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Validation Study to Establish a Standard Test Method for Phthalate in Indoor Air in Japan using Thermal Desorption

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The Japanese Ministry of Health, Labor, and Welfare has established guideline values for indoor air concentrations of eight volatile organic compounds (VOCs) and five semi-volatile organic compounds (SVOCs). In 2019, the Ministry updated the guidelines for three chemicals, including two phthalates, to reduce allowable concentrations. The Manual for the Measuring of Chemicals in Indoor Air proposes methods for analyzing these compounds; however, those for SVOCs, such as phthalates, are tentative. Therefore, in this study, inter-laboratory validation was performed in six facilities to propose a highly sensitive standard test method for the new guideline values of phthalate. The additional amount in this assessment was 5 ng, estimated based on the lowest concentration of the latest guideline values for phthalates (di-*n*-butyl phthalate: 17 µg/m³). The results showed that the average accuracy of each compound at each facility ranged from 73.7 to 150%, with bis(2-ethylhexyl) phthalate exceeding the validation criteria in the two facilities. However, the average accuracy across all facilities ranged from 96.5 to 119%, and repeatability and reproducibility varied from 1.4 to 17% and 10 to 17%, respectively. Therefore, the findings are largely consistent with the criteria for interlaboratory validation studies. This analytical method was proposed and published in the Manual for the Measuring of Chemicals in Indoor Air (Integrated Edition) as a standard test method for phthalates with guideline values established.

Key words indoor air, phthalate, inter-laboratory validation, thermal desorption, standard method

INTRODUCTION

The Japanese Ministry of Health, Labour, and Welfare has established guideline values for indoor air concentrations of eight volatile organic compounds (VOCs) and five semi-volatile organic compounds (SVOCs) to prevent health hazards caused by long-term exposure.[1)] The Committee on Indoor Air Pollution (CIAP) established and revised these rules based on the most recent information and reports. In 2019, the guideline values for xylene, di-*n*-butyl phthalate (DnBP), and bis(2-ethylhexyl) phthalate (DEHP) were revised to lower concentrations.[2)] In particular, the recommended value for DnBP is approximately 1/13th of the pre-revision value, necessitating a more sensitive test. On the other hand, CIAP proposed analytical methods in 2001 with the Manual for the Measuring of Chemicals in Indoor Air.[3)] However, SVOC measurement methods for DnBP and DEHP have been tentatively proposed and have not been fully validated. Therefore, in this study, a validation assessment was performed to verify the measurement method. The manual proposes two methods for measuring DnBP and DEHP: solid-phase adsorption solvent extraction gas chromatography/mass spectrometry (SE) and solid-phase adsorption thermal desorption gas chromatography/mass spectrometry (TD).[3)] The SE method is versatile and requires no equipment other than GC-MS and has been evaluated and published by Tanaka-Kagawa *et al*. for the phthalate method and Yoshitomi *et al*. for the insecticide method.[4,5)] However, this method has a risk of contamination from the environment in the process of extracting and concentrating the target compound, and greater caution is required when analyzing phthalates, which are abundant in the environment. In contrast, the TD method requires a thermal desorption device; however, because the compounds collected in a specialized tube are delivered directly into the GC-MS, there is little environmental contamination, and the sensitivity is high. This study used an inter-laboratory validation at six facili-

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ties, including approximately three types of thermal desorption devices from three manufacturers, to propose a highly sensitive standard test method for the revised phthalate guideline. Furthermore, phthalates with established guideline values, as well as phthalates detected in indoor air, have been evaluated.[6]

MATERIALS AND METHODS

Target Compounds The target compounds are two phthalates for which guideline values are currently set: DnBP and DEHP, and seven other phthalates: diethyl phthalate (DEP), di-*n*-propyl phthalate (DnPrP), diisobutyl phthalate (DiBP), di-*n*-pentyl phthalate (DnPP), di-*n*-hexyl phthalate (DnHP), benzyl butyl phthalate (BBP), dicyclohexyl phthalate (DCHP), for a total of nine compounds. It was optional to assess substances other than DnBP and DEHP in this validation study.

Reagent and Equipment Kanto Chemical Co., Inc. (Tokyo, Japan) provided the phthalates mixed standard stock solution for environmental analysis (mixture of 9 compounds, each 0.1 mg/mL in acetone) and the phthalates internal standard solution for water quality analysis (mixture of 5 compounds, each 0.1 mg/mL in hexane). The internal standard solution consisted of DnBP-d_4, DEHP-d_4, DEP-d_4, BBP-d_4, and di-2-ethylhexyl adipate-d_8. Acetone for phthalic acid ester analysis (Kanto Chemical Co., Inc.) was used to dilute the standard stock solution. The sample tubes were made of stainless-steel and filled with Tenax TA (Chemical Agent Monitoring Supply Co., Houston, TX, USA). The tubes were conditioned for 2 h at 300°C under high-purity nitrogen ventilation using STC-4000 (GL Sciences Inc., Tokyo, Japan).

Preliminary Experiments on Phthalate Stability for Validation A standard solution containing 5 ng of each compound was injected into 15 sample tubes using a microneedle syringe and aerated with high-purity nitrogen gas at 100 mL/min for 1 min. Five tubes were examined immediately after preparation to ensure that each compound was recovered as a 0-day sample. The remaining tubes were sealed in a special container, wrapped in aluminum foil, and stored in aluminum bags. For 7 days, tubes were stored at room temperature (RT, approx. 20°C) or 4°C, and they were analyzed again after storage (n = 5). Table 1 lists the analytical conditions.

Table 1. Analytical Conditions for Sample Stability and Homogeneity Tests

Thermal Desorption Condition	
Instrument	TurboMatrix 650 (Perkin Elmer)
Purge time (min)	10
Purge rate (mL/min)	30
Carrier gas	Helium
Cold trap temp. (°C)	5
Trap desorption (°C)	280
Desorption time (min)	20
Line temperature (°C)	290
Valve temperature (°C)	280
Injection ratio (%)	7.5
GC-MS Condition	
Instrument	GCMS-QP2010 ultra (Shimadzu)
Column	DB-5MS (Agilent) 0.25 mm i.d.×30 m, 0.25 µm
Oven temperature	80 °C (2 min) → 30 °C/min→180°C (5 min) → 10 °C/min→ 250 °C (10 min)
Interface temperature (°C)	260
Ion source temperature (°C)	250

Preparation for Sample Distribution In this assessment, the amount added was based on 17 μg/m^3 (DnBP), which is the lowest concentration in the phthalate guideline values as of 2020, when this study was conducted.[2] Assuming 3 L of indoor air was collected at the residence, the absolute amount equal to 1/10 of the guideline value was 5 ng, which was the amount to be added. This was the smallest volume of air sample at 100-200 mL/min for 30 min or 10-100 mL/min for 24 h. To prepare the tubes for distribution, they were injected with 5 ng of each compound and aerated with high-purity nitrogen gas at 100 mL/min for 1 min. After preparing 35 distribution sample tubes, five were immediately analyzed to ensure homogeneity. Table 1 contains a list of analytical conditions.

Inter-laboratory Validation of Phthalates The analysis was validated in six laboratories: the Hokkaido Institute of Public Health (Sapporo, Hokkaido), Tokyo Metropolitan Institute of Public Health (Shinjuku, Tokyo), Yokohama City Institute of Public Health (Yokohama, Kanagawa), National Institute of Health Sciences (Kawasaki, Kanagawa), Nagoya City Public Health Institute (Nagoya, Aichi), and Meijo University (Nagoya, Aichi). Each facility was assigned an identifying letter (A-F) in no particular order. The concentrations added to the samples were blinded, and the samples were analyzed within seven days of preparation. Six sample tubes were sent: five contained a mixed standard solution and one was a travel blank. After being wrapped with aluminum foil and stored in aluminum bags, the tubes were sealed in a special container. Six sample tubes were analyzed at each of the six facilities using the established method.

Analytical Method Quantitative analysis was performed using the internal standard method, with either DnBP-d_4 (except for DEHP) or DEHP-d_4 (for DEHP) serving as the internal standard. The concentration settings for the calibration curves, internal standard solutions, and analysis conditions for each validity evaluation were not specified; hence, each facility's usual methods were used. In the analytical procedure, the quantitative value was calculated by subtracting the travel blanks. Each facility reported the quantification values of travel blanks, added samples, and the signal-to-noise (S/N) ratio at the lowest concentration of the calibration curve. The ranges of accuracy (recovery rate) and precision (RSD$_r$ and RSD$_R$) values in this investigation were obtained from the organic matter section of the "guideline for the validation of testing method for drinking water",[7] which target chemicals in similar environmental and also serve as validation criteria for instrumental analysis. The targets were 70.0 to 130% accuracy, 20% or less repeatability (RSD$_r$), and 25% or less reproducibility (RSD$_R$).

RESULT AND DISCUSSION

Selection of Sample Tubes According to the manual, the sample tube material should be glass.[3] When choosing stainless-steel tubes for this study, the influence of various materials on desorption during heating was studied. Stainless-steel and glass tubes filled with Tenax TA were used, and the calibration curve findings were compared. Analysis of the standards (1-20 ng) showed that the correlation coefficients of the calibration curves lay between 0.998 and 1.000 for both tubes. The slope of the calibration curve was slightly less for the

Table 2. Sample Stability and Homogeneity Test Results ($n = 5$)

	(a) Stability						(b) Homogeneity	
	0 day		Stored at RT, 7 days		Stored at 4 °C, 7 days			
	Recovery (%)	RSD$_r$ (%)	Recovery (%)	RSD$_r$ (%)	Recovery (%)	RSD$_r$ (%)	Recovery (%)	RSD$_r$ (%)
DnBP	102	3.1	96.6	7.9	94.8	4.4	96.4	4.9
DEHP	103	5.4	99.4	4.9	97.7	4.9	97.7	6.1
DEP	100	4.5	97.5	8.6	90.0	4.3	95.6	4.5
DnPrP	98.3	3.8	93.5	7.9	93.7	3.7	97.0	4.6
DiBP	99.6	3.2	96.5	7.9	94.6	4.8	96.9	4.2
DnPP	96.7	4.0	94.1	8.7	96.1	4.7	96.8	6.4
DnHP	94.7	4.1	93.7	7.6	96.1	4.3	95.5	4.5
BBP	102	7.0	90.0	13	96.6	4.1	98.6	5.0
DCHP	102	5.7	97.2	7.5	102	5.3	100	4.2

stainless-steel tubes than for the glass tubes, ranging from 75 to 89% (data not shown). This degree of sensitivity difference was deemed not to interfere with the performance of this test, and stainless-steel tubes were chosen for this study to avoid damage during transport.

Stability of Phthalate in the Sample Tubes The recovery of compounds after seven days of storage at RT or 4°C following inclusion of the standards was studied. The average recovery immediately after addition and after seven days of storage was 90.0 to 103% for all compounds, with the RSD$_r$ of the five tubes being less than 13% (Table 2(a)). These results suggest that each compound in the sample tubes was still stable after seven days of storage at either temperature. All inter-laboratory studies were completed within seven days of preparing the sample tubes.

Homogeneity Test of Distribution Sample Tubes Random sampling and examination of sample tubes with standard solutions revealed good recovery and accuracy, with average recovery rates of 95.5 to 100% and RSD$_r$ less than 6.4% (Table 2(b)). Five sample tubes were distributed to each participating facility.

Inter-laboratory Validation Study Table 3 lists the analysis conditions for each facility, and Table 4 lists the calibration information, S/N ratio, and travel blank concentration obtained under these conditions.

Although the equipment and analytical settings varied between facilities, the correlation coefficients of the calibration curves and S/N ratio at the lowest concentrations were acceptable. The travel blanks were below the lowest concentrations on the calibration curve for most sample tubes. However, concentrations above the lower limit of quantitation were detected at some facilities, raising concerns regarding contamination during transport. Particularly at Facility D, the DEP values exceeded the quantitative values of the added samples, so they were treated as missing data. Table 5 lists the accuracy, RSD$_r$, and RSD$_R$ values derived from the quantitative data for the six or five facilities. The accuracy of each compound at each facility ranged from 70.0 to 130%. In contrast, DEHP at Facilities E and F exceeded the criteria. DEHP has the highest boiling point of the nine compounds, and there is a concern that, depending on the structure of the thermal desorption device, DEHP may adsorb and desorb into it, affecting quantification. However, the thermal desorption devices used in these two facilities were not the same type. For example, the lengths of the transfer lines to the GC varied, with Facility E having several tens of centimeters and Facility F having approximately one meter. Other facilities that used these thermal desorption devices reported satisfactory results. This suggests that the higher DEHP concentrations at Facilities E and F were not due to the type of thermal desorption device but to other factors. One cause was the very low slope of the DEHP calibration curve at Facility E. This study also included mixed standard and mixed internal standard solutions. When these were used, the slopes of DnBP and DEHP were similar (Table 4). However, the slope of DEHP at Facility E was low at 76% of that for DnBP, suggesting that the quantitative DEHP concentration in the sample overestimated. It was also discovered that the range in DEHP area values in samples delivered to facilities E and F was greater than that of the other four facilities (17.8% and 19.5%, respectively, versus 3.0 to 12.0%, data not shown). One possible explanation is that the desorption temperature of the cold trap was not suitable for the sorbents. The cold trap in Facility F had a double layer of Tenax TA and carbon as a sorbent, but the desorption temperature was 280°C, the same as in facilities using a single layer of Tenax TA. Carbon-based sorbents absorb chemicals more strongly than Tenax TA; therefore, higher desorption temperatures, as found in facilities B and D, may have reduced the variability. It was anticipated that the quantification value would be affected by fluctuations in the amounts introduced into the GC or desorbed from the cold trap. Therefore, adjusting the analytical conditions and reviewing the facility's protocols may improve this situation. Other target validation criteria were met, with RSD$_r$ ranging from 1.4 to 17%, RSD$_R$ from 10 to 17%, and an accurate average of 96.5 to 119% across all six facilities. In conclusion, inter-laboratory validity tests of the TD method were performed on nine compounds, including two phthalates with established guideline values. The findings revealed that satisfactory results were obtained even for samples with concentrations less than 1/10 of the revised guideline values. The study was conducted in six facilities, including three types of thermal desorption devices, and the TD method proved practical in a variety of laboratory environments. On the other hand, it may be necessary to increase the prevalence of expensive thermal desorption devices in order to generalize the usefulness of this study. Moreover, while the TD method has generally been considered less susceptible to environmental contamination than the SE method, our results demonstrated that equivalent care is required. Although there is a challenge of instrument dissemination, the results of this test demonstrate that this analytical method can be presented as a standard test method for phthalates with predetermined guideline values. As a result, this method, which uses stainless-steel tubes, was included in the Manual for the Measuring of Chemicals in Indoor Air (Integrated Edition), which was released as an administrative notification document in 2025.[8)]

Table 3. Conditions for Each Facility

	A	B	C	D	E	F
Thermal Desorption Condition						
Instrument	TurboMatrix 650 (Perkin Elmer)	TD100-xr (Markes)	TD-30 (Shimadzu)	TD100 (Markes)	TD-20 (Shimadzu)	TurboMatrix 650 (Perkin Elmer)
Purge time (min)	10	10	8	10	8	10
Purge rate (mL/min)	30	50	50	50	50	50
Carrier gas	Helium	Helium	Helium	Helium	Helium	Helium
Cold trap sorbent	Tenax TA	Graphitised Carbon	Tenax TA	Graphitised Carbon	Tenax TA	Tenax TA/Carboxen 1000
Cold trap temp. (°C)	5	-10	-20	4	-20	5
Trap desorption (°C)	280	310	280	320	280	280
Desorption time (min)	20	12	5	10	5	20
Line temperature (°C)	290	200	250	250	250	290
Valve temperature (°C)	280	200	250	250	250	280
Injection ratio (%)	7.5	5	10	17.7	10	5
GC-MS Condition						
Instrument	GCMS-QP2010 ultra (Shimadzu)	JMS-Q1500GC (Agilent + JEOL)	GCMS-TQ8030 (Shimadzu)	5977A MSD (Agilent)	GCMS-QP2010 ultra (Shimadzu)	GCMS-QP2010 plus (Shimadzu)
Column	DB-5MS (Agilent)	HP-5MSI (Agilent)	Rtx-5ms (RESTEK)	DB-5MS (Agilent)	DB-1 (Agilent)	DB-5MS (Agilent)
	0.25 mm i.d .× 30 m, 0.25 µm	0.25 mm i.d .× 30 m, 0.25 µm	0.25 mm i.d. × 30 m, 0.25 µm	0.25 mm i.d. × 30 m, 0.25 µm	0.25 mm i.d. × 15 m, 0.1 µm	0.25 mm i.d. × 30 m, 0.25 µm
Oven temperature	80 °C (2 min) → 30 °C/min→ 180°C (5 min) → 10 °C/min → 250 °C (10 min)	50 °C (2 min) → 20 °C/min → 210°C → 4 °C/min→ 280 °C (15 min) → 10 °C/min → 300 °C	60 °C (2 min) → 30 °C/min → 150°C → 6 °C/min → 300 °C (5 min)	80 °C (2 min) → 30 °C/min → 180°C (5 min) → 10 °C/min → 250 °C (10 min)	80 °C (2 min) → 8 °C/min → 210°C (3 min) → 20 °C/min → 300 °C (2 min)	40 °C (2 min) → 25 °C/min → 200°C → 40 °C/min → 280 °C (5 min)
Interface temperature (°C)	260	250	250	250	260	280
Ion source temperature (°C)	250	250	200	200	200	280
Quantitative Ion / Qualifying Ion (*m/z*)						
DnBP	149 / 223	149 / 205, 223	149 / 205, 223	149 / 223	149 / 223, 57	149 / 205, 223
DEHP	149 / 167	149 / 167, 279	149 / 167, 279	149 / 167	149 / 167, 279	149 / 167, 279
DEP	149 / 177	149 / 177	149 / 176, 177	149 / 177	149 / 177, 105	149 / 65, 177
DnPrP	149 / 191	149 / 191	149 / 191, 209	149 / 191	149 / 191	149 / 65, 209
DiBP	149 / 223	149 / 167	149 / 167, 223	149 / 223	149 / 57, 167	149 / 57, 104
DnPP	149 / 237	149 / 219	149 / 150, 237	149 / 237	149 / 219	149 / 43, 237
DnHP	149 / 251	149 / 233	149 / 150, 251	149 / 251	149 / 251, 233	149 / 43, 251
BBP	149 / 206	149 / 206	149 / 206	149 / 206	149 / 206, 91	149 / 104, 206
DCHP	149 / 167	149 / 167, 249	149 / 167, 249	149 / 167	149 / 167, 249	149 / 167, 249
DnBP-d_4	153	153 / 209, 227	153	153	153 / 227	153 / 209, 227
DEHP-d_4	153	153 / 171, 283	153	153	153 / 171	153 / 171, 283

Table 4. Calibration Information, S/N Ratio, and Travel Blank Concentration for the Six Facilities Used for Validation

	A	B	C	D	E	F
Curve range (ng)	1-20	1-25	1-20	1-20	1-20	1-20
Coefficient of correlation	0.991-0.999	0.981-1.000	0.987-0.999	0.995-1.000	0.997-1.000	0.992-0.998
Slope						
DnBP	0.212	0.022	0.130	0.265	0.207	0.125
DEHP	0.208	0.024	0.122	0.250	0.158	0.119
Signal-Noise ratio (S/N ratio, 1 ng)						
DnBP	269	1,690	2,091	1,327	662	180
DEHP	173	279	784	136	107	88
DEP	112	1,161	1,881	286	389	188
DnPrP	96	675	658	504	652	130
DiBP	202	77	1,163	361	621	58
DnPP	198	610	1,357	335	360	73
DnHP	200	52	1,175	272	253	100
BBP	34	30	813	153	109	27
DCHP	96	138	766	116	162	62
Travel blank concentration (ng)						
DnBP	<1	<1	<1	2.2	<1	<1
DEHP	<1	<1	<1	1.1	<1	<1
DEP	<1	<1	<1	-	<1	<1
DnPrP	<1	<1	<1	<1	<1	<1
DiBP	<1	1.1	<1	<1	<1	<1
DnPP	<1	1.6	<1	<1	<1	<1
DnHP	<1	1.6	1.2	1.1	<1	<1
BBP	<1	<1	<1	<1	<1	<1
DCHP	<1	1.4	<1	<1	<1	<1

-: indicates missing data

Table 5. Inter-laboratory Validation Tests

	Accuracy (n = 5 average*, %)						RSD$_r$ (n = 5*, %)						Six facilities**	
	A	B	C	D	E	F	A	B	C	D	E	F	Accuracy (%)	RSD$_R$ (%)
DnBP	101	100	100	73.7	111	119	4.8	5.2	2.2	4.8	10	6.6	101	15
DEHP	104	114	104	104	150	136	4.2	11	1.4	4.2	14	10	119	17
DEP	102	100	103	-	103	126	3.9	5.7	2.3	-	11	6.5	107	10
DnPrP	100	90.9	98.2	84.9	107	125	4.3	4.9	2.1	4.3	11	6.0	101	14
DiBP	101	88.9	98.5	92.9	111	125	4.5	5.8	2.1	4.5	11	5.9	103	13
DnPP	99.0	88.4	91.5	90.4	103	119	6.4	3.4	2.4	6.4	11	8.9	98.6	12
DnHP	94.7	89.4	85.2	94.3	104	115	4.3	3.1	3.4	4.3	9.6	10	97.1	11
BBP	101	100	78.5	92.3	90.8	117	3.9	5.5	7.1	3.9	7.3	17	96.5	13
DCHP	102	94.7	88.9	90.7	104	119	3.7	5.5	3.6	3.7	10	11	100	11

* D: n = 4 average
** The DEP has been assessed with the results of five facilities.
-: indicates missing data

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Conflict of interest The authors declare no conflict of interest.

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