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A review of phthalate test methods

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Abstract
Phthalates are a class of environmental endocrine disruptors that are widely distributed and pose certain hazards to human health. Because phthalates volatilize under certain conditions, they are found in many different types of samples, including water, dust, food and its packaging, personal care products, etc. There are different detection methods for phthalates in samples with different morphologies and properties. This paper briefly introduces phthalates and their health hazards, and summarizes the test methods and content calculation methods of phthalates in different types of samples, so as to provide scientific basis for the detection and exposure level analysis of phthalates.
Keyword: *Phthalates; Health hazards; Test methods; Content calculation*

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Ⅰ. INTRODUCTION

1.1 Introduction to phthalates

A few days ago, Taiwan's food safety incident "clouding agent" caused great social repercussions, and some illegal businesses illegally added di(2-ethylhexyl) phthalate to food additives, which is a harmful plasticizer for people, resulting in the contamination of a variety of drinks. Phthalates are one of the most commonly used plasticizers, it is a class of environmental endocrine disruptors, environmental endocrine disruptors refer to exogenous interference with biological endocrine chemicals, in recent years, environmental endocrine disruptors are paying more and more attention, its related research is also a lot, as one of the important organic pollutants in the environment, its pollution prevention and health risk evaluation has been widely concerned[1, 2]. In addition to the daily use of phthalates, because phthalates and polyolefin molecules are connected by hydrogen bonds or van der Waals forces, it is easy to diffuse from plastics, thereby polluting water, air, etc., endangering people's production and life[3].

Phthalates generally consist of a rigid plane of aromatics and two plastic nonlinear fat side chains, room temperature for very low volatility viscous liquid, with special odor, insoluble in water, toxic, soluble in most organic solutions, is phthalic anhydride and alcohol in the presence of acid (such as sulfuric acid) catalyst esterification, because can enhance the ductility of plastic products, it is widely used in toys, clothing, cosmetics food packaging materials, medical blood bags and hoses, ethylene flooring. Human exposure to phthalates includes food ingestion, breathing air exposure, drinking water, skin absorption, and intravenous injection. Table 1 lists information on 14 common phthalates.

Table 1: 14 kinds of phthalates physical and chemical properties

Phthalates	Molecular Formula	Abbreviation	Properties
Di(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	DEHP	Colorless and odorless liquid. Insoluble in water, soluble in ether, ethanol, mineral oil, etc.
Dibutyl phthalate	$C_{16}H_{22}O_4$	DBP	Colorless, oily liquid, flammable, aromatic odor, moderately toxic, eye or skin contact can trigger sensitization.
Benzyl butyl phthalate	$C_{19}H_{20}O_4$	BBP	Colorless transparent oily liquid freezing point of -35 ° C, boiling point of 370 ° C, soluble in organic solvents and hydrocarbons, insoluble in water, flammable, slightly toxic.
Dipentyl phthalate	$C_{18}H_{26}O_4$	DPP	The density was 1.025g/mL at 20° C; Flash point> 100° C; Melting point<-55° C; The boiling point is 342° C.
Diethyl phthalate	$C_{12}H_{14}O_4$	DEP	It is a colorless to yellowish clear oily liquid. The product is extremely soluble in ethanol, almost insoluble in water, and the relative density is 1.117-1.121.
Diisobutyl phthalate	$C_{16}H_{22}O_4$	DIBP	Diisobutyl phthalate is slightly soluble in water, compatible with cellulose acetate and other resins, and is mainly used as a plasticizer.
Di-n-octyl phthalate	$C_{24}H_{38}O_4$	DNOP	Light yellow oily liquid. Slightly odorous. Stable to light and heat. Miscible with organic solvents, insoluble in water. The

			freezing point is -40 ° c. The boiling point is 340 ° c.
Diisononyl phthalate	$C_{26}H_{42}O_4$	DINP	Colorless liquid with a slight ester odor. Density 0.975g/mL at 20° C; The boiling point at normal pressure is 252° C.
Diisodecyl phthalate	$C_{28}H_{46}O_4$	DIDP	The boiling point is 420 ° c; Flash point is 232 ° C, insoluble in water, miscible in most organic solvents. In case of open flame, high heat can be flammable. Mainly used as plasticizer.
Dimethyl phthalate	$C_{10}H_{10}O_4$	DMP	It can be compatible with a variety of cellulose resin, rubber and vinyl resin, and has good film-forming, adhesion and waterproofness.
Dihexyl phthalate	$C_{20}H_{30}O_4$	DHP	Colorless or slightly yellowish oily liquid, aromatic odor, soluble in ethanol and ether, insoluble in water. Used in resin synthesis and toughener.
Dicyclohexyl phthalate	$C_{20}H_{26}O_4$	DCHP	White crystalline powder, melting point of 65 ° C, boiling point of 220~228 ° C insoluble in water, slightly soluble in ethylene glycol and some amines, soluble in acetone, methyl ethyl ketone, cyclohexanone, ether, carbon tetrachloride, toluene and other organic solvents. It has good compatibility with many resins.
Diisooctyl phthalate	$C_{24}H_{38}O_4$	DIOP	Colorless or light yellow viscous liquid. Slightly odorous. Soluble in aliphatic hydrocarbons, aromatic hydrocarbons and most organic solvents, slightly soluble in glycerol, ethylene glycol and some amines, insoluble in water. Melting point is -43° C; The boiling point is 284.9° C.
Di-n-nonyl phthalate	$C_{26}H_{42}O_4$	DNNP	Colorless liquid with slight ester odor, density of 0.975g/mL at 20° C; The boiling point at normal pressure is 252° C.

1.2 Regulations on the use of phthalates

Studies have found that phthalates have certain toxicity, and the use of phthalates has been restricted in many countries. In the European Union, the 1999/815/EEC directive published in 1999 states that the content of six plasticizers (DEHP, DBP, BBP, DINP, DIDP and DNOP) in polyvinyl chloride (PVC) related children's toys and related articles placed in the mouths of three-year-old children shall not exceed 0.1%.On December 27, 2005, the European Union issued a new Directive 2005/84/EC requiring that the content of DEHP, DBP and BBP in all toys and childcare articles should not exceed 0.1%; All toys and parenting articles that can be placed in children's mouths should not contain more than 0.1% DINP, DIDP and DNOP. In the United States, California proposed AB1108 in February this year, which is expected to be implemented on January 1, 2009, and its requirements are as follows: the content of DEHP, DBP and BBP in all toys and parenting articles should not exceed 0.1%; All toys and parenting articles that can be placed in children's mouths under the age of three must not contain more than 0.1% DINP, DIDP and DNOP. In Denmark, in addition to the six content requirements set by the EU above, the content of any other phthalates for toys and childcare articles used by children under three years of age shall not exceed 0.05%. China's priority pollutant blacklist includes: dimethyl phthalate, di-n-butyl phthalate, and dioctyl phthalate. However, there are currently no clear regulations on the content of phthalates in China, so it is difficult for ordinary consumers to see the content of this substance from the domestic product label. Phthalates have a wide range of uses, and now they have become an indispensable part of our lives, and human beings will inevitably come into contact with phthalates through various ways, so their health hazards should not be underestimated[4, 5].

1.3 Health hazards

Phthalates are a class of chemicals that act as a softening agent. A number of animal experiments have confirmed that some types of phthalates have carcinogenic effects on animals. Experimental reports from the National Toxicology Program showed that rats and mice caused liver cancer after absorbing bis-ethylhexyl phthalate through food. Kluwe et al. found that the incidence of hepatocellular carcinoma increased significantly after eating DEHP-containing feed in female mice and male mice after chronic toxicity experiments on mice for up to 103 weeks[6]. Ruslyn et al. also found through experiments that DEHP can lead to the expansion of many hepatocyte organelles, such as peroxisomes and mitochondria, causing the development of animal liver adenoma and cancer[7]. After injecting a trace amount of DEHP's hydrolysate DPE to mice, Ape et al. found that the liver, kidney, gastrointestinal lesions of mice occurred, DPE was detected in urine and feces, weight was decreasing, skin was loose, albumin and phosphate content increased, cholesterol decreased, and subchronic toxicity appeared in the dermis of mice[8].

A large number of data prove that PAEs can lead to sperm malformation, sperm malformation and germ cell mutation are highly correlated, sperm morphology has multiple gene control, and the increase in sperm malformation rate reflects the genetic damage of male germ cells to a certain extent. Cheng Changmei, a professor in the Department of Chemistry at Tsinghua University, said that studies have shown that phthalates play a role

similar to estrogen in the human body and animals, which can interfere with endocrine, reduce men's semen volume and sperm count, low sperm motility, abnormal sperm morphology, and serious testicular cancer, which is the "culprit" of male reproductive problems. After oral administration to adolescent mice, Kondo et al. found that immature testicles were more sensitive to DBP contamination, which can lead to apoptosis of a large number of seminiferous tubules in germ cells, causing testicular atrophy in adolescent young rats[9].

For women, nail polish has the highest content of phthalates in cosmetics, and many cosmetic aromatic ingredients also contain this substance. After Shirota et al. administered oral administration to pregnant mice and found that the male piglet germ cells of their fetuses degenerated, thin seminiferous ducts that were scattered and atrophied or dilated were found in the testicles, local testes stromal cells were hyperplasia, and sperm were malformed[10]. Professor Cheng Changmei and others found that this substance in cosmetics will enter the body through women's respiratory system and skin, and if used too much, it will increase the chance of breast cancer in women and endanger the reproductive system of their future male babies.

For children, soft plastic toys and children's products containing phthalates may be put into the mouth by children, if left for a long time, it will cause the dissolution of phthalates to exceed safe levels, which will harm children's liver and kidneys, and can also cause precocious puberty in children. Xiao et al. found that the serum DBP and DEHP of precocious girls were higher than those of normally developing girls, and the content of DBP and DEHP in the uterus and ovaries was also significantly higher than that of normally developing girls[11].

Ⅱ. Overview of test methods

2.1 Test meaning

Phthalates (PAEs) are a collective term for esters formed by phthalic acid and alcohols. As plasticizers, PAEs are widely used in toys, cosmetics, textiles, food packaging materials, medical blood bags and hoses. Studies have shown that PAEs play a role similar to estrogen in humans and animals, disrupting human endocrine and is one of the main causes of male reproductive problems. The content of PAEs reflects the exposure level of the human body to a certain extent, so it is of great significance to detect the content of different PAEs.

2.2 Review of test methods for phthalates in different samples

Table 2 summarizes the test methods for phthalates in samples of different types and properties, including environmental samples such as water samples, soil samples, etc., but also food and its packaging, personal care products, textiles, dust and atmospheric particulate matter.

Table 2: Testing methods for phthalate esters of different types of samples

Samples	Compounds	Usage Method	Advantages and Disadvantages	Typical Conclusion
Water and soil samples[12]	DEP	fluorescence	This method is easy to operate, with high sensitivity and accuracy.	Analyzing the sample can calculate the total amount of PAEs in the soil sample.
Dust samples from roads such as residential areas and urban traffic arteries[13]	DMP, DEP, DnBP, DiBP, DnOP, DEHP	Gas chromatography: USE-PA8061 series method	Simple operation and high accuracy. It is generally suitable for measuring the PAEs content in dust.	DEHP, DnBP, and DiBP are the main PAEs in road dust.
Sediment samples from 5 equidistant locations along the Huangpu River coast[14]	DMP, DEP, DIBP, DBP, DMEP, BMPP, DEEP, DPP, DHxP, BBP, DBEP, DCHP, DEHP, DPHP, DNOP, DNP	Rapid solvent extraction/gas chromatography-mass spectrometry (GC-MS)	This method improves extraction efficiency and reduces the amount of organic extraction solvent used. The preprocessing method is simple, and the qualitative and quantitative analysis is accurate and reliable.	The pollution distribution of PAEs in sediment samples in this area is relatively widespread. The highest content of DEHP is the main pollutant, followed by DIBP and DBP. This characteristic may be related to the situation of plasticizers in China.
Plant protein beverage samples[15]	DMP, DEP, DIBP, DBP, DMEP, BMPP, DEEP, DHXP, BBP, DCHP, DEHP, DBEP, DNOP, DPP, DNP	Gas chromatography-mass spectrometry determination method	This measurement method is simple, fast, and highly sensitive, meeting the needs of practical applications.	A gas chromatography-mass spectrometry method has been established for the determination of phthalate esters in plant protein beverages.
Three types of plastic bottle caps that have been sliced into thin pieces[16]	DMP, DEP, DIBP, DBP, DMEP, BMPP, DEEP, DHXP, BBP, DCHP, DEHP, DBEP, DINP,	GC-MS MS Internal Standard Curve Method	Compared with the national standard, this method has better repeatability, higher accuracy, and lower detection limit.	In this experiment, only 4 phthalate ester compounds were detected in plastic bottle caps, and the content was relatively low. The use of qualified food

	DNOP, DPP			packaging materials is beneficial for food safety.
Ham sausage[17]	DEP, BBP, DBP, DEHP, DOP	high-performance liquid chromatography	The method has good reproducibility and high accuracy.	The preliminary experimental results indicate that the content of PAEs in the surface layer of meat products tightly attached to the plastic is much higher than that in the inner layer.
Representative textile sample[18]	DMEP, DBP, DIBP, BBP, DHP, DIHP, DEHP, DNOP, DINP, DIDP	high-performance liquid chromatography	High performance liquid chromatography is simple, easy to operate, less polluting, and has high accuracy and precision.	A method for simultaneously extracting 10 phthalates from textiles using a fat analyzer has been established for the first time, which meets the technical requirements for residue analysis.
Milk tea, instant noodles seasoning bag[19]	DMP, DEP, DIBP, DBP, DMEP, BMPP, DEEP, DPP, DHXP, BBP, DCHP, DEHP, DBEP, DINP, DNOP, DPP, DHP	Dispersive solid-phase extraction gas chromatography-mass spectrometry	This method is simple and has a high recovery rate, which is conducive to the detection of PAEs in samples.	The dispersed solid-phase extraction gas chromatography mass spectrometry method can meet the requirements for the detection of phthalates in high-fat foods.
Cosmetics[20]	DMP, DEP, DBP, DEHP	Ultra high performance liquid chromatography with UPLC and PDA detector	This method has good separation effect, high sensitivity, and can meet the relevant detection needs.	115 cosmetics samples were analyzed and tested using this analysis method, with 29 cosmetics detecting DEP, 14 cosmetics detecting DBP, and 25 cosmetics detecting DEHP.
Water samples from different points in the Xuanwu Lake tourist area[21]	DMP, DEP, DBP, DOP, DEHP, BBP	Solid Phase Membrane Extraction - High Performance Liquid Chromatography	The recovery rate is higher, the detection limit is lower, the amount of organic solvents is greatly reduced, and the time consumption is shorter.	The mass concentration of PAEs in Xuanwu Lake far exceeds the standard limits specified in the national standard GB 3838-2002, and the pollution of PAEs is very serious.
PM2.5[22]	DMP, DMEP, DEEP, DEP, DBEP, DBP, DPP, BBP, DCHP, DAP, DMPP, DHXP, DEHP, DNOP, DINP	Ultrasonic extraction ultra high performance liquid chromatography tandem mass spectrometry method	Effectively controlling blank interference, low solvent usage, high sensitivity, and strong selectivity.	Most PAEs have good linear relationships within the range of 0.1 to 450μg/L.
Paint samples[23]	DMP, DPrP, DEP, BBP, DBP, DEHP, DnOP, DAP, DiDP, DHP, DiNP	High performance liquid chromatography analysis and determination	This method has the characteristics of simplicity, rapid analysis, and high accuracy and precision.	According to this method, all of them contain DBP substances.

2.3 Summary of test methods

At present, for different samples and their different properties, the detection technology of phthalates mainly includes liquid chromatography, gas chromatography, high performance liquid chromatography, thin layer chromatography, gas chromatography-mass spectrometry, etc. The analytical instruments are mainly high performance liquid chromatograph (HPLC) and gas chromatograph (GC). Sample preparation methods mainly include solid phase extraction (SPE), solid phase microextraction (SPME) and liquid-liquid extraction, among which organic solvent liquid phase extraction usually uses n-hexane. Due to the complex composition of some samples, organic interference needs to be removed. Take meat food as an example, because it contains a lot of fat, it needs to be refrigerated at high speed at -10°C. For environmental samples, preparation methods include Soxhlet extraction (SE), supercritical fluid extraction (SFE), accelerated solvent extraction and ultrasonic assisted extraction (UAE).

Ⅲ. Calculation method for phthalate content of different kinds of samples

According to the preamble, a variety of daily products contain phthalates, and due to its heat volatilization nature, it is also detected in dust, air and water, and the human exposure level is closely related to the phthalate content in the environment, Table 3 summarizes the calculation method of phthalate content in typical samples.

Table 3: Calculation method for phthalate content in typical samples

Type	Content calculation formula and symbol explanation	Remarks
Phthalate esters in gas, including DMP, DEP, BBP, DBP, DCHP, DEHP, DnOP[24].	$M = \frac{V_0 \cdot C_0 \cdot A_i / A_0}{V_{air}} \times 10^{-3}$ M-Amount of phthalates per cubic meter of air (ng/m^3); C_0-Concentration of standard sample (mg/L); V_0-Constant volume (mL); A_0-Peak area of reference materials; A_i-Peak area of the sample; V_{air}-Sampling volume (m^3).	Use high-performance liquid chromatography to determine various parameters. The peak height and peak area are directly obtained from the chromatogram; Quantitative analysis of chromatograms using peak area method. A standard concentration close to the sample size was used in the experiment. Quantitative calculation is carried out using the one point external standard method.
Phthalate esters in dust, including DMP, DEP, BBP, DBP, DCHP, DEHP, DnOP[24].	$M = \frac{V_0 \cdot C_0 \cdot A_i / A_0}{\mathrm{m}} \times 10^{-3}$ M-Mass of phthalate esters per gram of dust (mg/g); m-Mass of dust reduction (g); C_0-Concentration of standard sample (mg/L); V_0-Constant volume (mL); A_0-Peak area of reference materials; A_i-Peak area of the sample.	Use high-performance liquid chromatography to determine various parameters. The peak height and peak area are directly obtained from the chromatogram; Quantitative analysis of chromatograms using peak area method. A standard concentration close to the sample size was used in the experiment. Quantitative calculation is carried out using the one point external standard method.
Phthalate esters in plastic food bags for fresh corn include - di (2-ethylhexyl) phthalate (DEHP)[25].	$W_i(C_i) = f_i A_i(h_i)$ $U = cV / 5$ W_i-Mass of component i; C_i-Concentration of component i; f_i-Correction factor for component i; A_i-Peak area of component i; C_i-Peak height of component i; U-Content of DEHP migration (mg/kg); c-The amount of DEHP calculated from the standard curve based on the chromatographic peak area (mg/kg); V-Quantitative volume after extracting DEHP (mL).	The parameters were determined using a gas chromatography-mass spectrometry (GC-MS) technique. The peak height and peak area are directly obtained from the chromatogram; Quantitative analysis of chromatograms using peak area method. Quantitative analysis is conducted using the external standard method, which is the standard curve method.
Phthalate esters in beverages include DMP, DEP, DBP, DAP, DEHP, and DnOP[26, 27].	$X = \frac{(\rho_x - \rho_0) \times V \times K}{m}$ X-Concentration of certain PAEs in beverage samples (mg/L); V-Fixed volume of beverage sample (mL); K-Dilution ratio; ρ_x-Concentration corresponding to peak area of a certain PAEs in beverage samples (mg/L); ρ_0-Concentration corresponding to the peak area of a certain PAEs in the blank sample (mg/L); m-Beverage sample mass (g).	Determine parameters using a gas chromatograph. Quantitative determination using external standard working curve method. Error analysis is required during calculation.
The phthalates in drinking water include DMP, DEP, BBP, DBP, DOA, DEHP, and DnOP[28].	$Q = \frac{A}{A_{RS}} \times \frac{Q_{RS}}{RRF_{RS}}$ $\rho_S = \frac{Q}{V}$ Q-mount of compound to be tested in the analysis sample (ng); A-The sum of the monitored ion peak areas of the compound to be tested on the chromatogram; ARS-Sum of monitoring ion peak areas for recycling internal standards; QRS-Addition amount of recycled internal standard (ng); RRFRS-Measure the relative response factor of compound relative recovery internal standard; ρs-Measured concentration of the compound to be tested in the sample (ng/L); V-Water sample volume (L).	Qualitative and quantitative detection using gas chromatography-mass spectrometry mass spectrometry. Calculate the absolute amount of target compounds detected in the analytical sample using the internal standard method. The recovery rate of the internal standard needs to be calculated.

Through the induction of the calculation of phthalate content and the review of relevant literature, it can be found that when calculating the content of phthalates, the relevant parameters are mainly obtained by gas chromatograph or liquid chromatograph (among which the gas chromatograph is used more times), and then the content of phthalates in the sample is calculated by formula, and then the standard curve is drawn by the external standard method according to the calculated sample content, and the peak map obtained on the chromatograph is compared with the standard curve drawn. Therefore, the content of the corresponding phthalate ester is obtained,

and a few literature uses the internal standard method to calculate the content.

Ⅳ. Summary and outlook

This paper briefly introduces the properties and hazards of phthalates, and summarizes the test methods and calculation methods of phthalate content in different types of samples. Through the study of phthalates, it can be found that phthalates have certain potential harm to the human body. This study will provide a strong scientific basis for current health research and phthalate pollution prevention and control, and its academic, social and practical significance is of great significance. Future research on phthalates can focus on the use of other items to replace the position of phthalates in the manufacture of daily necessities, toys and materials, etc., in order to reduce the harm of phthalates to the human body.

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