Maternal exposure to environmental endocrine disruptors during pregnancy is associated with pediatric germ cell tumors

Hou-Wei Lin1,2, Hai-Xia Feng3, Lin Chen4, Xiao-Jun Yuan5, and Zhen Tan5

1Department of Pediatric Urology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China
2Department of Pediatric Surgery, Jiaxing Maternity and Child Health Care Hospital, Zhejiang, China
3Department of Pediatric gastroenterology and nutrition, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China
4Ministry of Education-Shanghai Key Laboratory of Children's Environmental Health, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China
5Department of Pediatric Hematology/Oncology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China

ABSTRACT

Environmental endocrine disruptors (EEDs) are natural or synthetic chemical compounds that interfere with normal endocrine function in both wildlife and humans. Previous studies have indicated that EEDs may contribute to oncogenesis. This study explores the relationship between EEDs and pediatric germ cell tumors (GCTs). A case-control study was conducted in 84 pediatric patients from 2014 to 2017, including 42 subjects with immature teratoma, yolk sac tumor, or germinoma, and 42 controls who experienced pneumonia or trauma. Serum PFASs, including PFBS, PFHpA, PFHxS, PFOA, PFOS, PFNA, PFDA, PFUA, PFOSA, and PFDoA, were measured in each subject, and their history of possible EED exposure was reviewed. Six of the 10 measured PFASs were significantly increased in the GCT group relative to the control group. With respect to lifestyle history, only PFHxS levels were statistically significantly associated with GCTs as determined by logistic regression analysis. The odds ratio for a 1 ng/L increase in PFHxS was 19.47 (95% CI: 4.20–90.26). Furthermore, in the GCT and control groups, both parental consumption of barbecued foods and hair dye use among parents were significantly correlated with elevated serum PFHxS levels ($\rho$ = 0.383, 0.325 in the patient group and $\rho$ = 0.370, 0.339 in the control group; $p$ < 0.05). Our study confirmed that children with GCTs from our institute had relatively high serum levels of PFASs relative to those of tumor-free pediatric patients. Serum PFHxS levels were independently associated with germ cell tumor occurrence.

Keywords: serum environmental endocrine disruptors, pediatric germ cell tumor, parental exposure to environmental endocrine disruptors

Abbreviations:
EED: Serum environmental endocrine disruptors
GCT: pediatric germ cell tumor
PFAS: Per- and polyfluoroalkyl substance
PFBS: Perfluorobutane sulfonate
PFHpA: Perfluorohexanoic acid

Received: July 22, 2019; accepted: October 15, 2019
Corresponding Author: Zhen TAN, MD, PhD
Department of Pediatric Hematology/Oncology, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Kongjiang Road 1665, Shanghai, 200092, China
Tel: +86-21-25078999, E-mail: tanzhen@xinhuamed.com.cn
INTRODUCTION

Germ cell tumors (GCTs) are a group of benign or malignant neoplasms that arise from primordial germ cells and are capable of further differentiation into embryonic or extraembryonic cells.\(^1\) They may develop at sites other than the testicle and ovary. The 5-year overall survival (OS) for patients with GCTs ranges from 40% to 90%. Survival rates can be as high as 100% in seminomatous extragonadal GCTs, while much lower (40%) rates are observed in mediastinal nonseminomatous extragonadal GCTs.\(^2,3\) Moreover, GCTs can readily metastasize to lymph nodes, lung, liver, and brain.\(^4\) Thus far, the pathogenesis of germ cell neoplasms, including the contribution of environmental factors and genetic susceptibility, remains undefined.\(^5\)

Environmental endocrine disruptors (EEDs) are natural or synthetic chemical compounds that interfere with the normal function of the endocrine system in both wildlife and humans. They mimic or interrupt normal hormone function by binding or interfering with their respective receptors.\(^6\) The first recognized EED, diethylstilbestrol, is a highly potent, full agonist of both estrogen receptors. Studies have shown that diethylstilbestrol cannot effectively maintain pregnancy and may induce adenocarcinoma of the vagina. Therefore, research on the pathology and pharmacology of EEDs was initiated in the 1970s.\(^7\) Recently, EEDs have been found to contribute to the pathogenesis of various diseases including obesity,\(^6,8\) cancer,\(^9\) congenital malformation\(^10\) and infertility.\(^11\) It has been reported that EEDs may be involved in the acquired and congenital development of certain cancers including breast cancer, neuroblastoma, thyroid carcinoma, and GCTs.\(^12-15\)

The correlation between EEDs and GCTs has previously been reported in several studies.\(^3,16-18\) As a rapidly developing country, China has been significantly affected by environmental and food contamination for many years,\(^19\) and EEDs have been detected in water supplies for the last 15 years.\(^20\) EEDs are very stable in the serum because of their long term accumulation and half-lives.\(^21\) However, limited data are available regarding EED levels in Chinese children with GCTs, especially in those with a parental history of pollutant exposure during pregnancy. In this study, serum EED levels were measured for 10 perfluoroalkyl and polyfluoroalkyl substances (PFASs) in pediatric GCT and non-neoplasm subjects. In addition, we investigated the relationship between EEDs and exposure to physical or chemical factors during pregnancy.

METHODS AND MATERIALS

Study Population

A sex and age matched case-control analysis were performed in this study. All GCT and control group subjects were recruited from the Department of Pediatrics, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine during the period of October 1, 2014
Environmental endocrine disruptors and pediatric germ cell tumors

to April 30, 2017. Subjects in the GCT group were less than 16 years old and pathologically diagnosed with a GCT. Cases consisting of familial germ cell tumors (i.e. parents or grandparents) or cohabitation with tobacco smokers within the family were excluded. In total, 45 children with GCTs were enrolled, though three patients were not accessible for blood collection because of their disease condition. The remaining 42 patients were assigned to the GCT group, and the control group consisted of 42 patients with mycoplasma/bacterial pneumonia and asthma occurring at the same time (Table 1).

This study was approved by the Institutional Review Board of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine in accordance with the principles of Helsinki Declaration II. Written informed consent was obtained from each subject prior to the survey.

Blood Sample and Questionnaire Collection

Blood samples were collected one week following the pathological identification of the case group and on the day of discharge in the control group, since EEDs in the serum are extremely stable.21 For each patient, a 5 ml blood sample was obtained through a syringe and transferred to a tube containing EDTA. Serum was separated from whole blood samples by centrifugation at 1500 x g for 5 minutes and stored in a -80°C freezer prior to EED measurement. In-person interviews were conducted to acquire family history data. Incidents of possible contact with EED sources during pregnancy were collected by using an online questionnaire. The survey focused on 1) the use of cosmetic products such as hair dye during pregnancy, 2) eating habits, 3) surrounding environment in the living area, and 4) other basic information about the subject’s parents. Frequency of exposure was categorized as 1) none, 2) less than once a month, 3) once to 4 times a month, and 4) more than once a week.

Measurement of PFASs

The concentration of PFASs was measured by a protocol that was established at our institution.22,23 Briefly, samples were thawed at room temperature and homogenized for 15 seconds using a vortex. Then, serum samples (100 µl) were mixed with 10 µl of internal standard solution (50 ng/ml) and vortexed for 30 seconds. Afterwards, 150 µl of 1% formic acid in acetonitrile and 150 µl methanol were added to the prepared samples and vortexed for 30 seconds followed by 10 minutes of sonication. Following centrifugation at 12,000 rpm (Allegra X-15R, Beckman Coulter Inc., U.S.A.) for 10 minutes, 100 µl of supernatant was filtered through a 0.22 µm nylon syringe filter and collected for final analysis. A standard mixture of fetal bovine serum was used as a quality control. Detection of PFASs was performed using high performance liquid chromatography with tandem mass spectrometry (HPLC/MS-MS, Agilent Technologies Inc., U.S.A.). The HPLC/MS-MS procedures were conducted by strictly following the manufacturer’s guidelines. The present detection limit of the PFASs measurement was 0.02 to 0.09 ng/ml.

Statistical Analysis

The summarized data are presented as median (interquartile range) or frequency (%). Differences in demographic characteristics, living habits and serum PFAS levels were determined by Chi-Squared tests or Mann–Whitney U tests. The odds ratios of GCTs to increased PFAS levels were estimated by univariate and multivariate logistic regression methods. A correlation between PFAS levels with possible EED exposure frequencies was determined by Spearman’s rank-order correlation analysis. When the p value was less than 0.05, a significant result was considered. All calculations were done using PASW Statistics 18 (Chicago: SPSS Inc.) software.
RESULTS

Characteristics of the Study Population

Characteristics of the 84 enrolled participants in both the GCT and control groups are provided in Table 1. Of the 42 patients in the GCT group, 2 had germinoma, 7 had teratoma, and 33

Table 1  Characteristics of the cases enrolled in this study and lifestyles according to questionnaire responses

| Variables                          | Controls  | GCTs      | $\chi^2$ | P value |
|-----------------------------------|-----------|-----------|----------|---------|
| Age, months (range)               | 22 (11–47)| 29 (13–48)| /        | 0.558   |
| Cases by Sex                      | Male      | 19        | 23       |         |
|                                   | Female    | 23        | 19       | 0.762   | 0.383   |
| Diseases of cases                 | Germinoma | /         | 2        | /       |
|                                   | Yolk Sac Tumor | /      | 33       | /       |
|                                   | Teratoma  | /         | 7        | /       |
|                                   | Pneumonia | /         | 35       | /       |
|                                   | Cranial Trauma | /   | 7        | /       |
| Mother Age in Birth               | <30 years old | 14    | 32       | 15.570  | <0.001  |
|                                   | ≥30 years old | 28    | 10       |         |
| Father Age in Birth               | <30 years old | 8     | 28       | 19.444  | <0.001  |
|                                   | ≥30 years old | 34    | 14       |         |
| Emergency Contraceptive Usage     | Yes       | 8         | 12       | 1.050   | 0.306   |
|                                   | No        | 34        | 30       |         |
| Infectious Disease during Pregnancy | Yes     | 7         | 18       | 4.850   | 0.013   |
|                                   | No        | 35        | 26       |         |
| Hair Dye Usage during Pregnancy   | Yes       | 9         | 17       | 3.565   | 0.059   |
|                                   | No        | 33        | 25       |         |
| Cosmetics Usage during Pregnancy  | Yes       | 5         | 13       | 4.525   | 0.033   |
|                                   | No        | 37        | 29       |         |
| Barbecue during Pregnancy         | Yes       | 10        | 24       | 9.685   | 0.002   |
|                                   | No        | 32        | 18       |         |
| Filtered Water Drinking during Pregnancy | Yes   | 21        | 11       | 5.048   | 0.025   |
|                                   | No        | 21        | 31       |         |
| Indoor-decorating during Pregnancy | Yes     | 4         | 17       | 10.730  | 0.001   |
|                                   | No        | 38        | 25       |         |
| Insecticide Exposure during Pregnancy | Yes   | 0         | 0        | /       | N/A     |
|                                   | No        | 42        | 42       |         |
| Lead Exposure during Pregnancy    | Yes       | 0         | 1        | 1.012   | 0.500*  |
|                                   | No        | 42        | 41       |         |
| Living near Possible Pollution Sources | Farmland | Yes     | 2        | 8       | 4.086   | 0.043   |
|                                   | No        | 40        | 34       |         |
|                                   | Chemical Plant | Yes   | 0        | 0       | /       | N/A     |
|                                   |           | No        | 42        | 42       |         |
|                                   | Industrial Smog | Yes  | 3        | 0       | 3.111   | 0.241*  |
|                                   |           | No        | 39        | 42       |         |
|                                   | Generating Station | Yes | 7        | 1       | 4.974   | 0.057*  |
|                                   |           | No        | 35        | 41       |         |

GCT=Germ cell tumor.

* Fisher’s exact test.
had Yolk Sac tumors. The median age of the patients in the GCT group was 29 months and 19 (45.2%) of the subjects were boys. In the control group, the median age was 22 months and 23 (54.8%) of the subjects were boys. There were no significant differences in age and sex between the two groups. The GCT group contained younger parents (age less than 30 years at birth) as compared with the control group (mother: $\chi^2 = 15.570$, $p<0.001$; father: $\chi^2 = 19.444$, $p<0.001$). Differences between the GCT and control groups, respectively, were observed for multiple factors that existed during pregnancy including infectious disease, 7 (16.7%) vs. 18 (42.9%), cosmetics usage, 5 (11.9%) vs 13 (31.0%), barbecued food consumption, 10 (23.8%) vs. 24 (57.1%), indoor decorating, 4 (9.5%) and 17 (40.5%), living near farmland, 2 (4.8%) vs. 8 (19.0%), and drinking filtered water, 21 (50.0%) vs. 11 (26.2%). There were no statistical differences evident in other related living habits including the use of emergency contraceptives, hair dye, or insecticides. Also, no differences were observed with respect to lead exposure or living environment.

### Table 2 PFASs median concentrations in sera in two groups of cases in this study

| PFASs | Controls (ng/ml) | GCTs (ng/ml) | $P$ value |
|-------|------------------|--------------|-----------|
| PFHxS | 0.338 (0.233–0.553) | 1.787 (0.833–2.473) | $<0.001$ |
| PFBS  | 0.115 (0.094–0.198) | 0.165 (0.120–0.238) | 0.028 |
| PFHpA | 0.232 (0.163–0.510) | 0.268 (0.194–0.456) | 0.231 |
| PFOA  | 10.812 (5.534–20.101) | 15.915 (8.990–26.208) | 0.038 |
| PFOS  | 3.888 (1.976–6.944) | 5.202 (3.237–10.126) | 0.036 |
| PFNA  | 1.272 (0.761–2.372) | 1.663 (0.918–2.402) | 0.390 |
| PFDA  | 0.641 (0.342–1.353) | 0.794 (0.484–1.506) | 0.321 |
| PFUA  | 0.381 (0.184–0.741) | 0.575 (0.260–0.831) | 0.231 |
| PFOSA | 0.095 (0.093–0.113) | 0.115 (0.099–0.129) | $<0.001$ |
| PFDoA | 0.118 (0.087–0.159) | 0.140 (0.113–0.174) | 0.104 |

Parameter definition of PFASs concentrations: median (interquartile range), GCT: Germ cell tumor.

### Supplementary table  PFASs concentrations in sera (ng/mL) of all patients in this study

| PFASs | LOD | Percent | >LOD (%) | Minimum | 25th | Median | 75th | Maximum |
|-------|-----|---------|----------|---------|------|--------|------|---------|
| PFBS  | 0.009 | 100 | 0.07 | 0.10 | 0.14 | 0.22 | 2.38 |
| PFHpA | 0.03 | 100 | 0.09 | 0.17 | 0.25 | 0.48 | 0.95 |
| PFHxS | 0.02 | 100 | 0.13 | 0.34 | 0.62 | 1.82 | 4.96 |
| PFOA  | 0.09 | 100 | 2.05 | 8.05 | 13.89 | 21.37 | 360.50 |
| PFOS  | 0.09 | 100 | 0.73 | 2.48 | 4.47 | 8.26 | 76.76 |
| PFNA  | 0.02 | 100 | 0.38 | 0.83 | 1.54 | 2.37 | 15.72 |
| PFDA  | 0.02 | 100 | 0.08 | 0.35 | 0.74 | 1.38 | 14.60 |
| PFUA  | 0.02 | 100 | 0.03 | 0.20 | 0.51 | 0.75 | 5.22 |
| PFOSA | 0.12 | 46.43 | 0.09 | 0.09 | 0.11 | 0.12 | 0.83 |
| PFDoA | 0.05 | 98.81 | 0.04 | 0.10 | 0.13 | 0.17 | 1.08 |
Increased Serum PFAS Levels in GCT Subjects

Ten different PFAS compounds were detected in both the GCT and control groups (Supplementary Table). The median (interquartile) concentrations of 6 of the 10 PFASs in the GCT group were significantly increased as compared with the control group (Table 2). These included PFHxS [1.787 (0.833–2.473) vs. 0.338 (0.233–0.553) ng/ml, \( p < 0.001 \)], PFBS [0.165 (0.120–0.238) vs. 0.115 (0.094–0.198) ng/ml, \( p = 0.028 \)], PFOA [15.915 (8.990–26.208) vs. 10.812 (5.534–20.101) ng/ml, \( p = 0.038 \)], PFOS [5.202 (3.237–10.126) vs. 3.888 (1.976–6.944) ng/ml, \( p = 0.036 \)], PFOSA [0.115 (0.099–0.129) vs. 0.095 (0.093–0.113) ng/ml, \( p < 0.001 \)] and PFDOA [0.140 (0.113–0.174) vs. 0.118 (0.087–0.159) ng/ml, \( p = 0.104 \)].

PFHxS as a Potential Risk Factor in GCT Subjects

The association between PFASs and GCT in subjects was initially investigated using univariable logistic regression models. The odds ratio for each 1 ng/ml increase in serum PFHxS was 10.79 (95% CI: 3.78–30.84) in a crude analysis and 11.89 (95% CI: 3.47–40.75) after adjusting for confounding lifestyle-related factors (Table 3). Other PFASs measured in this study were not found to be significantly associated with GCT in both crude and adjusted models.

Irregular PFHxS Levels According to Categorical Covariates

Median serum levels of PFHxS in the study population, determined by categorical covariates, were compared between the GCT and control groups (Table 4). The median serum PFAS concentrations were consistently higher in most of the subgroups with frequent EED exposure, especially in the GCT subjects. More importantly, all participants with a history of hair dye usage or barbecued food consumption during pregnancy had significantly elevated serum PFHxS levels compared with those without either in the GCT [hair dye: 2.38 (1.72–3.29) vs. 1.37 (0.63–1.95), \( p = 0.010 \); barbecued food: 2.19 (1.27–3.03) vs. 1.42 (0.61–1.79), \( p = 0.007 \)] or control group [hair dye: 0.49 (0.34–0.67) vs. 0.32 (0.19–0.46), \( p = 0.020 \); barbecue: 0.58 (0.34–1.21) vs. 0.32 (0.19–0.46), \( p = 0.004 \)].

### Table 3

| Covariates | Crude Odds Ratio | Adjusted Odds Ratio |
|------------|------------------|---------------------|
| PFHxS      | 10.79 (3.78–30.84) | 19.47 (4.20–90.26) |
| PFBS       | 0.50 (0.08–3.24)   | 1.80 (0.20–16.61)   |
| PHHpA      | 1.84 (0.25–13.42)  | 4.78 (0.38–60.77)   |
| PFOA       | 1.03 (0.99–1.06)   | 1.03 (0.99–1.08)    |
| PFOS       | 1.07 (0.99–1.17)   | 1.08 (0.96–1.21)    |
| PFNA       | 1.08 (0.98–1.31)   | 1.00 (0.77–1.29)    |
| PFDA       | 1.25 (0.89–1.75)   | 1.15 (0.70–1.90)    |
| PFUA       | 1.49 (0.76–2.92)   | 0.90 (0.39–2.06)    |
| PFOSA      | 2006.99 (0.03–1.19×10⁶) | 3404.13 (0.04–2.69×10⁴) |
| PFDoA      | 6.73 (0.10–472.83) | 0.27 (0.01–24.19)   |

Odds ratio for each 1 ng/ml increase in serum, adjusted for infectious disease, cosmetics usage, barbecued food consumption, filtered water use, indoor decorating, living near farmland.

Parameter definition of Odds Ratios: median (interquartile range), GCT: Germ cell tumor.
Positive Correlation between PFHxS Levels and Repeated Exposure

The frequency of barbecued food consumption and hair dye usage during pregnancy in all study participants was analyzed. PFHxS levels were found to be positively correlated with barbecued food consumption (GCT and control group: Spearman’s $r=0.446$ and $0.405$, $p=0.003$ and 0.008) and hair dye usage (GCTs and control group: Spearman’s $r=0.350$ and 0.361, $p=0.023$ and 0.019) (Table 5).
As a developing country, China has been heavily polluted with EEDs from multiple sources over the last several decades, despite employing multiple methods of pollution control. According to previous reports, EEDs in the environment of the Shanghai metropolitan area have been detected in abundance. PFASs are a group of EEDs that are highly stable, widespread in humans and wildlife globally, exhibit high solubility and protein-binding ability, and are consistently associated with the genesis of some human diseases. They are considered potential threats to the public and scientific statements have been issued that urgently recommend further study of their impact.

In the present study, our results indicate that median concentrations of PFASs in the sera of Chinese children share a similar expression pattern to that observed worldwide. These results revealed that PFOA was the most abundant component, while PFOSA was the lowest. The effect of sample collection timing in cases and controls on PFAS concentrations was considered to be minimal, because of the stability of EEDs in sera. Among the 10 PFASs studied, significant increases in PFHxS, PFBS, PFOA, PFOS, PFOSA and PFDoA levels were observed in GCT subjects compared with controls. Few studies have reported increased levels of PFASs in children with malignant diseases, though similar results have been observed in adults, especially for PFOA toxicity in some cancers.

GCT subjects experienced more factors related to EED exposure during pregnancy including infectious disease, hair dye usage, barbecued food consumption, indoor decorating, as well as living near farmland and drinking less filtered water. In one report, a significant association was found between PFAS concentration in sera and the water supply, for at least half of the PFASs. The study indicated that environmental exposure was the biggest source of PFASs in the body. However, once we adjusted for these potential confounding covariates, only PFHxS levels were positively associated with GCTs. To further explore the possible origin and relationship of PFHxS with living habits during pregnancy, we used a stratification method followed by correlation analyses and found a positive correlation between PFHxS with hair dye usage or barbecued food consumption.

To our knowledge, this study is the first to report an association between PFHxS and GCTs.
Environmental endocrine disruptors and pediatric germ cell tumors

in children around the world, and that prenatal hair dye usage or barbecued food consumption may be possible sources. Similarly, other studies have suggested that circulating PFHxS in children from parents who ingested barbecued food during pregnancy may be a factor in causing malignancies. There has been a significant amount of evidence showing high levels of PFHxS in common barbecued foods, such as meat and seafood. Although there are no reports providing evidence that concentrations of PFHxS in foods are affected by cooking methods, it is believed that some PFASs are increased and detectable in foods that are grilled or fried. In addition, the increased PFHxS concentrations observed in the children’s GCT group may originate not only from foods themselves, but from packaging materials. In traditional Chinese-style cooking, such as Sichuan cuisine, high levels of EEDs have been detected, and PFHxS were identified previously in a population of pregnant Chinese women. Manufacturers of personal care products (cosmetics), such as hair dye, have been forced to list PFASs on package labels. It was also recently reported that the exposure of parents to EEDs in hair cosmetics is associated with a higher risk of hypospadias in their offspring, which may be the result of an effect of EED epigenetic programming of the germ line.

In the present study, concentrations of several PFASs were higher in GCT children, especially those with parents that used hair dye or consumed barbecued foods during pregnancy. However, the exact relationship and molecular mechanism of PFASs on carcinogenesis remain unclear. In addition, the questionnaire data are of great value in collecting lifestyle information on the subjects, but misclassification or recall bias remains a possibility in our case-controlled study design. Therefore, a future large-scale population-based cohort investigation is warranted.

CONCLUSIONS

This retrospective case-controlled study revealed elevated concentrations of serum PFHxS in children with GCTs. This was correlated with frequent barbecued food consumption or hair dye usage by their parents, thus indicating a potential risk factor of GCT occurrence in their children.

ACKNOWLEDGMENTS

This study was supported by Program of Shanghai Science and Technology Committee (18ZR1424700 to HW LIN), National Health and Family Planning Commission [ZY (2018-2020)-FWTX-3012 to Z TAN], and (in part) by the Early Life Plan Project at the Xinhua Hospital affiliated to the Shanghai Jiao Tong University School of Medicine. We also acknowledge Dr. Yi-Peng HAN from Department of Pediatric Neurosurgery in Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine for methodology, and Dr. Shen-Qi WANG from Department of Pulmonary in Shanghai Sixth People’s Hospital affiliated to Shanghai Jiao Tong University School of Medicine for statistical analysis. We thank Edanz Group (https://en-author-services.edanzgroup.com/) for editing a draft of this manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.
REFERENCES

1. Gobel U, Schneider DT, Calaminus G, Haas RJ, Schmidt P, Harms D. Germ-cell tumors in childhood and adolescence: GPOH MAKEI and the MAHO study groups. Ann Oncol. 2000;11(3):263–271 doi: 10.1023/A:1008360523160.

2. Bokemeyer C, Nichols CR, Droz JP, et al. Extragonadal germ cell tumors of the mediastinum and retroperitoneum: results from an international analysis. J Clin Oncol. 2002;20(7):1864–1873. doi: 10.1200/JCO.2002.07.062.

3. Makino T, Konaka H, Namiki M. Clinical features and treatment outcomes in patients with extragonadal germ cell tumors: a single-center experience. Anticancer Res. 2016;36(1):313–317.

4. Albany C, Adra N, Snively AC, et al. Multidisciplinary clinic approach improves overall survival outcomes of patients with metastatic germ-cell tumors. Ann Oncol. 2018;29(2):341–346. doi: 10.1093/annonc/mdx731.

5. Bahrami A, Ro JY, Ayala AG. An overview of testicular germ cell tumors. Arch Pathol Lab Med. 2007;131(8):1267–1280 doi: 10.1043/1543-2165(2007)131[1267:AOOTGC]2.0.CO;2.

6. Heindel JJ, Newbold R, Schug TT. Endocrine disruptors and obesity. Nat Rev Endocrinol. 2015;11(11):653–661. doi: 10.1038/nrendo.2015.163.

7. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. N Engl J Med. 1971;284(15):878–881. doi: 10.1056/nejm197104222841604.

8. Elobeid MA, Allison DB. Putative environmental-endocrine disruptors and obesity: a review. Curr Opin Endocrinol Diabetes Obes. 2008;15(5):403–408. doi: 10.1097/MED.0b013e3283e95c.

9. Soto AM, Sonnenschein C. Environmental causes of cancer: endocrine disruptors as carcinogens. Nat Rev Endocrinol. 2010;6(7):363–370. doi: 10.1038/nrendo.2010.87.

10. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet. 1993;341(8857):1392–1395. doi: 10.1016/0140-6736(93)90953-E.

11. Marques-Pinto A, Carvalho D. Human infertility: are endocrine disruptors to blame? Endocr Connect. 2013;2(3):R15–29. doi: 10.1530/EC-13-0036.

12. Knowler KC, To SQ, Leung YK, Ho SM, Clyne CD. Endocrine disruption of the epigenome: a breast cancer link. Endocr Relat Cancer. 2014;21(2):T33–55. doi: 10.1530/ERC-13-0513.

13. Zhu H, Zheng J, Xiao X, et al. Environmental endocrine disruptors promote invasion and metastasis of SK-N-SH human neuroblastoma cells. Oncol Rep. 2010;23(1):129–139. doi: 10.3892/or_00000614.

14. Hoffmann K, Lorenzo A, Butt CM, et al. Exposure to flame retardant chemicals and occurrence and severity of papillary thyroid cancer: a case-control study. Environ Int. 2017;107:235–242. doi: 10.1016/j.envint.2017.06.021.

15. Del-Mazo J, Brieno-Enriquez MA, Garcia-Lopez J, Lopez-Fernandez LA, De-Felici M. Endocrine disruptors, gene deregulation and male germ cell tumors. Int J Dev Biol. 2013;57(2-4):225–239. doi: 10.1387/ijdb.130042jd.

16. Hardell L, Van Bavel B, Lindstrom G, et al. Concentrations of polychlorinated biphenyls in blood and the risk for testicular cancer. Int J Androl. 2004;27(5):282–290. doi: 10.1111/j.1365-2605.2004.00489.x.

17. Beranger R, Le Cornet C, Schuz J, Fervers B. Occupational and environmental exposures associated with testicular germ cell tumors: systematic review of prenatal and life-long exposures. PLoS One. 2013;8(10):e77130. doi: 10.1371/journal.pone.0077130.

18. Morimoto LM, Zava D, McGlynn KA, et al. Neonatal hormone concentrations and risk of testicular germ cell tumors (TGCT). Cancer Epidemiol Biomarkers Prev. 2018;27(4):488–495. doi: 10.1158/1055-9965.EPI-17-0879.

19. Zhou Y, Wang H, Chen Y, Jiang Q. Environmental and food contamination with plasticisers in China. Lancet. 2011;378(9803):e4. doi: 10.1016/S0140-6736(11)61700-5.

20. Fan Y, Zhang M, Da SL, Feng YQ. Determination of endocrine disruptors in environmental waters using poly(acrylamide-vinylpyridine) monolithic capillary for in-tube solid-phase microextraction coupled to high-performance liquid chromatography with fluorescence detection. Analyst. 2005;130(7):1065–1069. doi: 10.1039/B502311D.

21. Siebenaler R, Cameron R, Butt CM, Hoffman K, Higgins CP, Stapleton HM. Serum perfluoroalkyl acids (PFAAs) and associations with behavioral attributes. Chemosphere. 2017;184:687–693. doi: 10.1016/j.chemosphere.2017.06.023.

22. Zhou W, Zhang L, Tong C, et al. Plasma perfluoroalkyl and polyfluoroalkyl substances concentration and menstrual cycle characteristics in preconception women. Environ Health Perspect. 2017;125(6):067012. doi: 10.1289/EHP1203.
Environmental endocrine disruptors and pediatric germ cell tumors

23. Chen Q, Huang R, Hua L, et al. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: a prospective birth cohort study. *Environ Health*. 2018;17(1):8. doi: 10.1186/s12940-018-0352-7.

24. Hu XY, Wen B, Shan XQ. Survey of phthalate pollution in arable soils in China. *J Environ Monit*. 2003;5(4):649–653. doi: 10.1039/B304669A.

25. Liu JL, Wong MH. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. *Environ Int*. 2013;59:208–224.doi: 10.1016/j.envint.2013.06.012.

26. Hu XY, Wen B, Shan XQ. Survey of phthalate pollution in arable soils in China. *J Environ Monit*. 2003;5(4):649–653. doi: 10.1039/B304669A.

27. Lv X, Xiao S, Zhang G, Jiang P, Tang F. Occurrence and removal of phenolic endocrine disrupting chemicals in the water treatment processes. *Sci Rep*. 2016;6:22860. doi: 10.1038/srep22860.

28. Yang B, Ying GG, Zhao JL, Liu S, Zhou LJ, Chen F. Removal of selected endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) during ferrate(VI) treatment of secondary wastewater effluents. *Water Res*. 2012;46(7):2194–2204. doi: 10.1016/j.watres.2012.01.047.

29. Yang Y, Ok YS, Kim KH, Kwon EE, Tsang YF. Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: a review. *Sci Total Environ*. 2017;596-597:303–320. doi: 10.1016/j.scitotenv.2017.04.102.

30. Nie M, Yang Y, Liu M, et al. Environmental estrogens in a drinking water reservoir area in Shanghai: occurrence, colloidal contribution and risk assessment. *Sci Total Environ*. 2014;487:785–791. doi: 10.1016/j.scitotenv.2013.12.010.

31. Wang Z, DeWitt JC, Higgins CP, Cousins IT. A never-ending story of per- and polyfluoroalkyl substances (PFASs)? *Environ Sci Technol*. 2017;51(5):2508–2518. doi: 10.1021/acs.est.6b04806.

32. Butt CM, Muir DC, Mabury SA. Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: a review. *Environ Toxicol Chem*. 2014;33(2):243–267. doi: 10.1002/etc.2407.

33. Scheringer M, Trier X, Cousins IT, et al. Helsingor statement on poly- and perfluorinated alkyl substances (PFASs). *Chemosphere*. 2014;114:337–339. doi: 10.1016/j.chemosphere.2014.05.044.

34. Blum A, Balan SA, Scheringer M, et al. The Madrid statement on poly- and perfluoroalkyl substances (PFASs). *Environ Health Perspect*. 2015;123(5):A107–111. doi: 10.1289/ehp.1509934.

35. Grandjean P, Clapp R. Perfluorinated alkyl substances: emerging insights into health risks. *New Solut*. 2015;25(2):147–163. doi: 10.1177/1048291115590506.

36. Boronow KE, Brody JG, Schaider LA, Peaslee GF, Havas L, Cohn BA. Serum concentrations of PFASs and exposure-related behaviors in African American and non-Hispanic white women. *J Expo Sci Environ Epidemiol*. 2019;29(2):206–217. doi: 10.1038/s41370-018-0109-y.

37. Noorlander CW, van Leeuwen SP, Te Biesebeek JD, Mengelers MJ, Zeilmaker MJ. Levels of perfluorinated compounds in food and dietary intake of PFOS and PFOA in the Netherlands. *J Agric Food Chem*. 2011;59(13):7496–7505. doi: 10.1021/jf104943p.

38. Authority EFS. Perfluoroalkylated substances in food: occurrence and dietary exposure. *EFSA journal*. 2012;10(6):2743. doi: 10.2903/j.efsa.2012.2743.

39. Vassiliadou I, Costopoulou D, Kalogeropoulos N, et al. Levels of perfluorinated compounds in raw and cooked Mediterranean finfish and shellfish. *Chemosphere*. 2015;127:117–126. doi: 10.1016/j.chemosphere.2014.12.081.

40. Jogsten IE, Perello G, Llebaria X, et al. Exposure to perfluorinated compounds in Catalonia, Spain, through consumption of various raw and cooked food-stuffs, including packaged food. *Food Chem Toxicol*. 2009;47(7):1577–1583. doi: 10.1016/j.fct.2009.04.004.

41. Wang L, Xiang Z, Stevanovic S, et al. Role of Chinese cooking emissions on ambient air quality and human health. *Sci Total Environ*. 2017;589:173–181. doi: 10.1016/j.scitotenv.2017.02.124.

42. Chen WL, Bai FY, Chang YC, Chen PC, Chen CY. Concentrations of perfluorooalkyl substances in foods and the dietary exposure among Taiwan general population and pregnant women. *J Food Drug Anal*. 2018;26(3):994–1004. doi: 10.1016/j.jfda.2017.12.011.

43. Haraux E, Braun K, Buisson P, et al. Maternal exposure to domestic hair cosmetics and occupational endocrine disruptors is associated with a higher risk of hypospadias in the offspring. *Int J Environ Res Public Health*. 2016;14(1). doi: 10.3390/ijerph14010027.

44. Anway MD, Skinner MK. Epigenetic programming of the germ line: effects of endocrine disruptors on the development of transgenerational disease. *Reprod Biomed Online*. 2008;16(1):23–25. doi: 10.1016/S1472-6483(10)60553-6.