

Standardization of environmental analysis methods of hazardous chemicals

— Contribution to international control of hazardous chemicals by using advanced technologies —

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The development and dissemination of reliable analysis methods and reference materials, and the accumulation of high-quality analytical data are important to: (1) understand the environmental impact of hazardous chemicals; (2) evaluate the safety and effectiveness of international treaties regarding these chemicals; and (3) formulate policies accordingly. We published an international standard method for analysis of hazardous chemicals, using recent data on hazardous chemical usage and environmental persistence, before international regulations came into force. Here, we describe the development of our method, its adoption as ISO and JIS standards, and the significance of these achievements.

Keywords : ISO 25101, PFOS, environmental analysis, Stockholm convention on persistent organic pollutants (POPs), hazardous chemicals

1 Introduction

Many chemical substances are manufactured and used to support our life in a convenient and efficient way. However, some of the chemical substances may affect humans or the ecological system even if they are present in the environment in small amounts. The nonylphenol (NP, Fig. 1), perfluorooctanesulfonic acid (PFOS, Fig. 2), and perfluorooctanoic acid (PFOA, Fig. 2) that are subjects of this research are recognized as endocrine disrupting chemicals (EDCs) and persistent organic pollutants (POPs), respectively. For NP, the environmental standard for water quality was set in 2012 in consideration of its effect on aquatic organisms. To take appropriate measures against the environmental issues that may be caused by such chemical substances, it is mandatory to understand the environmental load capacity and to clarify the global environmental dynamics. In order to prevent pollution and understand the distribution of such chemical substances in the environment, it is necessary to develop the analysis method which allows high sensitivity and precise detection. Particularly, in the measurement of ultra-trace substances, erroneous measurement may unnecessarily inflate social anxiety and may result in wrong countermeasures. Therefore, the accumulation of reliable analysis data for hazardous chemical substances in the environment is extremely important.

In fact, many literatures and reports are gathered for the risk assessment (document that summarizes the risk outline)

of hazardous substances which becomes the basis for the discussion in the Stockholm Convention on Persistent Organic Pollutants (hereinafter, POPs Convention). However, the individual analysis methods and the quality assurance and quality control (QA/QC) of each chemical are rarely discussed in detail, and presently, the quality and reliability of these analyses are not thoroughly considered.

In the global environmental issue, it is essential that any value, such as the concentration of CO₂ in the atmosphere causing global warming, measured in any part of the world, can be considered reliable and mutually comparable. To achieve this goal, we aimed to develop an analysis method with high reliability, and establish the international standard for the analysis method for the environmental researchers and analysts around the world. We also aimed to create a certified reference material (CRM), so the analysis value will be traceable to the SI unit. As a reference concerning the standardization of the environmental analysis method, the system of ISO and JIS will be briefly explained below.

The ISO standardization is conducted by the technical

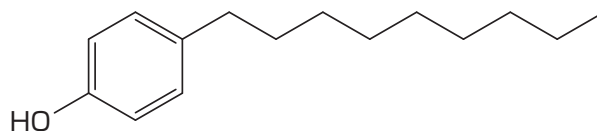


Fig. 1 Structural formula for linear nonylphenol (NP)

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committees (TC) of each field. There are two TCs for environmental measurement: TC146 (Atmosphere) and TC147 (Water quality). Each TC is divided further into subcommittees (SC) and working groups (WG), and each WG has an appointed convener who organizes the international meetings and writes the standardization draft. The NP (ISO 24293:2009^[1]) and PFOS/PFOA (ISO 25101:2009^[2]) were handled in the subcommittees ISO/TC147 (Water Quality) / SC2 (Physical, Chemical and Biochemical Methods). They were discussed and standardized in the working groups WG17 (Phenols) and WG56 (PFOS/PFOA), respectively.

The JIS for environmental measurement includes: K 0101 and K 0102 for environmental indices, inorganic ion and metals, etc; K 0125 for volatile organic compounds; K 0128 for agrochemicals; and K 0312 for dioxins. The NP (K 0450-60-10:2007^[3]) and PFOS/PFOA (K 0450-70-10:2011^[4]) that were standardized in this research were established in the K 0450 series. The K 0450 series were originally started in 1998 to standardize the measurement of organic chemical substances that may affect humans or the ecosystem at trace amount in irrigation water and wastewater, when the endocrine disrupting substances started to raise attention. Bisphenol-A (K 0450-10-10), alkylphenols (K 0450-20-10^[5]), phthalate ester (K 0450-30-10), bis (2-ethylhexyl) adipate (K 0450-40-10), and benzophenone (K 0450-50-10) were also standardized in the K 0450 series. The JIS standardization of NP was started at about the same time as the ISO, and the JIS standardization of PFOS/PFOA was set after ISO standardization as a MOD (partially modified standard).

In this report, the discussion on the environmental behavior of hazardous chemical substances and the development of their analysis method, as well as two case studies of ISO standards^{[1][2]} and JIS standards^{[3][4]} are described to illustrate the research process for standardization and the significance of such research.

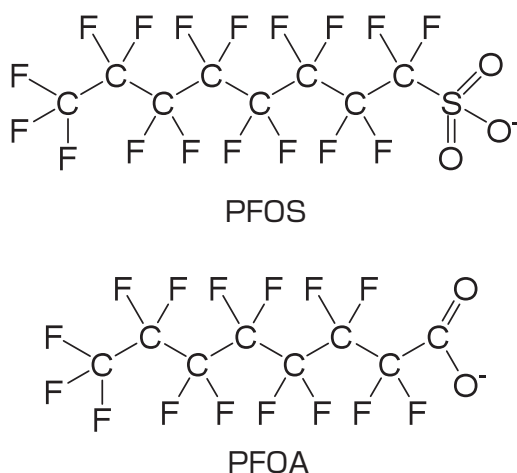


Fig. 2 Structural formulae for perfluorooctanesulfonic acid (PFOS), and perfluorooctanoic acid (PFOA)

2 Necessity and standardization of the environmental analysis technology that addresses the international regulation of hazardous chemical substances

In the international standardization for global environmental issues, the role of the public sectors, such as the international organizations and national research institutes, is important. Here, we engaged in the international standardization activities from this perspective. On the other hand, aside from international contribution, environmental analyses of the hazardous chemicals are useful in grasping the actual emissions from the Japanese industry and in executing appropriate environmental measures. Taking measures in response to the international regulation of chemical substances may become particularly essential for the continuation of a company. Hence, demands for standardization of environmental analyses were urged in Japan, and JIS standardization was promoted.

2.1 Necessity and standardization of the environmental analysis technology for NP

4-nonylphenol (NP, Fig. 1) is used as raw industrial material of nonylphenol ethoxylates (used as a nonionic surfactant in various industrial fields such as the rubber and plastic, textile, and metal processing industries), but it is strongly suspected to have an endocrine disrupting effect. Also, NP is produced by the successive breakdown of the ethoxy group of nonylphenol ethoxylate through the aerobic and anaerobic degradation in the sewage treatment process and aquatic environment. Therefore, the use of nonylphenol ethoxylate in household products was banned by the self-imposed regulation of industry. In recognition of this practice, the environmental standard pertaining to water pollution was revised in August 2012, and NP was newly added to the water quality environmental standard for the preservation of the aquatic organisms. We reported the vertical distribution of NP in outer seawater for the first time in 1998,^[6] and commenced the research to determine the environmental fate and toxicity, such as developing the analysis method for NP isomers and clarifying the estrogen-like activity of various isomers.^{[7][8]} In 2002, the necessity of the international standardization of the water analysis method for NP was explained to the Ministry of Economy, Trade and Industry. The research was selected as the Standard Certification Project (FY2002-2004) “Standardization of NP Analysis Method,” and we were able to start the research for the ISO and JIS standardizations for this analysis method.

NP is a type of alkylphenol. In 2002, JIS K 0450-20-10:2002^[5] existed, and for ISO, it was being drafted with Germany as the convener as ISO/CD 18857-1 (currently ISO 18857-1^[9]). Both analysis methods involved the total measurement of NP as a single compound, and there was no information on the isomer composition. However, the ISO

24293 developed and standardized in this research was a method that, for the first time, separated and measured NP into 13 types of isomers with varying degrees of endocrine disrupting effect. This is equivalent to the individual measurement and selection of 17 highly hazardous isomers with different hazardous levels among the 210 isomers of dioxins (collective name for polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran). Since the NP isomers showed different estrogen-like activities, it was necessary to understand the concentrations of each isomer in the environment for accurate risk assessment, and the accumulation of the concentration data for each isomer in the environmental samples was necessary.

To standardize the developed analysis method as ISO, it was necessary to make adjustments with Germany, who was acting as the convener and was working on the total measurement method for NP (ISO 18857-1^[9]) in ISO/TC147/SC2/WG17 (Phenols). Therefore, a meeting was set up for a preliminary discussion with the chairman of ISO/TC147 (Germany) and the convener for WG17 (Germany). We explained the necessity for the detailed analysis of individual isomers that enabled accurate assessment of hazards in the environment. The significance of such analysis was shared, and the new proposal by Japan on the analysis for individual isomers was approved at the ISO/TC147 meeting in 2003. As a result, the new analysis method was selected as the ISO New Work Item Proposal (NWIP) in 2005 before starting the JIS standardization,^[3] and international standardization was started by the the working group for phenols (WG17). In the ISO proposal, the analysis method proposed by Japan was generally accepted in the process of discussion with the TC members, but there were several arguments about the reference material and the suspended solids (SS) in the environmental water samples. The focus was placed on the effect of analysis precision due to the amount of SS in the environmental water sample. Hence, agreement was made to conduct the quality control studies using the environmental water samples with different amounts of SS and adding this result in the Annex (Informative). For the reference material, it was necessary for the measurers themselves to evaluate the commercially available mixtures. Therefore, measurements for the mixture of commercially available materials from the five manufacturers were listed in the Annex (Informative). The details will be described in subchapters 3.1 and 4.1. After the above discussion, the working draft (WD) was submitted in 2005, followed by the committee draft (CD) in 2006, the draft international standard (DIS) in 2009, and the final draft international standard (FDIS) in 2009. Finally, the ISO 24293 was issued as the international standard in July 2009.

2.2 Necessity and standardization of the environmental analysis technology for PFOS/PFOA

PFOS and PFOA are types of perfluoroalkyl substances (PFASs) that have fluoroalkyl groups as shown in Fig. 2.

Because PFOS/PFOA-related substances have extremely strong carbon-fluoride covalent bond, in addition to both hydrophobic groups (fluoroalkyl groups) and hydrophilic groups (sulfonate groups or carboxyl groups), they have excellent, diverse physical and chemical properties, such as chemical and thermal inertness, low surface energy and special surface active properties. Therefore, they have been used widely as functional industrial materials of fluoro-resin, state-of-the-art electronic device, semiconductors, coating, etching, photograph, emulsifiers, water repellents, soil release agents, firefighting foams, and their intermediary materials. Figure 3 shows the social and research trends of the PFOS/PFOA-related substances. The manufacturing method of the PFOS/PFOA-related substances was developed in the 1940s, and they became commercially available in the 1950s. However, PFOS was detected in high concentration in wild animals in February 2000, and the 3M Company of the United States announced the phase-out of the production of related materials in May 2000. Hence, the environmental issues due to PFOS-related substances have become widely studied and known. Thereafter, their environmental persistence, high accumulation in organisms, and concerns of the effects on humans and organisms became apparent, and they were even detected in high concentration from wild animals in the polar region.^[10] In such situations, international regulations on their use and treatment of their waste were considered, and PFOS and PFOA became “Type II Monitoring Chemical Substances” under the Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc. of Japan (CSCL) in December 2002. In January 2006, the US Environment Protection Agency (EPA) requested the major manufacturers voluntary reduction and elimination of PFOA and PFOA precursors by year 2015, and efforts on waste reduction started by setting up guidelines for drinking water around the water treatment plant. The considerations for regulations by the POPs Convention were started in June 2005 for PFOS. In 2010, PFOS and perfluorooctane sulfonyl fluoride (PFOSF) that is its raw material became the substances subject to the POPs Convention, and were designated “Type I Monitoring Chemical Substances” under the CSCL in Japan. Their production and use were banned worldwide except for certain essential use. However, measures would be delayed if the analysis method were standardized after the regulations. To execute measures efficiently in industry and society, it is necessary to ensure the appropriate risk profile by establishing a reliable analysis method before the danger of a new chemical substance becomes widely known in society. Figure 4 shows the scenario of the standardization of the PFOS/PFOA analysis method. The procedures taken in the standardization will be described below.

The Potential Pollutants Group, Research Institute for Environmental Management Technology, AIST, conducts basic researches on various potential pollutants based on the

findings and results obtained from numerous international joint researches using advanced analytical devices and technology. It has been engaged in researches focused on PFOS and its related chemicals before the international regulations were enforced. As part of the international joint research with the Wadsworth Center, USA, started in 1995, our group has conducted the development of the PFOS and PFOA analysis method from 1999, and in 2000, we started the PFOS project for the first time in Japan, through the Grant for Industrial Technology Research of the New Energy and Industrial Technology Development Organization (NEDO).

In 2001, the environmental concentration of PFOS and PFOA were surveyed for the surface water and fish in Japan, and the bioconcentration factor (transfer of chemical from water to the organism), which is an important factor in risk evaluation in the actual environment, was reported for the first time in the world.^[11] However, to understand the environmental fate such as long-range transport, it was mandatory to analyze the outer atmosphere and outer seawater with low concentration of 1,000 times less than the high-concentration samples of coastal water and organisms. The greatest issue in establishing the analysis method for the extremely low concentration level was the reduction of background contamination. This was because the target substance was used in various commercial products like water repellents, soil resisting agents, resin additives, and others. To reduce the contamination, contamination sources must be identified

and quantified systematically from our experimental environment, analysis equipment, device, and reference material. After elimination of all possible contamination sources, the level of contamination of PFOS and PFOA were reduced by over 1,000 times.^[12] The weak anion exchange solid phase extraction column (Oasis®WAX) was also deployed as the extraction method with high precision and high recovery rate.^[13] As a result, the establishment of the analysis technology at several pg/L level that could be applied to the outer seawater was achieved.

The measurements of outer surface seawater and deep seawater were started using this analysis method. Unlike the method where the PFOS/PFOA research was conducted from the perspective of hazardous chemical substances, we started this research by looking at their usability as chemical tracers in global material circulation satisfying the three requirements: persistence, water solubility, and detectability in ultra-trace analysis. In 2004, the outer seawater survey data was published for the first time in the world, and showed that the substances persisted in seawater at depth of 5,000 m.^[12] Through the joint collaboration with the world's top level research institutes, such as the Leibniz Institute of Germany and Wadsworth Center of the US that realized the importance of this research, international joint survey cruises were conducted to measure the vertical distribution from surface to deep seawater in the Sea of Japan, the Atlantic, the South Pacific, and the Labrador Sea. Particularly in the

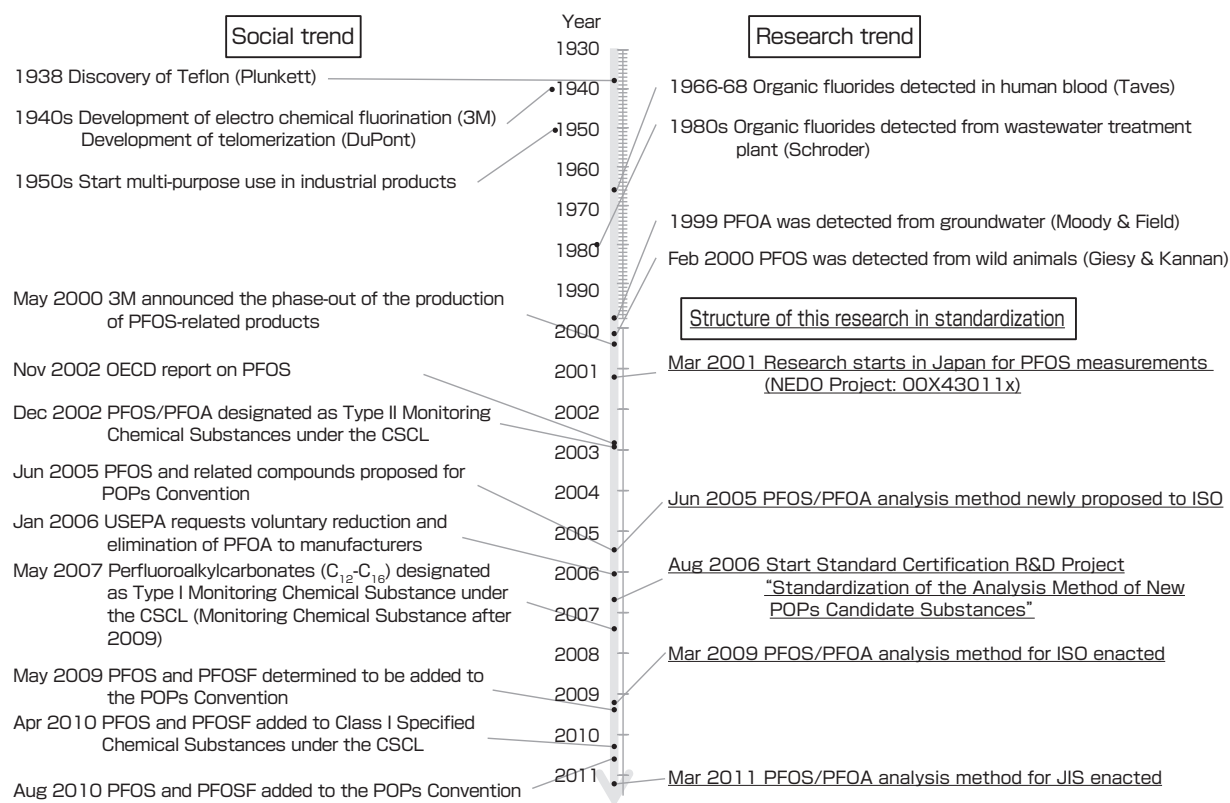


Fig. 3 Social and research trends of the PFOS/PFOA-related substances and their relationship to this research

Labrador Sea where the surface water dove straight into the depth and the surface and deep seawaters were mixed thoroughly, the vertical distribution at constant concentration was observed. It was found that PFOS was supplied to the deep seawater by the global general circulation mechanism of outer seawater by thermohaline convection.^{[14][15]} While the conventional long-range transport mechanism of POPs was discussed only in terms of atmospheric transportation, the importance of global long-range transport mechanism by ocean current was indicated in our study. Currently, this mechanism is under investigation mainly by the oceanographers of Europe and the United States. The outer sea survey method developed by AIST is now being used worldwide, such as, the report of the surface seawater distribution of the entire Atlantic by AIST and Environment Canada, which is the leading research institute for PFOS/PFOA study in Canada, in 2012.^[16]

The research accomplishments were highly evaluated in the international standardization of the analysis method, and Japan started up the working group WG56 (PFOS/PFOA) as the convener in the ISO/TC147 meeting held in June 2005, and commenced the international standardization process. The PFOS and 96 related substances were proposed as the substances subject to the POPs Convention in 2005. For standardization, research and development (R&D) (development of analysis method, improvement of analysis performance, maintenance of reliability by quality control study, etc.) and draft standard was written in the Standard Certification R&D Project (FY 2006-2008) “Standardization of the Analysis Method of New POPs Candidate Substances.” Almost all of the analysis technologies proposed by Japan were accepted, but there were a few issues raised in the discussion with the TC members. First issue was the

selection of the analytical device. Initially, at the start of the environmental measurement of PFOS and PFOA, liquid chromatograph mass spectrometer (LC-MS) or the so-called single MS was used instead of the liquid chromatograph tandem mass spectrometer (LC-MS/MS) that is generally used today, hence, the appropriateness of using the LC-MS was discussed. The British TC member proposed that the LC-MS should be included in the regulation in the draft process. However, LC-MS had lower selectivity compared to the LC-MS/MS and the separation from the interfering substances may be insufficient in some kinds of environmental samples. Since only one institute among the 23 institutes used it for international quality control study, the LC-MS method was only described in the Annex (Informative). Wastewater sample was proposed as a subject of analysis, but it was removed since the variation of analysis value was out of the guideline values in the international quality control study. For the related substances other than PFOS and PFOA, it was indicated that measurements could be done using the same analysis method, but only PFOS and PFOA were set as the subjects initially in the ISO standardization. The standardization would be delayed greatly if other related substances were added, and the subject substances were limited to PFOS and PFOA considering the demand for quick standardization. With the above deliberation, the WD was submitted in 2005, CD in 2006, DIS in 2007, and the FDIS in 2008. ISO 25101^[2] was established as the international standard in March 2009. PFOS was added to the international POPs Convention in 2010, and was also added to the “Class I Specified Chemical Substance” under the CSCL in Japan in 2010. The international standardization was completed before the regulations for the hazardous chemical substances.

Upon the establishment of the ISO standard, the JIS

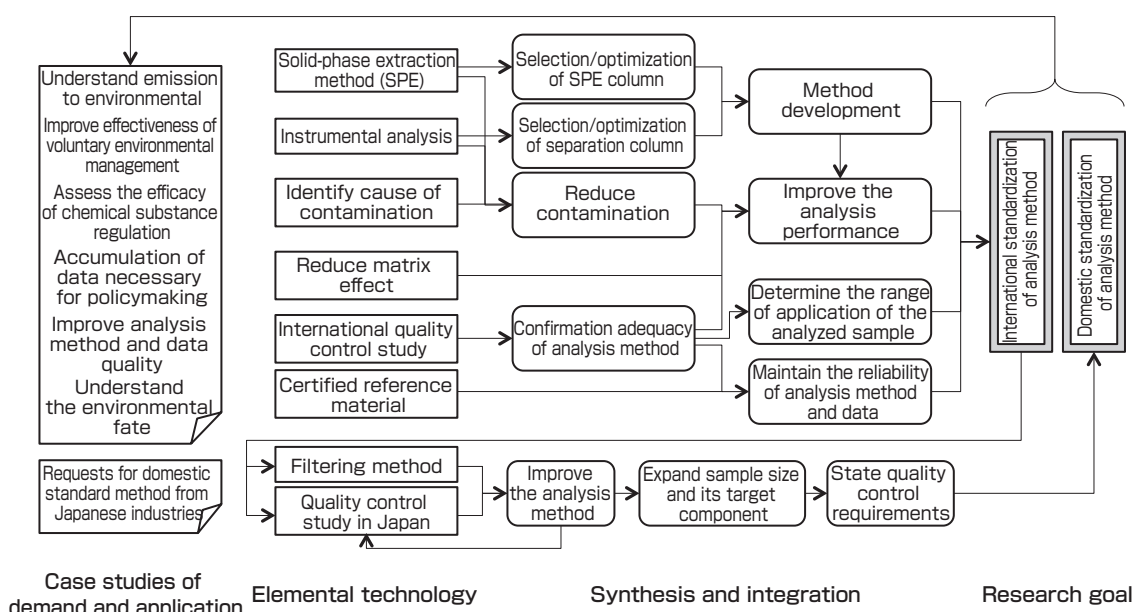


Fig. 4 Scenario for the standardization of PFOS/PFOA analysis method

standardization (in effect from March 22, 2011^[4]) was done to meet the international compliance according to the principle of domestic standardization (WTO/TBT Agreement) based on the international standard, and in response to the demands from the Japanese analysis laboratories for the establishment of the Japanese standard to ensure quality control and collaborative systems in Japan. The JIS standard is the addition, removal, and modification of the rules (MOD) based on the ISO standard. The modified points of the ISO standard include: not to use the LC-MS (written in Annex (Informative) of ISO 25101), addition of filtering procedures of samples that can be applied to SS rich water such as wastewater in JIS Appendix (Informative), and the addition of possible measurements of related substances other than PFOS and PFOA in JIS Appendix (Informative). For these modifications, quality control studies were conducted twice to check the adequacy of the new analysis method.

2.3 Timing of the standardization

The common points of ISO 24293 and ISO 25101 are that they started from scientific research on the environmental behavior, internationally acclaimed results published after developing a new analysis method, and the international standardization started at the stage when de facto standard was established as a consensus among the research community. This is in opposition to the conventional flow where the domestic standardization was done after the economic and social demands manifest and then fine adjustments are made with the international standard. In other words, the public sector decided that an environmental analysis method from a global viewpoint was necessary for the appropriate international use of chemical substances and for the global environmental protection. Furthermore, to ensure that the international chemical substance regulation could be done effectively, the awareness was raised among the research community by releasing the research results as soon as possible, and efforts were made to obtain a highly reliable analysis method by providing the international standard at the same time as the enforcement of the standard. The research was conducted with the point of view that “reliable analysis value and standard analysis technologies that ensure reliability are essential in the control of hazardous chemical substances.”

3 Development of the analysis method

3.1 Development of the analysis method for nonylphenol

The total analysis method for NP already existed in 2002 as JIS K 0450-20-10, and the analysis of general water samples could be done.^{[5][9]} However, other than the linear 4-NP shown in Fig. 1, there were theoretically 211 isomers of NP by different side chains and substitution sites,^[17] and dozens of isomers were detected with different environmental degradability and hazardousness from the environmental samples.^{[18][19]} Therefore, it was necessary

to develop a method to accurately quantify each isomer in order to conduct a highly reliable risk assessment. Using the gas chromatograph/preparative fraction collector (GC-PFC)^{Term 1} that was uncommonly used in Japan at that time, the NP isomers in the mixture were separated and refined. Individual isomers were collected in the glass tube cooled by liquid nitrogen, and this procedure was repeated about 100 times until six fractions were obtained for the amount necessary for a hormone activity test. It was confirmed that the endocrine disrupting activity differed greatly for different individual isomers.^{[6][7][18]} Next, the various commercially available capillary columns were compared to establish the optimal analysis condition for the advanced separation of the NP isomers. The method for maximum separation analysis of NP in the complex mixture was investigated using the two-dimensional gas chromatograph^{Term 2} mass spectrometry (GC×GC-MS), and we succeeded in separating 102 components in an NP product.^[20]

While it was possible to separate NP into multiple components if such state-of-the-art analysis technology was used, from the perspective of quality control, it was necessary to have a standard where the majority of the users could obtain the same data. Therefore, for the analysis of individual NP isomers in the ISO standard, 13 NP isomers that could be separated by general GC were set as the subjects of analysis. The styrene-divinylbenzene solid phase extraction method was used as the extraction method, since it could be used universally for water analysis. Since NP was used as the raw material of nonylphenol ethoxylate, which is a nonionic surfactant that can be detected from almost all water environments, utmost care was needed for contamination control. There were possibilities that NP might be present in the silica gel column cartridge and the anti-clogging agent (glass beads) in the solid phase extraction.

The selective ion monitoring (SIM) method of the gas chromatograph mass spectrometer (GC-MS) was used, and quantification was done using ions with good sensitivity and separability for each isomer (Fig. 5). Normally, five to six peaks were detected at m/z 135 (m/z is mass-to-charge ratio, or the value obtained by dividing the charge z with mass m), but in this analysis method, the assessment for individual isomers became possible by selecting an optimal monitor ion for each of the 13 isomers, and then calculating the relative response factor (RRF) of each NP isomer and the internal standard^{Term 3}. For the investigation of the ion selection for the NP isomer, refer to Horii *et al.* (2004).^[21] The reasons for the complicated quantification of individual NP isomers are because NP is a complex mixture, the commercially available standards for their isomers are limited, and the fragmentation patterns^{Term 4} differ greatly by isomers. In the situation at that time where the standards for individual isomers were not available, the only way was to use the NP mixture as a standard for quantification, and it was necessary

to investigate the isomer composition in the NP mixture before hand, using the gas chromatograph flame ionization detector (GC-FID)^{Term 5}. For the method development of NP, isomer compositions of the mixtures supplied by five reagent manufacturers were analyzed, and their coefficient of variation (value obtained by dividing the standard deviation by arithmetic mean; indicates relative variation) was 14 % (slightly different by isomers). To use the mixture for which the valuation of the isomer composition is done by the measurer him/herself as the quantifying standard substance is exceptional in the ISO standard. This point was discussed thoroughly in the DIS stage, and it was confirmed that there was hardly any variation in the composition among the reagent manufacturers,^[22] and the information for the composition of mixtures from the five commercial companies were included in the Annex (Informative).

3.2 Development of the analysis method for PFOS/PFOA

At the time when general research for PFOS/PFOA was started in 2000, most analysis was conducted in blood

samples which were considered as high concentration samples, and the analysis of environmental water was barely done. The reported analysis values had high detection limit due to background contamination, and there was no highly reliable analysis technology for low concentration environmental water. Therefore, research was done from 2001 in cooperation with the Wadsworth Center of the United States based on the solid phase extraction (SPE)^[23] method using the existing octadecyl group (C18).^[11] Until the new proposal of ISO 25101 in June 2005, we conducted the research to clarify the quality control condition to obtain highly reliable analysis data, by applying the developed PFOS/PFOA analysis method to various environmental samples.^{[11][12][14][24]-[29]} The first issue in PFOS/PFOA analysis was the reduction of background contamination.^[12] This was because the fluororesins such as the polytetrafluoroethylene (PTFE) that were used in various places around us as functional materials has become the contamination source, and also, system blank (contamination arising from the analysis device) tended to

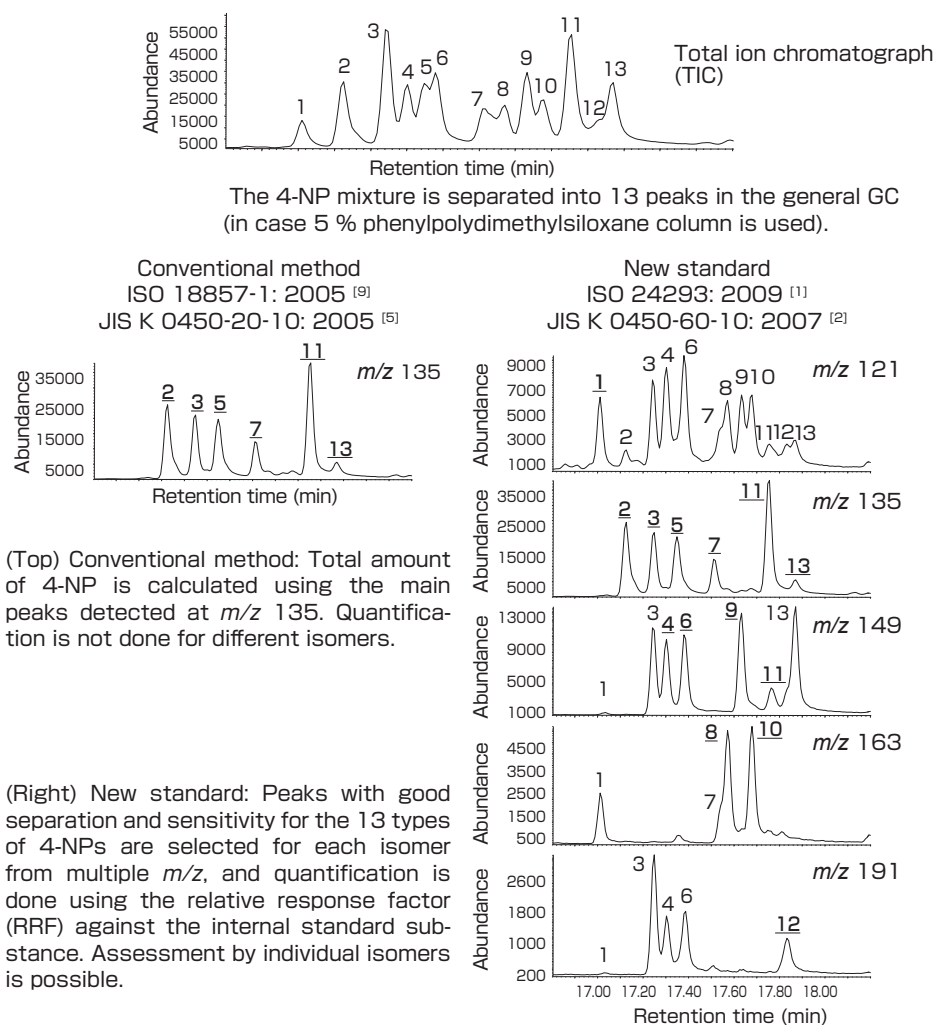


Fig. 5 Comparison of the NP analysis methods using GC-MS

The numbers above the chromatographs show the individual 4-NP isomers. The underlined numbers show the peaks designated by ISO standard to be used for quantification.

be high because the state-of-the-art analysis devices had fluororesin components. To solve this issue, the system blank was reduced to tens of fg ($1 \text{ fg} = 10^{-15} \text{ g}$) by eliminating parts such as the degassing device and switching valve that were not essential for measurement by the LC-MS/MS. Because the SPE cartridge^[23] that contained the existing octadecyl group (C18) was highly contaminated and not suitable for low concentration analysis, we developed the PFOS/PFOA analysis method using the Oasis[®]HLB cartridge that had both the hydrophilic and lipophilic properties, manufactured by the Waters Corporation. This was confirmed to have less contamination compared to the C18 SPE cartridge^[23] and could be applied to low concentration samples such as the outer seawater.^{[26][28]} However, the Oasis[®]HLB cartridge with excellent low contamination and high recovery rate is only limited to the measurement of PFOS/PFOA, and it was not suitable for the extraction of short-chain compounds such as perfluorobutanoic acid (PFBA). Therefore, we focused on the property of PFOS/PFOA as organic acids, and by reducing the contamination to a minimum by using the Oasis[®]WAX cartridge of Waters Corporation that had the anion exchange capability suitable for the adsorption and capture of acidic substance. We developed the analysis condition where carbonic acid with carbon chain length from C₂ to C₁₈ and sulfonic acid from C₂ to C₁₀ could be adsorbed and recovered, as well as PFOS and PFOA (Fig. 6).^{[13][30]} The reduction of contamination and system blank of the equipment and reagents used^{[12][24][25]} and its importance were also addressed in ISO 25101.

When an ordinary reverse phase HPLC column such as ODS (octadecylsilyl silica gel) column was used, elution occurred

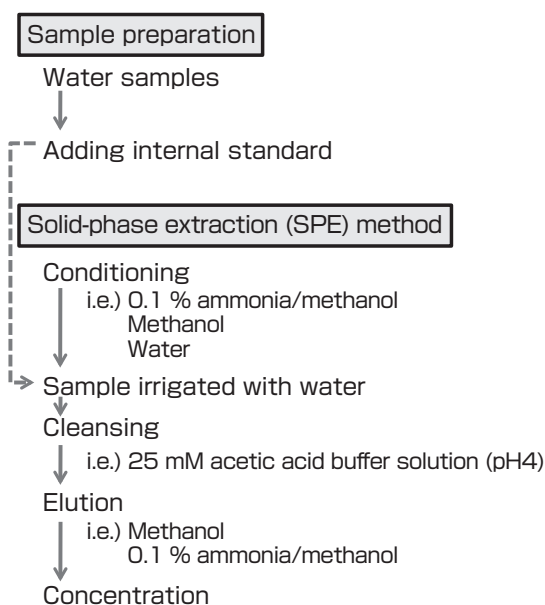


Fig. 6 Solid phase extraction method using the weak anion exchange cartridge (Oasis[®]WAX)

in the order of short to long chain, and the short-chain compound such as PFBA showed poor peak formation and the separation with the foreign substances was insufficient (Fig. 7a). On the other hand, in the separation column with ion exchange capacity, the order of elution reversed, and elution occurred from long to short chain, and the peak formation and foreign substance separation were improved (Fig. 7b).^[30] Crosschecking by two types of separation columns with different separation principles by concurrent check of the ion strength ratio of the measured mass showed that the reliability dramatically increased compared to the conventional single column measurement. In both ISO 25101 and JIS method, the reverse phase column is used as the separation column since only PFOS and PFOA are subjects of analysis. However, it is written in the JIS Annex (Informative) that the measurement of related chemical substances with short-chain compound other than PFOS and PFOA can be measured, the reverse phase column and the separation column with ion exchange capacity are both described, and the importance of crosschecking using the two types of separation columns is discussed.

In general, when the detection sensitivity is insufficient, the amount of the sample is increased. However, in our case, by reducing the amount of the sample, the effect of coexisting substances on the analysis sensitivity (matrix effect) decreased,^[12] and as a result, the measurement sensitivity was increased. With this technique, the limit of quantification can be increased by minimizing the contamination, even in the previous generation devices with low sensitivity. Since the latest measuring device uses a lot of fluororesin and has in general a higher system blank, what determines the methodological detection limit and precision for PFOS is, at present, not the sensitivity of the device, but how strictly the QA/QC is preformed. The highly sensitive analysis method is not truly necessary for rivers and coastal waters in Japan that are the usual monitoring targets. However, for outer sea environmental monitoring to understand the global dynamics, advanced QA/QC is essential in addition to high sensitivity. Such research papers were the basis of ISO 25101.

4 Reliability of the developed analysis method

In ISO /TC147 (water quality) /SC2 (physical, chemical and biochemical methods), the quality control studies (studies to determine whether the analysis method used is adequate) are conducted in multiple laboratories according to the standard operation procedure (SOP) for the analysis method to be used as a standard, and the performance data obtained must be comparable with the standard. On the other hand, in Japan, the performance data of the analysis method have not necessarily been released in the past. In the Research Project for Creating Standards to Meet Social Needs “Strategic Project to Build the JIS System for Environmental Measurement that Contributes to the Strengthening of

Industrial Competitiveness and Environmental Preservation,” the necessity for describing the results of the quality control studies was indicated from the perspective of meeting the ISO standard that requires the inclusion of the performance data of the standardized analysis method. This was indicated as part of the revision of the conventional JIS system for the measurements of water quality and atmosphere.

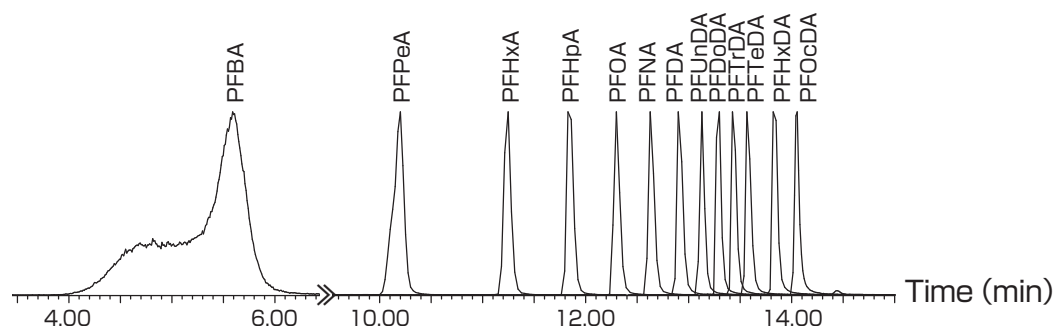
4.1 Quality control study for nonylphenol

For NP, participation in the quality control study for the analysis of NP isomers in the water sample was solicited under the project leadership of Japan for ISO/TC147/SC2/WG17 (phenols) from July to September 2008. There were applications from 17 institutes from Japan and overseas. After conducting statistical analysis of the study results, the results were organized and reported to the participating institutes as an interim report, and a report was submitted to the ISO/TC147/SC2/WG17 as the performance data for the ISO/DIS 24293 (draft standard) in December 2008. The intermediate precision coefficient of variation (CV_r) that looked at the variation of the analysis value within a laboratory was average of 10 % (minimum 4.4 % ~ maximum 21.6 %), and no difference was observed in intermediate precision due to the type of sample and difference in isomer composition. On the other hand, the reproducibility coefficient of variation (CV_R) that looked at the variation of the analysis results when

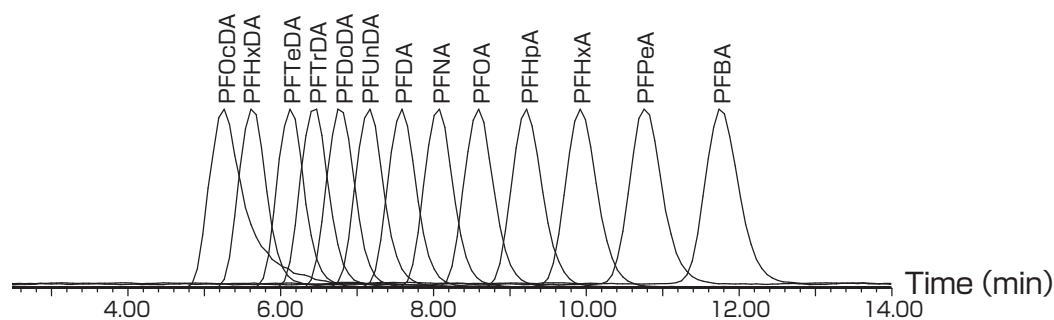
the measurements were done among different laboratories was approximately 30 % or less, but it surpassed 50 % in some isomers. Particularly, there was large variation in the inter-laboratory variation of isomers with small composition among the NP mixtures (NP8 and NP12, refer to Fig. 5), and the reproducibility tended to be poor. It was reported that there were slight differences in the isomer separation by different column manufacturers or due to the deterioration of the column even when equivalent separation columns were used. In this analysis method where the NP mixture was used as the quantifying standard, the effect of the slight difference in the peak separation capacity on the quantitative value could not be prevented for some isomers.^[22]

To check the effect on the analysis precision due to the difference of SS amount, river water (SS amount of 13 mg/L) and influx water of sewage treatment plant that contained large amount of SS (SS amount of 140 mg/L) were used as the quality control study samples. As described above, poor reproducibility was seen in some isomers (NP8 and NP12, refer to Fig. 5), but good results of CV_R within 30 % was obtained for other isomers.^[22]

This analysis method was published as the ISO 24293:2009^[1] in July 2009 after obtaining 15 votes of approval among 17 countries in the final vote. This analysis method was



a) Example of separation using the reverse phase column (Betasil C18)
(elution occurs in the order from short to long chain)



b) Example of separation using the multimode (reverse phase + anion exchange) column (JJ50 2D)
(elution occurs in the order from long to short chain)

Fig. 7 Example of the separation by chromatograph for perfluoroalkylcarboxylic acid

proposed as the ISO standard in February 2005. At that time, there were hardly any reference standards for the individual isomers of NP, but since the necessity of isomer analysis was recognized worldwide through the establishment of ISO 24293, several branched-chain isomers including the ^{13}C labeled standards are being sold by the reagent manufacturers today. Therefore, the improvement of reliability can be expected by using the commercially available standard materials in the next revision.

4.2 Quality control study for PFOS/PFOA

Before explaining the quality control performed in this research, the inter-laboratory study for the PFOS/PFOA-related substances conducted in early 2005 will be described. The inter-laboratory study was done using the in-house method (method that is not established as standard protocol) that each participant selected. The difference between the quality control study and the inter-laboratory study is that while the former is done for the purpose of assessing the analysis method and is conducted using a common SOP, the latter is done for the purpose of assessing the performance of laboratories on the analysis using in-house methods so there is no guideline for the analysis method used. The inter-laboratory study of 2005 was planned and managed by three organizations, Netherlands Institute for Fisheries Research, Örebro University, and Water Services Corporation. Thirty-seven international research institutes that have been working on the analysis technology development from the start of the PFOS problem, and five institutes from Japan (AIST, two companies, and two universities) participated. As a result, it was confirmed that the CV_R surpassed 100 % for the variation of analysis value. The reasons indicated were the use of standards with low purity, contamination by sample containers, and errors due to the difference in sensitivity and calibration curve of the measurement devices. It became apparent that the mutual comparison of the analysis values obtained by the in-house method was difficult, and there was a need for standardizing the analysis method.^[31] In this study, our group supplied the measurement data for PFBA with carbon number 4 for the first time in the world.

The quality control study organized by AIST for checking

the performance of ISO 25101 was done from November 2006 to February 2007 and 23 institutes from 9 countries participated. Since the quality control study was adopted to check the performance of the standard analysis method, the participants were required to use the draft of ISO 25101 as the SOP. The analyses were performed of river water, seawater, water containing low-concentration of the standard substance, water containing high-concentration of the standard substance, and the standard substance. The precision of CV_R of 27 % or less was successfully obtained for each sample, where the concentration of PFOS in the actual sample was 2.6-470 ng/L and PFOA was 9.4-4400 ng/L (Fig. 8a). Similar quality control study was conducted for wastewater samples, but the CV_R for PFOS reached 40 %, and the variation became greater than 30 % which was the guideline value set by ISO. Therefore, the wastewater sample was removed from the samples subjected to analysis in ISO 25101.

After the establishment of ISO 25101, two quality control studies organized by AIST were conducted for JIS standardization, as requests were made by the Japanese laboratories for the compliance of the standard according to the principle of domestic standardization based on the international standard. In the first study conducted from March to July 2008, studies were conducted for tap water, seawater, river water, water containing low-concentration of the standard substance, water containing high-concentration of the standard substance, and the standard substance, using the same analysis method as ISO 25101. There were 13 participating institutes, and the study results were analyzed using the reports submitted by 11 institutes. In most samples, good results were obtained where CV_R of PFOS/PFOA and their related substances was within 30 % (Fig. 8b), and it was confirmed that ISO 25101 could be used in the Japanese analytical laboratories. However, some points had to be reviewed, such as, the variation of the study results for low-concentration water samples and the low recovery rate of long-chain compounds. In the second quality control study conducted from September 2009 to January 2010, the industrial water and plant wastewater were used as the main subjects of measurement for the JIS standardization. There

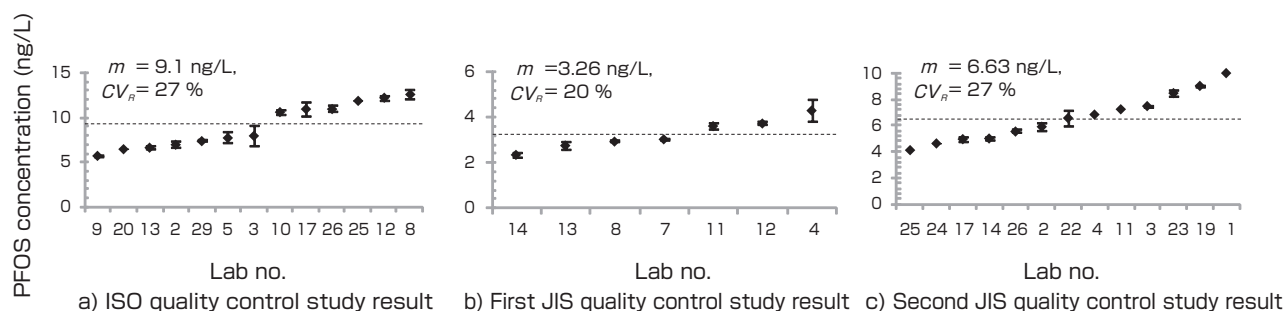


Fig. 8 Result of the PFOS in river water sample for the ISO and JIS quality control tests

Note) m = Average value of measurement, CV_R = Reproducibility coefficient of variation

were 30 participating institutes, and the analyses of the study results were done for the reports submitted by 23 institutes. In this study, satisfactory results ($CV_R < 30\%$) were obtained for all samples of PFOS/PFOA (Fig. 8c). It can be thought that the analysis error due to the difference in the in-house method was controlled by using the SOP, including the reference standard for creating the calibration curve.

5 Feedback for the effort on the international regulation of hazardous chemical substances using the ISO standard

How are these international standards being utilized?

First, for ISO 24293, the necessity of individual NP isomer analysis was recognized internationally, and the sales of reference standards for individual isomers were started by reagent manufacturers. Germany added the measurement for individual isomers when standardizing ISO 18857-2^[32] (analysis method for alkylphenols including NP using the solid phase extraction method and the derivatization method) that is the second part of ISO 18857-1^[9] (analysis method for alkylphenols including NP using the liquid-liquid extraction method; NP is the total measurement).

As an example of the use of ISO 25101, Company S, which is a Japanese manufacturer of semiconductors and assembled products, requested AIST to assess the environmental load and the percentage of PFOS in the chemicals used in their plants from 2003. Company S took measures against the PFOS issue before other companies and regulations were implemented. It obtained the information on the amount of PFOS used and the environmental load in 2006, and shifted to alternative substances, and sufficient safety measures were achieved by the time interviews and surveys were conducted by METI in 2008. On the other hand, the companies that did not take measures against PFOS by 2009 had to struggle to take action in a short period, as they had to determine the usage amount and shift to alternative substances while facing the countdown for regulations. If this was done by a private analytical laboratory without strict quality control, there might have been questions about the reliability of the values reported. The survey results in compliance with the ISO 25101 were readily accepted, and this helped the consideration of the exceptions and essential use according to the Chemical Substances Evaluation Act.

In the analysis, a reference material that has been accurately characterized is necessary to determine the measurement value. Previously, there was no choice but using the values provided by the chemical suppliers, but the National Metrology Institute of Japan (NMIJ), AIST, developed the certified reference material (CRM)^{Term 6} appropriate for ISO 25101 that is traceable to the SI. To strengthen the linkage of the reference material and the standards inside

and outside Japan as much as possible, the development of the reference material was performed concurrently with the international standardization for PFOS, and as a result, quick provision was possible for the CRM related to PFOS. For the development of CRM, NMIJ conducts a management system in compliance to the ISO Guide 34^[33] and ISO/IEC 17025^[34] which are the guidelines for the production of reference materials, and our CRM is produced accordingly. To ensure the traceability to the SI, the application of the primary method of measurement^{Term 7}^[35] is recommended. The freezing point depression method, one of the primary methods, is often chosen in the purity assessment of the organic reference materials. However, since the raw material used was potassium salt of PFOS (K-PFOS) that could be readily refined, the melting point was extremely high (about 300 °C), and it was difficult to obtain accurate results using the purity assessment by the freezing point depression method^{[36]-[39]} (about 150 °C or less) developed by NMIJ. The reproducibility of measurement was improved by using the high- pressure sample crucibles and by applying the reference material for the high-temperature melting point calibration to the freezing point depression method. Hence, it was possible to determine the purity traceable to the SI for the high melting point material such as the K-PFOS (Fig. 9). Therefore, it is expected that the purity assessment of many hazardous materials will become possible by combining this method with the conventional method.^[40] The preparation of solution of this reference material was applied to the

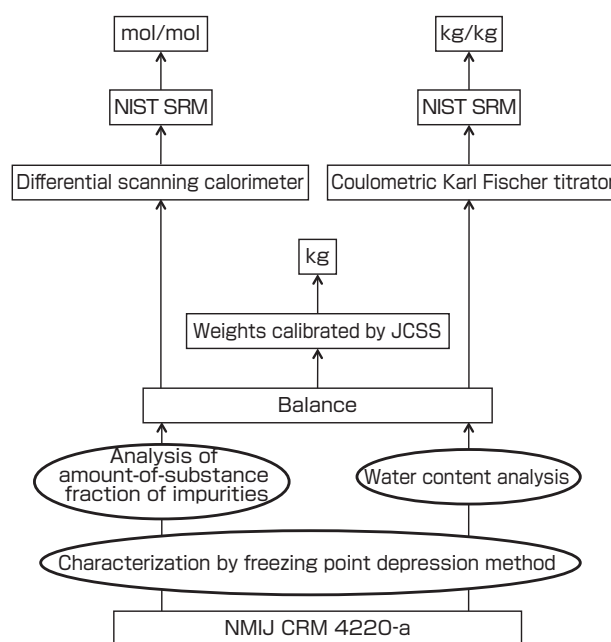


Fig. 9 Traceability diagram of potassium perfluorooctanesulfonate in methanol (NMIJ CRM 4220-a)

Note) JCSS = Japan Calibration Service System; NIST SRM = Standard Reference Material from the National Institute of Standards and Technology (USA).

Table 1. Factors of uncertainty on potassium perfluorooctanesulfonate in methanol

Factors of uncertainty	Relative standard uncertainty(%)
Purity assessment	0.059
Preparation of solution	0.515
Homogeneity	0.474
Stability	0.066
Solvent blank	0.001

Note) Relative standard uncertainty: The relative amount where the uncertainty arising from measurement result expressed by measurement standard deviation etc. (so-called standard uncertainty) is divided by the measurement result.

gravimetric blending method (one of the preparation methods used frequently by the national metrology institutes^[41]), and the concentration of the reference material was calculated by multiplying the dilution ratio and purity of K-PFOS. This concentration (certified value) is traceable to the SI. As described above, upon determining the certified value and uncertainty (Table 1), the development of the reference material of K-PFOS in methanol was completed in FY 2009^[42] (Fig. 10). The ISO international method (March 2009) and the world's first CRM (2010) became available at almost the same time as the addition of PFOS to the POPs Convention (August 2010).

On the other hand, we would like to point out the use of Oasis[®]WAX cartridge as the problem indirectly generated in this standardization. The analysis of the PFOS/PFOA-related substances by ISO 25101 assumes basic knowledge of the analysis method and strict quality control conditions, and the reliability and reproducibility of the analysis value may decrease when such conditions are insufficient. The Oasis[®]WAX cartridge used frequently in SPE can produce excellent results for the various PFOS/PFOA-related substances from C2 to C18, if the appropriate buffer solution

**Fig. 10 Potassium perfluorooctanesulfonate in methanol (NMIJ CRM 4220-a)**

is used as the cleansing fluid for low-concentration samples, as described in the original paper,^[13] which has become the basic technology for ISO 25101 and JIS K 0450-70. However, when the simplified elution method using formic acid (described in guidelines such as Guideline for Wastes Containing PFOS (2010)) was used, we found that the recovery varied greatly depending on the matrix. Therefore, reliability could not be maintained unless the elution condition was optimized sufficiently when the formic acid was used. The reasons for the decrease in the recovery and reproducibility of the short-chain (PFBA) were the same when the SPE cartridge that had similar property as the Oasis[®]WAX cartridge was used. Moreover, in recently conducted research, it was confirmed that particularly strict desalinization and elution condition controls were necessary in cases where the SPE cartridge was used for seawater analysis. If limited to the PFOS/PFOA measurement of carbon chain 8 in the high-concentration sample, the SPE for simple C18 or polymers was easier for less experienced people to use, although there were problems such as the contamination and low recovery of related compounds other than PFOS/PFOA. In the ISO 25101 and JIS K 0450-70, the C18^[23] and polymer SPE (such as the Oasis[®]HLB cartridge^{[26][28]}) were given as examples where SPE cartridges other than the Oasis[®]WAX cartridge could be used in the ISO Annex (Informative) and the JIS Annex (Informative).

At this point, there may be insufficient understanding for the most important essence of QA/QC of how to confirm the reliability of the “PFOS analysis technology using the solid phase extraction and liquid chromatograph tandem mass spectrometer” that is the basic concept of ISO 25101. The problem of ISO is that there is no “explanation” at the end like JIS, and there is hardly any detailed explanation such as the reasons and background for conducting a certain analysis procedure. JIS documents the items that were discussed in the process of JIS standardization and the detailed explanation of the reasons and background of standardization, not just the performance data, so the user can thoroughly understand each procedure. It is necessary to enforce the good laboratory practice (GLP)^{Term 8} by returning to the fundamentals of analytic chemistry; to obtain high quality study data and to ensure that the study data are of the same level and therefore allowing the mutual acceptance of the study data among different countries and laboratories.

6 Conclusions

The CSCL is controlled by three ministries: the Ministry of Environment; Ministry of Economy, Trade and Industry; and the Ministry of Health, Labour and Welfare. The POPs Convention is handled in the same framework. If the sharing of appropriate information and trans-ministry support for international standardization can be achieved, the establishment of several international standards in the environmental analysis field can be expected based

on the valuable findings and technologies of the Japanese environmental pollution science. This covers not only the environment in a narrow sense, but will become the basis for the international solutions for hazardous chemical substance issues including the hazardous substances in products, trans-boundary pollution, and the Basel Convention, etc. To create the risk and dynamic models and to establish policies, it is mandatory to obtain objective and reliable measurement values.

There are three ISO international standards on water quality measurement recently established with Japan as the convener, including ISO 24293^[1] and ISO 25101^[2] that were discussed in this paper, as well as ISO 22719.^[43] Moreover, the international standardization of pH measurement of seawater is in progress. It should be noticed that these are all international standards closely related to the global environmental problem that is one of the most important issues in the international community, including global warning and pollution. This is one of the examples where the ISO's main concept "to solve the international issues through international standards" is expanded to the global environmental issues, and where issues concerning domestic industry and environment policies as well as the international economy are being addressed. In the future, Japan must be responsible as an "advanced nation in environmental issues" for making international contributions by seeking solutions for the environmental issues through realizing the international standardization of many environmental measurements across the framework of different ministries. Also, standardization activity is needed to strengthen the industrial competitiveness through the advance acquisition of problem-solving technologies for environmental issues. In the future, standardization in close collaboration with industries will become essentially important.

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Terminology

Term 1. GC-PFC: Gas chromatograph with the preparative fraction collector system where trace target

substance in complex matrix is collected, concentrated, and purified using the high separation capacity of the GC capillary column.

- Term 2. Two-dimensional gas chromatograph: Method of separating and purifying the interfering substance or the target compound that co-elutes using two capillary columns. The separation capacity is determined by the length and diameter of the capillary column used and the combination of the liquid phase. This method enables advanced separation of the compounds, and also enables highly sensitive analysis by reducing the background of the detector by removing the organic compounds other than the ones subject to analysis from the analysis.
- Term 3. Internal standard: Substance added for the preliminary treatment of sample, the correction of yield in the analysis procedure, or for the confirmation of the recovery. Substance with similar chemical structure as the target substance can be used.
- Term 4. Fragmentation patterns: Pattern of ions produced by the cleavage of molecular ions. The fragmentation patterns reflect the chemical structure of the compound.
- Term 5. Flame ionization detector (FID): Standard detector used in gas chromatography. The backbone carbon of the organic compound is ionized using the combustion heat of hydrogen, and the change in ion current is measured.
- Term 6. Certified reference material (CRM): The reference material (standard substance) for which one or more property values have been certified using the procedure with established traceability (comes with a certificate). Each certified value has uncertainty of certain confidence level.
- Term 7. Primary method of measurement: The method defined as the "method that has the highest quality, of which the procedures are completely explained and understood, its uncertainty can be completely described using the SI unit, and the measurement result can be used as a standard without referring to other standards." The primary methods of measurement in chemical analysis include the gravimetric, titration, coulometric titration, isotope dilution mass spectrometry, and freezing point depression methods.
- Term 8. Good laboratory practice (GLP): The quality control system for the organizational procedures and the condition in planning, execution, monitoring, recording, storage, and reporting, for the purpose of maintaining the reliability and quality of the test performance and for ensuring the test data at certain level in various safety tests. It was established by the Organization for Economic Co-

operation and Development (OECD) in 1981, and was revised in 1997. In the Chemical Substances Evaluation Act, the GLP was introduced in March 1984, and it is in compliance with the GLP principle of OECD.

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Completed the doctor's course at the Graduate School of Natural Science and Technology, Kanazawa University in March 2006. Joined the Potential Pollutants Group, Research Institute for Environmental Management Technology, AIST in April 2006. Engages in the development of the analysis method for persistent organic halogen compounds and the research on the environmental fate. In this paper, was in charge of the development of analysis method, ISO standardization, and JIS standardization of PFOS and PFOA.



Nobuyasu HANARI

Completed the doctor's course in Agricultural Technology and Sciences at the Graduate School of Life and Environmental Sciences, University of Tsukuba in March 2001. Joined the Potential Pollutants Group, Research Institute for Environmental Management Technology, AIST in July 2004, and engaged in the development of the analysis method for dioxin analogs. Transferred to the Organic Analytical Chemistry Division, National Metrology Institute of Japan in October 2005. Engaged in the development of the national standard (NMIJ CRM) of organic reference material (particularly for persistent organic pollutants). Developed the reference materials essential for the operation of JCSS to ensure traceability, as well as general CRM. In this paper, was in charge of the development of potassium perfluorooctanesulfonate reference liquid (NMIJ CRM 4220-a).



Yuichi HORII

Completed the doctor's course at the Graduate School of Science and Engineering, Ibaraki University in March 2006. Post-doctorate researcher at the New York State Department of Health (Wadsworth Center) for two years from May 2006. Joined the Center for Environmental Science in Saitama in October 2008, and engages in the environmental monitoring and fate study of persistent organic pollutants. Current interest is the natural production of dioxin in kaolin clay. In this research, was in charge of the development of the analysis method for individual NP isomers.



Nobuyoshi YAMASHITA

Completed the doctor's course in Environmental Chemistry at the United Graduate School of Agricultural Sciences, Ehime University in 1992. Joined the National Institute for Resources and Environment, Agency for Industrial Science and Technology (current AIST) in 1992. Has been involved in the development of new technologies and their applications in the environmental analytic chemistry field. Has supervised various tests and researches on international



quality control to improve the reliability of analytical chemistry technologies both in Japan and overseas, as well as being various standardizing committee member and TC147/SC2/WG56 convener. Also contributed to the councils of chemical substance management policy such as the member of New POPs Review Committee. Highly Cited Author Award in 2010 (2 articles). Was the supervisor of this research.

Discussions with Reviewers

1 Overall evaluation

Comment (Akira Ono, AIST)

This paper is based on the authors' excellent research results on the analysis technology for hazardous chemical substances in the environment, and the effort was brought to international standardization through international joint collaboration. It can be seen that the synthetic and integrated researches were conducted based on a clear scenario that aims for international standardization, and this research is appropriate as a paper to be published in *Synthesiology*.

It should be noted that in this paper the authors conducted the research and standardization as a set. These standards have been used widely by people in the society after the international standard was published, and that should also be highly evaluated.

2 Expressions in consideration of the general readers

Comment (Akira Ono)

It is hoped that the papers of *Synthesiology* are read not only by specialists of certain technological fields, but also by researchers and engineers of wide-ranging fields. I think the intents of this paper are to introduce the thinking on international standardization, to present some case studies of environmental analysis to a wide range of readers, and to widely share this information.

From this perspective, I see some expressions that may be hard to understand for people whose specialties are not analytical chemistry, so please work on the written expression to ensure better communication.

Answer (Sachi Taniyasu)

As you indicated, there are many terminologies and expressions that may be hard to understand for the researchers and engineers outside the environmental analysis technology specialty, and I also realize that this may prevent understanding of the reader. I have added descriptions to the text and added explanations in "Terminology" to enhance the understanding of the standardization process in this research.

3 Composition of the paper (1)

Comment (Akira Ono)

Synthesiology requires the paper to have the following composition, so please consider revising.

- (1) First, how the research goal is linked to "social values" is explained,
- (2) next, the "research scenario" showing how the authors achieve the research goal is drawn, and then
- (3) the "synthesis process" of how elemental technologies were integrated to achieve the research goal is described.

I think the readers will have better understanding if you summarize the "research scenario" of (2) into a diagram that gives an overview of the entire research. The reviewer suggests a diagram of the "research scenario" within his understanding of this paper, so please consider it as a reference if it is useful.

Answer (Sachi Taniyasu)

- (1) The research goal of this paper is the "standardization of the

analysis method." Therefore, the main objective is to explain the four cases where the international and domestic standards such as ISO and JIS were established using the technological ideas developed by AIST. The chemical substances studied in this research began to be recognized as environmentally hazardous chemical substances when this research started. However, there was no analysis method for which reliability was assured, and it was difficult to understand the environmental load of the chemicals that was important in taking appropriate environmental measures. The need for standardization of the analysis method in such environmental analysis and the social background of the subject compounds were described specifically in chapters 1 and 2. The ISO and JIS systems for environmental analysis and the positioning of this report were described, and texts were added to enhance the understanding of this research.

- (2) In achieving the standardization, unlike the flow of "industrial demand → development of analysis method → quality control → JIS standardization → ISO standardization" that is commonly seen in ordinary standardization, the research was conducted in the flow of "understanding the environmental behavior and environmental loadings necessary to manage the chemical substances → development of analysis method → quality control → industrial demand → JIS standardization." Based on the diagram that you provided for us, we created Fig. 4 as the scenario of this research.
- (3) Details were described about the problems that were discussed in the process of ISO and JIS standardizations and the measures that were taken to solve them. Also, we added some points which were determined to be the normative items (rules to comply) and informative items (information provided for reference).

4 Composition of the paper (2)

Comment (Hiroaki Tao, Research Institute for Environmental Management Technology, AIST)

The composition of this paper is introduction, development of the analysis method, standardization, contribution to chemical substance management, and conclusion. I think the part on the clarification of environmental behavior is lacking. The reason this standardization was successful was because you published the paper on the clarification of environmental behavior and were highly evaluated internationally for conducting such excellent research. Therefore, I think you should add a chapter on the subject of clarification of environmental behavior.

Answer (Sachi Taniyasu)

I have added the text on the discussion of the environmental behavior, in the process of explaining the research elements and scenario in "Chapter 2 Necessity and standardization of the environmental analysis technology that addresses the international regulation of hazardous chemical substances."

5 Necessity of the standardization

Comment (Hiroaki Tao)

I think it will become easier to understand if you explain the chemical formula, properties, use, and what kind of environmental problem it is causing for each compound (such as nonylphenol), as well as the reasons why the existing standardization method cannot help solve the problem and the reasons why you proposed the new standardization.

Answer (Sachi Taniyasu)

I have added the background information on the use, environmental issues, and environmental fate for each compound because they are important in explaining the necessity of the environmental analysis technology, and modified the text so the

readers can understand the process of “standardization.”

6 Content of the standardization

Comment (Akira Ono)

In this paper, you describe how the international standard was created, but you do not describe details of the standard itself. Please describe what items were selected as “normative” and as “informative” respectively in the international standard. Also, please mention the reasons and background for those selections.

Answer (Sachi Taniyasu)

I have added the content of the standard and the background and process of the standardization, in addition to the response to comment 3 of discussion 3.

7 Necessity of the standardization and the standardization process

Comment (Hiroaki Tao)

In the international standardization, I think you should explain the role played by the Standard Certification R&D Project, as it may help the people who are trying for standardization using this system. This time, ISO standardization was first and JIS followed. I think if you explain this process and the problems in doing so, it will be helpful for people who are working on standardization by the reverse process.

Answer (Sachi Taniyasu)

I added the description of the work done in the Standard Certification R&D Project. As you indicated, in this research, the ISO standardization started before the JIS standardization. Also, for the ISO standardization of NP, Germany was investigating the standard for a similar analysis method, and it was necessary to do some preliminary adjustments with the convener country. Since such adjustments are important processes in standardization, I have added the description of this process. The points that were raised in the discussions in the process of ISO and JIS standardizations and the measures taken were described.

8 Relationship between the public institutes and private companies in standardization (1)

Question (Akira Ono)

I think it is often the case in the environmental field, but there is a reality that private companies (or related industrial organizations) are not very interested in the development of standards. The reviewer thinks that the public research institute should take action in the fields that private companies are not interested (or cannot be interested), and that the general public expects the public research institute to take on such a role. Considering such a role of the public research institute, what is the opinion of the authors about the relationship of public research institutes and private companies in the standardization in the environmental field?

Answer (Sachi Taniyasu)

In the standardization in the environmental field that is closely related to the global issues, it is often difficult to obtain support from the industry sector. The authors think that the role to be played by the public research institute is great, as you indicated, and we have engaged in the standardization activities from this perspective. On the other hand, seeking solution technology for the environmental issues will help strengthen industrial competitiveness. Therefore, in the future, I think it is important to develop standardizations in close collaboration with

industries, to contribute to the development of industry as well as to environmental preservation.

9 Relationship between the public institutes and private companies in standardization (2)

Comment (Hiroaki Tao)

One of the reasons that it is difficult to obtain support from industry is because the development of an environmental analysis method may require new measures to prevent environmental pollution, or it may mean more expense for industry. However, recently, quick discovery of the solution to global issues is important in maintaining industrial competitiveness. The standardization to strengthen industrial competitiveness through advance acquisition of problem solving technology is a hope in gaining support from industry for the standardization of environmental analysis methods. I think you should emphasize this point.

Answer (Sachi Taniyasu)

As indicated, it was difficult to gain support from the industrial organizations due to the image that new measures may be needed after standardization. However, taking measures quickly before something becomes a major social issue will encourage seeking a solution for an environmental issue, and this will lead to strengthening of the industrial competitiveness and the healthy growth of industry in the long run. Therefore, I suggested that the standardization in collaboration with industry is necessary in the future.

10 Effect of standardization

Comment (Akira Ono)

The question “How are these international standards being utilized?” in the beginning of chapter 5 is important. That’s because the important objective should not be making a standard, but how the standard is used.

It is important to investigate what aspects of society changed after the standards were delivered and used in the international community, and to compare the actual effect it brought about and the expected effect that was supposed to happen. Please address what were the actual effects in the society after the standards were enforced.

Answer (Sachi Taniyasu)

For the NP standards, two ISO standards were proposed by Germany and one was proposed by Japan. The first standard proposed by Germany (ISO 18857-1) is of total NP measurement, and the measurements of the individual isomers of NP were not described. However, in the Japanese proposed standard (ISO 24293), the necessity of analysis for individual isomers was widely recognized internationally, and the reagent manufacturers started the sales of reference standards for different isomers. The second Germany-proposed standard (ISO 18857-2) was also of total measurement of NP as in Part 1, but because the standards for individual isomers have become available and also because of the importance of the analysis for individual isomers, the measurements for different isomers were added and this enabled more detailed risk assessment using the analysis method. Most private analytical laboratories that subcontract the PFOS/PFOA analysis are in compliance with ISO 25101 and JIS K 0450-70-10, and this enables the provision of high quality and mutually comparable data. I think progresses have been made through this standardization.