suggests that these differences in susceptibility to developing chloracne may also extend to other dioxin toxicity endpoints.

In our study dioxin exposure was associated with up-regulation and down-regulation of genes in multiple pathways. Dioxin exposure significantly up-regulated several genes in the AHR pathway, which is the primary molecular target of dioxin. Previous studies from Seveso have observed a down-regulation of (Aryl Hydrocarbon Receptor) AHR at high dioxin blood levels. In theory, this may be due to a negative feedback mechanism mediated through an increased expression of AHRR (Aryl Hydrocarbon Receptor Repressor). While we did not observe a decrease in AHR expression at high dioxin blood levels, we did observe an increase in AHRR in those who had chloracne compared to those who did not.

We did not observe downstream effects of dioxin activation of AHR on drug metabolizing genes, though this may be due to the fact that we studied peripheral blood mononuclear cells rather than liver cells. Dioxin did affect multiple genes in the inflammation pathway suggesting that this is an important molecular mechanism for dioxin toxicity.

Outcomes/Impact

Despite numerous studies of dioxin toxicity in cell cultures and animal models, data on molecular targets of dioxin in highly exposed (e.g. occupationally-exposed) humans are scarce. This study provides important data on the molecular effects of dioxin on candidate genes and pathways. Dioxin exposure affected multiple genes in the inflammation pathway. These observations may be due in part to the type of cells studied, i.e. immune cells, but nevertheless support the hypotheses that inflammation is an important mechanistic pathway for dioxin toxicity. This study also suggests that there are important differences in susceptibility to the effects of dioxin in humans. This susceptibility is most likely mediated by genetic and perhaps even epigenetic mechanisms. Susceptibility to chloracne appears to provide an important avenue for further investigation. Archived DNA and RNA samples from the current study can be used to more fully investigate these gene-environment interactions.

SECTION 2

Hypotheses

Our primary hypotheses are:

- The frequency of t(14;18) translocations will increase with increasing TCDD level
- The frequency of t(14;18) translocations will increase with increasing TEQ

Our secondary hypotheses are:

- The frequency of t(14;18) translocations will increase with increasing back-extrapolated TCDD levels
- The frequency of t(14;18) translocations will increase with increase in current blood levels of individual and total dibenzofuran congeners

Our exploratory hypotheses are:

- The increase in the frequency of t(14;18) translocations associated with increasing blood TCDD level is mediated through increased expression of BCL2
- The increase in the frequency of t(14;18) translocation with increasing blood TCDD level is mediated through reduced expression of KLF4

Revised exploratory hypotheses:

- *When we did not observe the expected increases in the frequency of t(14;18) translocations with either current TCDD or TEQ or back-extrapolated TCDD, we decided to expand the number of gene expression analyses from two (BCL2 and KLF4) in the anti-apoptosis pathway to include 83 genes in multiple pathways. The pathways investigated were AhR, drug metabolism, anti-apoptosis, inflammation, lymphoma, lipid metabolism, cell-cycle, and WNT signaling.*

Study Populations

Exposed Population

The study population of 323 was recruited from former workers of a Russian Chemical Plant. The plant workers were occupationally exposed to dioxins during past production of chlorinated phenols and chlorophenoxy acids. The plant workers were involved in the manufacture of the N-butyl esters of trichlorophenoxyacetic acid (2,4,5-T) during the 1960's, and in production of trichlorphenol (TCP), TCP-Cu and 2, 4-dichlorophenoxyacetic acid (2,4-D) from the1960's through the 1980's. Because of the manufacturing process employed in the plant, commercial chlorophenol products contained considerable amounts of impurities. Some samples of technical 2,4,5-TCP contained up to 0.65 mg/kg of 2,3,7,8-TCDD. Workers at the plant were exposed to the CDDs and CDFs during routine production and also as a result of industrial accidents.

Unexposed Population

The comparison population of 150 was selected from the general population of a city located 250 km from the plant. Residents living in the city where the plant was located were environmentally exposed to dioxins, and we decided to select controls from a "cleaner" city with similar demographics. The unexposed were frequency-matched to the exposed on age, gender and ethnicity.

Methods

After obtaining informed consent, we recruited and examined 323 former workers from the chemical plant who had previous occupational exposure to dioxin while engaged in the manufacture of chlorinated phenols and their phenoxy-acid esters and 150 unexposed from a cleaner city with similar demographics, 250 km from the chemical plant. We obtained plant personnel records, medical and occupational histories, performed physical examinations, and collected blood samples for serum dioxin and biomarker studies from these participants. The subset of medical and occupational history variables that were related to our

hypothesis were entered into a database by our Data Management Center in Kyiv, Ukraine and the dataset was transferred to the University of Illinois at Chicago. We were able to obtain and transfer blood samples for dioxin, DNA, and RNA from 218 of the 323 exposed and all 150 or the 150 unexposed. Blood samples from these 218 exposed and 150 unexposed were transferred to our partner laboratories at CDC, Griefswald University in Germany, and the University of Milan in Italy.

For collection of the biomarker samples, trained and certified phlebotomists collected 88.5 ml of whole blood from each participant: 50 ml for blood dioxin determination, 14 ml for t (14; 18) translocation, and 14.5 ml for biomarker studies. For dioxin analysis blood was collected according the CDC Laboratory Procedure for PCDDs, PCDFs, and cPCBs, method HRGC/ID-HRMS, Method 28. Blood dioxin was processed, and serum has been frozen in a -80^{o}C freezer prior to shipment. For t(14;18) translocation, the Standard Operating Procedure was provided by Griefswald University. Whole blood was collected in two EDTA-vacutainer tubes (each 7 ml) and the blood was poured into leucosep tubes prefilled with Ficol solution; phosphate-buffered saline (PBS) was added to buffer up to a total volume (Ficoll + blood + PBS) of 45ml per tube. Mononuclear cells were separated by Ficoll Hypaque density gradient centrifugation at 1000 g for 10 min. The buffy coat containing the mononuclear cells was re-suspended in 45 ml PBS, centrifuged at 300 g for 5 min, the pellet was re-suspended again in 45 ml PBS. After centrifugation at 300 g for 5 min the cell pellet was re-suspended in 1 ml PBS and the cell count was determined. After centrifugation, cell pellets (supernatant liquid removed) containing at least 1*10^{7} cells in 1.5 ml centrifuge tubes were stored in at least 2 aliquots at -80°C. For RNA expression studies, an additional 28.5 ml of blood was collected in the following tubes: three PAX tubes (2.5 ml of blood each tube) for subsequent RNA extraction and one EDTA vacutainer tube (7 ml of blood) for DNA extraction. Blood for biomarker studies has been frozen in a -80^{o}C freezer prior to shipment. RNA was extracted from blood samples using PAXgene blood RNA kit (QIAGEN) according to the manufacturer's instructions.

Frozen peripheral blood mononuclear cell samples from 218 exposed individuals and 150 unexposed were transferred to the University of Griefswald for DNA extraction and analysis of t(14;18) translocations. Griefswald University has completed analysis of the t(14;18) translocations on the exposed population and unexposed populations. The University of Milan has received 218 sets of buffy coat samples from the exposed and 150 from the unexposed; they have also received PAX tubes from the 218 exposed and will soon be receiving PAX tube samples from the 150 unexposed.

The Organic Toxicology Branch, Division of Laboratory Science, National Center for Environmental Health, U.S. Centers for Disease Control and Prevention U.S. Centers for Disease Control has completed dioxin analyses of the serum samples for the 218 exposed and 150 unexposed. Serum samples were analyzed for seven polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 dibenzofurans (PCDFs), 4 non-ortho substituted or coplanar polychlorinated biphenyls (cPCBs), 38 ortho-substituted polychlorinated biphenyls (PCBs), 13 persistent chlorinated pesticides and selected pesticide metabolites are measured in serum by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (HRGC/ID-HRMS). http://carcin.oxfordjournals.org/cgi/content/full/24/4/673?ijkey=54f35984b4492f1075a134c1d507936d40c679f4 - B30#B30

For the gene expression analyses, we selected a random sample of 60 exposed subjects from the population of 218 exposed subjects for whom we had archived RNA samples. We also selected a random sample of 30 unexposed subjects from the population of 150 unexposed who were frequency matched to the 60 exposed on age, gender, nationality, and presence/absence of t(14;18) translocation. The PAXgne Blood RNA Kit was used to isolate total RNA (Qiagen-PreAnalytix, Hombrechtikon, Switzerland). Qiagen prepared a customized array for 83 genes selected by the investigators. The criteria for selecting candidate genes were: 1 Key components of the AhR, human lymphoma, and apoptosis pathways; or 2) > 1.3 fold up-regulation or 0.65 down-regulation in previous studies of dioxin-exposed humans; or 3) > 5 fold change in expression in previous studies of dioxin-exposed human cell lines. Gene expression was analyzed at the University of Milan using real-time PCR.

Exposure was classified dichotomously (exposed/unexposed); continuously, by current blood TEQ, current specific congeners (e.g. 2,3,7,8-TCDD); by back-extrapolated TCDD using a elimination half-lives of 7.2 years (from the literature) and 7.8 years (our data); by groupings of exposure (e.g. tertiles). Back-extrapolation of TCDD levels to date of last exposure was carried out under the direction of Dr. Scott Bartell.

Results

Dioxin Exposure and t(14;18) translocations

Demographics

The demographic characteristics of the examined exposed population and comparison population are presented in the following tables. Figures 1 and 2 compare the age, gender, and ethnicity of exposed and unexposed. Fig 3 compares their mean age. Fig 4 compares their ethnicity. *The demographics of exposed and unexposed are similar.*

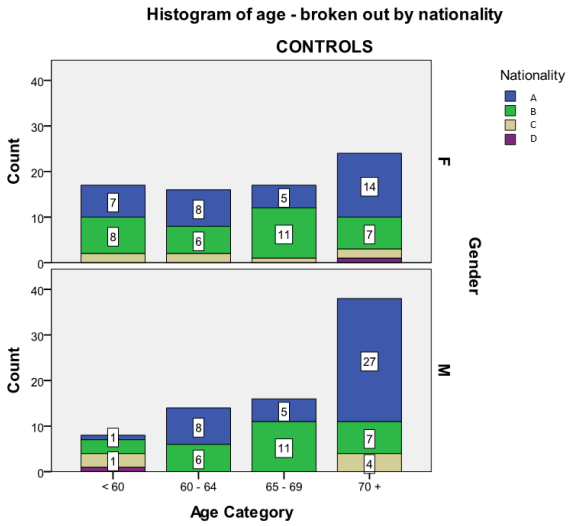


Figure 1

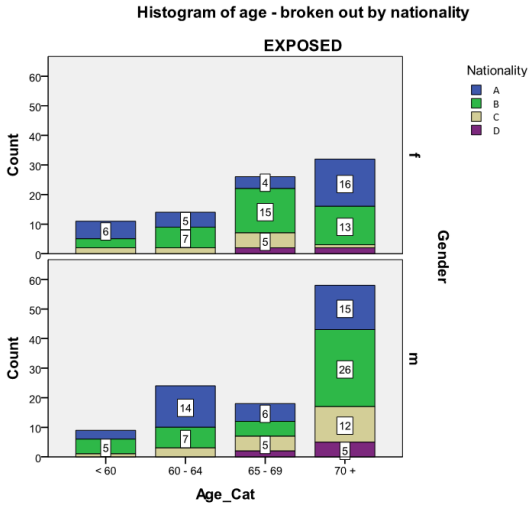


Figure 2

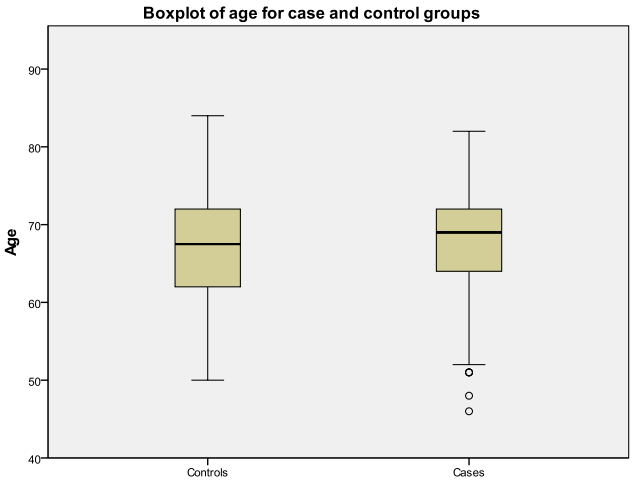


Figure 3

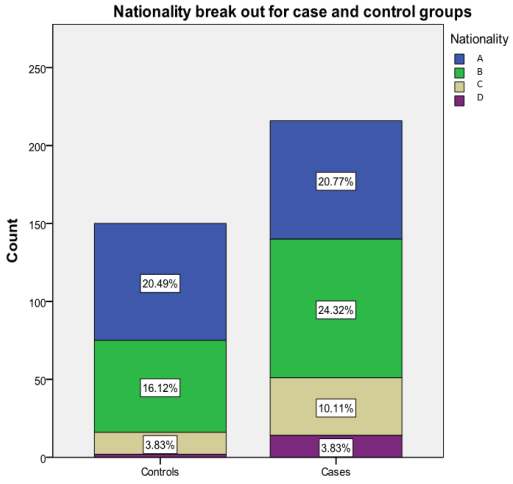


Figure 4

Dioxin Blood Levels

The results of serum dioxin analyses of the exposed population (labeled as cases) by congener are shown in Figure 5. *The exposed group is exposed to a complex mixture of PCDDs (D), PCDFs(F), and coplanar PCBs (P).*

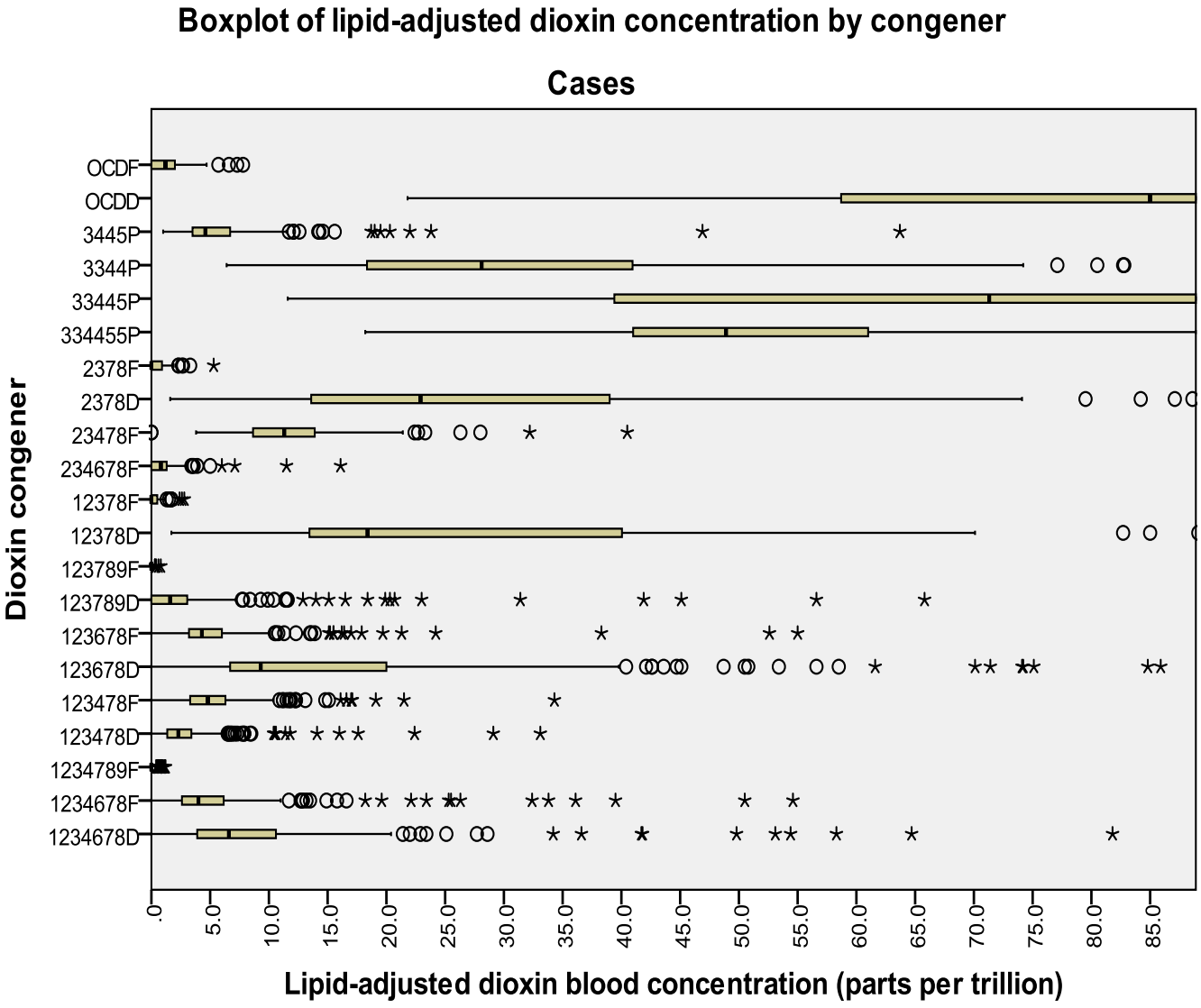


Figure 5

Figures 6 and 7 show dioxin levels by congener among the **exposed** stratified on gender. *Both male and female exposed have high exposures to this mixture of PCDDs, PCDFs, PCBs.*

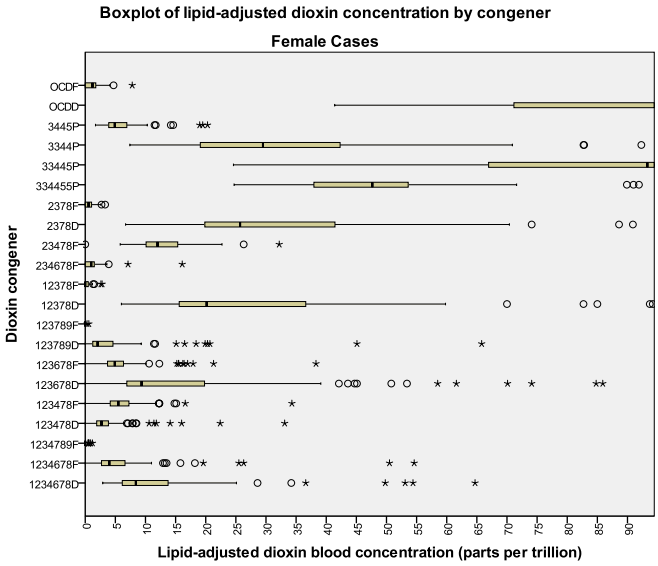


Figure 6

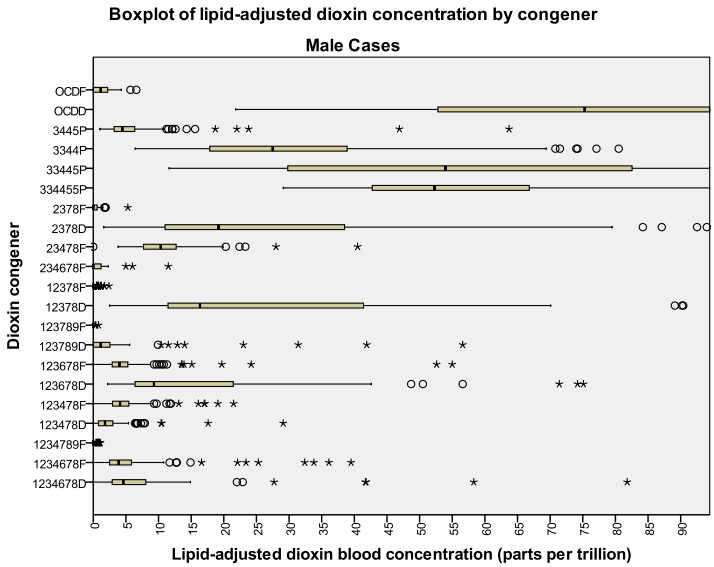


Figure 7

Figure 8 presents a boxplot of TEQ among the exposed by gender. *Results are similar.*

Figure 8

Dioxin levels in the unexposed group by congener are presented in Figure 9. *Except for the coplanar PCBs, the dioxin and dibenzofuran levels in the unexposed group are low.*

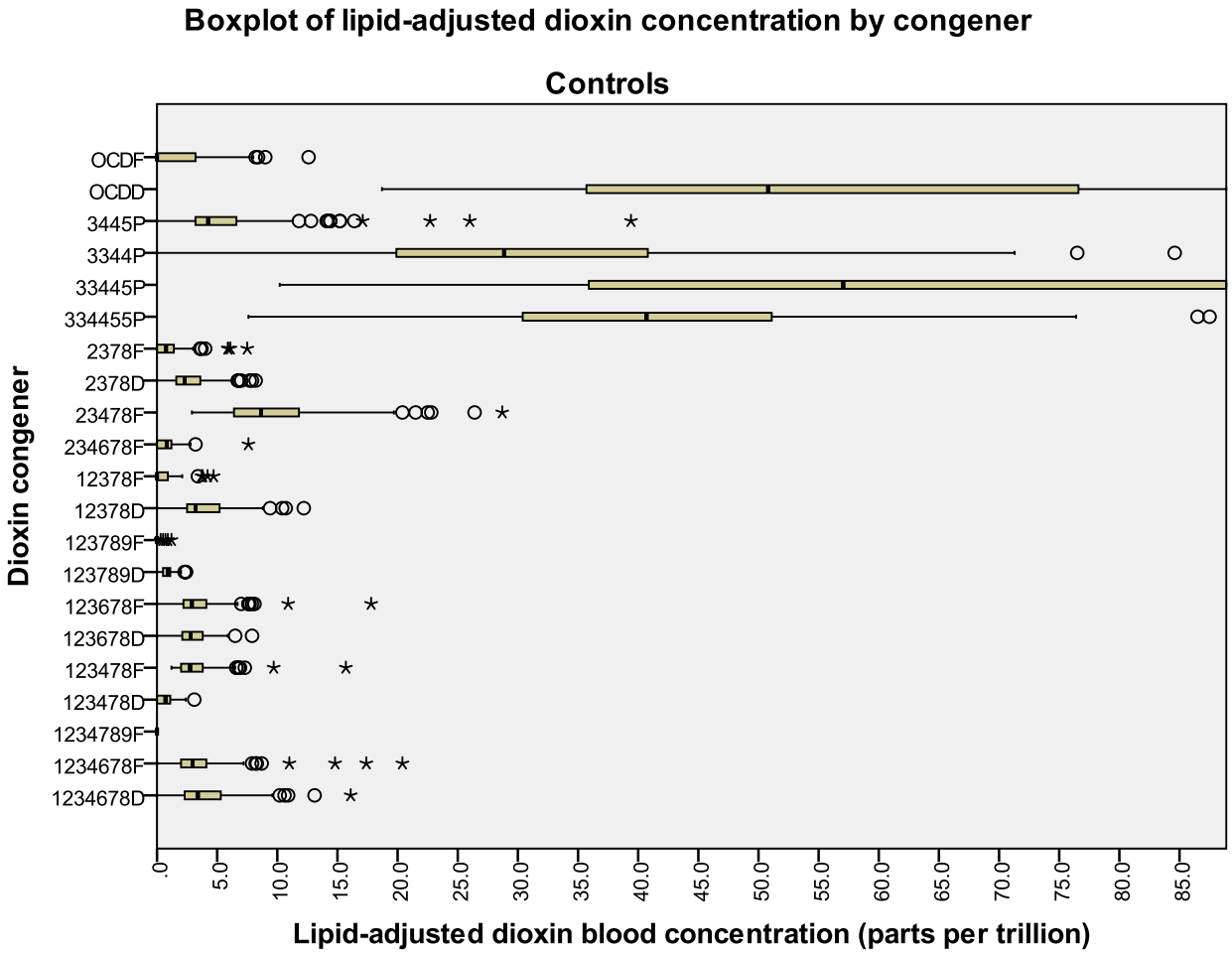


Figure 9

Figures 10 and 11 present dioxin levels in the unexposed group by gender.

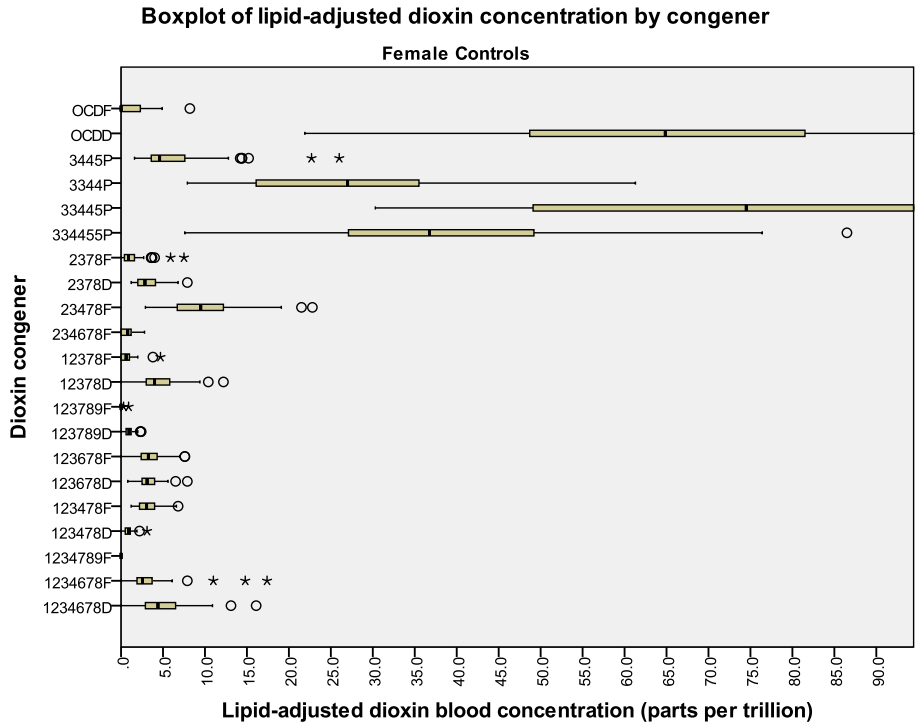


Figure 10

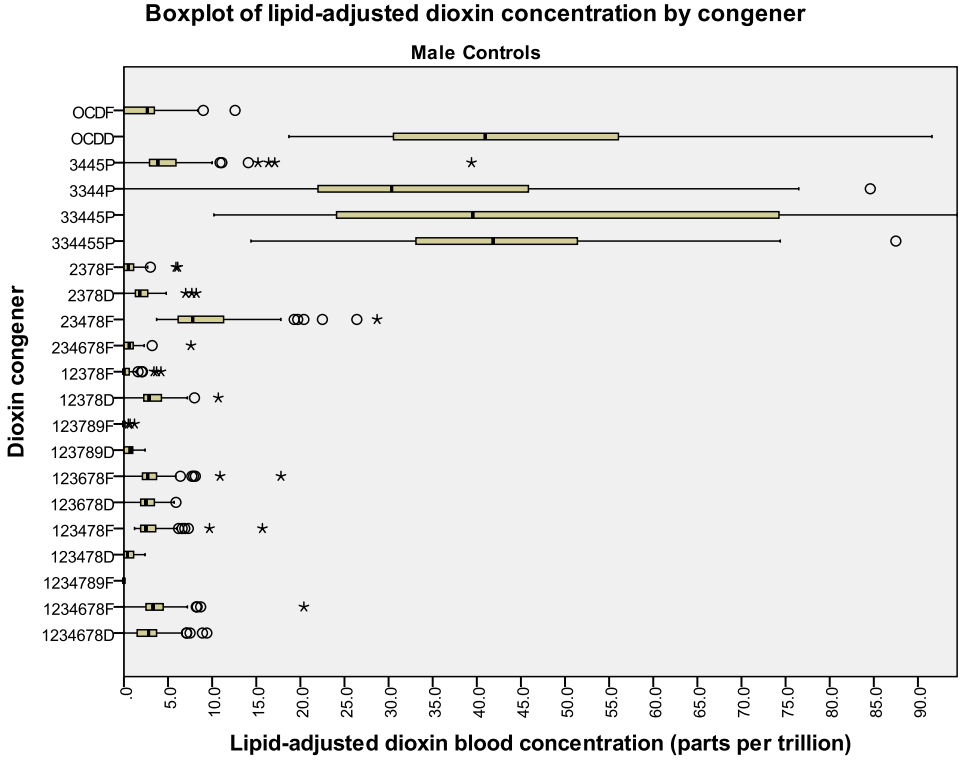
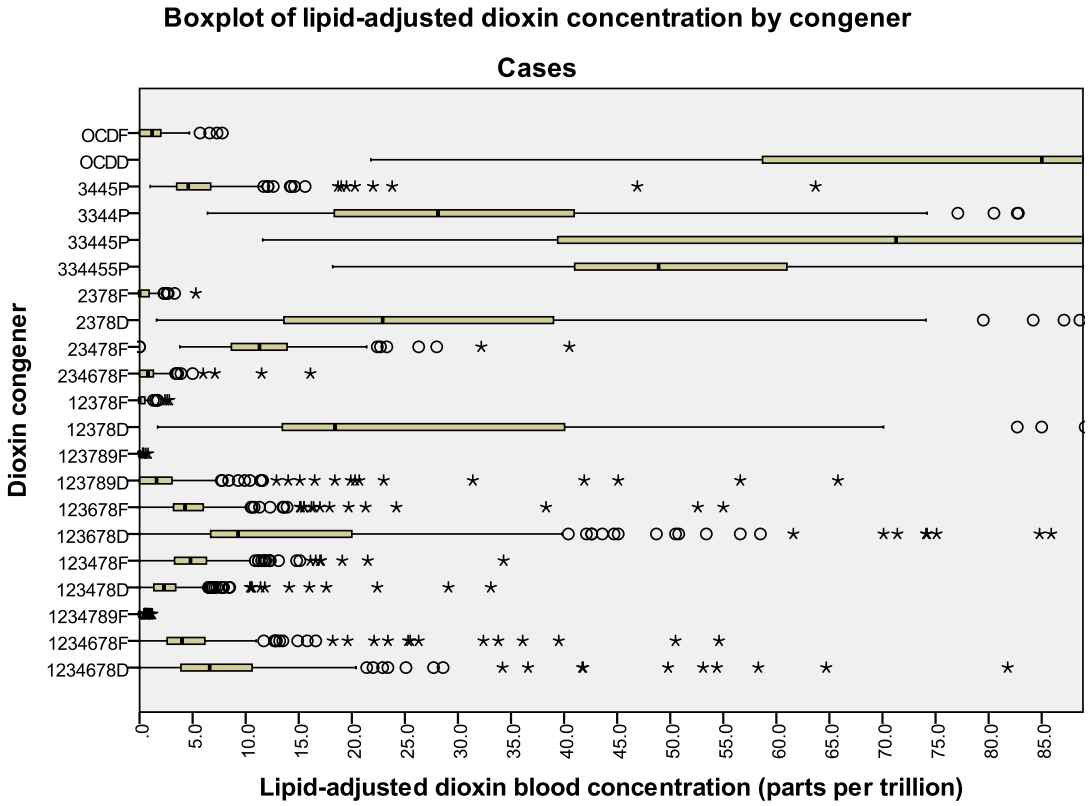


Figure 11

Figure 12 presents side-by-side comparisons of serum dioxin levels by congener between exposed and unexposed. Figure 13 presents these same data with mean, max, and min congener levels.



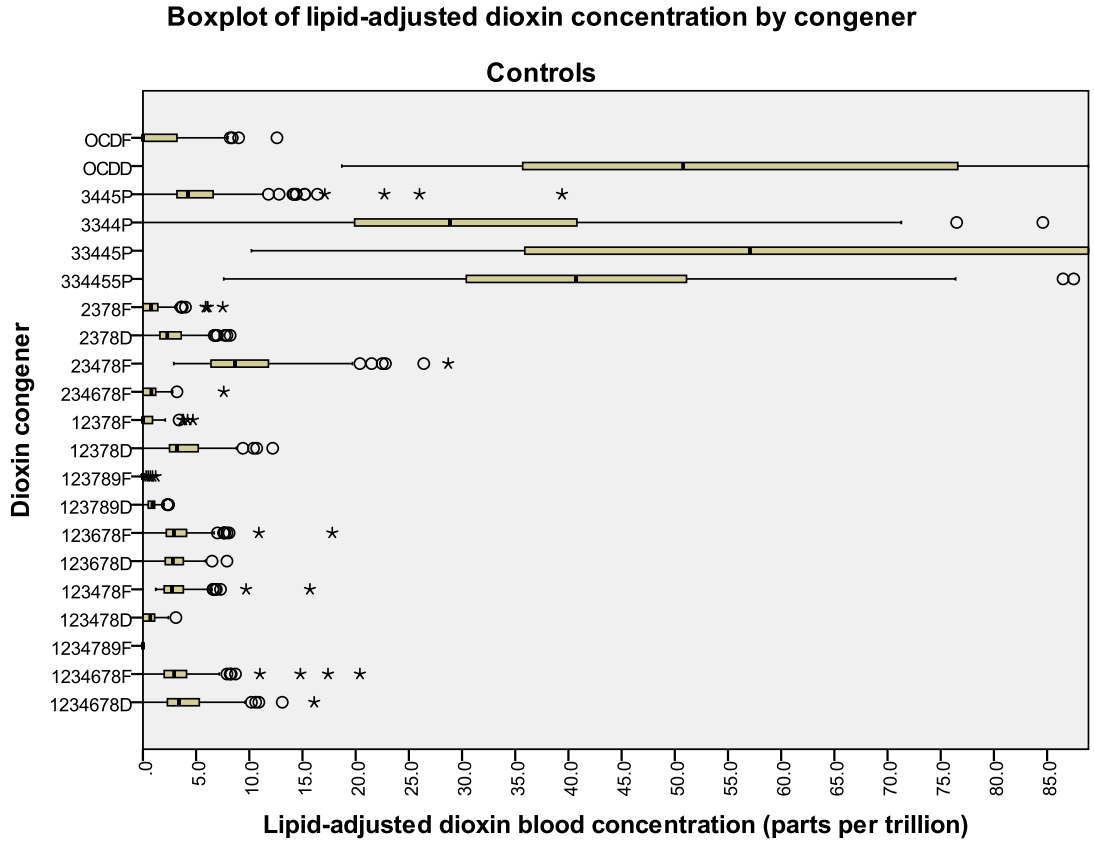
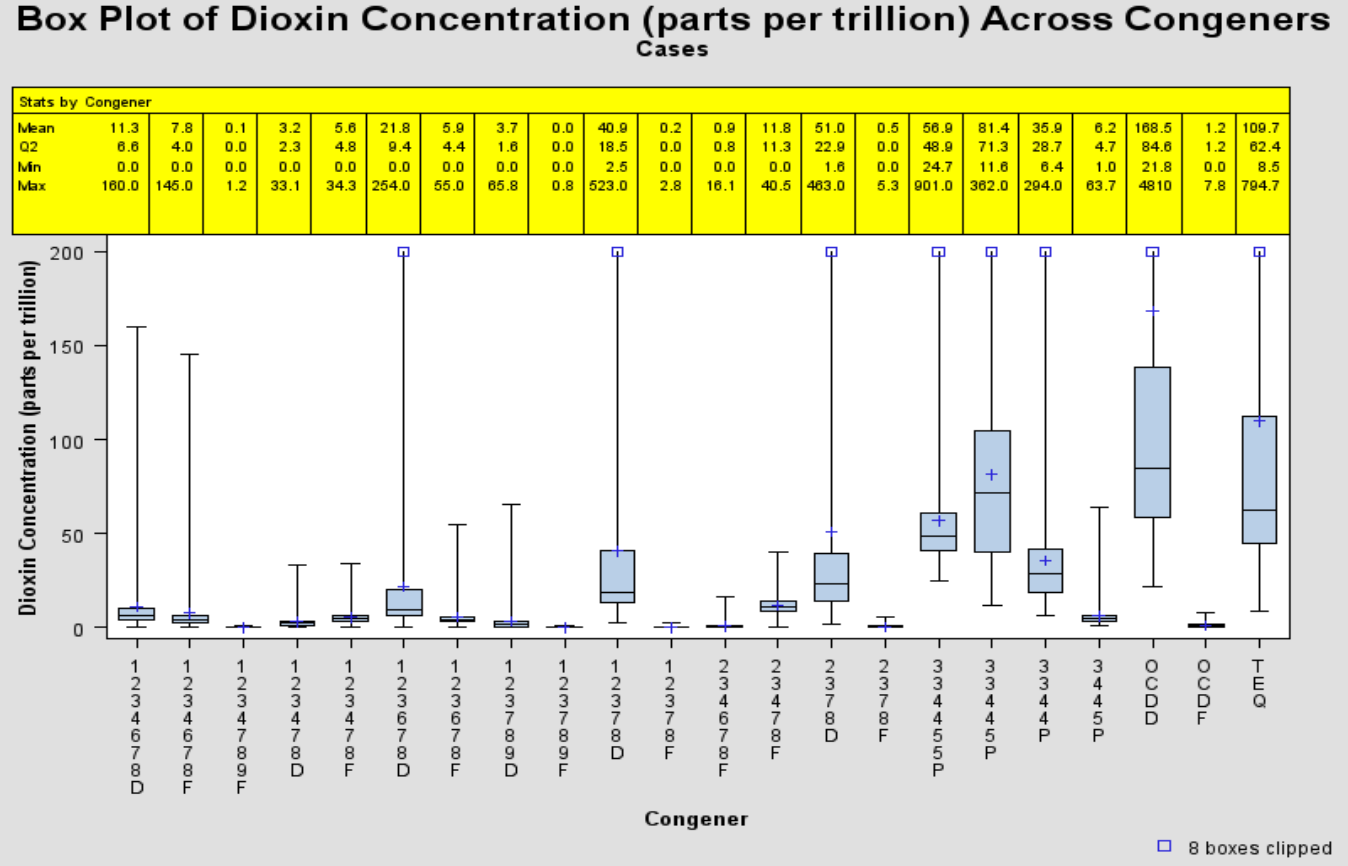
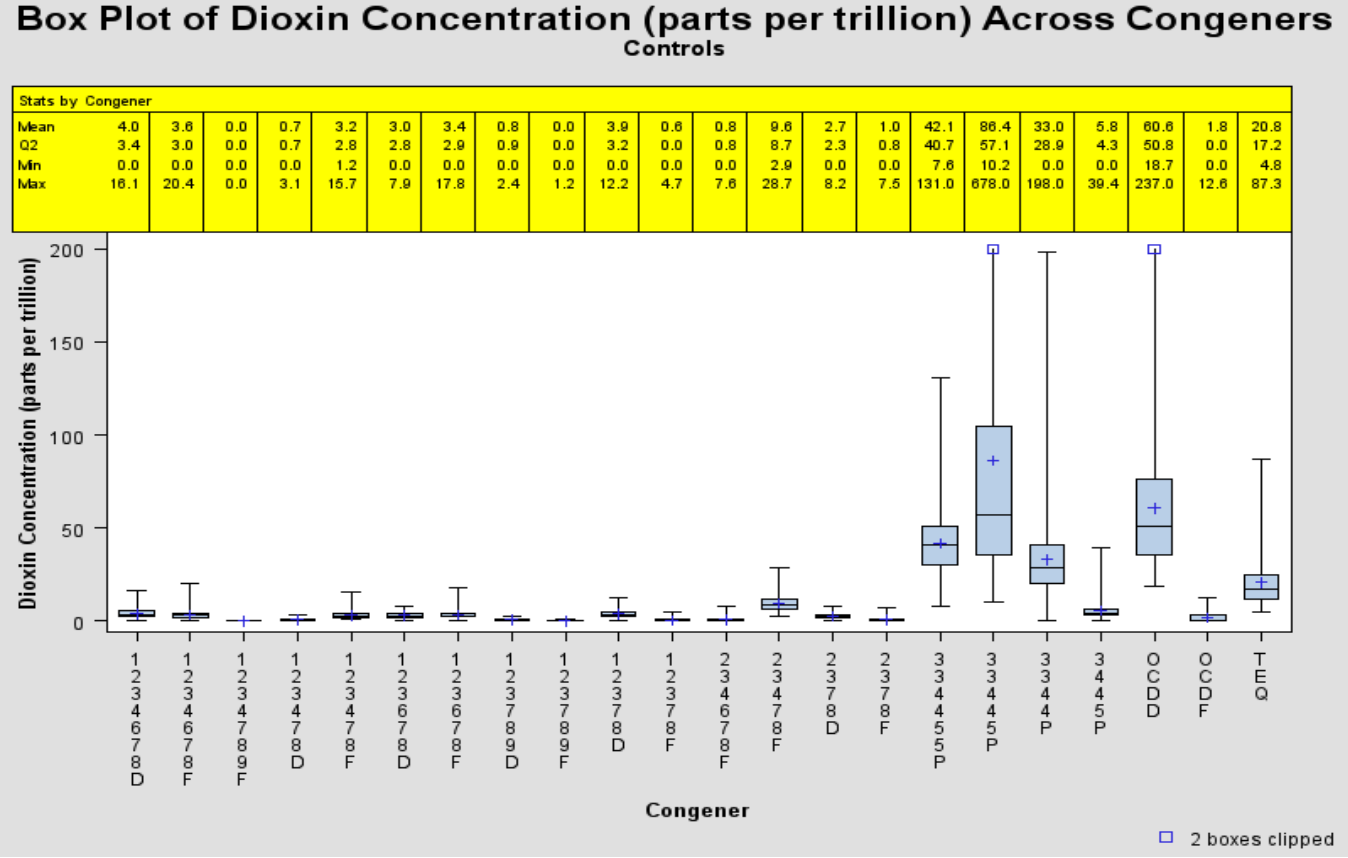


Figure 12


Box Plot of Dioxin Concentration (parts per trillion) Across Congeners
Cases
Stats by Congener
Mean 11.3 7.8 0.1 3.2 5.6 21.8 5.9 3.7 0.0 40.9 0.2 0.9 11.8 51.0 0.5 56.9 81.4 35.9 6.2 168.5 1.2 109.7
Q2 6.6 4.0 0.0 2.3 4.8 9.4 4.4 1.6 0.0 18.5 0.0 0.8 11.3 22.9 0.0 48.9 71.3 28.7 4.7 84.6 1.2 62.4
Min 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 2.5 0.0 0.0 0.0 1.6 0.0 24.7 11.6 6.4 1.0 21.8 0.0 8.5
Max 160.0 145.0 1.2 33.1 34.3 254.0 55.0 65.8 0.8 523.0 2.8 16.1 40.5 463.0 5.3 901.0 362.0 294.0 63.7 4810 7.8 794.7
Dioxin Concentration (parts per trillion)
0
50
100
150
200
1 2 3 4 6 7 8 D
1 2 3 4 6 7 8 F
1 2 3 4 7 8 9 F
1 2 3 4 7 8 D
1 2 3 4 7 8 F
1 2 3 6 7 8 D
1 2 3 6 7 8 F
1 2 3 7 8 9 D
1 2 3 7 8 9 F
1 2 3 7 8 D
1 2 3 7 8 F
2 3 4 6 7 8 F
2 3 4 7 8 F
2 3 7 8 D
2 3 7 8 F
3 3 4 5 5 P
3 3 4 4 5 P
3 3 4 4 P
3 4 4 5 P
O C D D
O C D F
T E Q
Congener
8 boxes clipped


Box Plot of Dioxin Concentration (parts per trillion) Across Congeners
Controls
Stats by Congener
Mean 4.0 3.6 0.0 0.7 3.2 3.0 3.4 0.8 0.0 3.9 0.6 0.8 9.6 2.7 1.0 42.1 86.4 33.0 5.8 60.6 1.8 20.8
Q2 3.4 3.0 0.0 0.7 2.8 2.8 2.9 0.9 0.0 3.2 0.0 0.8 8.7 2.3 0.8 40.7 57.1 28.9 4.3 50.8 0.0 17.2
Min 0.0 0.0 0.0 0.0 1.2 0.0 0.0 0.0 0.0 0.0 0.0 0.0 2.9 0.0 0.0 7.6 10.2 0.0 0.0 18.7 0.0 4.8
Max 16.1 20.4 0.0 3.1 15.7 7.9 17.8 2.4 1.2 12.2 4.7 7.6 28.7 8.2 7.5 131.0 678.0 198.0 39.4 237.0 12.6 87.3
Dioxin Concentration (parts per trillion)
0
50
100
150
200
1 2 3 4 6 7 8 D
1 2 3 4 6 7 8 F
1 2 3 4 7 8 9 F
1 2 3 4 7 8 D
1 2 3 4 7 8 F
1 2 3 6 7 8 D
1 2 3 6 7 8 F
1 2 3 7 8 9 D
1 2 3 7 8 9 F
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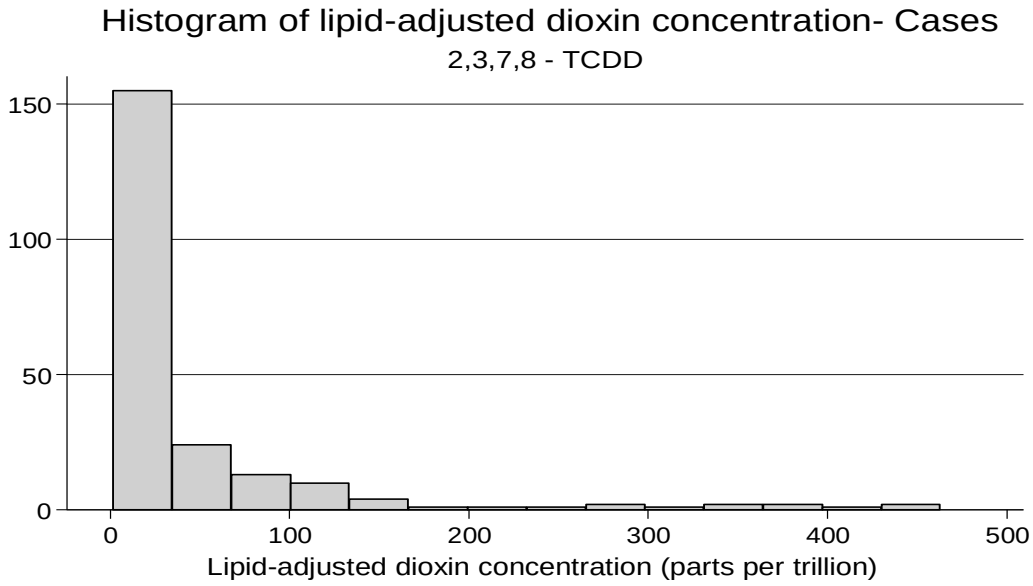
2 3 4 6 7 8 F
2 3 4 7 8 F
2 3 7 8 D
2 3 7 8 F
3 3 4 5 5 P
3 3 4 4 5 P
3 3 4 4 P
3 4 4 5 P
O C D D
O C D F
T E Q
Congener
2 boxes clipped

Figure 13

Figure 14 compares histograms of TCDD concentrations between exposed and unexposed. *Many of the exposed have high TCDD blood levels while TCDD levels in the unexposed are what would be expected among general population controls.*



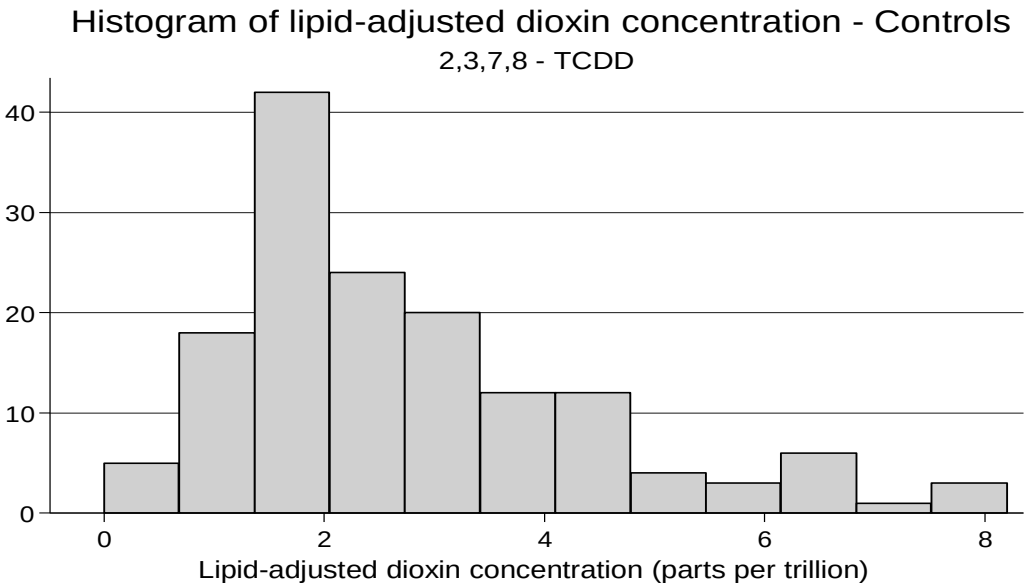


Figure 14

Figure 15 compares TEQ between exposed and unexposed stratified by gender.

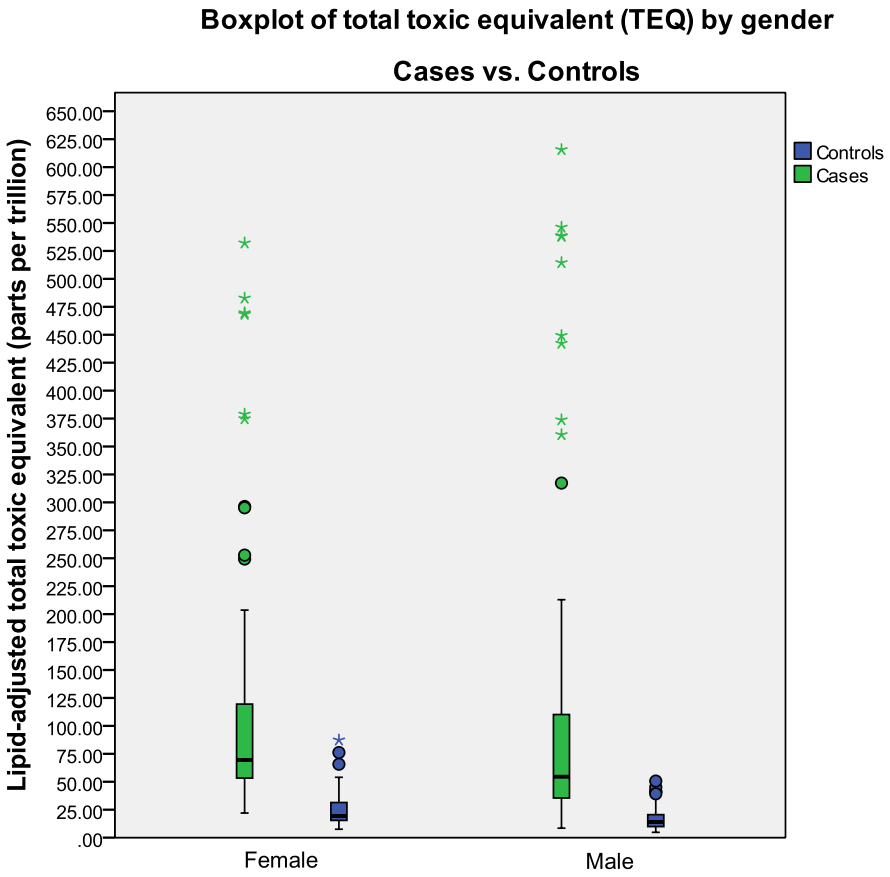


Figure 15

These data indicate that this occupational cohort was highly exposed to a mixture of PCDDs and PCDFs as we had anticipated while the unexposed were what we would expect from general population controls. The exposed had a mean 2,3,7,8 TCDD of 51.02 (median 22.9) vs 2.75 (median 2.30) for controls and mean TEQ of 109.71 (median 62.38) vs 20.83 (median 17.20) for the controls. Both the exposed and unexposed had similar serum levels of coplanar PCBs.

Prevalence and frequency of t(14;18) translocations among unexposed

We first examine the prevalence and frequency of t(14;18) translocations among German general population controls measured by Dr. Hirt and his laboratory (Figure 16, Shuler et al, Int J Cancer 2009, 124(4):958-63) and compare them to the prevalence and frequency of t(14;18) translocations measured in our unexposed population by this same laboratory (Figure 17).

Figure 16

Table I. Results of Quantitative Real-Time PCR Analysis of t(14;18)-MBR Translocation in Healthy Individuals (*n* = 715) Aged 0–91 Years with Respect to Prevalence and Frequency of Circulating t(14;18)-Positive Cells

Age (years)	*n*	Median age	Prevalence of t(14;18)-positive individuals (%)	Median frequency of t(14;18)-positive cells within the subgroups of all t(14;18)-positive individuals [10^{-6}]	Mean frequency of t(14;18)-positive cells within the subgroups of all t(14;18)-positive individuals [10^{-6}]	Median number of cells tested	Healthy individuals with > 40 t(14;18)-positive cells/10^6 PBMNC (%)
0 (cord blood)	36	0	0/36 (0)			993,000	0 (0)
0–9	48	3	0/48 (0)			493,000	0 (0)
10–19	47	16	9/47 (19)	3.8	9.3	437,000	0 (0)
20–29	63	25	21/63 (33)	3.8	7.3	631,000	0 (0)
30–39	130	36	60/130 (46)	3.5	8.2	769,000	1 (0.8)
40–49	140	44	92/140 (66)	5.4	26.1	796,000	7 (5)
50–59	81	54	47/81 (58)	5.1	17.9	817,000	4 (5)
60–69	85	63	51/85 (60)	13.2	25.1	742,000	9 (11)
70–91	85	74	47/85 (55)	9.2	26.4	681,000	10 (12)
All healthy individuals	715		327/715 (46)	5.8	19.8	644,000	31 (4)

Figure 17

Prevalence and frequency of t(14;18) translocations among 150 controls

Age	n	Median age	Prevalence of t(14;18) positive individuals (%)	Median frequency of t(14;18) positive cells per million	Mean frequency of t(14;18) positive cells per million	Median number of cells tested	Controls with >40 t(14;18) positive cells per million (%)
50-59	25	57.0	16/25 (64.0)	3.2	6.09	822504	0 (0.0)
60-69	63	65.0	31/63 (49.2)	2.8	5.28	775030	0 (0.0)
70-84	62	72.5	28/62 (45.2)	3.5	11.53	888968	2 (3.2)
All controls	150	67.5	75/150 (50.0)	3.4	7.79	814379	2 (1.3)
Note: Frequencies are for t(14;18) positive individuals only							

The prevalence of t(14;18) positive cells is similar between the German and our controls. The mean and median frequencies among our controls in similar age strata are lower.

Prevalence and Frequency of t(14;18) translocations by increasing serum dioxin level

We next compare population (exposed and unexposed) to the Seveso population (Baccarelli, et al. Carcinogenesis 2006). In this study, among those with 2,3,7,8-TCDD levels > 10, the geometric mean TCDD level was 44.5 ppt with a range of 10.5 to 475 ppt. These TCDD levels are comparable to those in our study, mean TCDD = 51 ppt and range 1.6 to 463 ppt. In Seveso (Figure 18) they observed in increase in the geometric mean frequency of t(14;18) translocations in the subgroup with > 0 translocations by increasing serum TCDD level.

Figure 18

t(14;18) translocations in lymphocytes of healthy dioxin-exposed individuals from Seveso, Italy

Table III
Prevalence and frequency of t(14;18) translocations by plasma TCDD levels, zone of residence and diagnosis of chloracne

	t(14;18)-positive subjects		t(14;18) frequency[a]	
	%	(Positive/total)	Mean	(95% CI)
Plasma TCDD				
<10 p.p.t.	34.7	(25/72)	4.2[b]	(2.9–6.2)
10.0–475.0 p.p.t.	34.7	(25/72)	9.9[b]	(6.8–14.5)
Zone of residence at the time of the accident				
Reference	42.4	(14/33)	4.3[c]	(2.3–8.0)
R	26.9	(7/26)	4.9[c]	(2.2–10.7)
B	29.4	(10/34)	7.2[c]	(3.8–13.6)
A	37.3	(19/51)	9.3[c]	(5.8–14.8)
Chloracne after the accident				
No	35.2	(32/91)	6.2	(3.7–10.6)
Yes	34.0	(18/53)	6.7	(4.7–9.6)

[a]Geometric means and 95% CIs of the number of t(14;18) translocations/10^6 lymphocytes among t(14;18)-positive subjects, adjusted for age, smoking status (never, ex or current smoker) and smoking duration in multivariable analysis.

[b]$P = 0.006$, test for difference in mean t(14;18) frequency between plasma TCDD categories.

[c]$P = 0.04$, test for trend in mean t(14;18) frequency across residence zones.

We next look at the geometric mean of t(14;18) translocations among our entire group (exposed and unexposed) with increasing quartile of TCDD serum levels (Figure 19). *We do not observe an increase in the geometric mean frequency of t(14;18) translocations among those with >0 translocations with increasing serum TCDD in the entire group of exposed and unexposed.*

Figure 19

Geometric mean t(14;18) frequency by current TCDD quartile among all of our participants (exposed and unexposed) among those who had at least one translocation

Quartiles

		t(14;18) translocation frequency			
	Exposure level*	n	Geometric mean	LCL	UCL
Males	≤ 2.4 ppt	29	3.24	2.27	4.64
	2.4-10.8 ppt	20	3.44	2.21	5.36
	10.8-25.2 ppt	25	2.59	1.65	4.08
	> 25.2 ppt	27	3.61	2.47	5.26
Females	≤ 2.4 ppt	17	3.08	2.05	4.62
	2.4-10.8 ppt	24	5.02	3.79	6.65
	10.8-25.2 ppt	20	2.95	2.03	4.28
	> 25.2 ppt	17	3.66	2.27	5.89
Total	≤ 2.4 ppt	46	3.18	2.45	4.14
	2.4-10.8 ppt	44	4.23	3.30	5.42
	10.8-25.2 ppt	45	2.74	2.05	3.68
	> 25.2 ppt	44	3.63	2.72	4.83

Note: Exposure level groups based on quartiles (all participants, >0 translocations)
Includes all participants with > 0 translocations
*ppt = parts per trillion, lipid-adjusted

We next look at the geometric mean of t(14;18) translocations among our entire group (exposed and unexposed) with increasing quintile of TCDD serum levels (Figure 20). *We do not observe an increase in the geometric mean frequency of t(14;18) translocations among those with >0 translocations with increasing serum TCDD in the entire group of exposed and unexposed.*

Figure 20

Quintiles by Current TCDD level in our data					
		t(14;18) translocation frequency			
	Exposure level*	n	Geometric mean	LCL	UCL
Males	≤ 2.0 ppt	26	3.24	2.19	4.81
	2.0-5.2 ppt	13	4.17	2.46	7.05
	5.2-16.8 ppt	23	2.50	1.69	3.70
	16.8-28.6 ppt	17	3.13	1.74	5.63
	> 28.6 ppt	22	3.52	2.25	5.50
Females	≤ 2.0 ppt	11	3.72	2.12	6.53
	2.0-5.2 ppt	22	4.24	3.10	5.78
	5.2-16.8 ppt	13	3.32	2.15	5.13
	16.8-28.6 ppt	19	3.27	2.04	5.24
	> 28.6 ppt	13	3.74	2.37	5.91
Total	≤ 2.0 ppt	37	3.38	2.47	4.61
	2.0-5.2 ppt	35	4.21	3.24	5.47
	5.2-16.8 ppt	36	2.77	2.08	3.69
	16.8-28.6 ppt	36	3.20	2.24	4.57
	> 28.6 ppt	35	3.60	2.63	4.94
Note: Exposure level groups based on quintiles (all participants, >0 translocations)					
Includes all participants with > 0 translocations					
*ppt = parts per trillion, lipid-adjusted					

We next look at the geometric mean of t(14;18) translocations among our entire group (exposed and unexposed) with increasing quintile of TEQ (Figure 21). *We do not observe an increase in the geometric mean frequency of t(14;18) translocations among those with >0 translocations with increasing serum TEQ in the entire group of exposed and unexposed.*

Figure 21

Quintiles by Current TEQ in our data					
		t(14;18) translocation frequency			
	Exposure level*	n	Geometric mean	LCL	UCL
Males	≤ 15.55 ppt	24	3.17	2.26	4.45
	15.55-31.15 ppt	18	4.40	2.53	7.64
	31.15-49.00 ppt	21	2.92	1.90	4.49
	49.00-81.98 ppt	16	2.25	1.36	3.72
	> 81.98 ppt	22	3.49	2.15	5.66
Females	≤ 15.55 ppt	12	2.93	1.93	4.43
	15.55-31.15 ppt	18	4.56	2.95	7.06
	31.15-49.00 ppt	15	3.99	2.69	5.91
	49.00-81.98 ppt	20	3.52	2.35	5.30
	> 81.98 ppt	13	3.25	2.00	5.30
Total	≤ 15.55 ppt	36	3.09	2.39	3.99
	15.55-31.15 ppt	36	4.48	3.20	6.27
	31.15-49.00 ppt	36	3.32	2.48	4.45
	49.00-81.98 ppt	36	2.89	2.12	3.94
	> 81.98 ppt	35	3.40	2.42	4.78
Note: Exposure level groups based on quintiles (all participants, >0 translocations)					
Includes all participants with > 0 translocations					
*ppt = parts per trillion, lipid-adjusted					

We next compare the prevalence of t(14;18) translocations between exposed and unexposed (Figure 22). *We do not see a difference in prevalence of t(14;18) translocations between our exposed and unexposed group.*

Figure 22

Exposed

	t(14;18) translocations	
	# with any translocations	% with any translocations*
Males	64	52.46%
Females	40	43.96%
Total	104	48.83%

*percentage within exposure group (ie: 52.46% of males had a translocation)

Unexposed

	t(14;18) translocations	
	# with any translocations	% with any translocations*
Males	37	48.68%
Females	38	51.35%
Total	75	50.00%

*percentage within exposure group (ie: 48.68% of males had a translocation)

We next compare the frequency of t(14;18) translocations among those with >0 translocations between the exposed and unexposed (Figuress 23 and 24). *We do not see differences between the exposed and unexposed but not that the standard deviations are very high.*

Figure 23

Exposed
t(14;18) translocations

	Mean	Std	Median	Min	Max
Males	6.28	9.31	2.45	0.40	56.60
Females	5.57	6.26	3.25	0.50	31.00
Total	6.00	8.25	2.60	0.40	56.60

Unexposed
t(14;18) translocations

	Mean	Std	Median	Min	Max
Males	9.61	22.81	3.10	0.80	127.20
Females	6.01	6.28	4.00	1.00	33.60
Total	7.79	16.62	3.40	0.80	127.20

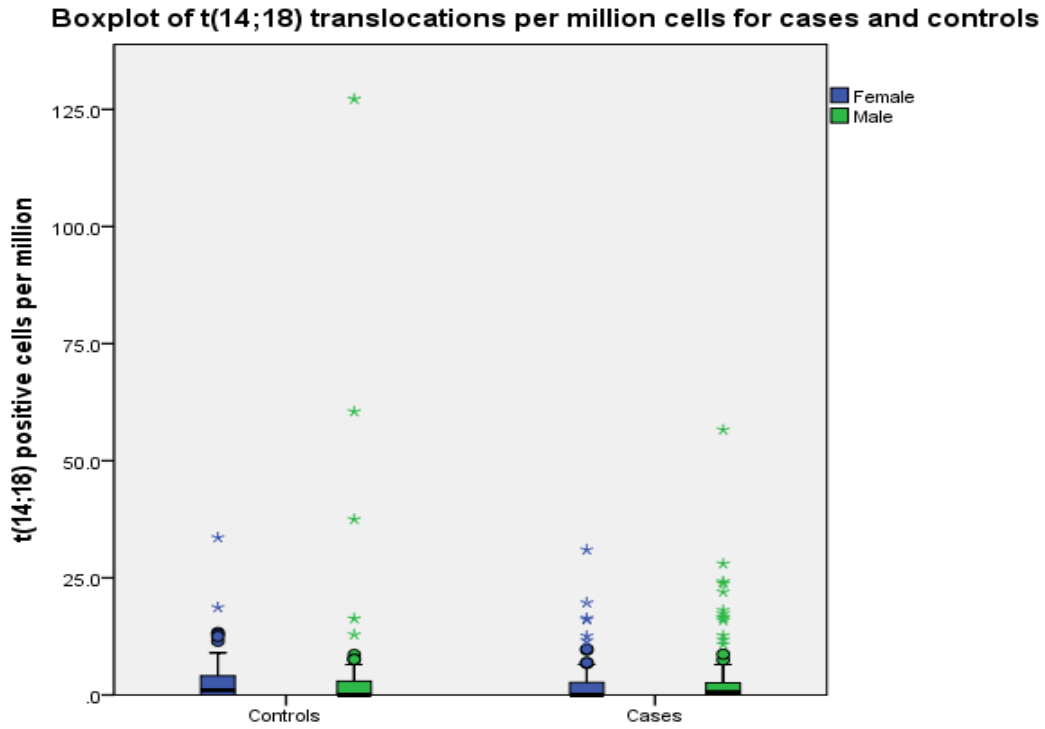


Figure 24

We next compare exposed and unexposed by frequency of t(14;18) translocations among those with >0 translocations and by PCDD, PCDF, PCB level (Figure 25)

Figure 25

ALL PARTICIPANTS WITH > 0 TRANSLOCATIONS

CONTROLS				CASES			
Dioxin and Furan Congeners	**Mean**	**Standard Deviation**	**Median**	**Dioxin and Furan Congeners**	**Mean**	**Standard Deviation**	**Median**
t(14;18) Translocations	**7.79**	**16.62**	**3.40**	**t(14;18) Translocations**	**6.00**	**8.25**	**2.60**
Total TEQ	**21.45**	**14.60**	**16.36**	**Total TEQ**	**101.53**	**122.81**	**59.79**
Dioxin Congeners				**Dioxin Congeners**			
1234678D	**4.04**	**2.85**	**3.20**	1234678D	**9.84**	**17.51**	**5.45**
123478D	**0.72**	**0.64**	**0.70**	123478D	**2.74**	**2.90**	**2.00**
123678D	**2.98**	**1.42**	**2.70**	123678D	**19.44**	**34.06**	**8.70**
123789D	**0.85**	**0.57**	**0.90**	123789D	**2.84**	**6.12**	**1.40**
12378D	**4.05**	**2.34**	**3.20**	12378D	**38.71**	**71.17**	**17.70**
2378D	**2.81**	**1.73**	**2.10**	2378D	**45.31**	**67.93**	**22.70**
OCDD	**62.89**	**42.30**	**48.40**	OCDD	**177.99**	**494.95**	**77.80**
Furan Congeners				**Furan Congeners**			
1234678F	**3.66**	**2.75**	**3.10**	1234678F	**7.19**	**15.72**	**3.90**
1234789F	**0.00**	**0.00**	**0.00**	1234789F	**0.07**	**0.22**	**0.00**
123478F	**3.21**	**1.97**	**2.70**	123478F	**5.29**	**3.48**	**4.40**
123678F	**3.47**	**2.34**	**2.80**	123678F	**5.60**	**6.25**	**4.20**
123789F	**0.03**	**0.18**	**0.00**	123789F	**0.01**	**0.07**	**0.00**
12378F	**0.55**	**0.82**	**0.00**	12378F	**0.18**	**0.37**	**0.00**
234678F	**0.79**	**1.03**	**0.80**	234678F	**0.80**	**1.14**	**0.70**
23478F	**9.59**	**5.10**	**8.20**	23478F	**11.82**	**5.32**	**10.90**
2378F	**0.90**	**1.09**	**0.60**	2378F	**0.47**	**0.60**	**0.00**
OCDF	**1.82**	**2.53**	**0.00**	OCDF	**1.11**	**1.29**	**1.10**
Other				**Other**			
334455P	**42.11**	**19.54**	**38.40**	334455P	**53.53**	**18.49**	**48.20**
33445P	**90.50**	**101.48**	**57.10**	33445P	**83.98**	**60.08**	**73.00**
3344P	**34.35**	**26.03**	**28.40**	3344P	**33.42**	**33.13**	**26.60**
3445P	**5.89**	**5.50**	**4.00**	3445P	**6.26**	**6.92**	**4.55**

*Numbers represent analyte concentrations in parts per trillion (pg/g) on a lipid-adjusted basis

Total n = 104; Males n = 64; Females n = 40

We now restrict our analyses to **exposed only** in the event there are differences between the exposed and unexposed that we have not accounted for. Figures 26 and 27 compare mean frequency of t(14;18) translocations among those with >0 translocations by dioxin serum TCDD. *We observe a non-significant trend in males and total exposed but not females.*

Figure 26

EXPOSED ONLY
2,3,7,8 TCDD

		t(14;18) translocation (yes/no)		t(14;18) translocation frequency		
	Exposure level*	# with any translocations	% with any translocations**	mean	std	median
Males	< 10 ppt	11	47.83%	4.63	6.22	2.40
	10-50 ppt	39	52.70%	5.72	7.17	2.30
	> 50 ppt	14	56.00%	9.11	15.12	2.95
Females	< 10 ppt	2	66.67%	6.30	4.95	6.30
	10-50 ppt	30	45.45%	5.12	5.22	3.10
	> 50 ppt	8	36.36%	7.08	9.91	3.90
Total	< 10 ppt	13	50.00%	4.88	5.89	2.60
	10-50 ppt	69	49.29%	5.46	6.36	2.60
	> 50 ppt	22	46.81%	8.37	13.24	3.50

Note: Exposure level groups based those used by Baccarelli et al, 2006

*ppt = parts per trillion, lipid-adjusted

**percentage within exposure group (ie: 47.83% of males exposed to < 10ppt had a translocation)

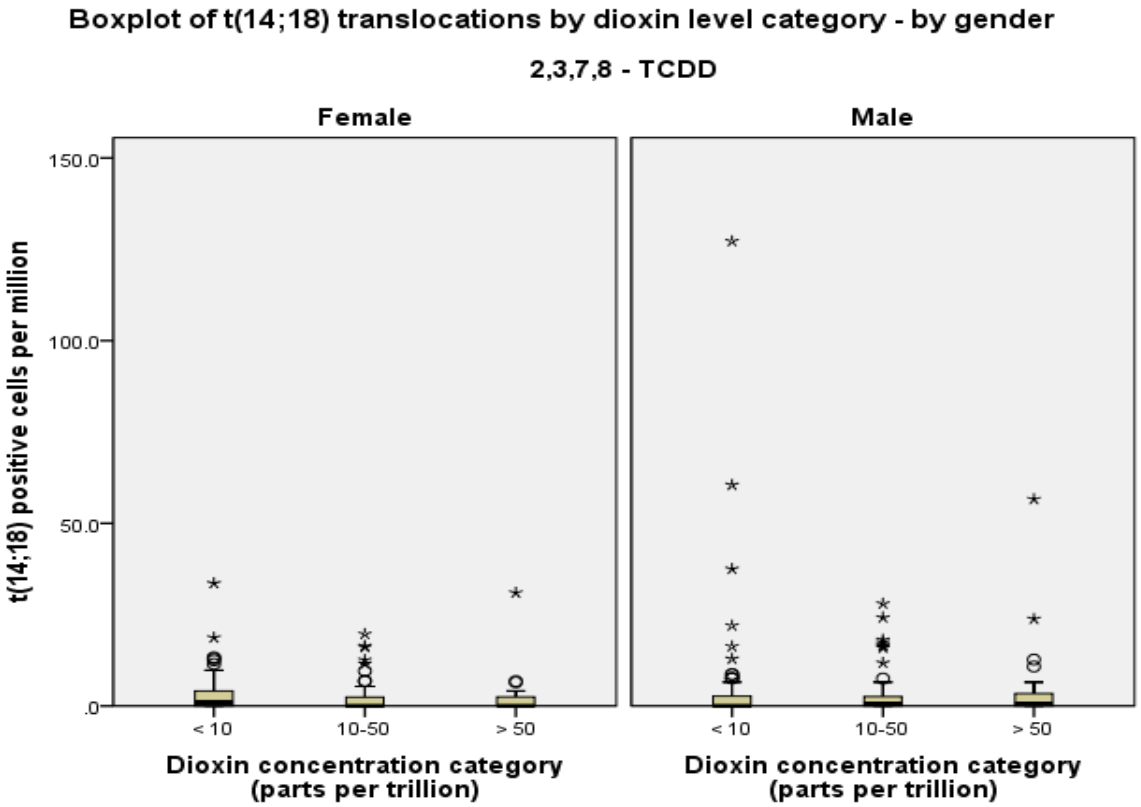


Figure 27

We now restrict our analyses to **unexposed only.** Figures 28 and 29 compare mean frequency of t(14;18) translocations among those with >0 translocations by dioxin serum TCDD using similar serum TCDD strata.

Figure 28

UNEXPOSED ONLY **2,3,7,8 TCDD**						
		t(14;18) translocation (yes/no)		t(14;18) translocation frequency		
	Exposure level*	# with any translocations	% with any translocations**	mean	std	median
Males	< 10 ppt	37	48.68%	9.61	22.81	3.10
	10-50 ppt	0	-	-	-	-
	> 50 ppt	0	-	-	-	-
Females	< 10 ppt	38	51.35%	6.01	6.28	4.00
	10-50 ppt	0	-	-	-	-
	> 50 ppt	0	-	-	-	-
Total	< 10 ppt	75	50.00%	7.79	16.62	3.40
	10-50 ppt	0	-	-	-	-
	> 50 ppt	0	-	-	-	-

Note: Exposure level groups based those used by Baccarelli et al, 2006

*ppt = parts per trillion, lipid-adjusted

**percentage within exposure group (ie: 48.68% of males exposed to < 10ppt had a translocation)

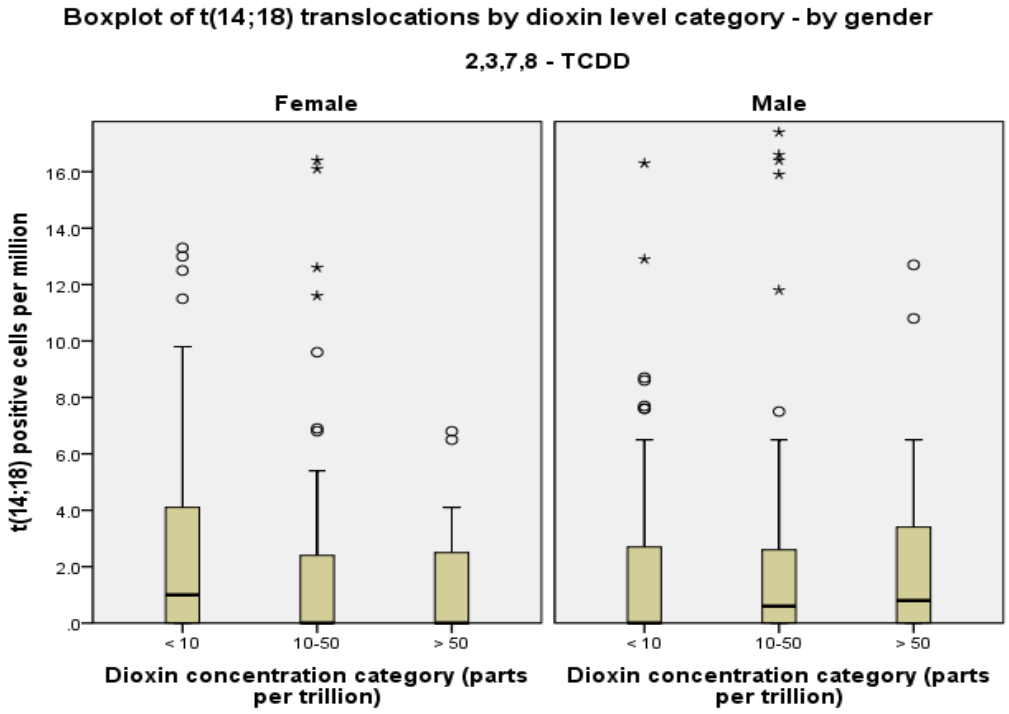


Figure 29

We next look at the frequency of t(14;18) translocations among those with >0 translocations by TEQ among the **exposed only** (Figures 30 and 31)**.** *We again observe a non-significant trend in males and total exposed but not females.*

Figure 30

EXPOSED ONLY
Total Toxic Equivalent (TEQ)

		t(14;18) translocation (yes/no)		t(14;18) translocation frequency		
	Exposure level*	# with any translocations	% with any translocations**	mean	std	median
Males	< 45 ppt	20	48.78%	4.52	5.49	2.35
	45-60 ppt	15	51.72%	6.68	7.85	3.10
	60-100 ppt	11	57.89%	5.39	5.77	3.50
	≥ 100 ppt	18	54.55%	8.43	14.43	2.35
Females	< 45 ppt	5	45.45%	4.34	3.87	2.80
	45-60 ppt	13	61.90%	3.38	5.02	2.20
	60-100 ppt	14	43.75%	7.59	4.89	5.95
	≥ 100 ppt	8	29.63%	6.38	10.15	3.10
Total	< 45 ppt	25	48.08%	4.48	5.14	2.40
	45-60 ppt	28	56.00%	5.15	6.78	2.30
	60-100 ppt	25	49.02%	6.62	5.30	5.30
	≥ 100 ppt	26	43.33%	7.80	13.09	2.50

Note: Exposure level groups based on quartiles for all cases

*ppt = parts per trillion, lipid-adjusted

**percentage within exposure group (ie: 48.78% of males exposed to < 45ppt had a translocation)

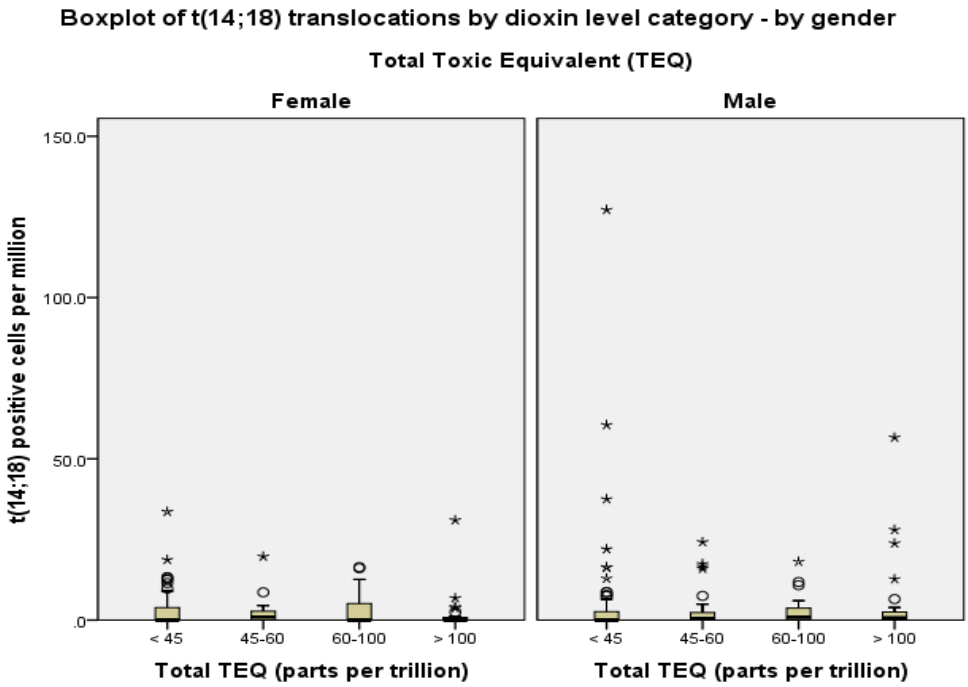

Boxplot of t(14;18) translocations by dioxin level category - by gender
Total Toxic Equivalent (TEQ)
Female
Male
t(14;18) positive cells per million
150.0
100.0
50.0
.0
< 45
45-60
60-100
> 100
Total TEQ (parts per trillion)
Total TEQ (parts per trillion)

Figure 31

We now restrict our analyses to **unexposed only.** Figures 32 and 33 compare mean frequency of t(14;18) translocations among those with >0 translocations by dioxin serum TCDD using similar TEQ strata.

Figure 32

UNEXPOSED ONLY
Total Toxic Equivalent (TEQ)

		t(14;18) translocation (yes/no)		t(14;18) translocation frequency		
	Exposure level*	# with any translocations	% with any translocations**	mean	std	median
Males	< 45 ppt	35	47.30%	10.10	23.38	3.20
	45-60 ppt	2	100.00%	1.10	0.42	1.10
	60-100 ppt	0	-	-	-	-
	≥ 100 ppt	0	-	-	-	-
Females	< 45 ppt	34	50.00%	6.26	6.52	4.00
	45-60 ppt	2	66.67%	6.55	2.90	6.55
	60-100 ppt	2	66.67%	1.25	0.35	1.25
	≥ 100 ppt	0	-	-	-	-
Total	< 45 ppt	69	48.59%	8.20	17.25	3.40
	45-60 ppt	4	80.00%	3.83	3.57	2.95
	60-100 ppt	2	66.67%	1.25	0.35	1.25
	≥ 100 ppt	0	-	-	-	-

Note: Exposure level groups based on quartiles for all cases

*ppt = parts per trillion, lipid-adjusted

**percentage within exposure group (ie: 47.30% of males exposed to < 45ppt had a translocation)

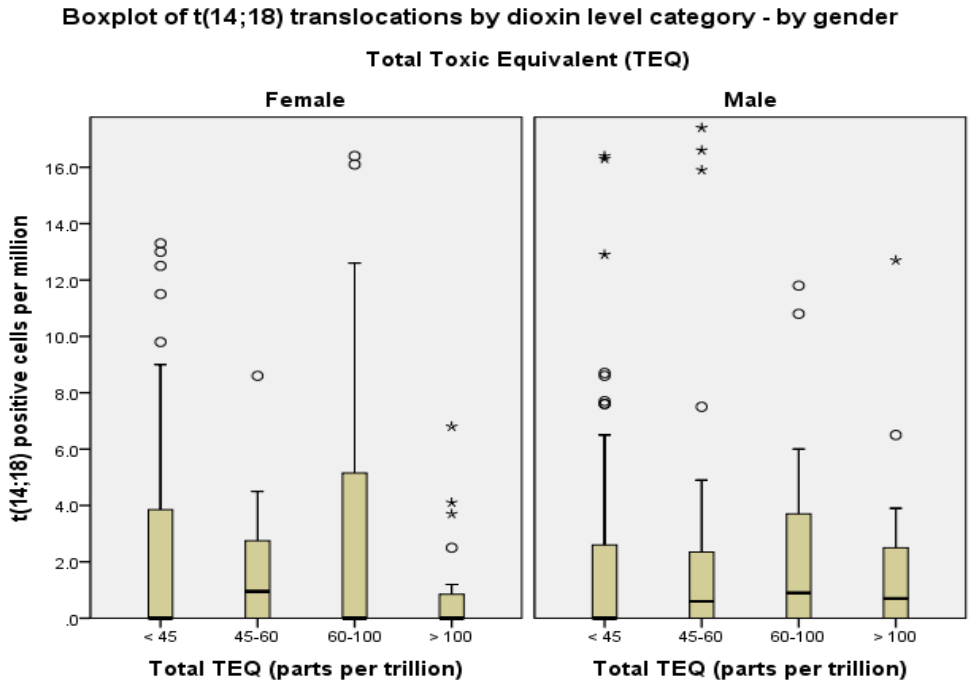

Boxplot of t(14;18) translocations by dioxin level category - by gender
Total Toxic Equivalent (TEQ)
Female
Male
t(14;18) positive cells per million
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
.0
< 45
45-60
60-100
> 100
Total TEQ (parts per trillion)
Total TEQ (parts per trillion)

Figure 33

Frequency of t(14;18) translocations by increasing "back-extrapolated" serum dioxin level

We extrapolated serum 2,3,7,8-TCDD levels to the last date of employment by"
1. Calculating TCDD half-lives for a subset (n=10) of exposed workers on whom we had 3 or more 2,3,7,8 TCDD levels over time
2. Took the average of these half-lives (= 7.8 years)
3. Back-extrapolated to date of last employment using the following formula:

Dioxin concentrations in this workbook (with the exception of the "DioxinBoxplots" tab) are back-extrapolated to the last day of employment. The analysis of the relationship between dioxin concentration and t(14;18) translocations in this file only includes exposed cases (not controls).
Dioxin Concentrations were back-extrapolated using the following formula:
$C_t = C_o e^{-kt}$
C_t = concentration at time of sample collection
C_o = peak concentration (last day of employment)
-k = -0.693 / 7.8 years
t = time from last day of employment to sample collection (years)

The mean frequency of t(14;18) translocations among those with >0 translocations by increasing back-extrapolated TCDD level is shown in Figures 34 and 35. *We again see a non-significant trend of increasing frequency of translocations with increasing back-extrapolated TCDD level in males and total but not in females.*

Figure 34

		t(14;18) translocation frequency**			
	Exposure level*	n	mean	std	median
Males	< 54.7 ppt	14	4.88	6.40	2.20
	54.7-126.2 ppt	18	5.88	7.12	3.50
	126.2-302.0 ppt	15	6.26	7.66	2.10
	> 302.0 ppt	17	7.86	14.09	2.50
Females	< 54.7 ppt	5	4.56	3.68	2.80
	54.7-126.2 ppt	12	4.15	4.01	3.25
	126.2-302.0 ppt	13	8.79	9.32	5.30
	> 302.0 ppt	10	3.60	2.38	3.10
Total	< 54.7 ppt	19	4.79	5.71	2.40
	54.7-126.2 ppt	30	5.19	6.05	3.25
	126.2-302.0 ppt	28	7.44	8.41	3.25
	> 302.0 ppt	27	6.28	11.34	2.50

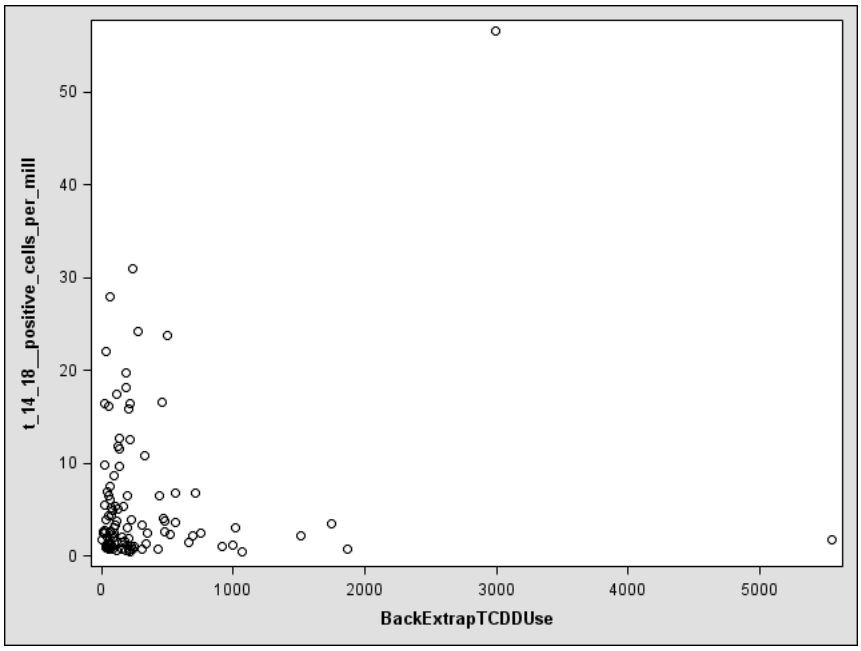
Note: Exposure level groups based on quartiles

Dioxin concentrations are back-extrapolated to last day of employment

*ppt = parts per trillion, lipid-adjusted

**Only includes cases who had at least one t(14;18) translocation

Figure 35



We next examine the relationship between increasing TCDD levels and increasing frequency of t(14;18) translocations among those with >0 translocations among exposed only while excluding outliers. *We now see a non-significant trend of increasing frequency with increasing back-extrapolated TCDD except for the highest TCDD level >357.3* (Figures 36 and 37).

2,3,7,8 TCDD - Excluding Outliers

t(14;18) translocation frequency**

	Exposure level*	n	mean	std	median
Males	< 56.5 ppt	14	4.88	6.40	2.20
	56.5-136.6 ppt	18	5.88	7.12	3.50
	136.6-357.3 ppt	15	6.26	7.66	2.10
	> 357.3 ppt	15	5.01	6.78	2.50
Females	< 56.5 ppt	5	4.56	3.68	2.80
	56.5-136.6 ppt	12	4.15	4.01	3.25
	136.6-357.3 ppt	13	8.79	9.32	5.30
	> 357.3 ppt	10	3.60	2.38	3.10
Total	< 56.5 ppt	19	4.79	5.71	2.40
	56.5-136.6 ppt	30	5.19	6.05	3.25
	136.6-357.3 ppt	28	7.44	8.41	3.25
	> 357.3 ppt	25	4.45	5.43	2.50

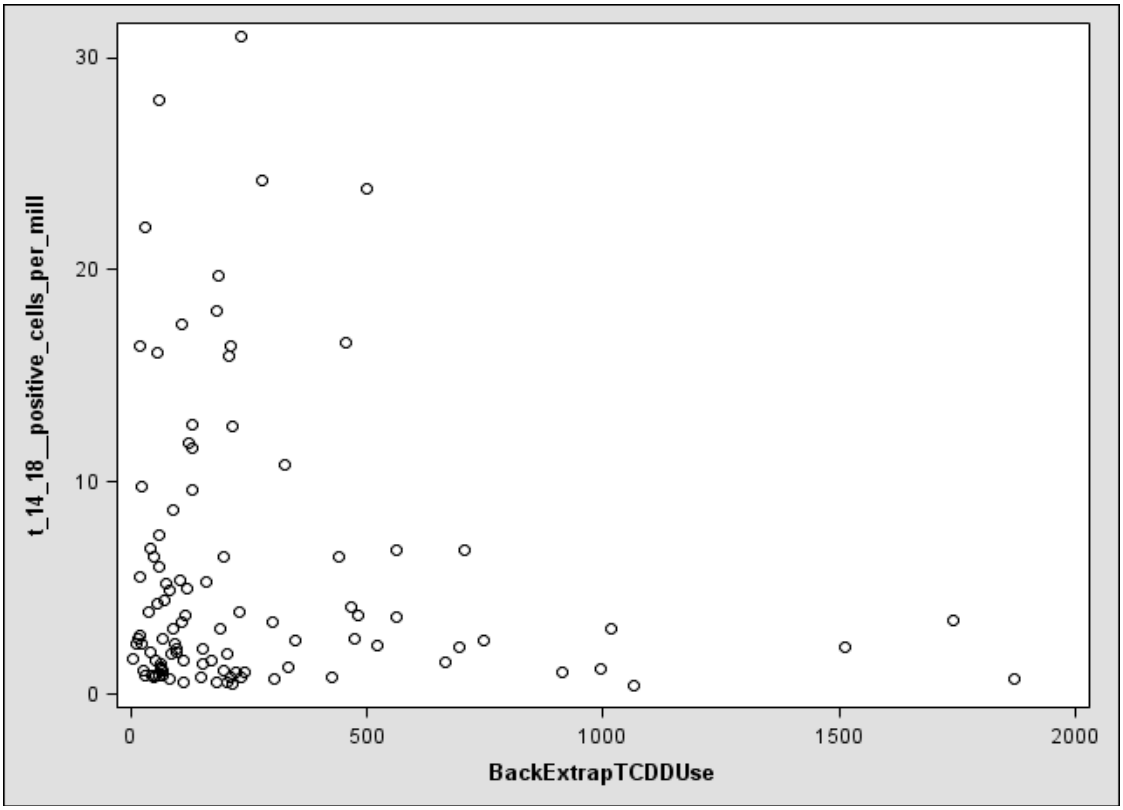

t_14_18__positive_cells_per_mill
30
20
10
0
0
500
1000
1500
2000
BackExtrapTCDDUse

Figure 37

Prevalence and Frequency of t(14;18) translocations by chloracne status

Next (Figure 38) we examine the prevalence and frequency of t(14;18) translocations by chloracne status (i.e. dermatologists assessment as to whether the individual currently has or has ever had chloracne). *Among the exposed group, those who had chloracne had a higher frequency of t(14;18) translocations than those without chloracne.*

Figure 38

Prevalence and Frequency of t(14;18) translocations by chloracne status

	Control Group	Exposed Group	
		No Chloracne	Chloracne
t(14;18) Prevalence			
#	75	76	23
%	50.0	46.6	52.3
Frequency (all subjects)			
n	150	163	44
mean	3.89	2.62	4.22
median	0.40	0.00	0.60
Frequency (> 0 translocations only)			
n	75	76	23
mean	7.79	5.62	8.07
median	3.40	3.10	2.60

When we stratify the participants on chloracne status, we see that, in the chloracne group, the frequency of translocations among those with at least one translocation increases with increasing TEQ (Figure 39) and increasing TCDD (Figure 40). *The correlation coefficients between TCDD/TEQ and frequency of translocations are only significantly positive for the subgroup with chloracne (Figures 39 – 46).*

Prevalence and Frequency of t(14;18) translocations by chloracne status and TEQ

Figure 39

		Total Toxic Equivalent (TEQ) Quartile			
Chloracne Status		≤ 19.2	19.2-41.2	41.2-72.8	> 72.8
Chloracne	t(14;18) Prevalence				
	#	0	7	6	9
	%	0.0	63.6	42.9	56.3
	Frequency (all subjects)				
	n	2	11	14	16
	mean	0.00	1.18	2.67	7.61
	median	0.00	1.30	0.00	1.15
	Frequency (> 0 translocations only)				
	n	0	7	6	9
	mean	-	1.86	6.23	13.52
	median	-	2.00	2.50	6.50
No Chloracne	t(14;18) Prevalence				
	#	48	36	36	31
	%	53.3	46.2	50.7	42.5
	Frequency (all subjects)				
	n	90	78	71	73
	mean	2.88	5.08	2.81	2.13
	median	1.00	0.00	0.40	0.00
	Frequency (> 0 translocations only)				
	n	48	36	36	31
	mean	5.40	11.01	5.55	5.02
	median	2.75	4.30	3.10	2.50

Prevalence and Frequency of t(14;18) translocations by chloracne status and TCDD

Figure 40

		2,3,7,8-TCDD Quartile			
Chloracne Status		≤ 2.8	2.8-10.8	10.8-25.6	> 25.6
Chloracne	t(14;18) Prevalence				
	#	1	5	8	9
	%	100.0	45.5	42.1	69.2
	Frequency (all subjects)				
	n	1	11	19	13
	mean	2.60	2.01	2.15	9.23
	median	2.60	0.00	0.00	2.20
	Frequency (> 0 translocations only)				
	n	1	5	8	9
	mean	2.60	4.42	5.10	13.33
	median	2.60	2.40	2.35	6.50
No Chloracne	t(14;18) Prevalence				
	#	48	36	39	28
	%	52.8	46.2	55.7	37.8
	Frequency (all subjects)				
	n	91	78	70	74
	mean	4.05	3.51	3.16	1.99
	median	1.00	0.00	0.65	0.00
	Frequency (> 0 translocations only)				
	n	48	36	39	28
	mean	7.69	7.61	5.67	5.25
	median	2.70	4.45	2.40	3.45

Figure 41

Pearson Correlation Coefficients for dioxin exposure and t(14;18) frequency

All subjects (n=365)

	Total Toxic Equivalent (TEQ)	2,3,7,8 - TCDD	t(14;18) frequency
Total Toxic Equivalent (TEQ)	1.00	0.86*	-0.02
2,3,7,8 - TCDD		1.00	< 0.01
t(14;18) frequency			1.00

* Pearson Correlation Coefficient statistically significant ($p < 0.05$)

Figure 42

Pearson Correlation Coefficients for dioxin exposure and t(14;18) frequency

All subjects with > 0 translocations (n=180)

	Total Toxic Equivalent (TEQ)	2,3,7,8 - TCDD	t(14;18) frequency
Total Toxic Equivalent (TEQ)	1.00	0.86*	0.01
2,3,7,8 - TCDD		1.00	0.04
t(14;18) frequency			1.00

* Pearson Correlation Coefficient statistically significant ($p < 0.05$)

Figure 43

Pearson Correlation Coefficients for dioxin exposure and t(14;18) frequency

All subjects without chloracne (n=313)

	Total Toxic Equivalent (TEQ)	2,3,7,8 - TCDD	t(14;18) frequency
Total Toxic Equivalent (TEQ)	1.00	0.85*	-0.07
2,3,7,8 - TCDD		1.00	-0.07
t(14;18) frequency			1.00

* Pearson Correlation Coefficient statistically significant ($p < 0.05$)

Figure 44

Pearson Correlation Coefficients for dioxin exposure and t(14;18) frequency

All subjects without chloracne and > 0 translocations (n=151)

	Total Toxic Equivalent (TEQ)	2,3,7,8 - TCDD	t(14;18) frequency
Total Toxic Equivalent (TEQ)	1.00	0.84*	-0.07
2,3,7,8 - TCDD		1.00	-0.07
t(14;18) frequency			1.00

* Pearson Correlation Coefficient statistically significant ($p < 0.05$)

Figure 45

Pearson Correlation Coefficients for dioxin exposure and t(14;18) frequency

All subjects with chloracne (n=44)

	Total Toxic Equivalent (TEQ)	2,3,7,8 - TCDD	t(14;18) frequency
Total Toxic Equivalent (TEQ)	1.00	0.94*	0.37*
2,3,7,8 - TCDD		1.00	0.42*
t(14;18) frequency			1.00

* Pearson Correlation Coefficient statistically significant ($p < 0.05$)

Figure 46

Pearson Correlation Coefficients for dioxin exposure and t(14;18) frequency

All subjects with chloracne and > 0 translocations (n=23)

	Total Toxic Equivalent (TEQ)	2,3,7,8 - TCDD	t(14;18) frequency
Total Toxic Equivalent (TEQ)	1.00	0.94*	0.38
2,3,7,8 - TCDD		1.00	0.41*
t(14;18) frequency			1.00

* Pearson Correlation Coefficient statistically significant ($p < 0.05$)

Dioxin Exposure and Gene Expression

The following Figures (Tables) present data on gene expression for the sample of 60 exposed and 30 unexposed whose peripheral blood mononuclear cells were tested for up-regulation or down-regulation of 83 candidate genes.

Figure 47 presents the percent missing data for specific genes (undetectable). Undetectable results for some of these genes are most likely due to their expression being tissue-specific, i.e. not highly expressed in peripheral blood mononuclear cells.

Figure 47. Percent missing by gene

Gene	n	% missing	Gene	n	% missing
AHR	90	0.0	HSP90AA1	90	0.0
AHRR	88	2.2	IGFBP7	89	1.1
AICDA	87	3.3	IL17RB	89	1.1
AIP	89	1.1	IL1A	60	33.3
ALDH1A3	83	7.8	IL1B	89	1.1
ALDH3A1	16	82.2	IL6	80	11.1
ALDH3A2	88	2.2	IL8	89	1.1
ALDH6A1	89	1.1	JUN	90	0.0
ALOX15B	88	2.2	KLF4	89	1.1
AREG	90	0.0	MTMR7	89	1.1
ARNT	89	1.1	NFIL3	88	2.2
BACH2	87	3.3	NFKB1	90	0.0
BAX	90	0.0	NRIP1	89	1.1
BCL2A1	90	0.0	PAPPA	56	37.8
BCL2L1	90	0.0	PARP1	90	0.0
BCL2L10	14	84.4	PAX5	90	0.0
BCL2L2	88	2.2	PDK4	89	1.1
BRCA1	89	1.1	PIK3R1	90	0.0
BTN1A1	89	1.1	PRDM1	90	0.0
Bcl2_syber	90	0.0	PTGS2	89	1.1
CCL2	71	21.1	PTN	12	86.7
CD36	90	0.0	RB1	90	0.0
CDCA5	87	3.3	RSP01	14	84.4
CEBPB	90	0.0	RSP02	16	82.2
CEBPD	90	0.0	RSP03	21	76.7
CREM	88	2.2	SERPINB2	89	1.1
CRYlL1	90	0.0	SERPINE2	89	1.1
CTBP2	90	0.0	ST8SIA1	90	0.0
CTGF	75	16.7	STAT3	90	0.0
CTNNB1	89	1.1	THRSP	76	15.6
CXCL12	10	88.9	TIPARP	90	0.0
CYP1A1	38	57.8	TNF	89	1.1
CYP1A2	27	70.0	TP53	90	0.0

CYP4F8	5	94.4	TRIP11	87	3.3
EIF2S1	90	0.0	UGT1A1	87	3.3
ESR1	89	1.1	WNT5A	31	65.6
FST	57	36.7			
GAL	33	63.3			
GRN	90	0.0			
GSTM1	35	61.1			
GSTM3	88	2.2			
HBEGF	90	0.0			
HIST1H2AM	90	0.0			
HIST1H2BE	90	0.0			
HSD3B1	7	92.2			

Figure 48 compares blood dioxin levels among the sample of 60 exposed and 30 unexposed. *Except for comparable levels of PCB 126, TCDD and TEQ are much higher in the sample of exposed than in the unexposed, as expected.*

Figure 48. Exposure levels in Gene Expression Study Groups

	n	mean	std	median	min	max
Exposed						
TCDD	60	51.9	76.4	23.8	10.6	456.0
PCB 126	60	97.6	66.9	85.2	14.1	360.0
TEQ	60	108.4	99.4	72.0	30.6	532.0
PCB 180	60	254.9	100.9	245.7	88.9	598.0
Unexposed						
TCDD	30	2.8	1.4	2.3	1.1	6.8
PCB 126	30	118.1	131.0	73.1	27.5	678.0
TEQ	30	24.4	17.4	18.6	9.4	87.3
PCB 180	30	131.6	66.7	121.2	39.5	334.0

Note: Dioxin and PCB levels measured in parts per trillion (ppt), lipid adjusted

Figure 49 presents the fold changes and significance levels for the exposed vs controls for the 83 candidate genes by pathway.

Figure 49. Fold change in specific gene pathways exposed vs unexposed

	Gene	Exposed/ Unexposed	n	mean ΔCt	std. dev.	median ΔCt	min	max	Fold change	p-value
AhR Pathway	AHR	Exposed	603	3.2	0.5	3.1	1.9	4.9	1.2	0.008
		Unexposed	30	3.5	0.5	3.5	2.3	4.8		
	AHRR	Exposed	592	10.2	1.0	10.3	7.1	12.1	1.1	0.535
		Unexposed	29	10.4	1.2	10.3	7.7	13.3		
	AIP	Exposed	593	3.1	1.7	2.7	2.3	12.1	0.8	0.153
		Unexposed	30	2.8	0.2	2.8	2.2	3.3		
	ARNT	Exposed	593	5.6	0.4	5.5	4.4	7.7	1.2	0.001
		Unexposed	30	5.9	0.4	5.8	4.5	6.6		
	BRCA1	Exposed	602	7.0	0.3	7.0	6.1	7.7	1.1	0.053
		Unexposed	29	7.2	0.4	7.1	6.6	8.0		
	ESR1	Exposed	602	10.0	0.9	9.8	8.3	12.5	1.2	0.188
		Unexposed	29	10.3	0.9	10.2	7.9	12.6		
	HSP90AA1	Exposed	603	2.5	1.0	2.4	1.5	9.7	0.5	< 0.001
		Unexposed	30	1.5	0.3	1.5	1.0	2.0		
	NRIP1	Exposed	593	4.7	0.3	4.7	4.0	5.3	1.4	< 0.001
		Unexposed	30	5.2	0.5	5.2	4.0	6.1		
	TIPARP	Exposed	603	4.3	0.3	4.3	3.1	4.7	1.1	0.012
		Unexposed	30	4.5	0.4	4.5	3.7	5.3		
	TRIP11	Exposed	582	6.6	0.3	6.6	5.8	7.7	1.3	< 0.001
		Unexposed	29	7.0	0.5	7.0	5.9	7.9		

In the AhR pathway,AHR, ARNT, NRIP1, TIPARP, and TRIP11 were significantly up-regulated while HSP90AA1 was down-regulated. BRAC1 was borderline up-regulated.

Drug Metabolism	CYP1A1	Exposed	24	13.3	0.7	13.3	12.0	14.4	0.8	0.245
		Unexposed	14	13.0	0.9	13.3	10.9	14.2		
	CYP1A2	Exposed	18	12.6	0.6	12.7	11.0	13.8	1.1	0.798
		Unexposed	9	12.7	1.0	12.6	11.0	14.3		
	CYP4F8	Exposed	3	13.2	1.2	13.2	12.0	14.4	1.2	0.821
		Unexposed	2	13.5	1.1	13.5	12.7	14.2		
	ALDH3A1	Exposed	13	13.5	0.4	13.5	12.4	14.1	0.4	0.209*
		Unexposed	3	12.2	1.0	12.1	11.6	13.5		
	ALDH3A2	Exposed	58	4.4	1.0	4.4	3.2	11.3	1.2	0.068*
		Unexposed	30	4.7	0.4	4.6	3.9	5.9		
	ALDH1A3	Exposed	58	11.0	0.7	11.0	9.8	12.9	0.9	0.358
		Unexposed	25	10.8	0.7	10.8	9.8	12.3		
	ALDH6A1	Exposed	60	6.2	1.4	5.9	5.2	13.2	0.9	0.375*
		Unexposed	29	6.0	0.5	6.0	4.9	6.9		
	GSTM1	Exposed	28	8.4	0.7	8.3	6.6	10.8	0.9	0.788
		Unexposed	7	8.3	0.8	8.3	7.0	9.5		
	GSTM3	Exposed	60	8.9	1.8	9.5	5.7	12.8	0.9	0.742
		Unexposed	28	8.8	1.5	8.9	6.5	11.8		
	UGT1A1	Exposed	60	9.8	1.1	9.8	7.1	13.4	0.7	0.068
		Unexposed	27	9.3	1.3	9.3	7.0	13.0		

None of the candidate genes in the drug metabolism pathway were significantly up-regulated. ALDH3A2 was borderline up-regulated while UGT1A1 was borderline down-regulated. The absence of AHR activation downstream effects on drug metabolism may be due to the tissue studied (PBMNs) rather than liver where most drug metabolism occurs.

Anti-Apoptosis	BAX	Exposed	60	2.7	0.6	2.7	1.6	7.0	0.9	0.044*
		Unexposed	30	2.6	0.3	2.6	1.7	3.0		
	BCL2	Exposed	60	4.0	0.5	4.1	2.5	5.1	1.0	0.885*
		Unexposed	30	4.0	0.7	4.1	2.4	5.0		
	BCL2A1	Exposed	60	3.9	0.7	3.9	2.0	5.6	0.6	< 0.001
		Unexposed	30	3.2	0.9	3.0	1.9	5.6		
	BCL2L1	Exposed	60	0.5	0.9	0.5	-1.6	2.5	0.7	0.019
		Unexposed	30	0.0	1.0	-0.1	-1.9	1.9		
	BCL2L10	Exposed	10	13.7	0.6	13.9	12.6	14.4	1.1	0.598
		Unexposed	4	13.9	0.4	13.9	13.4	14.4		
	BCL2L2	Exposed	59	5.6	0.3	5.6	4.7	6.6	1.4	< 0.001
		Unexposed	29	6.0	0.4	6.0	5.6	7.3		
	PTGS2	Exposed	59	4.6	1.2	4.5	3.3	10.9	1.3	0.040*
		Unexposed	30	5.0	0.6	4.9	4.0	6.0		
	CEBPB	Exposed	60	1.5	0.6	1.5	0.0	4.8	1.0	0.642

		Unexposed	30	1.6	0.5	1.5	0.5	2.8		
	KLF4	Exposed	59	4.0	1.4	3.7	2.9	12.4	1.0	0.850*
		Unexposed	30	4.0	0.5	4.1	3.2	4.9		

In the Anti-Apoptosis pathway, BAX, BCL2AI and BCL2L1 were significantly down-regulated while PTGS2 was significantly up-regulated.

Lymphoma	AICDA	Exposed	59	11.0	1.0	10.6	9.4	13.7	1.1	0.591
		Unexposed	28	11.1	1.1	10.9	8.8	13.7		

Inflammation	TNF	Exposed	60	4.8	0.4	4.8	4.0	5.7	1.1	0.063
		Unexposed	29	5.0	0.3	4.9	4.3	5.7		
	IL1A	Exposed	48	11.7	0.9	11.6	9.6	13.6	0.8	0.309
		Unexposed	12	11.4	1.2	11.5	9.4	13.1		
	IL1B	Exposed	60	3.3	0.7	3.3	1.7	7.5	1.1	0.181
		Unexposed	29	3.5	0.6	3.5	2.6	4.7		
	IL6	Exposed	55	10.8	0.8	10.9	8.8	13.3	1.4	0.103*
		Unexposed	25	11.3	1.4	11.2	8.6	14.1		
	IL8	Exposed	59	5.3	0.9	5.3	2.9	8.0	1.6	0.003
		Unexposed	30	6.0	1.1	5.8	3.8	8.4		
	IL17RB	Exposed	59	4.5	1.0	4.5	2.6	7.4	0.7	0.020
		Unexposed	30	3.9	1.3	3.8	1.7	6.9		
	PARP1	Exposed	60	4.2	0.4	4.3	3.2	5.0	1.3	< 0.001
		Unexposed	30	4.7	0.4	4.6	4.1	5.7		
	BACH2	Exposed	58	4.4	0.6	4.3	2.9	5.9	1.2	0.051
		Unexposed	29	4.6	0.7	4.7	2.7	6.0		
	CCL2	Exposed	52	12.8	1.0	12.9	9.2	14.3	0.6	0.096*
		Unexposed	19	12.1	1.8	12.7	7.8	14.2		
	SERPINB2	Exposed	60	7.2	0.9	7.1	6.1	12.1	1.6	< 0.001*
		Unexposed	29	7.9	0.6	7.9	7.1	9.2		
	CXCL12	Exposed	7	13.8	0.6	13.9	12.6	14.3	0.8	0.493
		Unexposed	3	13.5	0.6	13.4	13.0	14.1		
	NFKB1	Exposed	60	4.0	0.3	4.0	3.4	5.6	1.3	< 0.001
		Unexposed	30	4.4	0.3	4.4	3.8	4.9		
	JUN	Exposed	60	6.9	0.6	6.9	5.1	9.2	1.0	0.916*
		Unexposed	30	6.9	0.9	6.9	3.1	8.9		
	STAT3	Exposed	60	0.5	0.4	0.6	-0.4	1.3	1.3	< 0.001
		Unexposed	30	0.9	0.4	0.9	0.2	1.5		

In the inflammation pathway, dioxin exposure was associated with significant up-regulation of IL8, PARP1, SEPINB2, NFKB1, and STAT3 and significant down-regulation of IL17RB. TNF and BACH2 were borderline up-regulated while CCL2 was borderline down-regulated.

Lipid Metabolism	CRYIL1	Exposed	60	4.6	0.4	4.6	3.4	5.4	1.0	0.373
		Unexposed	30	4.5	0.4	4.5	3.3	5.1		
	MTMR7	Exposed	60	6.1	0.2	6.1	5.6	6.6	1.4	< 0.001*
		Unexposed	29	6.6	0.4	6.7	5.6	7.5		
	ST8SIA1	Exposed	60	6.3	0.6	6.3	4.9	7.8	0.8	0.055
		Unexposed	30	6.1	0.5	6.0	5.2	7.0		
	GRN	Exposed	60	0.5	0.3	0.5	-0.2	1.5	1.3	0.006*
		Unexposed	30	0.9	0.6	0.8	-0.4	2.3		
	THRSP	Exposed	58	12.4	0.7	12.5	10.7	13.8	1.1	0.560
		Unexposed	18	12.5	0.6	12.4	11.4	14.0		
	HSD3B1	Exposed	7	13.7	0.5	13.7	13.1	14.4	-	-
		Unexposed	0	-	-	-	-	-		
	ALOX15B	Exposed	60	9.3	1.0	9.2	7.6	13.5	1.5	0.007
		Unexposed	28	9.9	0.9	9.7	8.3	11.9		

In the lipid metabolism pathway, MTMR7, GRN, and ALOX15B were significantly up-regulated, while ST8SIAI was borderline down-regulated.

Glucose Metabolism	CD36	Exposed	60	2.8	1.6	2.5	1.4	11.4	1.2	0.267*
		Unexposed	30	3.0	0.5	2.9	2.3	4.8		
	PDK4	Exposed	60	6.0	0.7	5.8	4.9	8.6	1.1	0.417
		Unexposed	29	6.1	0.6	6.0	5.2	7.7		

Cell Cycle	PAPPA	Exposed	44	12.5	0.8	12.5	10.8	14.1	1.1	0.457
		Unexposed	12	12.7	0.5	12.8	11.7	13.4		
	CDCA5	Exposed	60	11.4	0.9	11.5	8.0	14.0	1.1	0.542
		Unexposed	27	11.6	0.7	11.7	10.3	13.2		
	SERPINE2	Exposed	60	9.0	0.8	8.9	7.4	11.5	1.0	0.941
		Unexposed	29	9.0	0.7	8.9	8.0	10.5		
	PTN	Exposed	8	13.5	0.5	13.6	12.7	14.0	0.4	0.003
		Unexposed	4	12.1	0.7	12.4	11.0	12.7		
	RB1	Exposed	60	3.9	0.3	3.9	3.4	5.2	1.2	0.007*
		Unexposed	30	4.2	0.5	4.3	3.3	6.5		
	CTBP2	Exposed	60	3.8	0.3	3.8	2.9	4.5	1.2	0.002
		Unexposed	30	4.0	0.3	4.0	3.1	4.7		

Translation	EIF2S1	Exposed	60	4.4	0.3	4.4	3.1	5.3	0.9	0.019*
		Unexposed	30	4.3	0.2	4.3	3.9	4.7		

In the cell cycle and translation pathways, RB1 and CTBP2 were significantly up-regulated while PTN and EIF2S1 were significantly down-regulated.

WNT Signaling	WNT5A	Exposed	25	13.2	0.7	13.2	11.9	14.3	0.7	0.074
		Unexposed	6	12.6	0.5	12.8	11.8	13.2		
	CTNNB1	Exposed	59	4.7	0.3	4.7	3.7	6.0	1.7	< 0.001*
		Unexposed	30	5.5	0.4	5.4	4.5	6.7		
	RSP01	Exposed	10	13.6	0.8	14.0	11.9	14.4	0.8	0.442
		Unexposed	4	13.3	0.8	13.2	12.4	14.2		
	RSP02	Exposed	11	13.6	0.9	14.0	11.0	14.4	0.8	0.541
		Unexposed	5	13.3	0.5	13.1	12.7	14.0		
	RSP03	Exposed	18	13.5	0.6	13.7	12.4	14.4	0.7	0.223
		Unexposed	3	13.0	0.9	13.5	11.9	13.6		

In the WNT signaling pathway, CTNNB1 was significantly up-regulated while WNT5A was borderline down-regulated.

Other	TP53	Exposed	60	3.0	0.4	3.0	1.4	3.6	1.2	0.014*
		Unexposed	30	3.3	0.6	3.2	2.6	5.7		
	PRDM1	Exposed	60	3.0	0.4	3.0	2.4	3.9	1.3	0.001*
		Unexposed	30	3.4	0.6	3.4	2.6	5.8		
	PAX5	Exposed	60	5.0	0.7	5.0	3.1	6.9	1.0	0.782*
		Unexposed	30	4.9	1.1	4.8	1.7	7.1		
	HIST1H2BE	Exposed	60	3.0	0.4	3.0	2.0	4.3	0.9	0.096
		Unexposed	30	2.8	0.3	2.8	2.3	3.5		
	HIST1H2AM	Exposed	60	3.2	0.5	3.2	1.6	4.8	0.8	< 0.001
		Unexposed	30	2.8	0.4	2.9	1.7	3.5		
	GAL	Exposed	22	12.0	1.7	12.2	9.4	14.4	1.0	0.987
		Unexposed	11	12.0	2.0	12.7	8.4	14.4		
	CREM	Exposed	59	8.2	0.4	8.2	7.0	9.2	1.0	0.608
		Unexposed	29	8.1	0.5	8.1	7.2	8.8		
	NFIL3	Exposed	59	2.9	0.6	2.8	1.7	5.4	1.4	< 0.001
		Unexposed	29	3.4	0.5	3.3	2.6	4.3		
	CEBPD	Exposed	60	1.2	0.5	1.2	0.1	3.1	0.8	0.013
		Unexposed	30	0.9	0.5	0.9	0.0	1.9		
	IGFBP7	Exposed	60	4.4	0.8	4.3	3.4	8.3	0.9	0.385
		Unexposed	29	4.3	0.8	4.2	2.9	7.3		
	PIK3R1	Exposed	60	3.1	0.4	3.1	2.2	4.5	1.0	0.662
		Unexposed	30	3.1	0.4	3.1	1.4	4.1		
	FST	Exposed	44	13.3	0.7	13.2	11.7	14.2	0.6	0.155*
		Unexposed	13	12.6	1.6	13.0	8.0	14.2		
	AREG	Exposed	60	8.5	1.6	8.8	3.6	11.5	0.9	0.722
		Unexposed	30	8.3	1.7	8.4	4.7	12.2		
	HBEGF	Exposed	60	8.4	0.6	8.3	7.1	9.8	0.9	0.515

	Unexposed	30	8.3	0.8	8.3	5.7	9.4		
CTGF	Exposed	54	11.8	1.2	11.8	9.1	13.9	1.1	0.558
	Unexposed	21	12.0	1.1	11.8	10.3	14.2		
BTN1A1	Exposed	60	8.7	0.6	8.7	7.5	10.5	1.3	0.018
	Unexposed	29	9.1	0.6	9.1	7.6	10.4		

* Unequal variances - Satterthwaite test p-values shown

Note: Cycles to threshold (Ct) represents PCR cycle number at which DNA amount reaches a threshold value

Housekeeping genes used in analysis: GAPDH, ACTB, GUSB, HPRT1, B2M, RPLPO

ΔCt = Ct(gene of interest) - Ct(mean of housekeeping genes)

ΔΔCt = ΔCt(experimental) - Ct(control)

For other pathways, dioxin exposure was associated with significant up-regulation of TP53, PRDM1, NFIL3, and BTN1A1 and significant down-regulation of HIST1H2BE and CDEBPD.

Figure 50 presents fold-changes in candidate genes by tertitles of blood TEQ.

Figure 50.

	Gene	TEQ: Tertile	n	mean ΔCt	std. dev.	median ΔCt	min	max	Fold change	p-value
AhR Pathway	AHR	≤ 38.6	30	3.4	0.5	3.4	2.8	4.8	-	< 0.001
		38.6-73.3	31	3.4	0.6	3.3	2.6	4.9	1.0	
		> 73.3	29	3.0	0.4	3.1	1.9	3.6	1.4	
	AHRR	≤ 38.6	29	10.3	1.2	10.1	7.7	13.3	-	0.776
		38.6-73.3	30	10.2	1.0	10.4	7.7	12.1	1.0	
		> 73.3	29	10.3	1.0	10.4	7.1	11.9	0.9	
	AIP	≤ 38.6	30	2.8	0.2	2.8	2.2	3.1	-	0.409
		38.6-73.3	30	3.2	1.8	2.8	2.5	11.9	0.8	
		> 73.3	29	3.1	1.8	2.7	2.3	12.1	0.8	
	ARNT	≤ 38.6	30	5.8	0.4	5.8	4.5	6.6	-	0.027
		38.6-73.3	31	5.6	0.5	5.5	4.7	7.7	1.2	
		> 73.3	28	5.6	0.3	5.6	4.4	6.0	1.2	
	BRCA1	≤ 38.6	29	7.1	0.4	7.1	6.6	8.0	-	0.121
		38.6-73.3	31	7.0	0.4	7.0	6.1	7.7	1.1	
		> 73.3	29	7.0	0.3	7.1	6.4	7.5	1.1	
	ESR1	≤ 38.6	29	10.3	1.0	10.4	7.9	12.6	-	0.513
		38.6-73.3	31	9.8	0.9	9.6	8.3	12.1	1.4	
		> 73.3	29	10.2	0.9	10.2	8.4	12.5	1.1	
	HSP90AA1	≤ 38.6	30	1.6	0.5	1.5	1.0	2.7	-	0.001
		38.6-73.3	31	2.5	1.4	2.3	1.5	9.7	0.5	
		> 73.3	29	2.4	0.4	2.4	1.5	3.1	0.6	
	NRIP1	≤ 38.6	30	5.2	0.4	5.2	4.0	6.1	-	< 0.001
		38.6-73.3	31	4.7	0.3	4.7	4.0	5.3	1.4	
		> 73.3	28	4.7	0.2	4.8	4.2	5.1	1.4	
	TIPARP	≤ 38.6	30	4.5	0.4	4.5	3.7	5.3	-	0.012

	38.6-73.3	31	4.3	0.3	4.3	3.3	4.7	1.1	
	> 73.3	29	4.3	0.3	4.3	3.1	4.7	1.2	
	≤ 38.6	29	6.9	0.5	7.0	5.9	7.9	-	
TRIP11	38.6-73.3	30	6.6	0.4	6.6	5.8	7.7	1.3	0.002
	> 73.3	28	6.6	0.3	6.6	6.1	7.0	1.2	
Drug Metabolism	≤ 38.6	16	13.2	0.7	13.3	11.8	14.2	-	
	CYP1A1	38.6-73.3	13	13.2	1.0	13.5	10.9	14.4	1.0	0.991
	> 73.3	9	13.2	0.4	13.2	12.6	14.1	1.0	
	≤ 38.6	9	12.8	1.0	13.0	11.0	14.3	-	
	CYP1A2	38.6-73.3	5	11.8	0.6	11.8	11.0	12.5	2.0	0.941
	> 73.3	13	12.8	0.4	12.8	12.1	13.8	1.0	
	≤ 38.6	2	13.5	1.1	13.5	12.7	14.2	-	
	CYP4F8	38.6-73.3	1	13.2	-	13.2	13.2	13.2	1.2	0.828
	> 73.3	2	13.2	1.7	13.2	12.0	14.4	1.2	
	≤ 38.6	4	12.9	1.2	12.8	11.6	14.1	-	
	ALDH3A1	38.6-73.3	5	13.4	0.7	13.6	12.4	14.0	0.7	0.248
	> 73.3	7	13.4	0.2	13.5	13.2	13.6	0.7	
	≤ 38.6	30	4.7	0.4	4.6	3.9	5.9	-	
	ALDH3A2	38.6-73.3	29	4.6	1.3	4.4	3.6	11.3	1.0	0.097
	> 73.3	29	4.3	0.3	4.3	3.2	4.8	1.3	
	≤ 38.6	26	10.9	0.6	10.8	9.8	12.3	-	
	ALDH1A3	38.6-73.3	28	10.9	0.7	11.0	9.8	12.9	1.0	0.414
	> 73.3	29	11.0	0.7	11.0	9.8	12.6	0.9	
	≤ 38.6	29	6.0	0.5	6.0	4.9	6.9	-	
	ALDH6A1	38.6-73.3	31	6.3	1.6	6.0	5.2	13.2	0.8	0.996
	> 73.3	29	6.0	1.2	5.7	5.3	12.2	1.0	
	≤ 38.6	8	8.4	0.7	8.4	7.0	9.1	-	
	GSTM1	38.6-73.3	11	8.1	0.6	8.2	6.6	8.8	1.3	0.359
	> 73.3	16	8.6	0.8	8.3	7.8	10.8	0.9	
	≤ 38.6	28	9.0	1.4	9.6	6.5	11.4	-	
	GSTM3	38.6-73.3	31	9.1	1.8	9.5	5.7	12.8	0.9	0.510
	> 73.3	29	8.7	1.8	8.1	5.9	11.9	1.2	
	≤ 38.6	27	9.4	1.2	9.4	7.3	13.0	-	
	UGT1A1	38.6-73.3	31	9.9	1.0	10.0	7.0	12.2	0.7	0.471
	> 73.3	29	9.7	1.2	9.6	7.1	13.4	0.8	

	≤ 38.6	30	2.6	0.3	2.7	1.7	3.0	-	
BAX	38.6-73.3	31	2.6	0.3	2.7	1.6	3.1	1.0	0.119
	> 73.3	29	2.8	0.8	2.7	2.2	7.0	0.9	
	≤ 38.6	30	4.2	0.6	4.3	2.4	5.0	-	
BCL2	38.6-73.3	31	3.9	0.5	4.0	2.5	5.1	1.2	0.185
	> 73.3	29	4.0	0.4	4.0	3.0	4.7	1.1	
	≤ 38.6	30	3.1	0.9	2.9	1.9	5.6	-	
BCL2A1	38.6-73.3	31	3.9	0.7	3.9	2.1	5.6	0.6	< 0.001
	> 73.3	29	4.0	0.6	3.9	2.9	5.6	0.6	
	≤ 38.6	30	0.3	1.1	0.2	-1.6	2.5	-	
BCL2L1	38.6-73.3	31	0.3	0.9	0.5	-1.6	1.9	1.0	0.827
	> 73.3	29	0.3	0.8	0.5	-1.9	1.7	1.0	
	≤ 38.6	4	13.9	0.4	13.9	13.4	14.4	-	
BCL2L10	38.6-73.3	7	13.6	0.7	13.9	12.6	14.4	1.2	0.952
	> 73.3	3	13.9	0.1	13.9	13.8	13.9	1.0	
	≤ 38.6	29	5.9	0.4	5.9	5.1	7.3	-	
BCL2L2	38.6-73.3	30	5.7	0.4	5.6	4.7	6.8	1.2	< 0.001
	> 73.3	29	5.6	0.3	5.6	4.9	6.2	1.3	
	≤ 38.6	30	5.0	0.7	4.9	3.4	6.0	-	
PTGS2	38.6-73.3	30	4.8	1.6	4.6	3.7	10.9	1.1	0.049
	> 73.3	29	4.4	0.5	4.5	3.3	5.8	1.5	
	≤ 38.6	30	1.5	0.5	1.5	0.5	2.8	-	
CEBPB	38.6-73.3	31	1.5	0.7	1.5	0.0	4.8	1.0	0.555
	> 73.3	29	1.6	0.5	1.6	0.3	2.6	0.9	
	≤ 38.6	30	4.0	0.5	4.0	3.1	4.9	-	
KLF4	38.6-73.3	31	4.2	1.9	3.7	2.9	12.4	0.8	0.447
	> 73.3	28	3.7	0.3	3.7	3.1	4.5	1.2	

Lymphoma		≤ 38.6	29	11.0	1.1	10.8	8.8	13.7	-	
	AICDA	38.6-73.3	30	11.1	1.0	11.1	9.7	13.0	0.9	0.832
		> 73.3	28	10.9	1.1	10.7	9.4	13.7	1.0	

Inflammation		≤ 38.6	29	5.0	0.3	5.0	4.5	5.7	-	
	TNF	38.6-73.3	31	4.8	0.4	4.8	4.0	5.7	1.1	0.006
		> 73.3	29	4.7	0.3	4.7	4.1	5.2	1.2	
		≤ 38.6	14	11.6	1.1	11.8	9.4	13.1	-	
	IL1A	38.6-73.3	22	11.5	0.9	11.5	9.6	12.9	1.0	0.616
		> 73.3	24	11.7	0.9	11.7	9.9	13.6	0.9	
		≤ 38.6	29	3.5	0.6	3.5	2.1	4.7	-	
	IL1B	38.6-73.3	31	3.2	0.5	3.3	1.7	4.5	1.2	0.841
		> 73.3	29	3.4	0.9	3.4	2.3	7.5	1.0	
		≤ 38.6	25	11.5	1.2	11.2	9.4	14.1	-	
	IL6	38.6-73.3	29	10.8	1.0	10.8	8.8	13.3	1.7	0.006
		> 73.3	26	10.7	0.7	10.9	8.6	11.8	1.7	
		≤ 38.6	30	5.9	1.2	5.6	3.8	8.4	-	
	IL8	38.6-73.3	31	5.3	0.9	5.4	2.9	6.7	1.5	0.070
		> 73.3	28	5.4	1.0	5.3	3.7	8.0	1.4	
		≤ 38.6	30	4.0	1.2	4.0	1.7	6.9	-	
	IL17RB	38.6-73.3	31	4.7	1.1	4.7	2.5	7.4	0.6	0.275
		> 73.3	28	4.3	1.0	4.1	2.6	6.8	0.8	
		≤ 38.6	30	4.7	0.4	4.7	4.1	5.7	-	
	PARP1	38.6-73.3	31	4.3	0.3	4.3	3.3	4.9	1.3	< 0.001
		> 73.3	29	4.2	0.4	4.1	3.2	5.0	1.5	
		≤ 38.6	29	4.7	0.7	4.7	2.7	6.0	-	
	BACH2	38.6-73.3	30	4.3	0.5	4.4	2.9	5.8	1.3	0.022
		> 73.3	28	4.3	0.5	4.3	3.0	5.4	1.3	
		≤ 38.6	20	12.2	1.8	12.8	7.8	14.2	-	
	CCL2	38.6-73.3	25	12.9	0.9	13.1	11.1	14.3	0.6	0.230
		> 73.3	26	12.7	1.1	12.8	9.2	14.0	0.7	
		≤ 38.6	29	7.7	0.6	7.8	6.3	9.2	-	
	SERPINB2	38.6-73.3	31	7.4	1.1	7.3	6.1	12.1	1.3	0.008
		> 73.3	29	7.2	0.6	7.0	6.2	9.0	1.5	
		≤ 38.6	4	13.6	0.5	13.6	13.0	14.1	-	
	CXCL12	38.6-73.3	4	13.6	0.7	13.8	12.6	14.1	1.0	0.318
		> 73.3	2	14.2	0.1	14.2	14.1	14.3	0.7	
		≤ 38.6	30	4.4	0.3	4.4	3.5	4.9	-	
	NFKB1	38.6-73.3	31	4.0	0.3	4.0	3.4	4.7	1.3	0.003
		> 73.3	29	4.1	0.4	4.0	3.6	5.6	1.2	
		≤ 38.6	30	6.8	0.8	7.0	3.1	7.9	-	
	JUN	38.6-73.3	31	6.9	0.8	6.8	5.1	9.2	0.9	0.412
		> 73.3	29	7.0	0.5	6.9	5.9	8.2	0.9	
		≤ 38.6	30	0.9	0.4	0.9	0.0	1.5	-	
	STAT3	38.6-73.3	31	0.5	0.4	0.6	-0.4	1.2	1.3	0.009
		> 73.3	29	0.6	0.3	0.6	-0.3	1.3	1.2	

Lipid Metabolism	CRYIL1	≤ 38.6	30	4.6	0.4	4.5	3.3	5.3	-	0.816
		38.6-73.3	31	4.6	0.4	4.6	3.4	5.1	1.0	
		> 73.3	29	4.6	0.4	4.5	3.8	5.4	1.0	
	MTMR7	≤ 38.6	29	6.6	0.4	6.6	5.6	7.5	-	< 0.001
		38.6-73.3	31	6.1	0.3	6.0	5.6	6.7	1.4	
		> 73.3	29	6.1	0.2	6.1	5.8	6.7	1.4	
	ST8SIA1	≤ 38.6	30	6.2	0.5	6.2	5.2	7.0	-	0.861
		38.6-73.3	31	6.3	0.6	6.2	5.3	7.5	0.9	
		> 73.3	29	6.2	0.6	6.2	4.9	7.8	1.0	
	GRN	≤ 38.6	30	0.7	0.6	0.7	-0.4	2.3	-	0.135
		38.6-73.3	31	0.6	0.4	0.5	-0.1	1.7	1.1	
		> 73.3	29	0.6	0.4	0.6	-0.2	1.8	1.1	
	THRSP	≤ 38.6	19	12.5	0.5	12.6	11.4	13.5	-	0.750
		38.6-73.3	29	12.3	0.8	12.2	11.2	14.0	1.1	
		> 73.3	28	12.4	0.7	12.5	10.7	13.8	1.1	
	HSD3B1	≤ 38.6	0	-	-	-	-	-	-	0.257
		38.6-73.3	5	13.9	0.5	13.7	13.4	14.4	-	
		> 73.3	2	13.4	0.4	13.4	13.1	13.7	-	
	ALOX15B	≤ 38.6	28	9.8	1.0	9.6	7.8	11.9	-	0.062
		38.6-73.3	31	9.4	1.2	9.2	7.6	13.5	1.3	
		> 73.3	29	9.3	0.7	9.3	8.0	10.5	1.4	
Glucose Metabolism	CD36	≤ 38.6	30	2.9	0.5	2.8	2.2	4.8	-	0.547
		38.6-73.3	31	2.9	1.4	2.6	1.4	8.7	1.0	
		> 73.3	29	2.7	1.7	2.5	1.7	11.4	1.2	
	PDK4	≤ 38.6	29	6.0	0.7	6.0	4.9	7.7	-	0.724
		38.6-73.3	31	6.1	0.7	6.1	5.1	7.6	0.9	
		> 73.3	29	5.9	0.7	5.8	5.1	8.6	1.0	
Cell Cycle	PAPPA	≤ 38.6	15	12.7	0.5	12.8	11.7	13.4	-	0.653
		38.6-73.3	21	12.5	0.8	12.4	10.8	13.9	1.2	
		> 73.3	20	12.6	0.9	12.6	11.1	14.1	1.1	
	CDCA5	≤ 38.6	28	11.7	0.8	11.7	10.3	13.2	-	0.066
		38.6-73.3	30	11.4	1.0	11.4	8.0	14.0	1.2	
		> 73.3	29	11.3	0.6	11.4	10.3	13.0	1.3	
	SERPINE2	≤ 38.6	29	9.0	0.7	8.9	8.0	11.5	-	0.997
		38.6-73.3	31	8.9	0.9	8.8	7.4	10.5	1.1	
		> 73.3	29	9.0	0.8	8.9	7.6	10.8	1.0	
	PTN	≤ 38.6	3	12.5	0.2	12.5	12.3	12.7	-	0.473
		38.6-73.3	3	13.5	0.7	13.7	12.7	14.0	0.5	
		> 73.3	6	13.1	1.1	13.6	11.0	13.8	0.7	
	RB1	≤ 38.6	30	4.2	0.5	4.3	3.3	6.5	-	0.001

		38.6-73.3	31	3.9	0.4	3.9	3.4	5.2	1.2	
		> 73.3	29	3.9	0.2	3.9	3.3	4.4	1.3	
		≤ 38.6	30	4.0	0.4	4.0	3.1	4.7	-	
	CTBP2	38.6-73.3	31	3.8	0.3	3.9	3.1	4.5	1.1	0.015
		> 73.3	29	3.8	0.3	3.8	2.9	4.5	1.2	
Translation		≤ 38.6	30	4.3	0.2	4.3	3.9	4.7	-	
	EIF2S1	38.6-73.3	31	4.4	0.3	4.4	3.7	5.3	0.9	0.515
		> 73.3	29	4.4	0.3	4.4	3.1	4.9	1.0	
WNT Signaling		≤ 38.6	7	12.6	0.5	12.7	11.8	13.2	-	
	WNT5A	38.6-73.3	16	13.3	0.7	13.3	11.9	14.3	0.6	0.265
		> 73.3	8	13.0	0.7	12.8	12.2	14.0	0.7	
		≤ 38.6	30	5.4	0.5	5.4	4.6	6.7	-	
	CTNNB1	38.6-73.3	30	4.7	0.4	4.7	3.9	6.0	1.6	< 0.001
		> 73.3	29	4.7	0.3	4.7	3.7	5.1	1.7	
		≤ 38.6	7	13.7	0.8	14.1	12.4	14.4	-	
	RSP01	38.6-73.3	3	13.7	0.5	13.9	13.2	14.0	1.0	0.301
		> 73.3	4	13.1	1.0	13.3	11.9	14.0	1.5	
		≤ 38.6	5	13.6	0.6	13.7	13.0	14.3	-	
	RSP02	38.6-73.3	8	13.6	0.6	13.5	12.7	14.4	1.0	0.382
		> 73.3	3	13.0	1.7	14.0	11.0	14.0	1.5	
		≤ 38.6	4	13.2	0.9	13.5	11.9	14.0	-	
	RSP03	38.6-73.3	9	13.5	0.7	13.4	12.4	14.4	0.8	0.737
		> 73.3	8	13.4	0.6	13.7	12.5	14.2	0.9	
Other		≤ 38.6	30	3.2	0.6	3.2	2.6	5.7	-	
	TP53	38.6-73.3	31	2.9	0.4	3.0	1.7	3.5	1.2	0.033
		> 73.3	29	3.0	0.4	3.1	1.4	3.6	1.2	
		≤ 38.6	30	3.3	0.6	3.3	2.6	5.8	-	
	PRDM1	38.6-73.3	31	3.0	0.4	2.9	2.4	4.0	1.2	0.038
		> 73.3	29	3.1	0.4	3.1	2.4	3.9	1.2	
		≤ 38.6	30	5.1	1.1	5.2	1.7	7.1	-	
	PAX5	38.6-73.3	31	4.8	0.7	5.0	3.1	5.6	1.2	0.526
		> 73.3	29	4.9	0.8	4.8	3.2	6.9	1.1	
		≤ 38.6	30	2.9	0.3	2.8	2.3	3.6	-	
	HIST1H2BE	38.6-73.3	31	2.9	0.4	3.0	2.0	4.3	1.0	0.415
		> 73.3	29	3.0	0.3	2.9	2.5	3.5	0.9	
		≤ 38.6	30	2.9	0.4	2.9	2.2	3.9	-	
	HIST1H2AM	38.6-73.3	31	3.2	0.6	3.2	1.6	4.8	0.8	0.255
		> 73.3	29	3.1	0.4	3.1	1.7	3.6	0.9	
	GAL	≤ 38.6	12	12.2	2.0	12.9	8.4	14.4	-	0.488
		38.6-73.3	12	12.1	1.8	12.6	9.5	14.4	1.0	

	> 73.3	9	11.6	1.5	11.6	9.4	13.8	1.5	
	≤ 38.6	29	8.2	0.4	8.1	7.3	8.8	-	
CREM	38.6-73.3	31	8.1	0.5	8.2	7.2	9.2	1.0	0.802
	> 73.3	28	8.1	0.4	8.2	7.0	8.8	1.0	
	≤ 38.6	29	3.3	0.5	3.3	2.6	4.3	-	
NFIL3	38.6-73.3	30	3.0	0.7	2.8	1.7	5.4	1.3	0.003
	> 73.3	29	2.9	0.5	3.0	1.9	3.7	1.4	
	≤ 38.6	30	0.9	0.5	0.9	0.0	1.9	-	
CEBPD	38.6-73.3	31	1.2	0.6	1.2	0.2	3.1	0.8	0.010
	> 73.3	29	1.2	0.5	1.3	0.3	2.0	0.8	
	≤ 38.6	29	4.3	0.7	4.2	3.1	7.3	-	
IGFBP7	38.6-73.3	31	4.4	0.7	4.3	3.3	7.2	0.9	0.823
	> 73.3	29	4.3	0.9	4.2	2.9	8.3	1.0	
	≤ 38.6	30	3.2	0.4	3.3	1.4	4.1	-	
PIK3R1	38.6-73.3	31	3.1	0.3	3.1	2.3	3.7	1.1	0.466
	> 73.3	29	3.1	0.4	3.0	2.2	4.5	1.0	
	≤ 38.6	16	12.7	1.5	13.0	8.0	14.2	-	
FST	38.6-73.3	23	13.0	0.7	12.9	11.7	14.2	0.8	0.003
	> 73.3	18	13.6	0.5	13.8	12.6	14.2	0.5	
	≤ 38.6	30	8.2	1.7	7.9	4.7	12.2	-	
AREG	38.6-73.3	31	8.6	1.7	9.0	3.6	11.3	0.7	0.353
	> 73.3	29	8.6	1.5	8.6	4.2	11.5	0.8	
	≤ 38.6	30	8.3	0.8	8.3	5.7	9.5	-	
HBEGF	38.6-73.3	31	8.4	0.6	8.3	7.1	9.8	0.9	0.490
	> 73.3	29	8.4	0.5	8.3	7.6	9.8	0.9	
	≤ 38.6	22	12.1	1.0	12.1	10.3	14.2	-	
CTGF	38.6-73.3	28	11.7	1.4	11.6	9.1	13.9	1.3	0.709
	> 73.3	25	11.9	0.9	11.8	10.0	13.4	1.1	
	≤ 38.6	29	9.1	0.6	9.1	8.1	10.4	-	
BTN1A1	38.6-73.3	31	8.7	0.6	8.7	7.5	10.5	1.3	0.020
	> 73.3	29	8.7	0.5	8.7	7.9	10.0	1.3	

* p-values for Pearson correlation coefficients

Note: TEQ measured in parts per trillion (ppt), lipid-adjusted

Cycles to threshold (Ct) represents PCR cycle number at which DNA amount reaches a threshold value

Housekeeping genes used in analysis: GAPDH, ACTB, GUSB, HPRT1, B2M, RPLPO

ΔCt = Ct(gene of interest) - Ct(mean of housekeeping genes)

ΔΔCt = ΔCt(experimental) - Ct(control)

Figure 51 presents fold changes in gene expression by blood TCDD tertiles.

Figure 51.

	Gene	TCDD: Tertile	n	mean ΔCt	std. dev.	median ΔCt	min	max	Fold change	p-value
AhR Pathway	AHR	≤ 6.8	30	3.5	0.5	3.5	2.3	4.8	-	
		6.8-24.0	31	3.3	0.6	3.2	2.6	4.9	1.1	< 0.001
		> 24.0	29	3.0	0.4	3.1	1.9	3.6	1.4	
	AHRR	≤ 6.8	29	10.4	1.2	10.3	7.7	13.3	-	
		6.8-24.0	30	10.4	0.8	10.5	8.1	12.1	1.0	0.198
		> 24.0	29	10.0	1.2	10.2	7.1	11.6	1.3	
	AIP	≤ 6.8	30	2.8	0.2	2.8	2.2	3.3	-	
		6.8-24.0	30	3.2	1.8	2.8	2.5	11.9	0.7	0.508
		> 24.0	29	3.0	1.8	2.7	2.3	12.1	0.8	
	ARNT	≤ 6.8	30	5.9	0.4	5.8	4.5	6.6	-	
		6.8-24.0	31	5.5	0.5	5.5	4.7	7.7	1.3	0.007
		> 24.0	28	5.6	0.3	5.5	4.4	6.0	1.2	
	BRCA1	≤ 6.8	29	7.2	0.4	7.1	6.6	8.0	-	
		6.8-24.0	31	7.0	0.4	7.0	6.4	7.7	1.1	0.090
		> 24.0	29	7.0	0.3	7.0	6.1	7.5	1.1	
	ESR1	≤ 6.8	29	10.3	0.9	10.2	7.9	12.6	-	
		6.8-24.0	31	10.0	1.0	9.6	8.3	12.1	1.2	0.339
		> 24.0	29	10.1	0.9	9.9	8.4	12.5	1.2	
	HSP90AA1	≤ 6.8	30	1.5	0.3	1.5	1.0	2.0	-	
		6.8-24.0	31	2.6	1.4	2.4	1.5	9.7	0.5	0.001
		> 24.0	29	2.3	0.4	2.4	1.5	3.1	0.6	
	NRIP1	≤ 6.8	30	5.2	0.5	5.2	4.0	6.1	-	
		6.8-24.0	31	4.7	0.3	4.7	4.0	5.3	1.4	< 0.001
		> 24.0	28	4.7	0.2	4.7	4.2	5.1	1.4	
	TIPARP	≤ 6.8	30	4.5	0.4	4.5	3.7	5.3	-	
		6.8-24.0	31	4.3	0.3	4.3	3.3	4.7	1.1	0.030
		> 24.0	29	4.3	0.3	4.3	3.1	4.7	1.1	
	TRIP11	≤ 6.8	29	7.0	0.5	7.0	5.9	7.9	-	
		6.8-24.0	30	6.6	0.4	6.5	5.8	7.7	1.3	< 0.001
		> 24.0	28	6.6	0.2	6.6	6.1	7.0	1.3	
Drug Metabolism	CYP1A1	≤ 6.8	14	13.0	0.9	13.3	10.9	14.2	-	
		6.8-24.0	15	13.3	0.7	13.4	12.0	14.3	0.9	0.181
		> 24.0	9	13.5	0.5	13.3	13.1	14.4	0.7	
	CYP1A2	≤ 6.8	9	12.7	1.0	12.6	11.0	14.3	-	
		6.8-24.0	6	12.5	0.8	12.8	11.0	13.2	1.1	0.883
		> 24.0	12	12.6	0.6	12.6	11.6	13.8	1.0	

	≤ 6.8	2	13.5	1.1	13.5	12.7	14.2	-	
CYP4F8	6.8-24.0	1	14.4	.	14.4	14.4	14.4	0.5	0.483
	> 24.0	2	12.6	0.9	12.6	12.0	13.2	1.8	
	≤ 6.8	3	12.4	1.0	12.1	11.6	13.5	-	
ALDH3A1	6.8-24.0	5	13.5	0.7	13.9	12.4	14.1	0.5	0.066
	> 24.0	8	13.4	0.2	13.5	13.2	13.6	0.5	
	≤ 6.8	30	4.7	0.4	4.6	3.9	5.9	-	
ALDH3A2	6.8-24.0	29	4.6	1.3	4.4	3.6	11.3	1.1	0.038
	> 24.0	29	4.3	0.3	4.3	3.2	4.8	1.4	
	≤ 6.8	25	10.8	0.7	10.8	9.8	12.3	-	
ALDH1A3	6.8-24.0	29	11.0	0.7	11.0	9.8	12.9	0.9	0.422
	> 24.0	29	11.0	0.7	11.0	9.8	12.6	0.9	
	≤ 6.8	29	6.0	0.5	6.0	4.9	6.9	-	
ALDH6A1	6.8-24.0	31	6.3	1.6	6.0	5.2	13.2	0.8	0.989
	> 24.0	29	6.0	1.2	5.7	5.3	12.2	1.0	
	≤ 6.8	7	8.3	0.8	8.3	7.0	9.5	-	
GSTM1	6.8-24.0	14	8.3	0.6	8.3	6.6	9.1	1.0	0.526
	> 24.0	14	8.5	0.8	8.3	7.8	10.8	0.9	
	≤ 6.8	28	8.8	1.5	8.9	6.5	11.8	-	
GSTM3	6.8-24.0	31	9.3	1.8	9.9	5.7	12.8	0.7	0.622
	> 24.0	29	8.6	1.7	8.1	5.9	11.9	1.2	
	≤ 6.8	27	9.3	1.3	9.3	7.0	13.0	-	
UGT1A1	6.8-24.0	31	10.0	1.1	9.8	8.3	13.4	0.6	0.289
	> 24.0	29	9.7	1.0	9.8	7.1	11.2	0.8	
	≤ 6.8	30	2.6	0.3	2.6	1.7	3.0	-	
BAX	6.8-24.0	31	2.6	0.3	2.7	1.6	3.1	0.9	0.027
	> 24.0	29	2.9	0.8	2.7	2.4	7.0	0.8	
	≤ 6.8	30	4.0	0.7	4.1	2.4	5.0	-	
BCL2	6.8-24.0	31	4.0	0.5	4.1	2.5	5.1	1.0	0.953
	> 24.0	29	4.0	0.4	4.0	3.1	4.7	1.0	
	≤ 6.8	30	3.2	0.9	3.0	1.9	5.6	-	
BCL2A1	6.8-24.0	31	3.8	0.8	3.9	2.0	5.6	0.7	< 0.001
	> 24.0	29	4.1	0.6	3.9	3.1	5.6	0.6	
	≤ 6.8	30	0.0	1.0	-0.1	-1.9	1.9	-	
BCL2L1	6.8-24.0	31	0.4	1.0	0.5	-1.6	2.5	0.7	0.027
	> 24.0	29	0.5	0.7	0.6	-1.4	1.9	0.7	
	≤ 6.8	4	13.9	0.4	13.9	13.4	14.4	-	
BCL2L10	6.8-24.0	7	13.6	0.6	13.8	12.6	14.4	1.2	0.832
	> 24.0	3	14.0	0.2	13.9	13.9	14.2	0.9	
	≤ 6.8	29	6.0	0.4	6.0	5.6	7.3	-	
BCL2L2	6.8-24.0	30	5.6	0.4	5.5	4.7	6.6	1.4	< 0.001
	> 24.0	29	5.6	0.3	5.6	4.9	6.1	1.3	
	≤ 6.8	30	5.0	0.6	4.9	4.0	6.0	-	
PTGS2	6.8-24.0	30	4.7	1.6	4.4	3.4	10.9	1.2	0.078
	> 24.0	29	4.5	0.5	4.5	3.3	5.8	1.4	

Anti-Apoptosis

		≤ 6.8	30	1.6	0.5	1.5	0.5	2.8	-	
	CEBPB	6.8-24.0	31	1.4	0.8	1.3	0.0	4.8	1.1	0.766
		> 24.0	29	1.7	0.4	1.7	0.9	2.6	1.0	
		≤ 6.8	30	4.0	0.5	4.1	3.2	4.9	-	
	KLF4	6.8-24.0	31	4.2	1.9	3.8	2.9	12.4	0.9	0.284
		> 24.0	28	3.7	0.3	3.6	3.1	4.3	1.3	
Lymphoma		≤ 6.8	28	11.1	1.1	10.9	8.8	13.7	-	
	AICDA	6.8-24.0	31	10.8	1.0	10.6	9.4	13.0	1.3	0.743
		> 24.0	28	11.2	1.0	11.0	9.8	13.7	0.9	
Inflammation		≤ 6.8	29	5.0	0.3	4.9	4.3	5.7	-	
	TNF	6.8-24.0	31	4.8	0.4	4.8	4.2	5.7	1.1	0.046
		> 24.0	29	4.8	0.4	4.8	4.0	5.6	1.1	
		≤ 6.8	12	11.4	1.2	11.5	9.4	13.1	-	
	IL1A	6.8-24.0	25	11.8	0.9	12.0	9.6	13.0	0.8	0.663
		> 24.0	23	11.6	0.9	11.6	10.2	13.6	0.9	
		≤ 6.8	29	3.5	0.6	3.5	2.6	4.7	-	
	IL1B	6.8-24.0	31	3.2	0.6	3.1	1.7	4.5	1.3	0.763
		> 24.0	29	3.5	0.9	3.4	2.3	7.5	1.0	
		≤ 6.8	25	11.3	1.4	11.2	8.6	14.1	-	
	IL6	6.8-24.0	29	10.8	0.9	11.0	8.8	13.3	1.4	0.105
		> 24.0	26	10.8	0.7	10.8	9.1	11.9	1.4	
		≤ 6.8	30	6.0	1.1	5.8	3.8	8.4	-	
	IL8	6.8-24.0	30	5.1	0.9	5.2	2.9	6.6	1.8	0.060
		> 24.0	29	5.5	1.0	5.5	3.7	8.0	1.4	
		≤ 6.8	30	3.9	1.3	3.8	1.7	6.9	-	
	IL17RB	6.8-24.0	30	4.6	1.0	4.5	2.9	7.4	0.6	0.077
		> 24.0	29	4.4	1.0	4.2	2.6	6.8	0.7	
		≤ 6.8	30	4.7	0.4	4.6	4.1	5.7	-	
	PARP1	6.8-24.0	31	4.3	0.3	4.3	3.6	5.0	1.3	< 0.001
		> 24.0	29	4.1	0.4	4.2	3.2	4.6	1.5	
		≤ 6.8	29	4.6	0.7	4.7	2.7	6.0	-	
	BACH2	6.8-24.0	30	4.4	0.6	4.4	2.9	5.9	1.2	0.045
		> 24.0	28	4.3	0.5	4.3	3.0	4.9	1.3	
		≤ 6.8	19	12.1	1.8	12.7	7.8	14.2	-	
	CCL2	6.8-24.0	25	13.0	0.8	13.3	11.1	14.3	0.5	0.225
		> 24.0	27	12.6	1.1	12.7	9.2	14.0	0.7	
		≤ 6.8	29	7.9	0.6	7.9	7.1	9.2	-	
	SERPINB2	6.8-24.0	31	7.2	1.0	7.1	6.2	12.1	1.6	0.002
		> 24.0	29	7.2	0.6	7.0	6.1	9.0	1.6	
		≤ 6.8	3	13.5	0.6	13.4	13.0	14.1	-	
	CXCL12	6.8-24.0	3	13.4	0.8	13.9	12.6	13.9	1.0	0.183
		> 24.0	4	14.1	0.2	14.1	13.8	14.3	0.7	
	NFKB1	≤ 6.8	30	4.4	0.3	4.4	3.8	4.9	-	0.001
		6.8-24.0	31	3.9	0.3	3.9	3.4	4.6	1.4	

	> 24.0	29	4.1	0.4	4.1	3.6	5.6	1.2	
	≤ 6.8	30	6.9	0.9	6.9	3.1	8.9	-	
JUN	6.8-24.0	31	6.9	0.7	6.8	5.1	9.2	1.0	0.941
	> 24.0	29	6.9	0.5	6.9	5.9	7.8	1.0	
	≤ 6.8	30	0.9	0.4	0.9	0.2	1.5	-	
STAT3	6.8-24.0	31	0.5	0.4	0.5	-0.4	1.1	1.4	0.006
	> 24.0	29	0.6	0.3	0.6	-0.3	1.3	1.2	
Lipid Metabolism	≤ 6.8	30	4.5	0.4	4.5	3.3	5.1	-	
CRYIL1	6.8-24.0	31	4.6	0.4	4.6	3.4	5.4	0.9	0.477
	> 24.0	29	4.6	0.3	4.6	3.9	5.3	1.0	
	≤ 6.8	29	6.6	0.4	6.7	5.6	7.5	-	
MTMR7	6.8-24.0	31	6.1	0.3	6.0	5.6	6.6	1.5	< 0.001
	> 24.0	29	6.1	0.2	6.1	5.8	6.6	1.4	
	≤ 6.8	30	6.1	0.5	6.0	5.2	7.0	-	
ST8SIA1	6.8-24.0	31	6.3	0.6	6.3	5.3	7.8	0.8	0.164
	> 24.0	29	6.3	0.5	6.3	4.9	7.3	0.9	
	≤ 6.8	30	0.9	0.6	0.8	-0.4	2.3	-	
GRN	6.8-24.0	31	0.5	0.4	0.5	-0.2	1.5	1.2	0.003
	> 24.0	29	0.5	0.3	0.5	0.1	1.0	1.3	
	≤ 6.8	18	12.5	0.6	12.4	11.4	14.0	-	
THRSP	6.8-24.0	30	12.3	0.6	12.3	11.2	13.5	1.2	0.783
	> 24.0	28	12.5	0.8	12.6	10.7	13.8	1.0	
	≤ 6.8	0	-	-	-	-	-	-	
HSD3B1	6.8-24.0	4	13.7	0.4	13.6	13.4	14.3	-	0.966
	> 24.0	3	13.7	0.7	13.7	13.1	14.4	-	
	≤ 6.8	28	9.9	0.9	9.7	8.3	11.9	-	
ALOX15B	6.8-24.0	31	9.3	1.2	9.1	7.6	13.5	1.6	0.034
	> 24.0	29	9.4	0.6	9.3	8.3	10.5	1.5	
Glucose Metabolism	≤ 6.8	30	3.0	0.5	2.9	2.3	4.8	-	
CD36	6.8-24.0	31	2.8	1.4	2.5	1.4	8.7	1.2	0.419
	> 24.0	29	2.7	1.7	2.5	1.7	11.4	1.2	
	≤ 6.8	29	6.1	0.6	6.0	5.2	7.7	-	
PDK4	6.8-24.0	31	6.0	0.7	5.9	4.9	7.6	1.0	0.318
	> 24.0	29	5.9	0.8	5.8	5.1	8.6	1.1	
Cell Cycle	≤ 6.8	12	12.7	0.5	12.8	11.7	13.4	-	
PAPPA	6.8-24.0	23	12.5	0.8	12.5	10.8	13.9	1.1	0.522
	> 24.0	21	12.5	0.9	12.5	11.1	14.1	1.1	
	≤ 6.8	27	11.6	0.7	11.7	10.3	13.2	-	
CDCA5	6.8-24.0	31	11.5	0.7	11.5	9.8	13.1	1.0	0.329
	> 24.0	29	11.3	1.1	11.4	8.0	14.0	1.2	

		≤ 6.8	29	9.0	0.7	8.9	8.0	10.5	-	
	SERPINE2	6.8-24.0	31	8.9	0.9	8.8	7.4	11.5	1.1	0.780
		> 24.0	29	9.0	0.8	9.1	7.6	10.8	1.0	
		≤ 6.8	4	12.1	0.7	12.4	11.0	12.7	-	
	PTN	6.8-24.0	3	13.5	0.7	13.7	12.7	14.0	0.4	0.013
		> 24.0	5	13.5	0.4	13.6	12.8	13.8	0.4	
		≤ 6.8	30	4.2	0.5	4.3	3.3	6.5	-	
	RB1	6.8-24.0	31	3.9	0.3	3.9	3.4	5.2	1.2	0.002
		> 24.0	29	3.9	0.2	3.9	3.6	4.4	1.2	
		≤ 6.8	30	4.0	0.3	4.0	3.1	4.7	-	
	CTBP2	6.8-24.0	31	3.7	0.3	3.7	3.1	4.5	1.2	0.026
		> 24.0	29	3.8	0.3	3.8	2.9	4.5	1.1	
Translation		≤ 6.8	30	4.3	0.2	4.3	3.9	4.7	-	
	EIF2S1	6.8-24.0	31	4.5	0.3	4.5	3.7	5.3	0.9	0.535
		> 24.0	29	4.4	0.3	4.3	3.1	4.8	1.0	
WNT Signaling		≤ 6.8	6	12.6	0.5	12.8	11.8	13.2	-	
	WNT5A	6.8-24.0	14	13.1	0.7	13.1	11.9	14.3	0.7	0.054
		> 24.0	11	13.3	0.7	13.2	12.2	14.3	0.6	
		≤ 6.8	30	5.5	0.4	5.4	4.5	6.7	-	
	CTNNB1	6.8-24.0	30	4.7	0.3	4.7	3.9	6.0	1.7	< 0.001
		> 24.0	29	4.7	0.3	4.7	3.7	5.1	1.7	
		≤ 6.8	4	13.3	0.8	13.2	12.4	14.2	-	
	RSP01	6.8-24.0	7	13.8	0.6	14.0	12.7	14.4	0.7	0.886
		> 24.0	3	13.3	1.2	14.0	11.9	14.0	1.0	
		≤ 6.8	5	13.3	0.5	13.1	12.7	14.0	-	
	RSP02	6.8-24.0	8	13.7	0.4	13.8	13.2	14.3	0.7	0.963
		> 24.0	3	13.2	1.9	14.2	11.0	14.4	1.1	
		≤ 6.8	3	13.0	0.9	13.5	11.9	13.6	-	
	RSP03	6.8-24.0	9	13.5	0.6	13.8	12.4	14.4	0.7	0.435
		> 24.0	9	13.5	0.7	13.6	12.5	14.3	0.7	
Other		≤ 6.8	30	3.3	0.6	3.2	2.6	5.7	-	
	TP53	6.8-24.0	31	3.0	0.4	3.1	1.7	3.6	1.2	0.007
		> 24.0	29	2.9	0.4	2.9	1.4	3.3	1.3	
		≤ 6.8	30	3.4	0.6	3.4	2.6	5.8	-	
	PRDM1	6.8-24.0	31	3.0	0.4	2.9	2.4	3.9	1.4	0.002
		> 24.0	29	3.0	0.4	3.1	2.4	3.9	1.3	
		≤ 6.8	30	4.9	1.1	4.8	1.7	7.1	-	
	PAX5	6.8-24.0	31	4.9	0.8	5.1	3.1	6.6	1.0	0.748
		> 24.0	29	5.0	0.6	4.9	3.2	6.9	1.0	
	HIST1H2BE	≤ 6.8	30	2.8	0.3	2.8	2.3	3.5	-	0.111

	6.8-24.0	31	2.9	0.4	3.0	2.0	4.3	0.9	
	> 24.0	29	3.0	0.3	3.0	2.0	3.5	0.9	
	≤ 6.8	30	2.8	0.4	2.9	1.7	3.5	-	
HIST1H2AM	6.8-24.0	31	3.2	0.4	3.2	2.5	3.9	0.8	0.005
	> 24.0	29	3.1	0.5	3.1	1.6	4.8	0.8	
	≤ 6.8	11	12.0	2.0	12.7	8.4	14.4	-	
GAL	6.8-24.0	14	12.4	1.5	12.6	9.5	14.4	0.8	0.487
	> 24.0	8	11.3	1.8	11.5	9.4	14.1	1.6	
	≤ 6.8	29	8.1	0.5	8.1	7.2	8.8	-	
CREM	6.8-24.0	31	8.1	0.4	8.1	7.2	9.2	1.0	0.351
	> 24.0	28	8.2	0.4	8.2	7.0	8.8	0.9	
	≤ 6.8	29	3.4	0.5	3.3	2.6	4.3	-	
NFIL3	6.8-24.0	30	2.8	0.7	2.6	1.7	5.4	1.5	0.003
	> 24.0	29	2.9	0.4	3.0	1.9	3.7	1.4	
	≤ 6.8	30	0.9	0.5	0.9	0.0	1.9	-	
CEBPD	6.8-24.0	31	1.1	0.6	1.1	0.1	3.1	0.9	0.005
	> 24.0	29	1.3	0.4	1.3	0.6	2.0	0.8	
	≤ 6.8	29	4.3	0.8	4.2	2.9	7.3	-	
IGFBP7	6.8-24.0	31	4.4	0.7	4.3	3.7	7.2	0.9	0.616
	> 24.0	29	4.4	0.9	4.3	3.4	8.3	0.9	
	≤ 6.8	30	3.1	0.4	3.1	1.4	4.1	-	
PIK3R1	6.8-24.0	31	3.1	0.4	3.1	2.3	3.7	1.0	0.683
	> 24.0	29	3.1	0.4	3.1	2.2	4.5	1.0	
	≤ 6.8	13	12.6	1.6	13.0	8.0	14.2	-	
FST	6.8-24.0	24	13.0	0.6	13.0	11.7	14.1	0.8	0.002
	> 24.0	20	13.6	0.5	13.8	12.5	14.2	0.5	
	≤ 6.8	30	8.3	1.7	8.4	4.7	12.2	-	
AREG	6.8-24.0	31	8.2	1.8	8.8	3.6	11.3	1.1	0.365
	> 24.0	29	8.7	1.3	8.7	5.5	11.5	0.8	
	≤ 6.8	30	8.3	0.8	8.3	5.7	9.4	-	
HBEGF	6.8-24.0	31	8.4	0.7	8.3	7.1	9.8	1.0	0.393
	> 24.0	29	8.5	0.5	8.3	7.7	9.8	0.9	
	≤ 6.8	21	12.0	1.1	11.8	10.3	14.2	-	
CTGF	6.8-24.0	28	11.7	1.3	11.6	9.1	13.9	1.3	0.881
	> 24.0	26	12.0	1.0	11.9	10.0	13.8	1.0	
	≤ 6.8	29	9.1	0.6	9.1	7.6	10.4	-	
BTN1A1	6.8-24.0	31	8.7	0.6	8.7	7.5	10.5	1.2	0.041
	> 24.0	29	8.7	0.6	8.7	7.9	10.0	1.3	

* p-values for Pearson correlation coefficients

TCDD measured in parts per trillion (ppt), lipid-adjusted

Cycles to threshold (Ct) represents PCR cycle number at which DNA amount reaches a threshold value

Housekeeping genes used in analysis: GAPDH, ACTB, GUSB, HPRT1, B2M, RPLPO

ΔCt = Ct(gene of interest) - Ct(mean of housekeeping genes)

$\Delta\Delta Ct$ = ΔCt(experimental) - Ct(control)

Fold change = $2^{(-\Delta\Delta Ct)}$

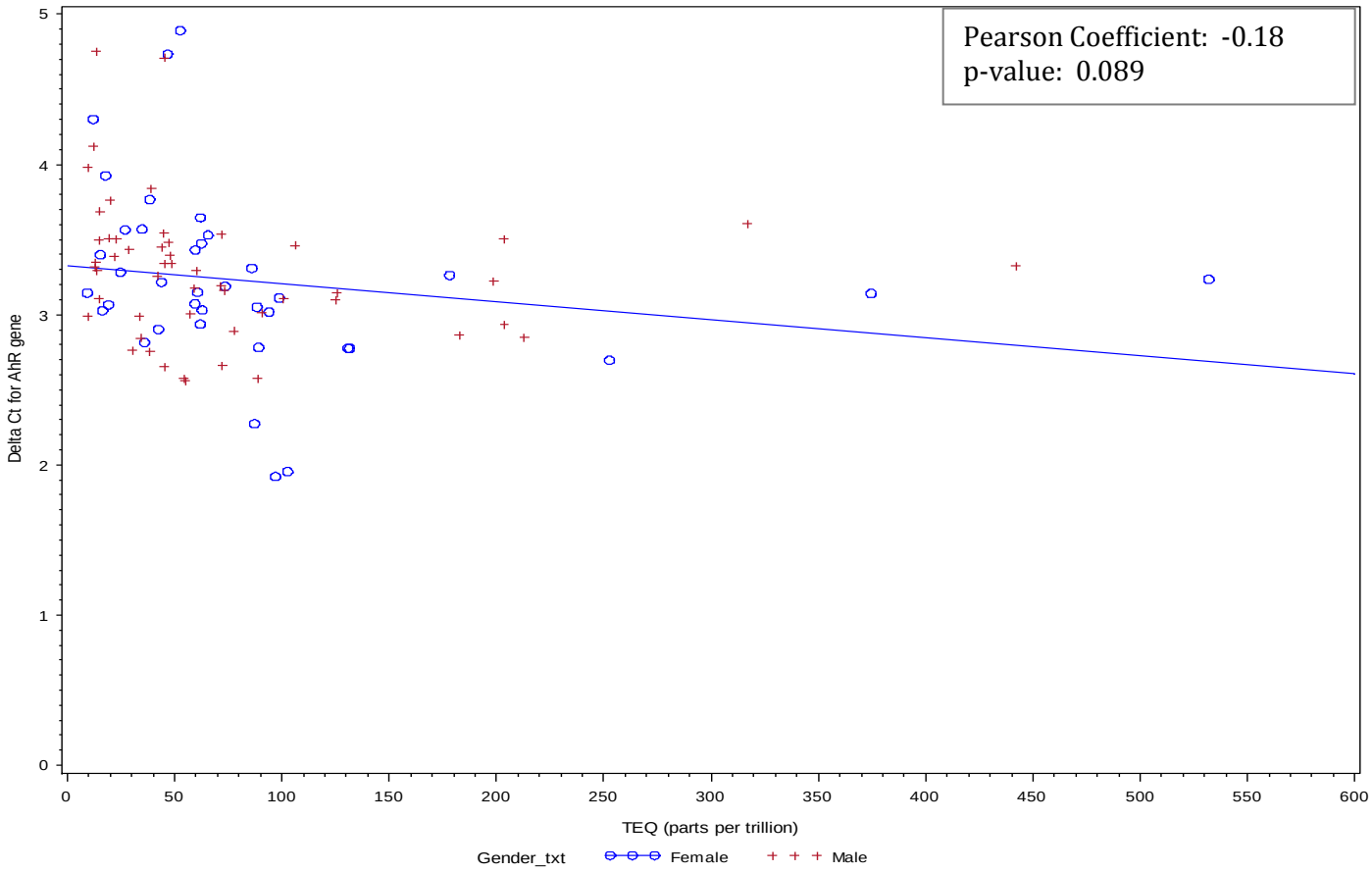


Figure 52. Scatter plot of TEQ vs AhR cycles to threshold

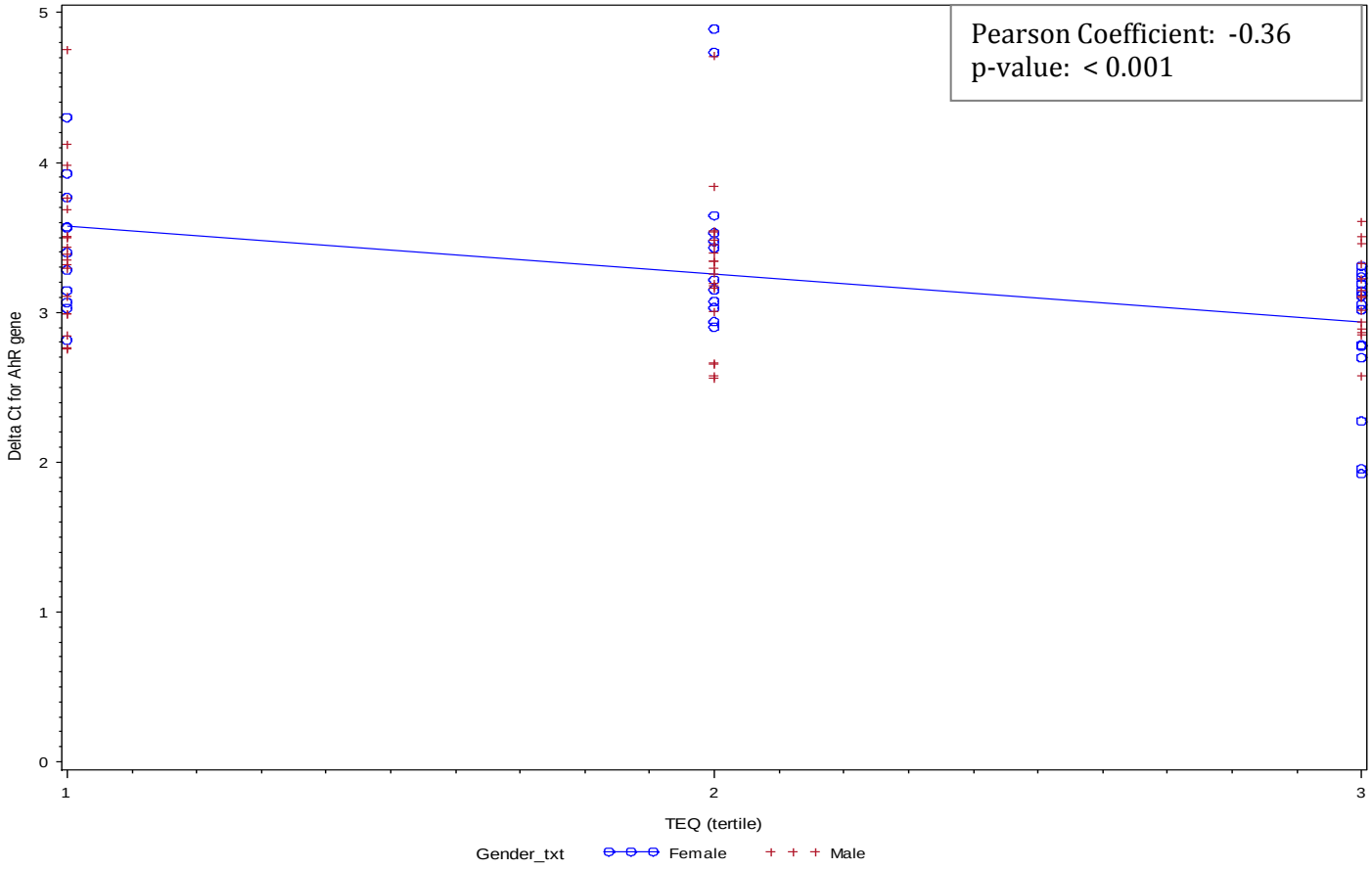


Figure 53. TEQ tertiles vs AhR cycles to threshold

Figures 52 and 53 present cycle to threshold values for AHR by TEQ blood levels and tertiles. Lower cycle to threshold corresponds with increase in fold-change. *These data show that blood TEQ levels are associated with up-regulation of AHR.*

One of our hypotheses was that increasing TEQ would be associated with increased expression of AHRR. *In our data (Figures 54 and 55) AHRR expression was not associated with blood TEQ in the entire group of 60 exposed and 30 unexposed. Interestingly (Figure 56), AHRR was inversely associated with chloracne status.*

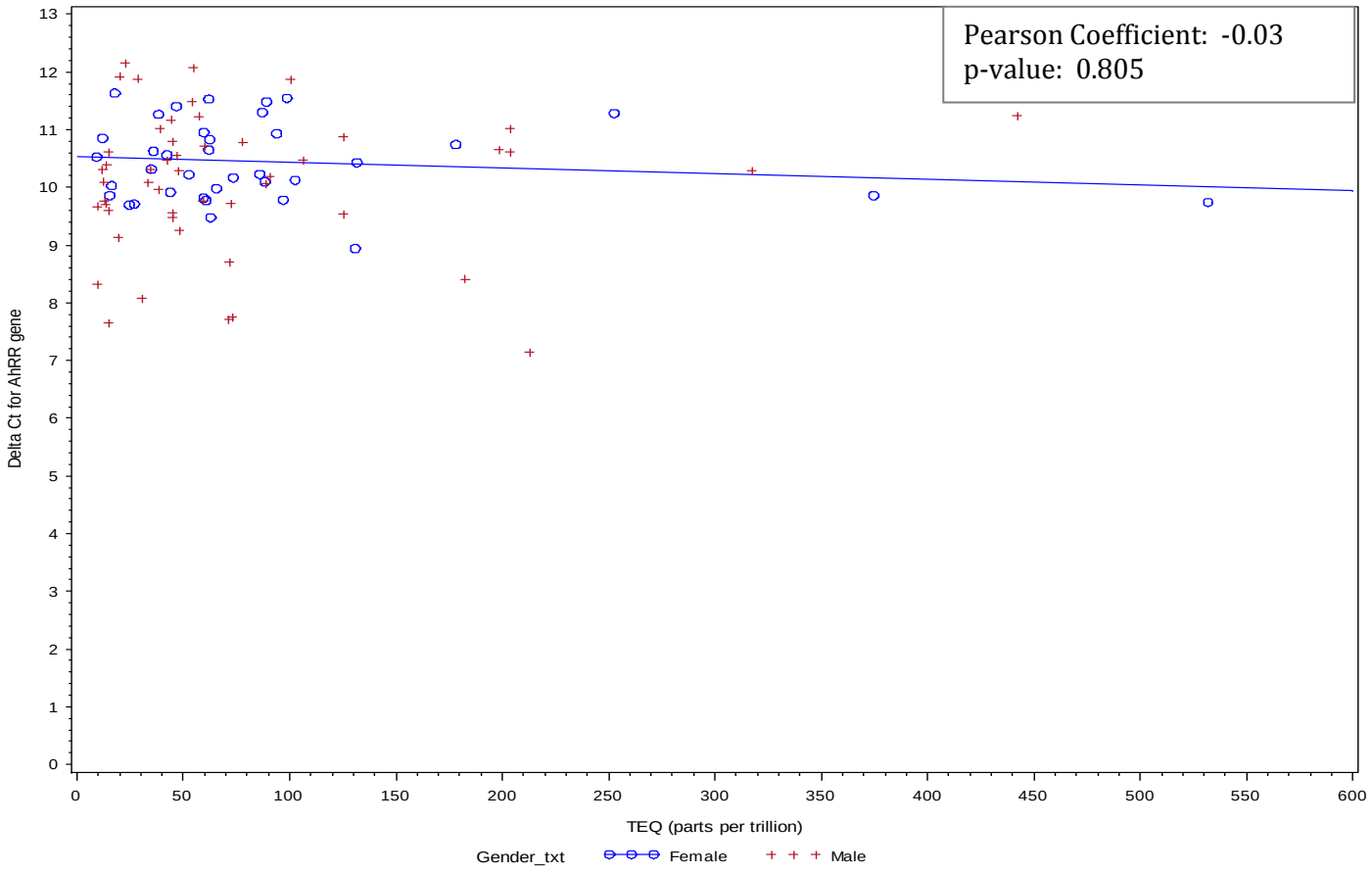


Figure 54. TEQ vs AHRR cycles to threshold

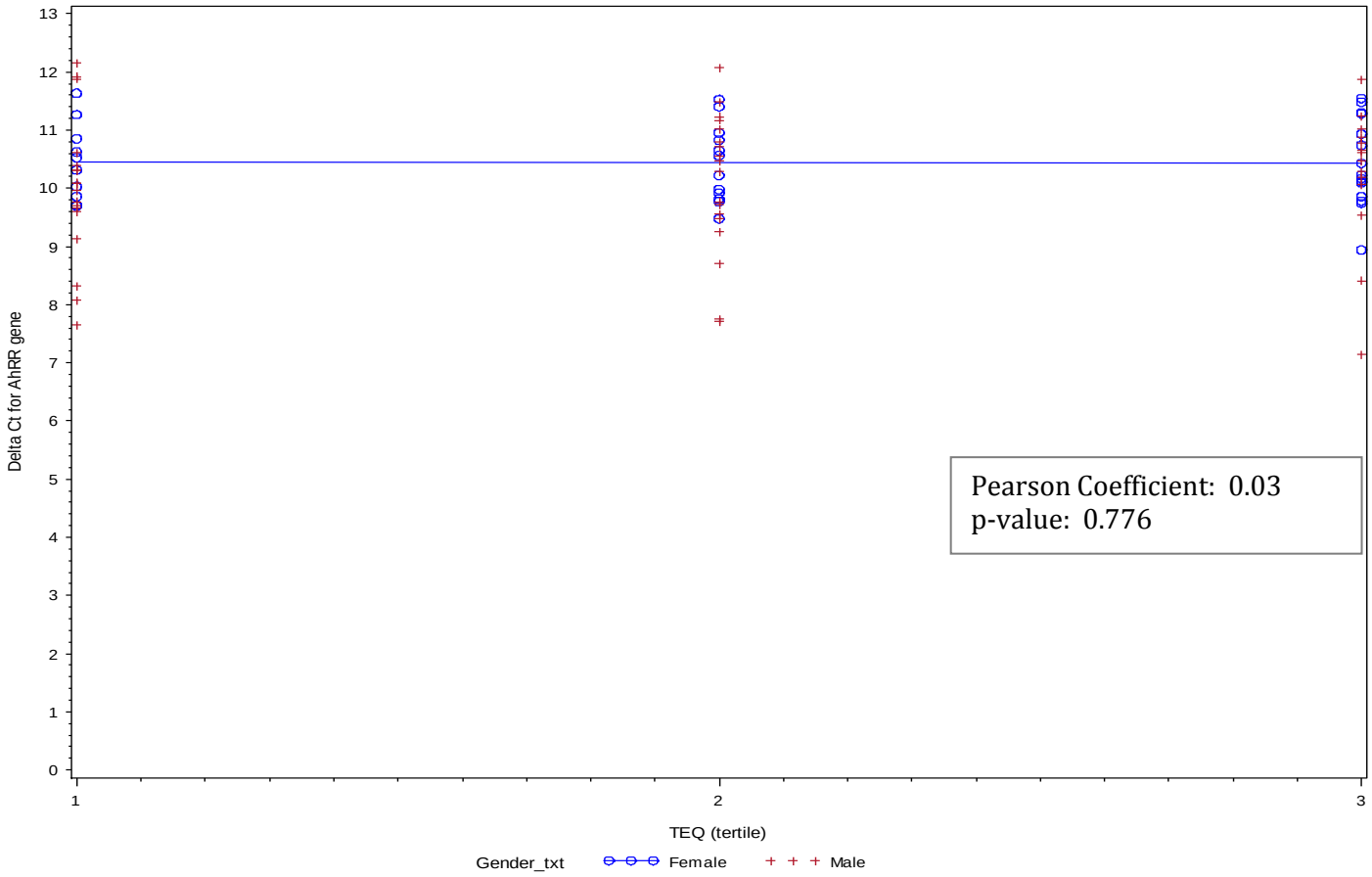


Figure 55. TEQ tertiles vs AHRR cycles to threshold

Figure 56. Fold change by chloracne status

	Gene	Chloracne: No/Yes	n	mean ΔCt	std. dev.	median ΔCt	min	max	Fold change	p-value
AhR Pathway	AHR	No	69	3.3	0.5	3.2	1.9	4.9	1.1	0.251
		Yes	13	3.1	0.6	2.9	2.0	4.7		
	AHRR	No	67	10.4	1.0	10.3	7.7	13.3	1.6	0.043
		Yes	13	9.8	1.3	10.1	7.1	11.4		
	AIP	No	68	2.9	1.2	2.8	2.2	11.9	0.7	0.486*
		Yes	13	3.5	2.6	2.8	2.5	12.1		
	ARNT	No	68	5.7	0.5	5.7	4.5	7.7	1.2	0.043
		Yes	13	5.4	0.4	5.6	4.4	5.9		
	BRCA1	No	68	7.1	0.3	7.0	6.4	7.7	1.1	0.083
		Yes	13	6.9	0.2	6.9	6.5	7.3		
	ESR1	No	68	10.0	0.9	10.0	7.9	12.5	0.9	0.439
		Yes	13	10.3	0.8	10.0	9.4	12.1		
	HSP90AA1	No	69	2.2	1.1	2.1	1.0	9.7	1.0	0.801*
		Yes	13	2.2	0.4	2.3	1.5	2.7		
	NRIP1	No	69	4.9	0.4	4.9	4.0	6.1	1.2	0.014*
		Yes	12	4.7	0.2	4.7	4.2	4.9		
	TIPARP	No	69	4.4	0.3	4.4	3.3	5.3	1.1	0.114
		Yes	13	4.2	0.4	4.3	3.1	4.7		
	TRIP11	No	67	6.7	0.4	6.7	5.8	7.9	1.3	< 0.001
		Yes	12	6.3	0.2	6.3	5.8	6.7		
Drug Metabolism	CYP1A1	No	28	13.2	0.8	13.3	10.9	14.4	1.0	0.886
		Yes	5	13.3	0.8	13.3	12.3	14.2		
	CYP1A2	No	19	12.6	0.9	12.8	11.0	14.3	0.9	0.846
		Yes	4	12.7	0.5	12.8	12.1	13.2		
	CYP4F8	No	5	13.3	1.0	13.2	12.0	14.4	-	-
		Yes	0	-	-	-	-	-		
	ALDH3A1	No	12	13.1	0.7	13.3	11.6	14.0	0.6	0.084
		Yes	4	13.8	0.3	13.7	13.5	14.1		
	ALDH3A2	No	67	4.6	0.9	4.4	3.6	11.3	1.3	0.033*
		Yes	13	4.2	0.4	4.3	3.2	4.6		
	ALDH1A3	No	62	10.9	0.7	10.9	9.8	12.6	0.9	0.301
		Yes	13	11.1	0.8	11.0	10.0	12.9		
	ALDH6A1	No	68	6.1	1.1	6.0	4.9	13.2	0.9	0.737*
		Yes	13	6.3	1.8	5.8	5.2	12.2		
	GSTM1	No	22	8.4	0.7	8.3	6.6	9.5	0.9	0.679
		Yes	11	8.5	0.8	8.3	7.7	10.8		
	GSTM3	No	67	9.0	1.7	9.5	5.7	12.8	1.9	0.074
		Yes	13	8.1	1.7	7.4	5.9	10.8		
	UGT1A1	No	66	9.6	1.2	9.5	7.0	13.4	0.8	0.452
		Yes	13	9.9	0.9	10.0	8.1	11.1		

Anti-Apoptosis	BAX	No	69	2.6	0.3	2.7	1.6	3.1	0.7	0.165*
		Yes	13	3.1	1.2	2.8	2.6	7.0		
	BCL2	No	69	4.0	0.6	4.1	2.4	5.1	1.0	0.805
		Yes	13	4.0	0.4	4.0	3.1	4.4		
	BCL2A1	No	69	3.7	0.9	3.8	1.9	5.6	0.9	0.559
		Yes	13	3.8	0.7	3.9	2.9	4.9		
	BCL2L1	No	69	0.3	1.0	0.5	-1.9	2.5	0.9	0.522
		Yes	13	0.5	0.9	0.6	-1.1	2.2		
	BCL2L10	No	11	13.9	0.4	13.9	13.1	14.4	1.9	0.009
		Yes	2	12.9	0.5	12.9	12.6	13.2		
	BCL2L2	No	67	5.7	0.4	5.7	4.7	7.3	1.1	0.187
		Yes	13	5.6	0.3	5.6	4.9	6.1		
	PTGS2	No	68	4.9	1.1	4.6	3.6	10.9	1.6	0.001*
		Yes	13	4.1	0.5	4.2	3.3	4.9		
	CEBPB	No	69	1.6	0.6	1.6	0.0	4.8	1.2	0.176
		Yes	13	1.3	0.5	1.3	0.7	2.6		
	KLF4	No	69	4.0	1.3	3.8	2.9	12.4	1.2	0.089*
		Yes	12	3.7	0.3	3.7	3.1	4.2		
Lymphoma	AICDA	No	67	11.1	1.1	10.8	9.6	13.7	1.4	0.109
		Yes	12	10.6	0.6	10.5	9.4	11.6		
Inflammation	TNF	No	68	4.8	0.4	4.8	4.0	5.7	1.0	0.720
		Yes	13	4.9	0.3	4.8	4.4	5.5		
	IL1A	No	47	11.7	1.0	11.7	9.4	13.6	1.2	0.344
		Yes	10	11.4	0.8	11.6	10.2	12.6		
	IL1B	No	68	3.4	0.6	3.3	1.7	4.7	0.9	0.836*
		Yes	13	3.4	1.3	3.2	2.1	7.5		
	IL6	No	62	10.9	1.1	11.0	8.6	14.1	1.0	0.904
		Yes	11	10.9	0.8	11.1	9.1	11.7		
	IL8	No	68	5.6	1.1	5.5	2.9	8.3	1.3	0.052*
		Yes	13	5.2	0.5	5.1	4.4	5.8		
	IL17RB	No	68	4.3	1.2	4.2	1.7	7.4	0.9	0.627*
		Yes	13	4.4	0.7	4.5	3.2	5.2		
	PARP1	No	69	4.4	0.4	4.4	3.6	5.7	1.2	0.037
		Yes	13	4.2	0.5	4.2	3.2	5.0		
	BACH2	No	66	4.4	0.6	4.5	2.7	5.9	1.2	0.203
		Yes	13	4.2	0.5	4.1	3.0	5.0		
	CCL2	No	52	12.6	1.3	12.8	7.8	14.3	1.0	0.997
		Yes	12	12.6	1.4	13.1	9.2	14.0		
	SERPINB2	No	68	7.5	0.9	7.4	6.1	12.1	1.5	0.002*
		Yes	13	6.9	0.5	7.0	6.2	7.5		

	CXCL12	No	7	13.6	0.6	13.9	12.6	14.1	0.6	-
		Yes	1	14.3	-	14.3	14.3	14.3		
	NFKB1	No	69	4.2	0.3	4.2	3.4	4.9	1.0	0.633
		Yes	13	4.1	0.5	4.0	3.6	5.6		
	JUN	No	69	7.0	0.8	7.0	3.1	9.2	1.2	0.063*
		Yes	13	6.7	0.4	6.8	5.9	7.6		
	STAT3	No	69	0.7	0.4	0.7	-0.4	1.5	1.2	0.012
		Yes	13	0.4	0.3	0.4	-0.3	1.0		
Lipid Metabolism	CRYIL1	No	69	4.6	0.4	4.6	3.3	5.3	1.0	0.815
		Yes	13	4.6	0.4	4.6	3.9	5.4		
	MTMR7	No	68	6.3	0.4	6.3	5.6	7.5	1.2	< 0.001*
		Yes	13	6.0	0.2	6.0	5.8	6.4		
	ST8SIA1	No	69	6.2	0.6	6.2	5.1	7.8	0.9	0.324
		Yes	13	6.3	0.6	6.3	4.9	7.4		
	GRN	No	69	0.7	0.5	0.6	-0.2	2.3	1.1	0.378
		Yes	13	0.6	0.3	0.6	0.1	1.0		
	THRSP	No	56	12.4	0.6	12.5	11.2	14.0	1.1	0.655
		Yes	13	12.3	0.9	12.5	10.7	13.8		
	HSD3B1	No	5	13.9	0.4	13.7	13.6	14.4	1.6	0.079
		Yes	2	13.2	0.2	13.2	13.1	13.4		
	ALOX15B	No	68	9.6	1.0	9.4	7.8	13.5	1.7	0.009
		Yes	13	8.8	0.7	8.8	7.6	10.3		
Glucose Metabolism	CD36	No	69	2.8	1.1	2.7	1.4	8.7	0.8	0.580*
		Yes	13	3.2	2.5	2.6	1.9	11.4		
	PDK4	No	68	6.0	0.7	5.9	4.9	7.7	0.9	0.462
		Yes	13	6.2	0.9	6.0	5.1	8.6		
Cell Cycle	PAPPA	No	48	12.6	0.7	12.8	10.8	14.1	1.6	0.039
		Yes	5	11.9	0.5	11.8	11.4	12.5		
	CDCA5	No	66	11.5	0.8	11.5	9.8	14.0	1.1	0.428
		Yes	13	11.3	0.6	11.2	10.4	12.3		
	SERPINE2	No	68	9.1	0.8	9.0	7.4	11.5	1.1	0.413
		Yes	13	8.9	0.8	8.8	7.7	10.3		
	PTN	No	12	13.0	0.9	13.2	11.0	14.0	-	-
		Yes	0	-	-	-	-	-		
	RB1	No	69	4.0	0.4	4.0	3.3	6.5	1.1	0.165*
		Yes	13	3.9	0.2	3.9	3.7	4.4		
	CTBP2	No	69	3.9	0.3	3.9	3.1	4.7	1.2	0.040
		Yes	13	3.7	0.4	3.7	2.9	4.3		
Translation	EIF2S1	No	69	4.4	0.2	4.4	3.7	5.3	1.0	0.572*
		Yes	13	4.3	0.4	4.4	3.1	4.9		

WNT Signaling	WNT5A	No	24	13.0	0.6	13.0	11.9	14.3	0.8	0.478
		Yes	4	13.3	0.8	13.5	12.2	13.9		
	CTNNB1	No	68	5.0	0.5	4.8	3.9	6.7	1.3	0.020
		Yes	13	4.6	0.4	4.7	3.7	5.1		
	RSP01	No	9	13.5	0.8	13.9	12.4	14.4	1.0	0.979
		Yes	4	13.5	1.1	14.0	11.9	14.2		
	RSP02	No	12	13.4	0.9	13.5	11.0	14.4	0.7	0.379
		Yes	3	13.9	0.6	14.0	13.2	14.3		
	RSP03	No	15	13.4	0.7	13.6	11.9	14.4	1.1	0.657
		Yes	4	13.3	1.0	13.2	12.4	14.2		
Other	TP53	No	69	3.1	0.5	3.0	1.7	5.7	1.1	0.432
		Yes	13	2.9	0.5	3.1	1.4	3.6		
	PRDM1	No	69	3.1	0.5	3.1	2.4	5.8	1.1	0.593
		Yes	13	3.1	0.4	3.1	2.5	3.9		
	PAX5	No	69	4.9	0.9	4.8	1.7	6.9	0.9	0.392
		Yes	13	5.1	0.8	5.0	3.2	6.6		
	HIST1H2BE	No	69	2.9	0.3	2.9	2.0	3.6	0.9	0.354
		Yes	13	3.0	0.4	3.0	2.5	4.3		
	HIST1H2AM	No	69	3.1	0.5	3.0	1.7	4.8	0.9	0.553
		Yes	13	3.1	0.5	3.1	1.9	3.9		
	GAL	No	25	12.0	1.7	12.3	8.4	14.4	0.6	0.446
		Yes	5	12.6	1.4	12.8	10.8	14.1		
	CREM	No	67	8.1	0.4	8.2	7.2	9.2	1.1	0.237
		Yes	13	8.0	0.4	8.0	7.0	8.5		
	NFIL3	No	67	3.1	0.6	3.2	1.7	5.4	1.3	0.024
		Yes	13	2.7	0.4	2.8	2.1	3.2		
	CEBPD	No	69	1.2	0.5	1.2	0.0	3.1	1.2	0.176
		Yes	13	0.9	0.6	0.9	0.1	1.9		
	IGFBP7	No	68	4.3	0.7	4.3	2.9	7.3	0.9	0.579*
		Yes	13	4.5	1.2	4.2	3.7	8.3		
	PIK3R1	No	69	3.1	0.4	3.1	1.4	4.1	1.0	0.727
		Yes	13	3.1	0.5	3.0	2.2	4.5		
	FST	No	42	13.1	1.1	13.2	8.0	14.2	1.0	0.951
		Yes	10	13.2	0.6	13.3	11.8	14.0		
	AREG	No	69	8.5	1.7	8.8	3.6	12.2	1.2	0.649
		Yes	13	8.3	1.7	8.3	5.5	11.5		
	HBEGF	No	69	8.4	0.7	8.3	5.7	9.8	1.2	0.291
		Yes	13	8.2	0.6	8.0	7.5	9.4		
	CTGF	No	57	11.9	1.1	11.8	9.6	14.2	1.0	0.942
		Yes	12	11.9	1.5	11.9	9.1	13.9		
	BTN1A1	No	68	8.8	0.6	8.7	7.5	10.5	1.1	0.127*
		Yes	13	8.6	0.4	8.7	7.9	9.1		

* Unequal variances - Satterthwaite test p-values shown

Cycles to threshold (Ct) represents PCR cycle number at which DNA amount reaches a threshold value

Housekeeping genes used in analysis: GAPDH, ACTB, GUSB, HPRT1, B2M, RPLPO

ΔCt = Ct(gene of interest) - Ct(mean of housekeeping genes)

DOSE RECONSTRUCTION FOR FUTURE STUDIES

While we collected occupational history, medical history, physical examination, and medical record abstraction data on 323 exposed, we were only able to collect and transport blood samples for 218. As a result, for future studies on health endpoints, we needed to reconstruct occupational exposures for those 105 workers on whom we did not have blood samples. The dose reconstruction was carried out by Dr. Nurtan Esmen from UIC with review by Dr. Kyle Steenland from Emory University. The methods used for dose reconstruction were as follows:

The exposure reconstruction was based on the estimate of an index blood dioxin concentration calculated from the estimates of this index for Job classes identified. The basis of the estimate was the blood samples obtained from 218 workers several years to decade after their last employment at the factory. Without exposure data, simultaneous exposure and biomarker measurements, and detailed description of the workplace or processes, the exposure reconstruction can be only in terms of a relative "exposure" parameter. We defined this parameter as an assigned value such that if a person is exposed to dioxin at levels which corresponds to this parameter, then the blood dioxin level would be equivalent to the measured levels. Even though this parameter cannot be used for representation of actual exposure with physicochemical significance or units, it is not at all different from classifying exposures qualitatively as "Low" to "High" with quantitative ordering. In addition, due to the fact that the exposure parameter was based on calculated blood levels from actual blood level measurements, there is no reason to believe that the concordance between the actual and estimated exposure parameter would be poor.

Using C_A as a nonspecific surrogate exposure for total exposure from all routes of entry, the characteristic surrogate exposure and blood body burden concentration may be expressed as:

$$C_i = \gamma C_A - C_i' e^{-\alpha_s \tau} \quad (1)$$

Where

C_i = Accumulated body burden increase due to exposure $C_A(\tau)$

$C`_i$ = Accumulated body burden over total time of employment (θ)

C_A = Characteristic surrogate exposure concentration

α_s = Short-term decay coefficient

τ = Exposure time

$C_i' e^{(-\alpha_s \tau)}$ = Body burden reduction (metabolization and/or excretion)

γ = Bodily absorption rate

Then the cumulative body burden is :

$$C_i = \gamma C_A \sum \tau - \int C_i' e^{(-\alpha_s \tau)} + \varepsilon \quad (2)$$

Where

ε = An error term representing individual and day to day workplace variability

$$C_i' = \int_0^{\theta} \frac{\partial C_i}{\partial \tau} d\tau + C_i\,(0) + \varepsilon \quad (3)$$

Therefore, the expected (average) value of C_A is:

$$E(C_A) = \frac{E(C_i)}{\gamma \sum \tau} + \frac{1}{\gamma \sum \tau} \int C_i'\, e^{(-\alpha_s \tau)} f(C_i') \delta f \quad (4)$$

Or assuming at least a piece-wise linear relationship between $E(C_A)$ and the integral, the relationship between these two entities (at the end of employment) may be simplified. For convenience, dropping E() and with the understanding that the "exposures" represent a sample
from a class of exposures the linearized estimation relationship is :

$$C_A \approx \beta_0 + \beta_1 C_i'(\theta_{MAX}) \quad (5)$$

The blood concentration at separation (B_T) can be back-calculated by using the known biological degradation rate (α) and the amount of time between the blood concentration for department i. τ_i be time last worked in department i to the termination. Then for worker j.

$$B_{T_j} = \sum B_{A_i} \exp(-\alpha \tau_{ij})\, \delta_{ij}, \quad (6)$$

With δ_{ij} – Kronecker delta = 1 if worker j worked in department i and 0 otherwise Equation 6 constitutes an over specified set of equations which can be solved by seeking optimum subject to all B_{Ai} are positive a hierarchy of preset order of B_{Ai} is preserved. This order is estimated by the examination of operations involved in each department. Utilizing the available data and information provided for each department, we were able to assign a relative characteristic exposure at least to the department and in many cases to the sub-classification of job titles. The solution was determined by inverting the matrix of normal equations using the method described in Phillips and Esmen (1999). The solutions obtained using equation 6 were used to classify job classes by relative exposure levels. Therefore, these results can also be used for assigning exposures to workers, whether they were tested or not.

There were 504 individual data points received which could be arranged into 306 possible job/department equations. However, considering the statistical requirements to ensure meaningful data, the received data included sufficient information for 25 department/job title analyses. For each of these analyses, the mean, median and geometric standard deviation were calculated. While two positions in department 19 showed elevated means, when the median was examined, only one of these elevated exposures remained. No other particular department or position appeared to show a consistent elevated exposure pattern. There were 49 job/department combinations which had four or less representations. These job/department representations show a high variability, as measured by the geometric standard deviation, indicating a large amount of heterogeneity in the tasks performed. The results of calculated exposures for the job classes (for those results which can be calculated and which are specific as to position and department) are shown on Table I. This constitutes 24 percent of the job titles with any data. The remaining job titles with any data for which the classes cannot be assigned, based on calculations, were estimated by comparison and/or expert opinion and are shown on Table II.

Since the job exposure matrix is complete, the assignment of exposures was straight forward. For cases and controls the assigned exposure will cease at the termination of employment. For all jobs, the cumulative exposure index is based on the job class and time spent in that class obtained by using equation 6 directly.

REFERENCES for Dose Reconstruction:

1. Esmen, NA (1981), "Limitations on dose estimation," Env. Health Perspectives 42: 3-7.
2. Esmen, NA, Kennedy, KJ, Hall, TA, Phillips, ML, and Marsh, GM (2007), "Classification of Worker Exposures," Chemico-Biological Interactions, 166: 245-253.
3. Phillips, M.L. and Esmen, NA (1999), "Computational Method for Ranking Task-Specific Exposures Using Multi-Task, Time-Weighted, Average Samples," Ann. Occup. Hyg. 47 (3): 201 – 213.

TABLE i - Calculated exposures

	DEPT / JOB TITLE	MEAN	MEDIAN	GSD	n	weightd seg	Exposure
AB1	Dept 19 - Repairman	102.73	25.34	5.33	13	0.355	300
AB3	Dept 19 - Operator	91.89	12.70	7.31	46	1.725	75
AB9	Dept 19 - Foreman	11.16	8.03	2.25	7	0.081	30
CD3	Dept 5 - Operator	53.94	22.96	3.70	28	0.531	75
CD5	Dept 5 - Laboratory Tech	56.49	21.52	4.01	8	0.165	75
EF3	Dept 10 - Operator	5.71	3.25	2.89	5	0.074	7.5
G1	Department 2 - Repairman	3.73	2.89	2.04	7	0.073	3
G3	Department 2 - Operator	5.50	2.63	3.37	35	0.605	7.5
G5	Dept 2 - Laboratory Tech	4.75	3.44	2.24	7	0.080	3
H1	Dept 3 - Repairman	4.36	2.82	2.55	5	0.065	3
H3	Dept 3 - Operator	42.78	20.57	3.35	17	0.292	30
H5	Dept 3 - Laboratory Tech	20.25	4.42	5.73	7	0.206	30
I3	Dept 11 - Operator	9.69	3.30	4.34	29	0.646	7.5
I5	Dept 11 - Laboratory Tech	7.40	5.05	2.40	9	0.111	7.5
J3	Dept 15 - Operator	2.63	0.33	7.69	7	0.276	3
L3	Dept 12 - Operator	17.82	6.27	4.24	8	0.174	30
O5	Central Lab - Laboratory Tech	3.34	1.81	3.03	7	0.109	3
O7	Central Lab - Chemical Worker	10.42	4.92	3.40	9	0.157	30
R1	Other - Repairman	33.66	4.02	7.86	27	1.369	30
R3	Other - Operator	60.91	4.81	9.52	45	2.762	75
R5	Other - Laboratory Tech	34.53	3.48	8.52	14	0.769	30
R7	Other - Chemical Worker	75.35	9.57	7.63	7	0.344	75
R9	Other - Foreman	39.96	0.73	16.97	22	2.409	30
R10	Other - Engineer	2.83	0.42	7.02	10	0.453	3
R11	Other - Trainee	139.34	32.02	5.56	5	0.179	300
R17	Other - Other	12.03	1.65	7.35	25	1.186	30

TABLE ii Expert opinion assigned exposures

	DEPT / JOB TITLE		Exposure			
AB2	Dept 19 - Op of Etherification		75.00			
AB4	Dept 19 - Briquette Operator		75.00		Range	Exposure
AB5	Dept 19 - Lab Tech		30.00		100 to 500 =	300
AB7	Dept 19 – Chemical Worker		75.00		50 to 100 =	75
AB16	Dept 19 - Welder		30.00		10 to 50 =	30
AB17	Dept 19 - Other		7.50		5 to 10 =	7.5
CD1,CD16	Dept 5 – Repairman, Welder		7.50		1 to 5 =	3
CD4	Dept 5 - Lab Tech		0.75		0.5 to 1.0 =	0.75
CD6,CD17	Dept 5 – Packer, Other		75.00		0.1 to 0.5 =	0.3
CD10	Dept 5 - Engineer		30.00			
EF6	Dept 10 - Packer		0.30			
EF7	Dept 10 - Chemical Worker		7.50			
EF9	Dept 10 - Foreman		0.75			
EF10	Dept 10 - Engineer		30.00			
EF17	Dept 10 - Other		7.50			
G2	Dept 2 - Op of Etherification		7.50			
G9	Dept 2 - Foreman		7.50			
G14	Dept 2 - Business Admin		0.75			
H2	Dept 3 - Op of Etherification		7.50			
H9	Dept 3 - Foreman		3.00			
H17	Dept 3 - Other		0.30			
I1,I9	Dept 11 – Repairman, Foreman		7.50			
I4	Dept 11 - Briquette Oper		3.00			
I11	Dept 11 - Trainee		30.00			
I12	Dept 11 - Filtration Oper		75.00			
J1, J5	Dept 15 – Repairman, Lab Tech		30.00			
L5, L7	Dept 12 - Lab Tech, Chem Wkr		30.00			
L9	Dept 12 - Foreman		7.50			
L10	Dept 12 - Engineer		0.75			
L11	Dept 12 - Trainee		3.00			
M8	Gas Rescue Svc - Worker		0.30			
N9,N10	Accident Prevention Dept		0.75			
O3	Central Lab - Operator		7.50			
O9, O10	Central Lab - Foreman		0.75			
O17	Central Lab - Other		3.00			
P13	Transport Dept - Driver		0.30			
Q1, Q17	Maint Dept - Repairman		7.50			
R4, R12	Other – Briquette, Filtration Oper		30.00			
R14	Other - Business Admin		0.30			
R16	Other - Welder		0.75			

Publications

1. Dardynskiy OA, Dardynskaia IV, Hryhorczuk D, Ruestow P, Kazakova EI. [2013] A study of cardiovascular outcomes in workers occupationally exposed to TCDD. Environmental Bulletin (article in English with summary in Russian). 26:43-49.

Other scientific papers, and a PhD dissertation by Peter Ruestow (UIC School of Public Health) are in the process of preparation.