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Abstract
Since the twentieth century, the health hazards caused by chemical pollutants have become increasingly prominent. Tens of thousands of high throughput chemicals have entered into the environment along with the massive production and use, raising emerging scientific issues for research in environmental science related fields. In this article, background and scientific significance of establishing the research direction on identification of novel organic pollutants are introduced. Basic principles of discriminating molecular structures and environmental behaviors of pollutant candidates with regard to the characteristics of persistent organic pollutants are discussed. Technical strategy and advantages of the established analytical frameworks, including quantitative structure-property relationship model prediction, suspect/non-targeted analysis, and biological effect-directed analysis, are further described. In-depth exploration on occurrence, behavior and effects of novel organic pollutants will play a crucial supportive role in leading the direction of discipline development, improving chemical risk assessment criterions, and revealing the toxicity and hazard mechanisms of environmental pollutants.

Keywords
persistent organic pollutants; artificial chemicals; risk assessment; health effects; screening and identification theory; analysis method; environmental behavior

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Basic Theory and Analytical Methodology for Identification of Novel Environmental Organic Pollutants

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Abstract: Since the 20th century, the health hazards caused by chemical pollutants have become increasingly prominent. Tens of thousands of high-throughput chemicals have entered the environment along with the massive production and use, raising emerging scientific issues in environmental science-related fields. In this article, background and scientific significance of establishing the research direction on identification of novel organic pollutants are introduced. Basic principles of discriminating molecular structures and environmental behaviors of pollutant candidates with regard to the characteristics of persistent organic pollutants are discussed. Technical strategy and advantages of the established analytical frameworks, including quantitative structure–property relationship model prediction, suspect/non-targeted analysis, and effect-directed analysis, are further described. In-depth exploration on occurrence, behaviors, and effects of novel organic pollutants will play a crucial supportive role in leading the direction of discipline development, improving chemical risk assessment criteria, and revealing the toxicity and hazard mechanisms of environmental pollutants.

Keywords: persistent organic pollutants; artificial chemicals; risk assessment; health effects; screening and identification theory; analysis method; environmental behavior

1 Health hazards caused by the rapidly growing chemicals

Since the 20th century, the synthesis and use of artificial chemicals have been increasing significantly. As of June 2015, the US Chemical Abstracts Service (CAS) had recorded 100 million chemicals, including organics, metals, coordination compounds, polymers, and salts. In 2019, the number increased to 150 million, with an average annual increase of over 12 million[1]. The Ministry of Ecology and Environment of the People's Republic of China published Inventory of Existing Chemical Substances in China in 2013, which has included more than 45,000 chemicals[2].

After being processed in industry or used in daily life, the chemicals mentioned above will inevitably enter the environment and produce complex chemical, ecological, and health effects. In fact, modern industrial pollution has driven development of environmental chemistry. In 1962, the publication of Silent Spring attracted the attention of academic circles to the damage of dichloro-diphenyl-trichloroethane (DDT) to the development of wildlife. In 1996, the publication of Our Stolen Future once again aroused people's concern to the health impact of environmental endocrine disruptors. The research directions of environmental protection have gradually changed from the treatment of conventional air pollutants (such as sulfur dioxide and dust) and water pollutants (as characterized by chemical oxygen demand, etc.) and the control of heavy metal pollution to the reduction of trace persistent organic pollutants (POPs).

Global convention marks the active prevention of POPs from passive response. In view of the persistence (P), long-range transport (LRT), bioaccumulation (B), and toxicity (T) of POPs, 179 countries and regions including China signed and joined Stockholm Convention on Persistent Organic Pollutants (hereinafter referred to as Stockholm Convention) in May 2001. The Stockholm Convention prohibits or restricts the use of 12 typical POPs including dioxin and DDT[3]. The number of contracting states reflects the international influence of Stockholm Convention and the emphasis of the states on POP pollution. Moreover, Stockholm Convention also marks the change of response to POPs from passive treatment into active prevention.

Along with the implementation of Stockholm Convention, the analysis methods of POPs, and environmental behaviors, ecological risks, and environmental health related to POPs have become research focuses in environmental science and the research direction has changed from the identification of typical POPs with significant ecological effects and the tracking of their homologues to the novel organic pollutants with characteristics similar to POPs, especially the risk...
assessment, management, and control of typical POP substitutes. The common characteristics of these novel environmental organic pollutants are as below: having at least one characteristic of POPs (P, LRT, B, and T), being in mass production and use, having high stock in the environment, and lacking data on ecological risks and health risks for standard management. The above-mentioned novel organic pollutants also pose challenges to the chemical safety assessment system.

The assessment of environmental behaviors and health risks of novel organic pollutants is also an important part of Stockholm Convention. Any contracting state can apply for the inclusion of a novel organic pollutant into the control list of the open Stockholm Convention. Since 2013, 16 novel organic pollutants such as polybrominated diphenyl ether and perfluorooctane sulfonate have been added to the control list of Stockholm Convention. At present, the candidate chemicals under review include perfluorohexane-1-sulfonic acid and its salts and related substances, DechloranePlus and methoxychlor [3]. These novel organic pollutants are produced, used, or discharged in a certain scale in China.

The novel POP pollution in China has unique characteristics. Chemical industry is one of the pillar industries in China. Statistics show that the output value of China's chemical industry has leaped to the top in the world since 2010, exceeding 10% of the gross domestic product (GDP) [4]. As a major chemical product producer, China witnesses more severe environmental pollution and higher health risks caused by novel POPs than other countries, and some novel pollutants that are not concerned by foreign countries may widely exist in the environment in China. The research on these compounds can help comprehensively assess the industrial economic costs of chemical safety supervision, lay a scientific basis for China to formulate policies to fulfill Stockholm Convention and carry out chemical risk management, and provide references for the development of chemical substitution technology. In addition, with the rapid economic development, the health problems caused by pollution are prominent in China, and the mechanisms for the T and health hazards of novel POPs have become research focuses in recent years.

2 Key scientific issues in the identification of novel organic pollutants

For a long time, POPs in the research of Chinese scholars have been identified by foreign experts and most of the studies in China are about the existing form, transferring rule, accumulation mechanism, T mechanism, health hazards, and techniques for elimination, reduction, and control of these pollutants in the environment in China. It will gradually change the passive tracking situation in China to identify novel pollutants with important influence in Chinese environment according to the chemical industry structure, geochemical characteristics, differences in use and emission, and other factors and characteristics in China.

In 2006, the State Key Laboratory of Environmental Chemistry and Ecotoxicology formally put forward the research direction of screening and identifying novel pollutants in the environment in China. Before the beginning of the research, we need to make the following preparations: (1) developing screening theory and method and formulating identification strategy and principle—how to judge and lock on the structures of compounds with pollutant characteristics? where to begin? (2) establishing a trace analysis method for environmental samples—how to analyze the concentration and determine the structure of trace compounds in the absence of standard substances? (3) determining the environmental chemical behaviors of pollutants—how to test and determine the P, B, and T of target compounds? what are their fates and environmental significance?

3 Basic principles of novel organic pollutant identification

For the screening and identification of novel organic pollutants, it is necessary to consider the influence of the discharge and use of chemicals on the environment and to clarify the environmental behavior characteristics of pollutants. In general, the commercial chemical products will have important impact on the natural environment and organisms if they have the following three characteristics [5]. (1) They should have at least one of the physico-chemical properties of POPs (P, LRT, B, T). (2) The production and use of them should reach a certain level. For example, after 3 000 t volatile organic compounds are released into the atmosphere and achieve equilibrium, the concentration in the air is only about 1 ng/m³. Chemicals with annual production of more than 454 t are regarded as high-throughput substances, and these substances should be emphasized. (3) They should have specific uses and pathways to release to the environment. For example, additive brominated flame retardant–polybrominated diphenyl ether which is used in the processing of plastic products and the residue of Perfluorinated alkyl substances in fluorine-containing surfactants can cause potential environmental problems.

Among the above three characteristics, physico-chemical properties have the most important influence on the environmental chemical behaviors of compounds. International organizations such as the UN Environment Programme have clearly defined the physico-chemical properties of the compounds with potential POP characteristics: (1) P [the half life (T1/2) in water, sediment, and soil > 180 days], (2) LRT [sub-cooled liquid vapor pressure (Pv) < 1 000 pa, atmospheric oxidation half life (AO T1/2) > 2 days], (3) B [octanol-water partition coefficient (logKOW) > 5, B factor (BAF) or bioconcentration factor (BCF) > 5 000] [6-8].
4 Guided discovery-based methods for identifying novel organic pollutants

Analysis method is the key to identifying novel organic pollutants. As the research object is the whole complex environment rather than a specific compound, diverse guided discovery-based methods are necessary. Combing sample pretreatment, instrument analysis, and data mining, the joint application of quantitative structure–property relationship (QSPR) model prediction, high resolution mass spectrometry (HRMS)-based suspect/non-targeted analysis, and effect-directed analysis (EDA) can achieve comprehensive screening of novel organic pollutants and ensure the discovery of novel pollutants (Figure 1).

Figure 1 Guided discovery-based methods for identifying novel pollutants

4.1 QSPR model

QSPR model was proposed on the basis of the hypothesis that the molecular structures of compounds determine their physico-chemical parameters. The physico-chemical parameters of compounds are closely related to actual environmental behaviors. Therefore, on the basis of the molecular structures of compounds, the physico-chemical parameters of them can be calculated, and according to the physico-chemical parameters, the potential POPs can be identified and screened.

The prediction of physico-chemical parameters is a classical branch of chemometrics. The structure-coded information of a series of molecules and actual physico-chemical property data obtained from experiments are required to establish the method. Through statistical analysis, a quantitative relationship model of the two can be established and used to predict the property parameters of novel compounds with similar functional groups. This method features ease of implementation, high accuracy, and high throughput. For example, $K_{ow}$ and $BCF$ have a linear regression relationship [9].

Environmental behaviors can also be predicted on the basis of the calculated physico-chemical parameters. The multimedia environmental fugacity model developed by the Organization for Economic Cooperation and Development (OECD) [10] uses air-water partition coefficient ($K_{ow}$), $K_{ow}$, and $T_{1/2}$ in air/water/soil to predict the $P$, LRT potential (LRTP), and transport efficiency (TE) of compounds, which achieves ideal result. The $T$ to aquatic organisms can also be predicted with the same method. The ECOSAR prediction model [9] developed by the United States Environmental Protection Agency quantitatively describes the functional relationship between the $K_{ow}$ of 130 compounds with different molecular structures and the half-lethal concentration ($LC_{50}$) of them to fish, water flea and green algae-like animals on the basis of a great deal of literature and experimental $T$ data.

Figure 2 Correlation between environmental behaviors of compounds and physico-chemical properties.
(a) Octanol-air partition coefficient and air-water partition coefficient; the area enclosed by dotted lines is the prediction result of fugacity model of compounds balanced in the air, water, and organic media (soil/vegetation); P-B-LRT score is a relative quantitative value that characterizes the similarity of the environmental chemical behaviors of compounds to those of the known POPs, and compounds with high P-B-LRT score show more obvious characteristics of POPs; (b) persistence; (c) bioaccumulation factor; (d) transport efficiency
Through the uniform coding of the information of molecular structures, the physico-chemical parameters of 1487 compounds \[^{[11]}\] in house dust and their correlations with the characteristics of POPs can be quickly calculated and predicted (Figure 2). In the compound database, the compounds with B, atmospheric stability, and LRT account for 8.6%, 9.0%, and 9.0%, respectively. A total of 35 compounds had both P and B, including 32 halogenated compounds. This result is of great significance for reducing the number of research objects and focusing on the environmental behavior investigation and T test of suspected substances with potential POP characteristics.

### 4.2 HRMS-based suspect/non-targeted analysis

In addition to the registered chemicals, novel organic pollutants may also include newly synthesized and unregistered artificial chemicals, compounds generated by reactions in the environment (such as polycyclic aromatic hydrocarbon analogues containing carbonyl and nitro groups generated by the oxidation reaction in the air), and compounds from natural resources (such as the estrogen-like substances and algal toxins). Research on such completely unknown compounds is mainly the summary of the common characteristics of their structures on the basis of analysis with instruments. HRMS can accurately determine the charge-to-mass ratio of charged ions and then analyze the element composition to obtain the molecular formula of compounds, which plays an important part in the structural analysis of novel chemical pollutants.

![Figure 3](image)

**Figure 3** Significant differences between high resolution mass spectrometry-based suspect/non-targeted analysis and conventional target analysis. The high resolution mass spectrometry-based suspect/non-targeted analysis and conventional target analysis are significantly different in the detection range and type of analyte detection and are also different in methodological flow of complex matrix pretreatment, instrument detection, and data processing.

The HRMS-based suspect/non-targeted analysis is significantly different from the conventional target analysis (Figure 3) \[^{[12]}\]. Target analysis must rely on standard substances to obtain the mass spectrometry signals of compounds and thus identify the target compounds. Target analysis is limited in the identification of novel pollutants as the targets are unknown and standard substances are difficult to obtain. However, the suspect/non-targeted analysis method comprehensively analyzes the compound information in an environment rather than a specific target without relying on the compound standards. Thus, it is widely applied in the identification of novel organic pollutants \[^{[13]}\]. For the suspect/non-targeted analysis method, sample preparation is relatively simple and universal pretreatment and accumulation methods are used. As for the analysis with instruments, the total ion chromatograms of samples and synchronously obtain the MS/MS fragmentation information of high-abundance ions are collected. Further mining of the acquired chromatographic and mass spectrometry data is the key step of non-targeted analysis, and appropriate data screening rules (such as accurate mass comparison, isotope abundance screening, matching with the databases of chromatographic retention time and MS/MS fragmentation information, and step-by-step exclusion) should be selected according to the specified experimental scheme and potential compound types.

For example, for perfluorinated or polyfluorinated compounds, the characteristic of the molecules is that the accurate mass of the fluorine atom is very close to its positive mass, so the overall difference in mass of the compound molecule will be in a specific range (\(\Delta m = -0.150–0.100 \) Da). Thus, the perfluorinated or polyfluorinated compounds can be distinguished from a large number of hydrocarbons on the basis of mass spectrometry signals. With the above method, we identified three novel pollutants of chlorinated perfluorinated ether sulfonic acids (CI-PFESAs) in the complex matrix of sludge in China \[^{[14]}\]. Further research shows that the residual concentration, B ability, and cytotoxicity of these three novel pollutants in environment are similar to the most concerned perfluorinated pollutant and perfluorooctane sulfonate in China.

### 4.3 EDA

EDA is driven by effects in bioassays, which combines sample treatment, chromatographic separation and purification, and compound characterization to determine the concentration, structure, and T of main effect pollutants \[^{[15]}\]. This method, combining analytical chemistry and biological detection system, shows high efficiency of detecting substances with positive biological effect as compared with the “from substance to risk” evaluation method, which can provide valuable basic data for screening the main pollutants and identifying unknown toxic components in specific polluted areas.

EDA includes four steps (Figure 4). (1) T test: It can test the T of a wide range of organisms, such as the biological individual test and the in vitro T test of cells and strains and “biological detector” is used for the testing, which is applied throughout the whole process. (2) Pollutant extraction: The key requirement is to extract all organic substances in the
sample. The multi-step separation can be adopted according to the polarity, molecular weight, and modified groups of the target compounds. (3) Identification of toxic pollutants: The structural identification and quantitative analysis of pollutants with active biological effects should be conducted with multiple characterization methods such as nuclear magnetic resonance, mass spectrometry, and spectroscopy. (4) T confirmation of pollutants: According to the measured total T of components, the T contribution of positive compounds of different concentration is quantitatively analyzed, and the detected products with a large T contribution in samples are regarded as key toxicants.

EDA has been applied to the screening of novel compounds and the identification of unknown organic pollutants. For example, our research group has identified the neurotoxic effectors in the sediment of a river near a flame retardant factory. After accelerated solvent extraction and purification by gel exclusion chromatography, the samples were divided into F1 and F2 components through silica gel solid-phase extraction columns. The cerebellar granule neurons of mice were used as the biological evaluation model and F2 component was found to have significant neurotoxicity. After purification by reverse preparative chromatography, the fractions were collected every 1 min and the results showed that F2.17 fraction was a toxic component. F2.17 was further analyzed by UV detector and mass detector, and tetrabromobisphenol A-diallyl ether was identified as the main pollutant [16].

4.4 Summary

With nearly 10 years of effort, the research group has preliminarily established an analytical framework for identifying novel organic pollutants and identified 161 monomers in 17 categories of novel environmental pollutants such as nitrogen heterocyclic brominated flame retardants, tetrabromobisphenol derivatives, and perfluoroalky iodide that have never been reported, which also leads the in-depth research on analysis methods, evolution trends, transformation rules, B, toxic effects and health risks of some novel structural pollutants. However, developing an efficient and universal analysis method system for identifying novel pollutants is still a frontier scientific issue in future environmental chemistry research, and the key is to guiding the continuous innovation of identification methods and strategies. This requires to further integrate the construction of compound information database and directed identification and characterization.

5 Prospects

(1) With the popularization of HRMS, suspect/non-targeted analysis is gradually influential. This method has obvious advantages in confirming the molecular structure of compounds, retrospective analysis of data independent of standards, and comprehensive analysis of environmental sample information. The suspect/non-targeted analysis will be more popular than the conventional target analysis in the trace analysis of pollutants, especially in big data acquisition and other disciplines (such as genomics, metabolomics, and epigenetics). The “Toxicology in the 21st Century” (TOX21) launched by the National Institutes of Health and the US Environmental Protection Agency [17], and the novel environmental substance monitoring reference laboratory, research center and related organization network (NORMAN Network) established by the European Union [18] emphasize the development of suspect/non-targeted analysis and its application in the characterization of the existing form, transport, and exposure risk of unconventional and novel organic pollutants. China also needs to strengthen the support in basic research and applied research of chemical safety assessment and gather the superior research institutions to develop an early warning research system for identifying novel pollutants with POP characteristics, so that the key role of advanced analysis technology in screening and management of priority control pollutants, screening of pollution sources, assessment of major environmental pollution incidents and emergency treatment can be brought into full play.

(2) Chemical analysis will be comprehensively combined with biological effect assessment. Because the toxic effect characteristics of chemicals are significantly different and the interaction of different substances will produce compound effects, it is an important direction of modern toxicology research to develop rapid and systematic methods for accurately identifying the main toxic effect components in complex matrices. Particularly, with the surge of novel environmental pollutants and complex interaction of pollutants in recent years, the current toxicological data are far from meeting the practical needs. Therefore, it is particularly
important to conduct T test and health risk assessment on a large number of pollutants and mixtures rapidly and accurately with low cost. An instrument automation test (ITA) platform integrating automatic and high-throughput chemical analysis and T effect assessment and continuous investment in its application and development are the key to achieving the above goals.

(3) Cross innovation of environmental chemistry and health sciences will be more important. The life science research shows that human health status or disease outcome is co-determined by genetic factors and environmental factors [19]. For example, a Nordic twin cohort study found that the contribution of environmental factors to tumors reached 80%, while the value of genetic factors was only about 10% [20]. The proposal of “exposome” reflects the emphasis on environmental factor evaluation, which covers the internal and external exposure of chemical pollutants in the whole cycle from embryo to the end of life. It is a technical difficulty for experts in environmental chemistry to develop efficient analysis techniques, find suitable exposure markers, and explore the mechanism of diseases caused by complex environmental processes with the help of exposure fingerprints.

References

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