Are Higher Exposures to Flame Retardant Chemicals Associated with Papillary Thyroid Cancer?

by

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Abstract

Papillary thyroid cancer (PTC) occurrence has been significantly increasing throughout the world, and particularly in the US, for several decades. At the same time the use of flame retardants (FR) chemicals has increased, as reflected by increasing concentrations in human tissues. In this study we sought to determine whether flame retardants exposures are higher in individuals recently diagnosed with papillary thyroid cancer relative to a healthy population. The study group included people diagnosed with PTC at the Duke Cancer Center, and controls were matched by age and sex who are recruited from the Duke Health System. Flame retardants (FRs) exposure were estimated from silicone wristband worn for 7 days by participants, which have been validated against traditional biomarkers of exposure. Results indicated that both obesity, and higher levels of the FR Tris (1,3-dichloro-isopropyl) phosphate (TDCPP), were related to increased odds of being a papillary thyroid cancer patient relative to a control. In adjusted statistical models, each log unit increase in TDCPPs on the wristband was found to be associated with a 57% increase in being a case vs a control, while each log unit increase in BMI will result in a 7.1% increase. Therefore, these results indicated that some FRs exposure may be associated with increased PTC incidence.

Introduction

In the United States, the number of people who diagnosed with thyroid cancer has been increasing over the last 4 decades, resulting in an increased incidence rate of 211% from 1975 to 2013 (Lim et al., 2017). Papillary thyroid cancer (PTC), which the most frequent and least aggressive type of thyroid cancer explains for most new cases. The increasing rate has been questioned by some researchers who hypothesize that improved surveillance and screening tests are contributing to this phenomenon (Lim et al., 2017). However, according to Lim et al. (2017), the overdiagnosis may account for the rapid increase incidence of small and localized PTC, but the annual incidence rates for advanced-stage and large (>4cm) PTCs and thyroid cancer mortality rates are consistent with a true increase rather than overdiagnosis. According to the statistics, the incidence rate and mortality rate have increased for advanced-stage and large PTC. Moreover, almost 30 years after new medical screening approaches have been introduced, thyroid cancer rates continue to increase, suggesting that there is a true increase in PTC occurrence and other factors are involved. (Aschebrook-Kilfoy et al., 2013).

In the meantime, the growing use of flame retardants chemicals (FRs) has also increased due to the implementation of flammability standards starting in the 1950s, such as the Flammable Fabric Act, California's Technical Bulletin 117, and standards implemented by the Consumer Product Safety Commission (Blum & Ames, 1977). FRs are a category of synthetic chemicals that are used in furniture, electronics, and construction materials and are intended to reduce the incidence and impact of fires. However, science now suggests that the increase use of FRs are resulting in unintended health consequences. FRs are used as additives in numerous products. FRs can be emitted from products, furniture, and buildings over time and therefore result in widespread exposure among the general population (Sjödin et al., 2001).

Today there is increasing concern about exposure to two common flame retardant classes, one class is polybrominated diphenyl ethers (PBDEs), the other is organophosphate flame retardants (OPFR). PBDEs have been frequently added to many consumer products like furniture and electronics; however, they were phased out in the early 2000s. Despite this phase-out, concerns remain regarding their toxic effects, environmental persistence, and continuous detection in humans after the phase out (Springer et al., 2012). PBDE have a quite similar chemical structure to the endogenous thyroid hormones 3,5,3',5'-tetraiodo-l-thyronine [thyroxine, (T4)] and 3,3',5-triiodo-l-thyronine [triiodothyronine (T3)]. PBDE can potentially cause thyroid homeostasis disruptions (Butt et al., 2011). OPFRs have been commonly used as a replacement for PBDEs after the phase out. However, a study published in 2015 showed that some OPFRs may also have adverse effects comparable to PBDEs; several OPFRs are associated with neurotoxicity, perhaps due to their structural similarities with organophosphate insecticides, which are also neurotoxic (Behl et al., 2016).

Thyroid hormones play an important role in body metabolism regulation, development and growth. They also influence proliferation of cancer cells. Therefore, chemicals that disturb thyroid hormone regulation may also increase thyroid cancer risk and severity. To determine if FRs exposure may be associated with the increase in PTC, Hoffman et al. (2017) conducted a case-control study that sought to compare exposures in recently diagnosed PTC patients and age and sex-matched healthy controls. They found that more exposure to some FRs in the household dust was associated with increased occurrence of PTC. Specifically, they found that PTC cases were more possible to have elevated concentrations of TCEP (one of OPFRs) and BDE-209 (one of PBDEs) in their home environment dust compared to the control population.

One limitation of this previous study was that exposure was only assessed in the home environment. Since people do not stay in their house for 24 hours per day, house dust may not reflect an individuals entire exposure to FRs. A personal exposure monitor may provide a better reflection of FR exposures compared to relying on household dust. Personal exposure montiors on the other hand, may provide average exposures insights across a certain time period and capture other exposure that is currently lacking in dust measurements (e.g. cars, work environment) (Hammel et al., 2016). Research conducted by Hammel et al. (2016; 2018) suggests that silicone wristbands can capture personal exposures to organophosphate and brominated flame retardants (OPFRs and PBDEs). They demonstrated that the concentrations of OPFRs and PBDEs measured on wristbands worn for one week were significantly correlate with levels measured in urine and serum, respectively. Wristbands are believed to capture exposures that occur via inhalation, dermal exposure and inadvertent dust ingestion. Based on these studies, the current study sought to determine if wristbands could be used to investigate exposure to FRs and to estimate odds of PTC in a case-controls tudy. Like the previous study by Hoffman et al. 2017, the hypothesis for the study was that FRs are related to increased odds of being a PTC case. However, one of the main differences compared to the Hoffman's study is that, the present study used silicone wristbands as an improved measurement of FR exposure.

Method

Study participants

Participants were recruited in 2017-2018 The study group are patients newly diagnosed with PTC at Duke University hospital or Duke Cancer Institute for treatment. The control group includes other Duke patients experience unrelated medical issues or commonly wellness care. To reduce the selection bias in the study, all participants had to live closer than 50 miles around Duke University. Pregnant women were not included in the study due to the thyroid hormone level variation during pregnancy. The control group are individuals who never had thyroid cancer or a history of thyroid disease. In total, 36 study case and 36 controls were recruited. The study involved human subject's research, therefore it undergone review and approved by Duke University Health System Institutional Review Board (IRB).

FRs in silicon wristband

Wristbands used in the study were purchased commercially (24hourwristbands.com) and cleaned with two 12-hour Soxhlet extractions, one with 1:1 ethyl acetate/hexane (v/v) and one with 1:1 ethyl acetate/methanol (v/v). After they were dried in a fume hood as described in Hammel et al. (2016). Then the wristbands were fully cover with aluminum foil and placed in a 40 mL amber jars. Both control group and study group participants were asked to wear the wrist band over a 7-day period all the time including when they are sleeping, eating, bathing, or playing sports. After the period is done, the wristbands were wrapped in aluminum foil and mailed back to the laboratory, and they were stored at 20°C until extraction. Field blank wristbands used in the study were also stored at the same room condition.

Wristband Extraction

The flame retardants quantified in the wristbands include polybrominated diphenyl ethers (PBDEs), TBB (2-ethylhexyl 2,3,4,5-tetrabromobenzoate), and TBPH bis(2-ethylhexyl) 3,4,5,6-tetrabromophthalate). The organophosphate FRs included TCEP (Tris(2-chloroethyl) phosphate), TCPP (tris (1-chloro-2-propyl) phosphate), TDCPP (Tris(1,3-dichloroisopropyl)phosphate) , and TPHP (Triphenyl phosphate). Each sample and field blank wristband were cut into 1-inch pieces for extraction, and the remaining part of the wristband was put back in the freezer. The mass of each piece was recorded and then transferred into a labeled 50 mL glass centrifuge tube. Internal standards were then added to the centrifuge tube. Each sample conducted 3 times extraction with 10 mL 1:1 hexane/acetone (v/v) via sonication extraction and evaporated to about 1 mL with a nitrogen evaporation unit (N- EVAP). Next, the 1 mL extracts were purified using 8.0 grams of activated Florisil®. FRs were eluted first with 35 mL hexane and 35 mL of ethyl acetate next. The eluent was concentrated to near-dryness with the N-EVAP and samples were transferred into auto sampler vials that specifically analyze with gas chromatography/mass spectrometry (GC/MS) using methods reported in Hammel et al. 2016, 2018.

Statistical Analysis

Descriptive Statistics and graphic analyses were used in the study to summarize the measurements. FRs' concentrations were not normally distributed, therefore non-parametric statistical analysis were used. "The method detection limit (MDL) was calculated using three times the standard deviation of the mass of each FR measured in the field blanks" (Hoffman et al., 2015). For statistical analyses, the MDLs were substituted by the MDL value for the FR/ (wristband weight * $\sqrt{2}$). Kruskal-Wallis tests were conducted to compare FR concentrations means between control and study groups. Spearman's rank correlation coefficients were used to assess the correlation among different FRs concentrations. Logistic regression models were appied to test the relationship between flame retardant concentrations and PTC status outcomes

(Case/Control). Besides FR concentrations, logistic regression models also included body mass index (BMI), age, and race (White/Non-White).

All statistical analyses are applied through R studio Version 1.1.456. Statistical significance is set at a p-value of 0.05.

Results

Table 1 shows demographic characteristics of study participants. Most of the participants were females, which is consistent with gender difference in thyroid cancer risk where the prevalence rate is 4 times higher among women than men (Lortet-Tieulent et al., 2019). Cases has a mean age of 52.1 which was similar to controls.

Table 1. Demographic characteristics of the participants.

	Cases (n=36)		Controls (n=36)	
	Mean ± st. dev.	Range or %	Mean ± st. dev.	Range or %
Age(years)	52.1 ± 12.6	27 - 77	42.4 ± 12.7	26 - 78
BMI	29.8 ± 7.5	21.2 - 50.8	27.2 ± 5.1	19.2 - 40.3
Sex				
Male	8	22.20%	8	22.20%
Female	28	77.80%	28	77.80%
Race				
White	25	69.40%	31	86.10%
Non-White	11	30.60%	5	13.90%
Annual Household Income				
Under $50,000	14	41.18%	9	26.47%
$50,000-$99,999	7	20.59%	10	29.41%
Over $100,000	13	38.24%	15	44.12%

FR concentration data was obtained from the GC/MS and is summarized in Table 2. The mass unit is nanogram (ng) of FRs per gram of wristband (ng/g). FRs were detected in all 72 silicone wristbands (36 Cases and 36 Controls). Most FRs test in the study were detected in over 90% of the samples.

Table 2. FRs concentrations measured on the wristbands.

	Geomean (ng/g)	Minimum (ng/g)	Maximum (ng/g)	> MDL%
BDE47	52.25	0.1	2356.8	94%
BDE100	5.24	0.17	562.5	79%
BDE154	2.31	0.02	118.0	93%
BDE153	3.84	0.49	203.6	94%
BDE209	21.97	4.3	276.3	81%
TBB	72.8	4.45	2033.2	100%
TBPH	61.47	2.89	814.0	96%
TCEP	8.2	1.21	257.5	67%
TCPP	269.44	13.5	7897.1	97%
TDCPP	351.98	25.84	7216.9	100%
TPHP	250.31	15.13	2161.4	100%

Figure 1 shows the Spearman correlation coefficients among the measured FRs. Among all the correlations, BDE 100, BDE 47, BDE 154, and BDE 153 have the strongest correlations with one another (0.59 -0.8). TBB and TBPH, TDCPP and TPHP, TBB and TPHP had moderate correlations (0.46 – 0.53). A Spearman correlation test was also used to determine if age, BMI, or race was associated with the concentration of flame retardants on the wristbands. None of the FRs concentrations were significantly correlated with either age or BMI. In other words, age and BMI are not confounding variables in this study. When evaluating differences in FRs based on race, the Wilcoxon rank sum test showed that there is a 0.81 ng/g lower BDE 153 concentration in white people (p-value = 0.11), and a 172.1 ng/g higher TBB concentration in white people (p-value = 0.04), compared to other races.

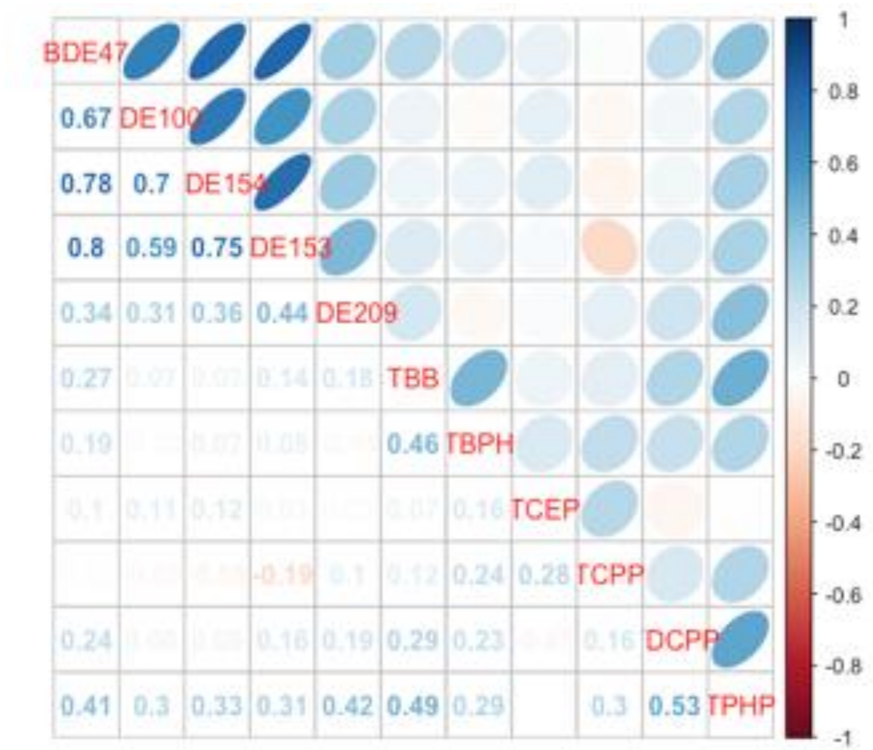

Figure 1. Spearman correlation coefficients among the FRs measured in wristbands.

Figure 2 shows the medians for each FRs stratified by case and control groups together with the p-value from the Wilcoxon tests. For example, TPHP has a concentration about 250 ng/g (parts per billion) in both case and control groups. The sum total of all 11 FRs is also plotted in the last figure. Among these comparisons, TDCPP has a significantly (p-value = 0.022) higher concentration on the wristband from cases compared to controls. BDE 209 and BDE 100 both have suggestive higher concentrations in the control group (p-value = 0.16) and (p-value = 0.16).

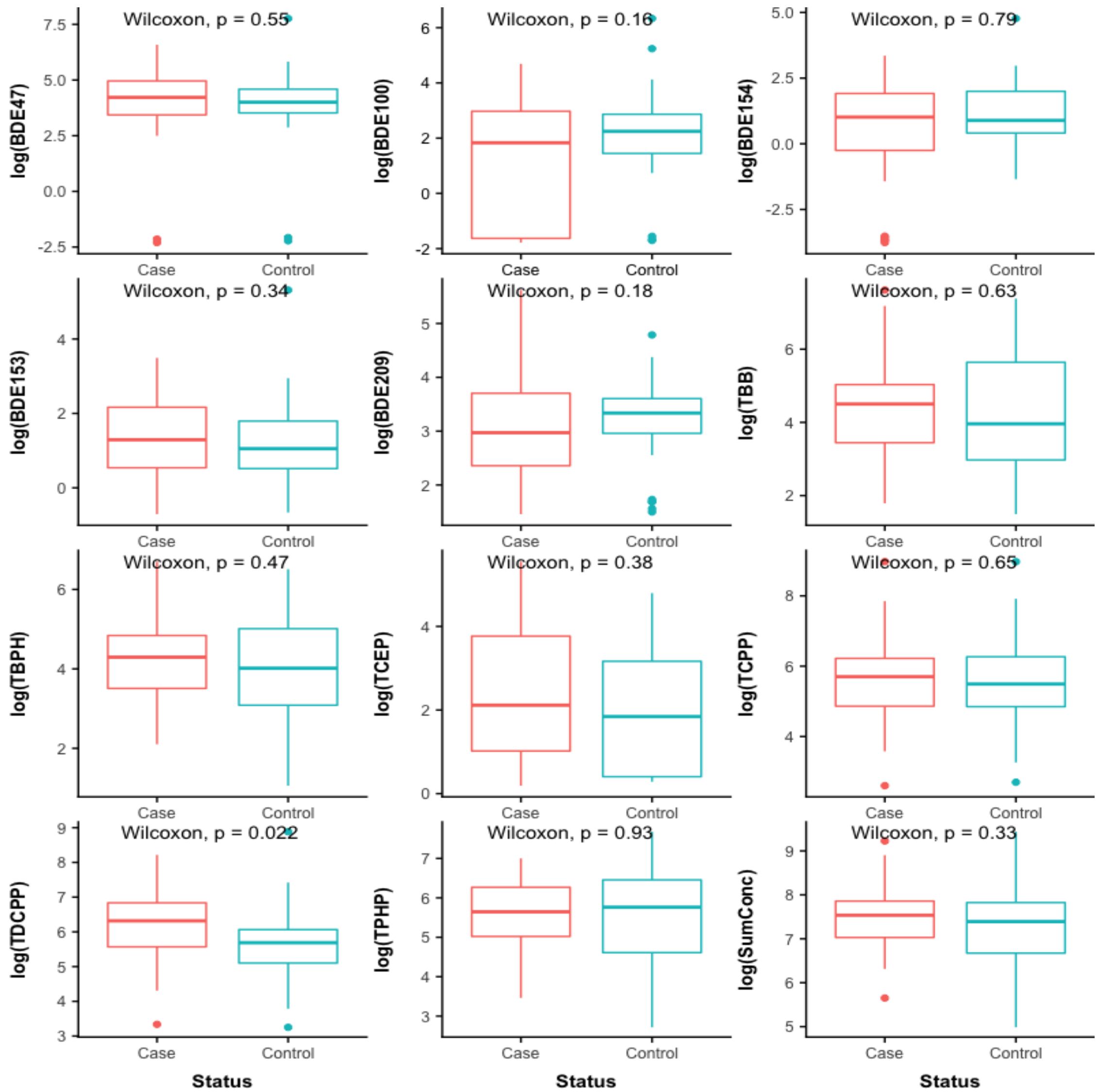

Figure 2. Box plots of log-scale FR concentrations (ng/g).

Table 3. Odds Ratio for PTC status for FR exposure and other important variables.

	Odds Ratio(OR) p-value
Individual FRs	
BDE-47	1.05, p = 0.73
BDE-100	0.67, p = 0.06
BDE-154	0.87, p = 0.32
BDE-153	1.21, p = 0.37
BDE-209	0.77, p = 0.32
TBB	1.03, p = 0.83
TBPH	1.18, p = 0.37
TCEP	1.19, p = 0.25
TCPP	1.07, p = 0.7
TDCPP	1.57, p = 0.05 *
TPHP	1.05, p = 0.83
Other Variables	
Age	0.99, p = 0.92
BMI	1.07, p = 0.08
Race (White)	0.37, p = 0.09

* p = 0.05 statistical significant

Using a logistic regression model, odds ratio between cases and controls were calculated and are presented in Table 3. Besides FRs, BMI and race were also included in the logistic regression model since the coefficients of these variables are different from 0, implying that BMI and race may be significant variables in predicting the outcome. BMI was included as a continuous variable while race was included as a factor with two levels: white and non-white. The results are suggestive of an association between BMI and PTC, such that a 1 unit increase in BMI will result in 7% increase in the odds of being a papillary thyroid cancer case (p = 0.08) compared to a control. In this study we found that a white race was associated with a 63% less likely odds of PTC compare to non-white (p = 0.09). For flame retardants, each log unit increase in TDCPP concentration on the wristband resulted in a 57% increase in the odds of being a case vs a control.

Discussion

In conclusion, the result in the study support our hypothesis and suggest that exposure to some FRs may related to elevated risk of PTC. In this study, we considered a p-value less than 0.2 to be suggestive due to the small sample size. We do see some suggestive findings that relate to PTC occurrence in our study including exposure to FR TDCPP, BMI, and Race. In our models, TDCPP concentrations were significant and associated with PTC. This result is consistent with findings from Dr. Hoffman et al (2017)'s study where they found that exposure to some FRs was higher in PTC cases. Comparing the results of these two studies, TDCPP exposure in cases was found to be higher than controls in both studies. In Hoffman's study, the odds ratio of TDCPP was 1.49 ($p = 0.31$). An odds ratio of 1.49 means that exposure to each unit increase of TDCPP will result in a 49% chance of being a PTC case vs a control, therefore, the risk may also be elevated but it was not statistically significant. In Hoffman's study, they found that BDE 209 was statistically significant with an odds ratio of 2.29 ($p=0.04$), but higher exposure to BDE 209 in cases was not observed in our study. These differences may result from the different exposure measurements. Silicones wristband capture exposures from multiple microenvironments such as working environments, and cars, compared to the home environment alone.

There are some limitations in this study that should be considered when interpreting the results. When recruiting control group participants, demographic characteristics are matched. According to Rose and Laan (2009), matched case and control study is often used in public health research, for it is able to eliminate confounding effects and be more efficient at identifying potential causative factors. However, cases and controls in this study were not perfectly matched. The white and non-white sample sizes are not equal. This study included 56 white people in the participants compared to 16 non-white participants, and therefore, the association of race with PTC may lack statistical power. Moreover, income could also be matched better when recruiting control participants, although income is not a significant indicator in this study. Studies have shown that thyroid cancer rate varies between high income and low income areas due to overdiagnosis in high income populations that have better access to medical care. In other words, there is a higher diagnosis rate among high income populations, but a high mortality thyroid cancer rate in low income populations (Lortet-Tieulent et al., 2019). Also, the age range is similar in cases and controls, but the age mean is 10 years higher in cancer group compared to the control group. A disadvantage in using matched case and control studies is that matching factors cannot be studied anymore, and the difficulties in recruiting well-matched controls can jeopardize the efficiency of the statistical analysis.

Another limitation of this study is the small sample size. We were only able to recruit 36 cases and 36 controls in this study. From the box plot for other FR concentrations (besides TDCPP) in Figure 2 we see that there are some higher concentrations in cases (e.g BDE 154, BDE 153, TBB) but the p-values are not statistically significant. Several extreme values may cause the boxplots to skew due to small sample size effects, and the estimation based on a small sample size is unstable. Additional research assessing exposure to these FRs with silicone wristbands may provide important additions to this study. Based on the hypothesis that exposure to endocrine-disrupting chemicals and radiation may be risk factors for PTC, future studies should be conducted to determine if exposure to other endocrine-disrupting chemicals can be detectable using wristbands.

Conclusion

In summary, results from this study suggest that higher exposure to the flame retardant TDCPP may result in an increased odds of PTC. This conclusion is consistent with previous studies' result by Hoffman et al. (2017). In addition, we noticed that BMI and race are two significant variables that may be associated with PTC occurrence. A higher BMI and non-white race was related to PTC case-status in our study. More research is warranted in the future with sufficient sample sizes and comprehensive measurement of FR exposures to determine if these chemicals represent a true risk for developing PTC.

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