Analytical Methods for the Quantification of Bisphenol A, Alkylphenols, Phthalate Esters, and Perfluorinated Chemicals in Biological Samples

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Our modern society has created a large number of chemicals that are used for the production of everyday commodities including toys, food packaging, cosmetic products, and building materials. We enjoy a comfortable and convenient lifestyle with access to these items. In addition, in specialized areas, such as experimental science and various medical fields, laboratory equipment and devices that are manufactured using a wide range of chemical substances are also extensively employed. The association between human exposure to trace hazardous chemicals and an increased incidence of endocrine disease has been recognized. However, the evaluation of human exposure to such endocrine disrupting chemicals is therefore imperative, and the determination of exposure levels requires the analysis of human biological materials, such as blood and urine. To obtain as much information as possible from limited sample sizes, highly sensitive and reliable analytical methods are also required for exposure assessments. The present review focuses on effective analytical methods for the quantification of bisphenol A (BPA), alkylphenols (APs), phthalate esters (PEs), and perfluorinated chemicals (PFCs), which are chemicals used in the production of everyday commodities. Using data obtained from liquid chromatography/mass spectrometry (LC/MS) and LC/MS/MS analyses, assessments of the risks to humans were also presented based on the estimated levels of exposure to PFCs.

Keywords Chromatography, hazardous chemicals, endocrine disrupting chemicals

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food packaging materials and processing equipments are made of plastics, or they contain a polymeric layer that comes in direct contact with food, such as a laminate or coating.

In the 1960s and 1970s, many reports of phenomena in humans emerged that were difficult to explain using existing biological and toxicological knowledge. Abnormal reproductive functions and behaviors, demasculinization, and deleterious effects on the immune and nervous systems of fish, reptiles, birds, and other wildlife were also reported. In 1962, R. Carson issued a wake-up call concerning the biogeocenosis effect of agrichemicals in her book entitled “Silent Spring”. In 1996, R. Carson published “Our Stolen Future” with a warning regarding chemicals released into the environment from existing materials, and proposing a new concept for the risk assessment of chemicals affecting human health at trace levels. Specifically, the authors reported that some chemicals disrupt hormonal secretion by the endocrine system, which is essential for the maintenance of homeostasis in living organisms. That finding sparked a series of local and international studies with a focus on chemicals that have since come to be known as endocrine disrupting chemicals (EDCs).

Notably, when human blood was analyzed, chemicals that may have been derived from high-molecular-weight compounds were detected. However, the extent of the endocrine-disrupting effects of chemicals on successive generations of living organisms (the “low” dosage problem), which is a dose-behavior relationship, remains to be known. Many of these studies can, in fact, be considered as low-dose studies, regardless of whether the cutoff point for a low dose was based on the range of typical human exposures, doses used in traditional toxicology, or doses that use an internal measure of body burden.

This global concern about EDCs has led to a need for highly sensitive and specific analytical tools for their determination in food, environmental, and biological samples at very low concentrations. Although enzymatic methods can serve as tools for the rapid in situ screening of a large number of samples in a short period of time, biological analyses are too specific to cover a wide range of different EDCs, and have limits of detection on the order of ng/mL. For the reliable identification and quantification of compounds at extremely low levels, chromatographic methods are thus required. Therefore, to conduct risk assessments of EDCs we developed precise and accurate analytical methods based on liquid chromatography (LC), and gas chromatography (GC), and applied them to the analysis of everyday products. Based on the results of these analyses, liquid chromatography/mass spectrometry (LC/MS) and gas chromatography/mass spectrometry (GC/MS) techniques were employed to quantify various hazardous chemicals in human biological samples, including urine and blood. In this review, we focus on methods developed for the analysis of bisphenol A (BPA), alkylphenols (APs), phthalate esters (PES), and perfluorinated chemicals (PFCS) using mass spectrometry (MS), and present our estimation of the level of human exposure to these compounds. Note that in all of these studies permission was obtained from all persons contributing samples, and all analyses were conducted in accordance with the relevant laws and rules approved by the collaborative institutional committees.

2 Bisphenol A Overview

Bisphenol A (BPA) is prepared via condensation of acetone and phenol. It is used as a raw material for the production of polycarbonates via copolymerization with phosgene or diphenyl carbonate. It is also a key monomer for the production of epoxy resins (Fig. 1), which are used in many types of adhesives and coatings, including those applied to the interior of food cans. These phenolic resins and polyesters are also added as stabilizers to vinyl chloride, which is another commonly used plastic. BPA has been shown to migrate from cans and cap-sealing resins, and has been detected in drinks, vegetables, fruits, and honey. In addition, BPA and tetrabromobisphenol A (a fire retardant derived from BPA) have also been found in indoor air and river water. Stir bar sorptive extraction (SBSE) with in situ derivatization and liquid phase microextraction (LPME) with in situ derivatization were applied for the measurement of BPA in river-water samples using GC/MS. Moleurally imprinted solid-phase extraction (SPE) using stable isotope-labeled compounds as templates was used for the trace analysis of BPA in water by LC/MS.

BPA in biological samples was measured using several methods, such as fluorescence (FL) and electrochemical detection (ECD) (Table 1). However, because BPA exists in trace amounts in biological samples, highly sensitive and selective methods are required for its analysis. Recently, MS has become a major analytical tool, because it has increased the reliability of compound identification with the use of surrogates.
purified using an Oasis HLB cartridge prior to analysis by LC/MS.\textsuperscript{35} Hollow fiber assisted LPME (HF-LPME)\textsuperscript{33} was also investigated as a pretreatment method for urine samples containing BPA, and a simple, accurate, and highly sensitive method for the determination of BPA in human urine was achieved using a miniaturized HF-LPME system with in situ derivatization and subsequent GC/MS analysis.

### 3 Alkylphenol Overview

Alkylphenols (APs), such as nonylphenol (NP) and octylphenol (OP), are well known as xenoestrogen compounds.\textsuperscript{36–39} Alkylphenol ethoxylates (APEOs), which are AP derivatives, are widely used nonionic surfactants. They are employed as emulsifying, wetting, dispersing, and stabilizing agents in industrial, agricultural, and consumer products.\textsuperscript{38,40,41} For many years, 4-NP and 4-OP were extensively used as raw materials for the production of APEOs and phenolic resins in both E.U. and United States.\textsuperscript{37,38} However, since then, the biodegradation of APEOs has been shown to be an important source of 4-NP and 4-OP in the environment,\textsuperscript{42} APs can also be released into the environment during the manufacturing process.\textsuperscript{43}

Since 2000, 4-NP and 4-OP have been included on the European list of priority hazardous substances established by Directive 2000/60/EC.\textsuperscript{44} In addition, a reduction policy was implemented in 2003 for 4-NP\textsuperscript{45} in the E.U. Today, in fact, only a few countries continue to use APEOs.\textsuperscript{42} Nevertheless, 4-NP and 4-OP are still widespread in the environment, and continue to be detected in industrial wastewater, rivers, sediment, and so on.\textsuperscript{42,46,47} In addition to BPA, 4-NP and 4-OP also exhibit multiple modes of endocrine disruption activity; the major pathways involve binding to estrogen receptors\textsuperscript{48} and acting competitively with natural hormones, even though the affinity of these APs for estrogen receptors is much weaker than the affinity of natural hormones within living organisms.\textsuperscript{37,38,49} In addition, 4-NP and 4-OP were reported to exhibit reproductive and developmental toxicity in aquatic organisms and animals.\textsuperscript{37,38,50–52} Notably, 4-NP is a mixture of many isomeric compounds, and the estrogen activity may differ for the various isomers, depending on the alkyl chain structure.\textsuperscript{53} Therefore, numerous groups have

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**Table 1 Analysis of BPA in biological samples**

| Instrument | Analyte | Sensitivity | Sample | Preparation | Reference |
|------------|---------|-------------|--------|-------------|-----------|
| LC/ECD | BPA, BPA β-estradiol, estrone, estradiol | 0.2 ng/mL (LOQ) | Human urine | On-line SPE (column switching system) | 19 |
| LC/FL | BPA, BPB | 0.5 ng/mL (LOQ) | Human serum | Liquid-liquid extraction | 20 |
| GC/MS | BPA, tricosan | 0.5 ng/mL (LOQ) | Human urine and serum | SPE (HLB), PFB derivatization | 21 |
| LC/MS/MS | BPA, BPA β-estradiol, genistein, genistein 4′-β-d-glucuronide | 0.087 ng/mL (LOQ) | Maternal and umbilical cord blood serum | Two step SPE (Florisil and HLB), MSTFA derivatization | 23 |
| GC/MS/MS | Bisphenol AF | 1.0 ng/g (LOQ) | Rat liver, muscle, kidney, serum and urine | SPE (MAX) | 24 |
| LC/UV | BPA, 17β-estradiol, estrone, estradiol, 17β-ethynylestradiol, diethylstilbestrol, dienestrol, 4-OP | 0.055 ng/mL (LOQ) | Sediment and fish | Hollow fiber liquid-liquid-liquid microextraction (HF-LLLLE) | 25 |

*Note that while the removal of the other components in samples intended for BPA analysis is essential, it is necessary to consider the possibility of contamination during sample pretreatment using SPE. BPA and bisphenol A diglycidyl ether metabolites (Fig. 2) in human plasma have been successfully monitored in vivo.*

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For LC/MS analysis, ionization was enhanced using derivatization reagents to reduce interference from the sample matrix.\textsuperscript{26} For example, Yang et al.\textsuperscript{27} developed and validated an LC/MS method for the quantitative determination of derivatized BPA in human saliva. Völkel et al.\textsuperscript{28} administered deuterium-labeled BPA (BPA-d\textsubscript{16}) to healthy volunteers, and monitored its pharmacokinetics and metabolism. It was found that the half-life of BPA within the human body is approximately six hours, and that most of the BPA is excreted into urine as its glucuronic acid conjugate. In our studies, BPA and related compounds in biological samples were measured using various high-throughput chromatographic techniques. For example, the analysis of trace BPA in human urine was achieved using GC/MS\textsuperscript{29–33} and LC/MS.\textsuperscript{34}
focused on the development of methods for the separation and detection of the different isomers, as well as the determination of their toxicity.54–56

Our group reported on the analysis of NP and OP in biological fluids, such as serum, plasma, and urine.59,60 For example, SBSE was used in combination with thermal desorption (TD) GC/MS for the determination of NP and OP in human urine and plasma samples.60 When five human urine samples and three human plasma samples from healthy volunteers were analyzed, NP and OP were not detected in any of the urine samples. However, NP and OP were found at levels of 0.2 – 0.3 ng/mL and 0.1 – 0.2 ng/mL, respectively, in the human plasma samples. The limits of quantitation (LOQs) of this method were 0.2 and 0.02 ng/mL for NP and OP, respectively. When the urine sample was deconjugated by adding β-glucuronidase and sulfatase, followed by protein precipitation, the limits of detection (LODs) were 10 pg/mL (OP) and 50 pg/mL (NP) in human urine samples using SBSE combined with in situ derivatization and TD-GC/MS analysis.62

A list of methods developed in the last 2 – 3 years for the determination of NP and OP in biological fluids is presented in Table 2. For sample cleanup, liquid-liquid extraction70,71 and/or SPE59,72 were mainly employed, and most analyses were conducted on a mass spectrometer. In addition, the determination of BPA was often conducted simultaneously with the analysis of NP and OP.

### 4 Phthalate Ester Overview

Polyvinyl chloride (PVC) resin is widely used on a daily basis, and is an essential plastic used in the manufacture of protective gloves, medical devices, furniture, flooring, etc. Phthalate esters (PEs) are extensively used as plasticizers to increase the flexibility of PVC products. The migration of PEs from cap-sealing resins and PVC tubes used in the preparation of food was investigated using GC/MS.73,74 Also, analyses of plasticizer phthalates and adipate esters in stationary, cosmetics, and household and nutrition products were conducted using GC and/or GC/MS.75,76 In addition, the levels of five common phthalates in the feed and bedding of laboratory animals was determined using GC/MS,77 so that exposure to these chemicals through diet and the environment can be considered when conducting comprehensive animal toxicity studies for phthalates. Of the common phthalates, di-2-ethylhexyl phthalate (DEHP) is considered to exhibit reproductive and developmental toxicity,78,79 carcinogenicity, and testicular toxicity,79,80 and has been shown to affect reproductive organs and fertility.81 Thus, the analysis of PEs in human biological samples may be useful for evaluating the risks associated with exposure to phthalates.

PEs are mainly metabolized to phthalate monoesters and excreted into the urine (Fig. 3).82 The US Centers for Disease Control and Prevention (CDC) has continuously promoted studies on human exposure to PEs, its National Health and Nutrition Examination Survey (NHANES) Program conducted a large-scale investigation of human exposure to PEs83 by analyzing human urine samples for phthalate monoester metabolites.84 In our studies, highly sensitive and accurate LC/MS and LC/MS/MS methods for the determination of PEs were developed using deuterated surrogate standards. An on-line SPE-LC/MS/MS method was then developed as a column switching system, which enabled both the avoidance of contamination during sample preparation and the automation of a continuous analysis process. This system was also applied to the determination of phthalate metabolites in human urine for assessment of exposure levels.85 In addition, GC/MS was used for the determination of five phthalate monoesters in human urine.86 A list of methods developed in the last 2 – 3 years for the determination of PEs in biological fluids is given in Table 3.

The analytical methods described above were used to evaluate the exposure to PEs of healthy individuals working in an industrial setting. For example, urinary mono-2-ethylhexyl phthalate (MEHP) and mono-butyl phthalate (MBP) concentrations of workers in a phthalate ester manufacturing facility were determined.87,90,91,92 The results clearly indicated that the workers in the plant were exposed to PEs at significantly higher levels,87 compared to the exposure levels of previously described healthy individuals.

Measurements of PEs in blood samples were also conducted using GC/MS,89 LC/MS,100,101,102 and LC/MS/MS103 methods with various sample pretreatment techniques. Accurate detection of DEHP in blood samples is challenging because DEHP metabolism in human blood is accelerated in the presence of blood enzymes, such as esterases, and thus its metabolites are frequently detected. DEHP contamination was avoided in the analysis of phthalate and adipate esters in plasma and beverages by using equipment originally designed for the quantitative determination of essential oils via steam distillation and extraction.89 The column switching method was also developed in order to avoid DEHP contamination during the analysis of blood samples.103 When DEHP and MEHP concentrations in blood plasma samples from six healthy volunteers were analyzed using LC/MS with the column switching system as a pretreatment, they were found to be equal to, or less than, the quantitative limit.103 In addition, the DEHP and MEHP

| Instrument | Analyte              | Sensitivity       | Sample          | Preparation            | Ref. |
|------------|----------------------|-------------------|-----------------|------------------------|------|
| LC/MS/MS   | BPA, NP, OP          | 0.18 ng/mL (NP; LOD) | Urine           | Deconjugate, SPE       | 65   |
| LC/MS/MS   | BPA, NP, OP          | 0.15 ng/mL (OP; LOD) | Unspecified     | Colostrums             | 66   |
| UPLC/MS/MS | BPA, BP-3, 4-NP, 4-OP | 0.02 ng/mL (NP, OP; LODs) | Maternal urine | Deconjugate, SPE       | 67   |
| UPLC/MS/MS | BPA, BP-3, FCP, triclosan, 4-OP, 4-n-OP, 4-n-NP | 0.34 ng/mL (4-OP; LOD) | Urine and semen | Deconjugate, SPE       | 68   |
| UPLC/MS/MS | BPA, NP, OP          | 0.10 ng/mL (NP; LOD) | 0.15 ng/mL (OP; LOD) | Enzyme hydrolysis, SPE | 69   |

Abbreviations: Bisphenol A (BPA), nonyl phenol (NP), octyl phenol (OP), benzophenone-3 (BP-3), pentachlorophenol (PCP), limit of detection (LOD), limit of quantitation (LOQ), solid phase extraction (SPE).
concentrations in the blood sera of four healthy volunteers after consuming food in containers that were made in part of plastic, the concentration of DEHP was again found to be equal to, or lower than, the quantitative lower limit (trace levels) in all of the volunteers. Therefore, the blood concentration of PEs is very low, and quantification is difficult at normal exposure levels. As a result, urine samples are more suitable for evaluating the exposure index of PEs.

High-throughput analytical methods for the determination of PEs and their metabolites in human breast milk, which is one of the most important sources of nutrition for infants, have also been developed. In addition, in order to assess the risks and benefits of PEs in medical applications, the migration of phthalates from medical polyvinylchloride materials was studied. Perfluorinated Chemicals Overview

Perfluorinated chemicals (PFCs) have received significant attention as new EDCs. Perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorooctane sulfonamide (PFOSA), perfluorononanoic acid (PFNNA), and perfluorohexanesulfonate (PFHxS) are representative examples of PFCs. These compounds are widely used in everyday products as water repellants for fabrics, surfactants, leveling agents, fire extinguishing agents, lubricants, and anticoagulants. PFCs are straight chain carbon compounds with fluorine atoms bonded to all of the carbon atoms, except at the ends of the chain, which bear sulfonate or carboxylic acid substituents (Fig. 4). The 3M Company was the major producer of perfluorooctane sulfonyl fluoride (POSF). It began production in 1949, and the total cumulative production of POSF is estimated to be approximately 96000 t in the peak years from 1970 to 2002. After 3M discontinued production in 2002, other companies began manufacturing POSF to meet existing market demands, with an estimated 1000 t being produced each year since 2002. PFCs, which possess hydrophobic and lipophobic characteristics, are extremely thermally, biologically, and chemically stable compounds, and are poorly metabolized with half-lives of greater than two years in human serum. For that reason, PFOS has been listed since May 2009 as a type of persistent organic pollutant (POP) under the Stockholm Convention. Several publications regarding the determination of PFCs in biological samples have appeared in the literature in recent years (Table 4). Han et al. reported that PFCs bind to proteins in the blood and tissues, rather than to lipids, unlike most other persistent organic chemicals. The monitoring of PFCs levels in blood is thus useful for the evaluation of human exposure to these EDCs. Therefore, we developed analytical methods based on LC/MS and LC/MS/MS for the determination of PFCs in human biological samples, such as blood and breast milk, with high sensitivity and reliability. As with phthalates, analytical scientists must be careful to avoid potential contamination of samples with PFCs from other sources, such as laboratory appliances, solvents, and other commonly used items that contain fluorinated materials. Some strategies for minimizing PFCs contamination include the replacement of poly(tetrafluoroethylene) (PTFE) tubing and

![Fig. 3 Metabolic pathway of di-2-ethylhexyl phthalate (DEHP).](image)

Major metabolites for biomonitoring analysis are highlighted.
fittings with those made from materials that do not contain PFCs, such as polyether ether ketone (PEEK) or stainless steel.14 Other equipment, such as pressurized liquid extraction devices that contain sufficient amounts of PTFE tubing, such that refitting may not be feasible, should be avoided.125 The installation of an extra guard column before the injection port of an LC system to remove any PFCs that originate from the instrument prior to sample introduction126 should also be considered. In addition, solvents that may contain PFCs can be passed through an SPE cartridge before use in order to avoid PFC contamination of samples.127

Human exposure to these chemicals is widespread, but estimated daily intakes are generally below their guideline levels. PFOS and PFOA contamination in adult human blood was reported in various countries. Because fetuses might be more vulnerable than adults to potential harmful effects of chemicals, exposure assessment studies for PFCs in human fetuses are urgently needed. Therefore, PFCs levels in maternal and cord blood samples from 15 healthy pregnant volunteers were measured to assess the exposure level of this susceptible population to PFOS.121 PFOS was found in all of the maternal population to PFOS.121 PFOS was found in all of the maternal

| Instrument | Analyte | Sensitivity | Sample | Preparation | Reference |
|------------|---------|-------------|--------|-------------|-----------|
| UPLC/MS/MS | DEHP    | 0.1 μg/mL (LOQ) | Rat plasma | Protein precipitation | 91 |
| LC/MS/MS   | DEHP metabolites (MEHP, MEHPP, 5cx-MEPP, 2cx-MMHPP) | 1.0 pg/mg (MEHP, MEHPP, MEOHP; LOQs) | Human hair | Liquid-liquid extraction, stable isotope dilution | 92 |
| LC/MS/MS   | MBBP, MEHP, MEHPP, MEOHP | 5.0 pg/mg (5cx-MMHPP, 2cx-MMHPP; LOQs) | Urine and serum | On-line SPE (column switching system) | 93 |
| LC/MS/MS   | Phthalate metabolite (MMP, MEP, MBzBP and MEHP), BPA | 0.9 – 1.1 ng/mL (MEHP, LOD) | Urine | On-line SPE (column switching system) | 94 |
| LC/MS/MS   | MEHP, SOH-MEHP, 5oxo-MEHP, 5cx-MEHP, 5oxo-MEHP, OH-MiNP, o xo-MiNP, ex-MiNP | 0.5 μg/L (SOH-MEHP, 5oxo-MEHP, 5cx-MEHP, OH-MiNP, oxo-MiNP, ex-MiNP; LOQs) | Urine | Enzymatic hydrolysis, on-line SPE (column switching system) | 95 |
| LC/MS/MS   | MEHP, OP, 4-NP | 2.0 μg/L (MEHP, MBP, LOQs) | Serum and meconium | Enzymatic deconjugation, SPE | 96 |
| UPLC/MS/MS | Fourteen phthalate metabolites (free and conjugated) | 0.03 – 0.24 ng/mL (LODs) | Serum | Enzymatic hydrolysis, SPE | 97 |
| GC/MS      | DMP, DEP, DBP, BBP, DCHP, DEHP, DiNP, DOP | 0.2 ng/mL (MEHP; LOD) | Serum, sweat and urine | Enzymatic hydrolysis, SPE, liquid-liquid extraction | 98 |
| LC/MS      | MMP, MEP, MBzBP, MBzP, MCHP, MEHPP, MEOHP, MiBP, MBzP | 0.2 ng/mL (phthalate metabolites; LODs) | Serum, sweat and urine | Enzymatic hydrolysis, SPE | 99 |

Abbreviations: di-2-ethylhexyl phthalate (DEHP), mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyloxyl)phthalate (MEHHP), mono-(2-ethyl-5-oxo-oxyl)phthalate (MEOPH), mono-(2-ethyl-5-carboxyhexyl)phthalate (5cx-MEPP), monon-(2-carboxymethyl)hexyl)phthalate (2cx-MMHPP), mono-butyl phthalate (MBP), mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-benzyl phthalate (MBzBP), bisphenol A (BPA), mono-(2-ethyl-5-hydroxyhexyloxyl)phthalate (SOH-MEHP), mono-(2-ethyl-5-oxo-oxyl)phthalate (5oxo-MEHP), mono-(2-carboxymethylhexyl)phthalate (2cx-MEHP), monohoixylated-MiNP (OH-MiNP), mono-oxidized-MiNP (oxo-MiNP), mono-cyclohexylphthalate (MCHP), methyl-5-dimethylhexyl phthalate (monoisononylphthalate; MiNP), mono-n-octyl phthalate (MOP), limits of detection (LODs), limits of quantitation (LOQs), instrument detection limits (IDLs).

![Fig. 4](image-url) Chemical structures of representative PFCs. (A) Perfluorooctanesulfonate (PFOS). (B) Perfluororononanoic acid (PFNA). (C) Perfluorohexanesulfonate (PFHxS). (D) Perfluorooctanoic acid (PFOA).
were supplemented with serum or albumin. Therefore, we analyzed IVF media using LC/MS/MS, and determined that the concentrations of PFOS and PFOA were 0.42 and 0.19 ng/mL, respectively. In addition, it was found that the concentrations of PFOS and PFOA in protein sources were higher than those in other IVF media samples.

Exposure to PFCs may have deleterious effects on fetal growth. Thus, the PFOS and PFOA concentrations in blood samples from 428 pregnant volunteers living in Hokkaido were analyzed to investigate the relationship between the PFCs concentration in their blood and physical parameters of newborns, such as birth weight and birth size, including length, chest circumference, and head circumference. PFOS and PFOA were detected in the ranges from 1.3 to 16.2 ng/mL and N.D. (< 0.5 ng/mL) to 5.3 ng/mL, respectively. In utero exposure to relatively low levels of PFOS negatively correlated with birth weight, while there was no correlation between PFOA levels and the birth weight. However, the cord blood IgE levels of female infants decreased significantly at higher maternal PFOA levels compared to those of male infants. These new findings thus clarified the influence of prenatal exposure to PFCs on the health outcomes for infants and children.

6 Conclusions

EDCs present complex and wide-ranging issues. Therefore, it is important to evaluate from various perspectives the risks to our health associated with exposure to trace amounts of these potentially harmful chemicals. One of the issues that must be immediately discussed is how accuracy control and analytical method validation should be achieved to ensure reliable results. It is necessary to not only depend on the latest analytical equipment, but to also obtain reliable data, achieve reproducibility, and analyze and disclosed results.

"Regulatory science" is gaining more attention as a field in which methods are devised that can more precisely provide accurate control.
information about the origins, conditions, and effects of the chemicals and phenomena that surround us.\textsuperscript{14} In order to be able to predict and evaluate each advantage, i.e., efficacy and disadvantage, i.e., safety of various substances based on outcomes, analytical chemistry is essential. In addition, the core technique is an analytical method that fully utilizes “high throughput chromatography.” Furthermore, it is important to share the outcomes of research projects and the knowledge gained by actively presenting study results at conferences, and publishing them in academic journals. If this is done properly, the application of analytical science can aid in the advancement of regulatory science and thus contribute to the improvement of human health through the development and implementation of appropriate policies by administrative agencies.

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