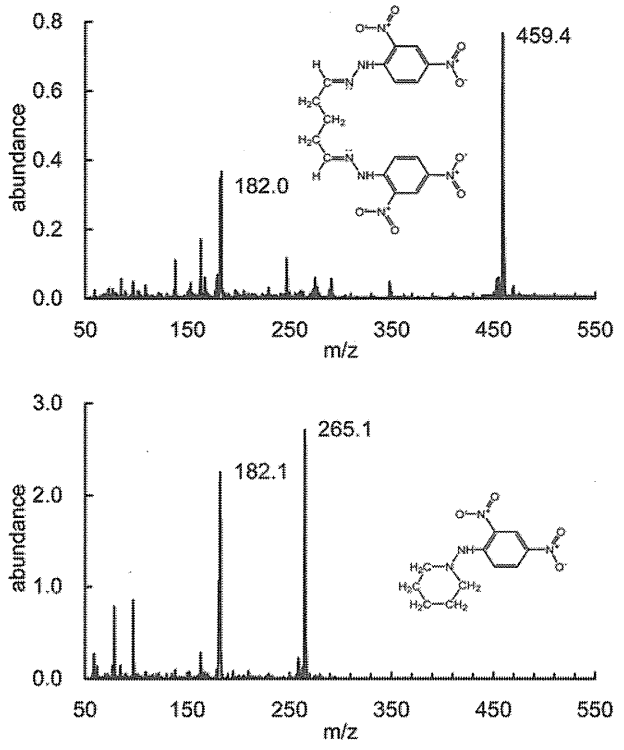

Fig. 3 Mass spectra of the GLA–DNPhydrazone derivative (upper) and its corresponding reduced form (lower). The upper spectrum is for signal C, and the lower spectrum is for signal E.

peaks were converted to their respective reduced form. This reduced form is very stable and did not change when stored for four weeks at room temperature. The synthesized reduced form (described above) was identified as peak E and was confirmed as *N*-(2,4-dinitrophenyl)-1-piperidinamine (DNPPA) from the NMR and LC-APCI-MS data. ^{1}H-NMR data of the derivatives were as follows: *N*-(2,4-dinitrophenyl) piperidin-1-amine. ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 9.08 (1H, d, $J = 2.6$ Hz), 8.95 (1H, s), 8.25 (1H, dd, $J = 2.6$, 9.6 Hz), 7.72 (1H, d, $J = 9.6$ Hz), 3.13 (2H, broad s), 2.52 (2H, broad s), 1.79 (5H, broad s), 1.25 (1H, broad s); ^{13}C NMR ($CDCl_3$, 100 MHz, δ; ppm) 148.0, 136.8, 130.4, 128.9, 124.2, 116.1, 57.2, 25.8, 23.2. The mass spectrum of the peak E in Fig. 2 contains the deprotonated molecule $[M - H]^-$ at *m/z* 265.1 with a molecular weight of 266.2 for DNPPA.

Peak D is DNPH and formed in equal molar amounts to DNPPA (peak E). These results indicate that the reductive amination reaction of GLA–DNPhydrazone using 2-picoline borane proceeds as indicated in Fig. 4.

Catalytic amounts of phosphoric acid to amination of GLA–DNPhydrazone

A 10 mL vial was charged with the GLA–DNPhydrazone derivative (2 mL of a 500 μmol L^{-1} solution in acetonitrile). 2-Picoline borane solution (200 μL of a 0.1 mol L^{-1} solution in acetonitrile) was added, together with phosphoric acid (20–1000 μL of 1 mol L^{-1} solution in acetonitrile). The solution was then diluted to a 10 mL volume with additional acetonitrile. Immediately, the solutions were analyzed by HPLC in isocratic mode at 12.5 min intervals. The chromatographic peaks of the isomers of GLA–DNPhydrazones were initially observed as an inevitable result of the unsymmetrical carbonyl compounds in the acetonitrile solution. When 2-picoline borane was added to the solution, new peaks of DNPPA appeared gradually in front of the corresponding DNPhydrazone peak, and then finally all the GLA–DNPhydrazone peaks were converted to their respective reduced forms. This reductive amination reaction is accelerated by the presence of catalytic acid. Fig. 5 shows the amination reaction of GLA–DNPhydrazone with 2-picoline borane at various concentrations of phosphoric acid. Increasing the concentration of phosphoric acid dramatically increased the reaction rate. The amination reaction of GLA–DNPhydrazone with 2-picoline borane was complete in 10 minutes in the presence of 100 mmol L^{-1} phosphoric acid. The facts that the derivatization reaction requires a catalytic amount of acid and that amine formation is irreversible explain the behavior observed in Fig. 5.

Limit of detection and applications to real sample analysis

The limit of detection (LOD) and the limit of quantitation (LOQ) for the reductive amination analysis of GLA were calculated using linear regression theory.[43] GLA standard (10 μL of a 1 μmol L^{-1}) was introduced into DNPH cartridges. These were eluted with acetonitrile (4 mL each). 2-Picoline borane (100 μL of a 0.1 mol L^{-1} solution in acetonitrile) and phosphoric acid (500 μL of a 1 mol L^{-1} solution in acetonitrile) were added to the eluents. These were then diluted to a final volume of 5 mL with

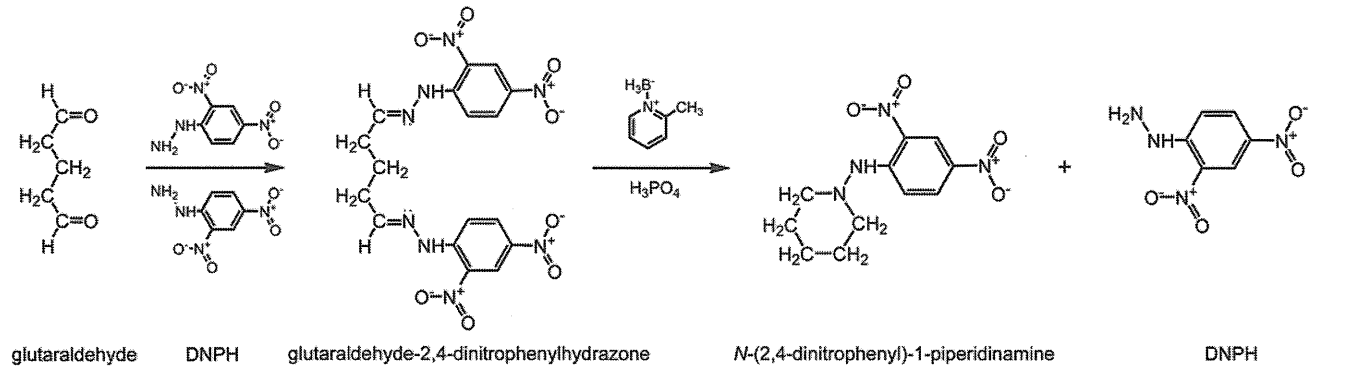

Fig. 4 Reductive amination of GLA–DNPhydrazone with 2-picoline borane.

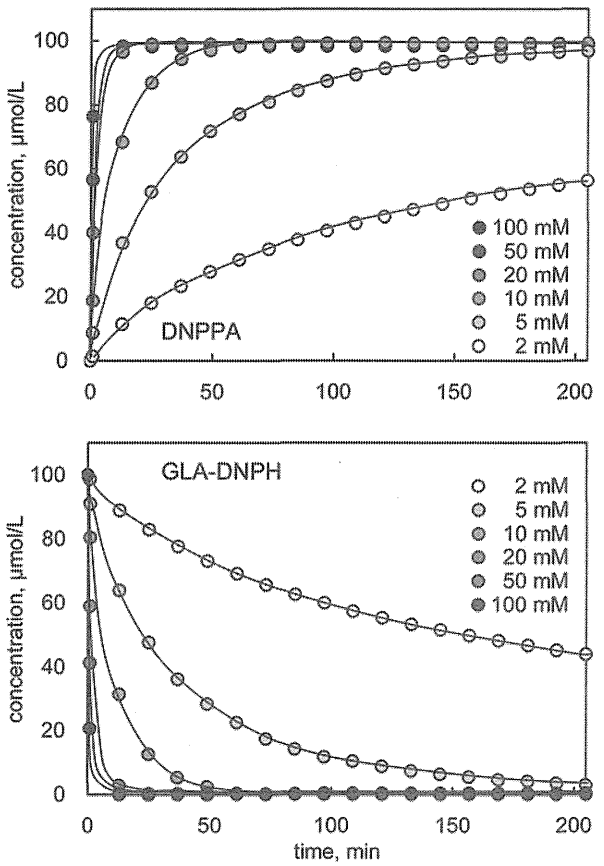

Fig. 5 Changes in GLA–DNPhydrazone (lower) and its reduced form (upper) concentrations with time at various concentrations of phosphoric acid.

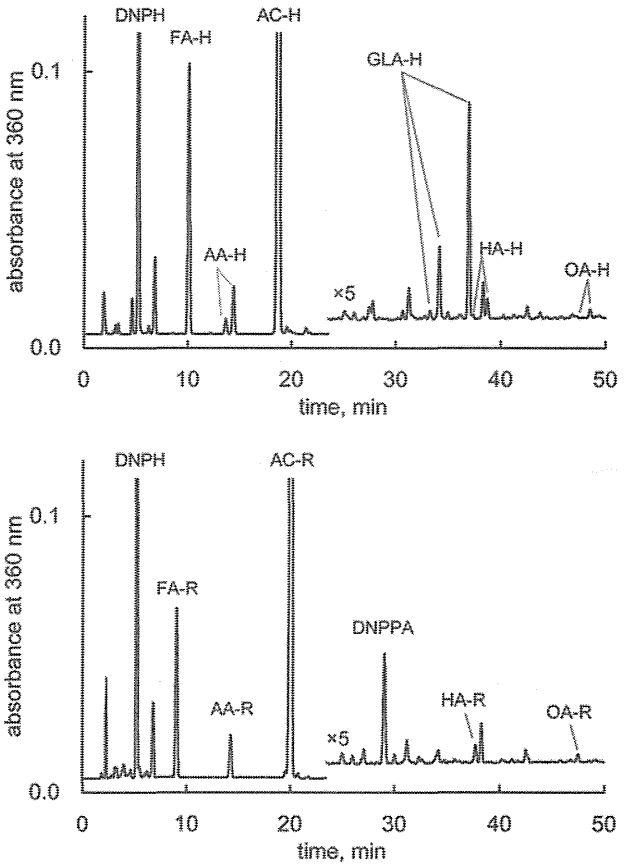

Fig. 6 Chromatographic profiles of DNPhydrazones (upper panel) and their reduced forms (lower panel). The separation was performed in the gradient mode. FA, formaldehyde; AA, acetaldehyde; AC, acetone; HA, hexanal; OA, octanal. "H" indicates the DNPhydrazone derivative and "R" indicates the reduced form of the DNPhydrazone derivative.

additional acetonitrile. After 20 min, the solutions were analyzed using the analytical conditions described above. The LODs were calculated as three times the standard deviation obtained from the data of 10 replicate measurements. The reproducibility of the reductive amination analysis was estimated from data of ten DNPH cartridges spiked with 10 μL of GLA standard solution (50 μmol L^{-1}). LOD, LOQ and the relative standard deviation (RSD) are shown in Table 1. GLA-A, GLA-B and GLA-C indicate the geometric isomers of GLA–DNPhydrazone. GLA-T indicates the sum of isomers.

Workplace air from a pathology laboratory was collected using a DNPH cartridge at a flow rate of 500 mL min^{-1} for 6 hours.[42] After collection, the DNPH-cartridge was eluted with acetonitrile (4 mL). 2-Picoline borane (100 μL of a 0.1 mol L^{-1} solution in acetonitrile) and phosphoric acid (500 μL of a 1 mol L^{-1} solution in acetonitrile) were added to the eluent. This was then diluted to a final volume of 5 mL with additional acetonitrile. After 20 min, the solution was analyzed using the analytical conditions described above. Concentrations (mg L^{-1}, μg m^{-3}) in the sample solution (mg L^{-1}) and in the air (μg m^{-3}) determined by the reductive amination method were (2.5, 35) for formaldehyde, (0.84, 12) for acetaldehyde, (38, 530) for acetone, (0.77, 11) for GLA, (0.13, 1.8) for hexanal and (0.072, 1.0) for octanal, respectively. Fig. 6 shows the chromatographic profiles of the DNPhydrazones and corresponding reduced forms obtained from workplace air. As can be clearly seen, adding 2-picoline borane solution to the eluate caused all DNPhydrazone derivatives, including GLA's three geometric isomers of *E–E*, *E–Z* and *Z–Z*, to be completely converted to their respective reduced forms (lower panel) producing a simpler chromatographic profile.

Table 1 LOD, LOQ and reproducibility for the reductive amination analysis. GLA-A, -B, -C and -T correspond to signals in Fig. 2 and total

	LOD, nmol	LOQ, nmol	RSD, %
DNPPA	0.14	0.48	0.52
GLA-A	1.00	3.40	4.90
GLA-B	0.15	0.51	1.10
GLA-C	0.12	0.40	0.94
GLA-T	0.31	1.00	1.00

Conclusions

The traditional method for the measurement of GLA, using DNPH to form the corresponding DNPhydrazone derivative, is susceptible to analytical errors because DNPhydrazones form three geometric isomers (*E–E*, *E–Z* and *Z–Z*) as a result of the two C=N double bonds. To overcome this issue, a method for transforming the C=N double bond into a C–N single bond,

using reductive amination of DNPhydrazone derivatives, has been applied. The reduction product is unique and identified as DNPPA. DNPPA is very stable and has high solubility in acetonitrile. The reductive amination method developed in this study overcomes analytical errors caused by *E–E*, *E–Z* and *Z–Z* geometrical isomers.

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Short communication

Ozone removal in the collection of carbonyl compounds in air

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ABSTRACT

The most widely used method for measuring carbonyl compounds in air is 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by high-performance liquid chromatography (HPLC). However, substantial negative interference caused by the presence of ozone in air has been reported. To avoid the influences of ozone, a potassium iodide scrubber (KI-scrubber) is commonly used. However, when air sampling using a DNPH-cartridge and a KI-scrubber is performed under conditions of high humidity, moist potassium iodide in the KI-scrubber traps carbonyls before they reach the DNPH-cartridge. Moreover, wet KI reacts with I_2 to form KI_3 and this oxidative reagent moves to the DNPH-cartridge and destroys the DNPH and DNPhydrazone derivatives. In order to alleviate these problems, new ozone scrubbers (BPE-scrubber, HQ-scrubber) have been developed. BPE-scrubber and HQ-scrubber consist of silica gel particles impregnated with *trans*-1,2-bis-(2-pyridyl) ethylene (BPE) and hydroquinone (HQ), respectively. BPE reacts with ozone to form pyridine aldehyde and HQ reacts with ozone to form benzoquinone. The amounts of reducing agent in silica gel (130 mg) for ozone scrubber are 1% (w/w) for BPE-cartridge; 0.2% (w/w) for HQ-scrubber. These scrubbers can be used in air containing 140 $\mu g/m^3$ of ozone for 24 h at a flow rate of 200 mL/min. When the relative humidity exceeded 80%, KI in the KI-scrubber was gradually moistened and changed to yellow in color. Peak abundance of formaldehyde, acetaldehyde and acetone DNPhydrazones was diminished to 25%, 15%, and 2%, respectively, compared with the BPE-scrubber or HQ-scrubber. When using a BPE-scrubber or HQ-scrubber, decomposition of DNPH and DNPhydrazones was not observed at a wide range of relative humidities (3–97%).

1. Introduction

Formaldehyde, acetaldehyde and other carbonyl compounds are ubiquitous pollutants that are formed through oxidation of hydrocarbons by ozone in the troposphere [1–3] and by the reaction between ozone and terpenoid in indoor air [4–6]. Long-term exposure to relatively high levels of carbonyl compounds such as formaldehyde and acetaldehyde is known to increase the risk of asthma [7] and cancer [8]. Accurate aldehyde measurements are therefore important both for determining the formation mechanism of aldehydes and for evaluating the implications for human health.

The most widely used method for qualitative and quantitative analyses of carbonyl compounds is 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by high-performance liquid chromatography (HPLC). Sampling can be performed using acidic solutions of DNPH in impingers or with acidic DNPH-coated solid sorbents in a cartridge. At the present time, a number of cartridge devices packed with DNPH-coated silica gel particles are commercially available for sampling aldehydes in air. Due to the importance of the method, it has been introduced as a standard procedure by several national and international standardization bodies.

While the derivatization reaction at first glance appears straightforward, substantial negative interference caused by the presence of ozone in the air sample has been reported [9–11]. Ozone decomposes DNPH and DNPhydrazone derivatives to form 2,4-dinitrophenol, 2,4-dinitroaniline and 1,3-dinitrobenzene [12]. Additionally, Rodier and Birks reported that sampling atmospheres containing isoprene and ozone lead to the formation of artifact carbonyl peaks in a system using DNPH or dansylhydrazine-coated C18 cartridges [13]. The peaks were purportedly due to a reaction of isoprene with ozone on the cartridge surface, which led to positive artifacts for a number of compounds including formaldehyde.

To avoid the influences of ozone, a potassium iodide scrubber (KI-scrubber) can be used to destroy ozone before sampling the carbonyl compounds. In this case the air sample is first drawn over a surface on which solid KI is adsorbed. Ozone reacts with KI to form iodine and potassium hydroxide (Fig. 1). At the present time, KI-scrubbers are commercially available from many suppliers and are widely used. However, KI-scrubbers have two disadvantages. When air sampling using a DNPH-cartridge with a KI-scrubber is performed at high humidity, moist potassium iodide in the

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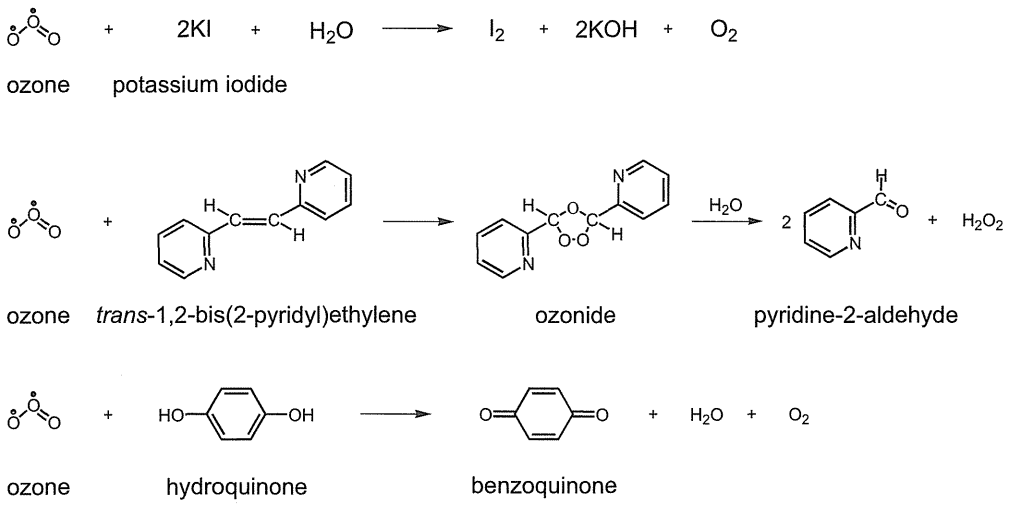

Fig. 1. Reactions of ozone with reducing agents.

KI-scrubber traps carbonyl compounds before they can reach the DNPH-cartridge. Moreover, wet KI reacts with I_2 to form KI_3 and this oxidative reagent moves to the DNPH-cartridge and destroys the DNPH and DNPhydrazone derivatives.

We have previously developed a method for the simultaneous determination of ozone and carbonyls in air using a two-bed cartridge system [14,15]. Each bed consists of reagent-impregnated silica particles. The first contains *trans*-1,2-bis-(pyridyl) ethylene (BPE), while the second contains 2,4-dinitrophenylhydrazine (DNPH). Air samples are drawn through the cartridge first through the BPE and then through the DNPH. Ozone in the air sample is trapped in the first bed by the BPE-coated silica particles and produce pyridine aldehyde (Fig. 1). In this method, BPE acts as an ozone scrubber. We have also developed a method for the determination of acrolein and other carbonyls in cigarette smoke using a dual cartridge system [16]. Each cartridge consists of reagent-impregnated silica particles. The first contains hydroquinone (HQ) for the inhibition of acrolein polymerization, while the second contains DNPH for the derivatization of carbonyls. HQ is a radical and ozone-trapping reagent and is used to inhibit acrolein radical polymerization and to remove ozone. Ozone reacts with HQ to form benzoquinone (Fig. 1). Thus, both BPE and HQ can function as ozone scrubbers. In this study, the effectiveness of KI-scrubber, BPE-scrubber and HQ-scrubber as ozone removers was investigated.

2. Experimental

2.1. Apparatus and reagents

The HPLC system (Shimadzu, Kyoto, Japan) used included two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photodiode array detector. The analytical column was an Ascentis Express RP-Amide, 2.7 μm particle size, 150 mm × 4.6 mm i.d. column (Supelco Inc, Bellefonte, PA, USA). Solution A of the mobile phase mixture was acetonitrile/water (45/55, v/v) containing 5 mmol/L ammonium acetate and solution B was acetonitrile/water (75/25, v/v). HPLC elution was carried out with 100% A for 5 min, followed by a linear gradient from 100% A to 100% B in 50 min and then held for 10 min. The flow rate of the mobile phase was 0.7 mL/min. The column temperature was 40 °C, the autosampler temperature was 25 °C and the injection volume was 10 μL.

The environmental test chamber, supplied by Ohnishi Netsugaku Co., Ltd., Tokyo, Japan, was used for the sampler exposure tests. The test chamber had a volume of 34.8 m^3 (4.2 m × 3.6 m × 2.3 m) and was equipped with an adjustable constant temperature and humidity controller. Ozone gas was generated using an Ozone Generator (TGO-1, Funatech Ltd., Tokyo, Japan). Air pumps (MP-Σ30N, Shibata Scientific Technology Ltd., Tokyo, Japan) and wet gas meters (WS D-1A; Shinagawa Co., Tokyo, Japan) were used for air sample collection. The humidity and temperature of standard ozone gas were recorded using a TR-72U data logger (T&D Corporation, Japan).

Water used for HPLC and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA, USA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) and *trans*-1,2-bis(2-pyridyl)ethylene (BPE, >97%) were purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade, >99.9%), hydroquinone (HQ, >99%), 2-pyridinecarboxaldehyde (pyridine-2-aldehyde, 99%), phosphoric acid (85% solution in water), hydrochloric acid (37%), and ammonium acetate (99.999%) were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Rezorian Ozone Scrubbers and LpDNPH Rezorian cartridges were obtained from Supelco Inc. Silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was obtained from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan). Pyridine-2-aldehyde 2,4-DNPhydrazone was synthesized according to previously reported methods [15].

2.2. Preparation of a *trans*-1,2-bis(2-pyridyl)ethylene-impregnated silica cartridge (BPE-scrubber) and a hydroquinone-impregnated silica cartridge (HQ-scrubber)

Silica gel (50 g) was washed with water (3× 500 mL), methanol (2× 500 mL), and lastly acetonitrile (2× 500 mL). The solvent was then completely evaporated to dryness at 100 °C for 30 min under vacuum on a rotary evaporator. After cooling to room temperature, acetonitrile (200 mL) was added to the washed silica gel. BPE (0.5 g) or HQ (0.1 g) was dissolved in 50 mL acetonitrile. This solution was added to the washed silica gel, the mixture was stirred and the solvent was evaporated to dryness at 40 °C under vacuum on a rotary evaporator. BPE-impregnated silica (130 mg) or HQ-impregnated silica (130 mg) was packed into polyethylene cartridges (Rezorian tube, 1 mL, Supelco Inc, Bellefonte, PA) and stored in a refrigerator at 4 °C.

The commercially available ozone scrubbers used in this study contained 1.5 g of potassium iodide.

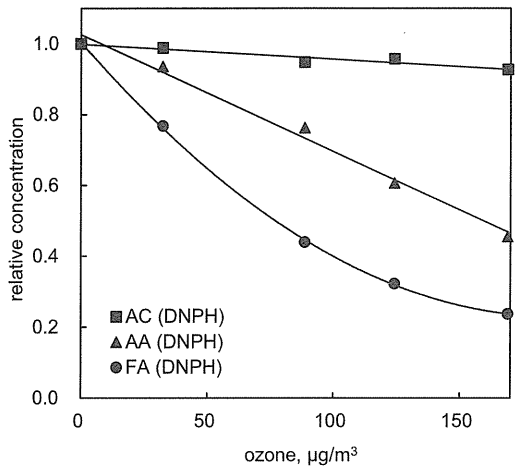

Fig. 2. Changes in measured carbonyl concentrations with the coexistent ozone concentration. DNPH-cartridges were used without an ozone scrubber. FA: formaldehyde; AA: acetaldehyde; AC: acetone.

2.3. Air sampling and analysis

Prior to air sample collection, each KI-scrubber, BPE-scrubber or HQ-scrubber was connected to a DNPH-cartridge to construct a dual-cartridge sampling train (KI-DNPH, BPE-DNPH and HQ-DNPH). Air was drawn through a coupled cartridge pair from the ozone scrubber to the DNPH-cartridge at a flow rate of 200 mL/min. After collection, the coupled cartridges were extracted. In the case of KI-DNPH, the KI-cartridge was discarded and the DNPH-cartridge was eluted with acetonitrile to a final volume of 5 mL. With BPE-DNPH, elution was performed in the reverse direction to air sampling. An intact, coupled cartridge pair was eluted with 30% dimethyl sulfoxide in acetonitrile solution containing 0.085% (v/v) phosphoric acid to a final volume of 5 mL. The HQ-DNPH cartridge pair was also eluted intact and in the reverse direction to air sampling. Acetonitrile was used as the elution solvent to a final volume of 5 mL. After 30 min of elution, the eluates were analyzed by HPLC.

3. Results and discussion

3.1. Decomposition of DNPhydrozones by ozone

An ozone generator was operated in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 50%. The concentrations of formaldehyde, acetaldehyde and acetone in the environmental test chamber were 14, 15 and 8.0 μg/m^3, respectively. Air sampling was performed by using a DNPH-cartridge without an ozone scrubber for 24 h at a flow rate of 200 mL/min. After collection, DNPH-cartridges were eluted with acetonitrile and analyzed by HPLC. Fig. 2 shows changes in the concentrations of carbonyl compounds with changes in ozone concentration. Carbonyl concentrations are expressed as relative concentrations in Fig. 2. Concentrations of formaldehyde and acetaldehyde decreased dramatically with increased ozone concentration. When the concentration of ozone is 170 μg/m^3, the measured concentrations of formaldehyde and acetaldehyde are 20% and 45%, respectively, of the concentrations measured when no ozone is present. Alternatively, the measured concentration of acetone remained within 5% of the concentration when no ozone was present.

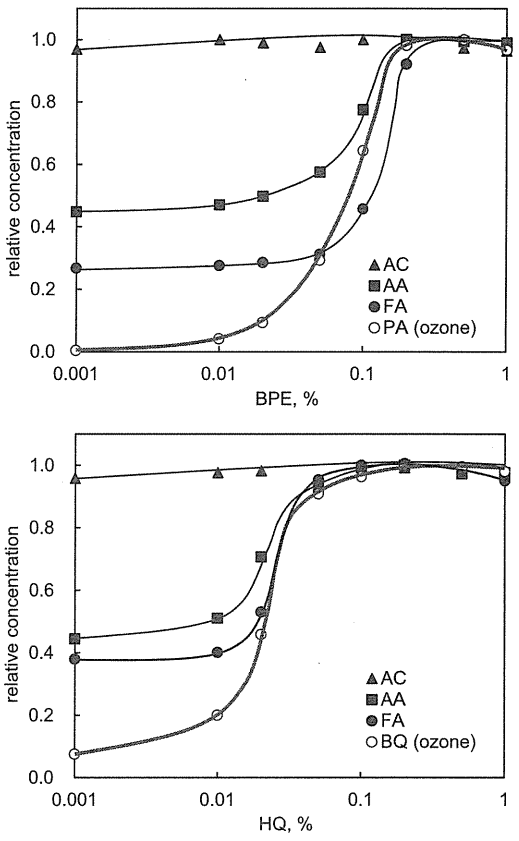

Fig. 3. Changes in the concentrations of carbonyl compounds with the contents of BPE (upper panel) and HQ (lower panel) in the ozone scrubber at a temperature of 25 °C and a relative humidity of 50%. FA: formaldehyde; AA: acetaldehyde; AC: acetone; PA; pyridine-2-aldehyde; BQ: benzoquinone.

3.2. Preferable contents of BPE and HQ in silica gel

An ozone generator was operated in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 50%. The ozone concentration reached a constant value of 140 μg/m^3 after 24 h. The concentrations of formaldehyde, acetaldehyde and acetone in the environmental test chamber were 13, 11 and 8.0 μg/m^3, respectively. BPE-scrubbers containing 0–1% (0–7.1 μmol) of BPE and HQ-scrubbers containing 0–1% (0–12 μmol) of HQ were connected to DNPH-cartridges. Air sampling was performed for 24 h at a flow rate of 200 mL/min. After collection, BPE-DNPH-cartridges were eluted with 25% dimethyl sulfoxide in acetonitrile solution containing 0.085% (v/v) phosphoric acid and HQ-DNPH-cartridges were eluted with acetonitrile. After 30 min following elution, the eluate was analyzed by HPLC. Fig. 3 shows changes in the relative concentrations of carbonyl compounds with the loading of BPE (upper panel) and loading of HQ (lower panel) in the ozone scrubber.

In the case of BPE-DNPH cartridge, concentrations of formaldehyde, acetaldehyde, acetone, and pyridine-2-aldehyde increased with increasing BPE concentration, and reached a maximum value when the BPE concentration exceeded 0.5% (3.6 μmol). HQ-DNPH cartridges exhibited similar behavior. Measured concentrations of carbonyls reached a plateau when the HQ concentration exceeded 0.1% (1.2 μmol). HQ may react more efficiently with ozone than BPE. Based on the data presented in Fig. 3, appropriate ozone scrubbers with 130 mg silica support packing should contain 1% BPE-cartridge and 0.2% HQ-cartridge.

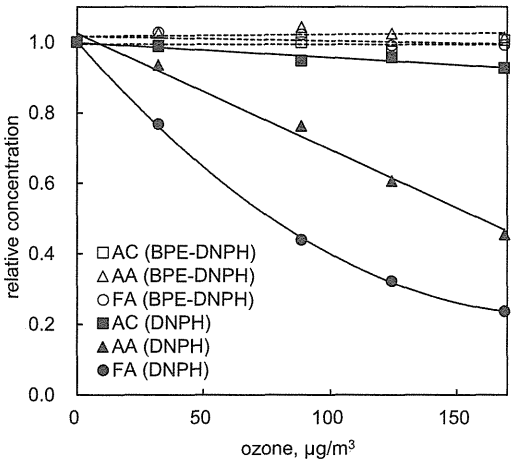

Fig. 4. Changes in measured carbonyl concentrations with the coexistent ozone concentration. DNPH-cartridges were used with BPE-scrubber and without an ozone scrubber. FA: formaldehyde; AA: acetaldehyde; AC: acetone.

Under the same conditions described in Fig. 2, KI-DNPH, BPE-DNPH and HQ-DNPH methods showed good performance in ozone removal and analysis of carbonyls. Broken lines of Fig. 4 show changes in measured carbonyl concentrations with the coexistent ozone concentration by using BPE-DNPH. Decreases of formaldehyde, acetaldehyde and acetone were not observed at a wide range of ozone concentrations (0–170 μg/m^3). HQ-DNPH and KI-DNPH exhibited similar behavior to BPE-DNPH.

3.3. Influence of humidity on the ozone scrubbers

The environmental test chamber was set to a temperature of 25 °C and relative humidity was varied from 3% to 97%. The concentrations of ozone, formaldehyde, acetaldehyde and acetone in the environmental test chamber were 70, 40, 12 and 9.0 μg/m^3, respectively. Air sampling was performed by using KI-DNPH, BPE-DNPH and HQ-DNPH for 24 h at a flow rate of 200 mL/min. Fig. 5 shows the chromatographic profiles of the eluates eluted from KI-DNPH, BPE-DNPH and HQ-DNPH cartridges. In the case of KI-DNPH, when the relative humidity exceeded 80%, KI in the KI-scrubber was gradually wetted and changed to yellow in color (KI_3). The liquefied, wet KI migrated into the DNPH-cartridge where the DNPH-silica was discolored to brown. DNPH was decomposed by the wet KI and the DNPH peak was not detected in the chromatogram. Peak abundance of FA-D, AA-D and AC-D was diminished to 25%, 15%, 2%, respectively, relative to the same peaks when BPE-DNPH or HQ-DNPH was used. It is suggested that carbonyl compounds dissolve in the wet KI because carbonyl compounds are polar, hydrophilic and water-soluble. In the case of BPE-DNPH and HQ-DNPH, decomposition of DNPH was not observed and large unreacted DNPH peaks were detected. Peak abundance of FA-D, AA-D and AC-D is of the same order of magnitude as

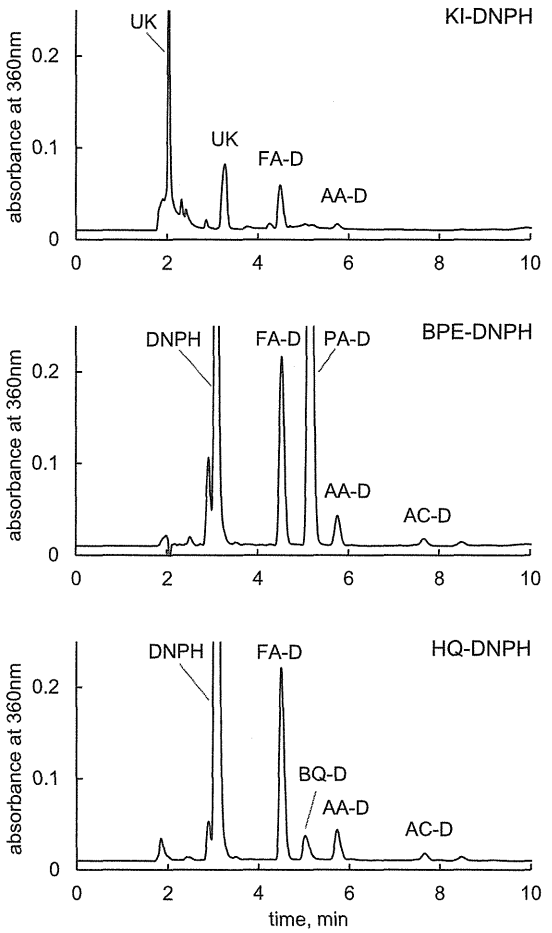

Fig. 5. Chromatographic profiles of DNPhydrazones obtained from KI-DNPH (upper panel), BPE-DNPH (middle panel) and HQ-DNPH (lower panel) methods. FA-D: formaldehyde DNPhydrazone; AA-D: acetaldehyde DNPhydrazone; AC-D: acetone DNPhydrazone; PA-D: pyridine-2-aldehyde DNPhydrazone; BQ-D: benzoquinone DNPhydrazone; UK: unknown compound.

those measured by both BPE-DNPH and HQ-DNPH under dry conditions.

The large PA-D peak in the BPE-DNPH chromatogram is the derivative derived from PA and DNPH. PA is formed by the reaction of BPE with ozone [15]. Therefore, it is possible to determine ozone concentration by measuring PA concentration quantitatively [15]. By the same token, BQ-D peak in the HQ-DNPH is the derivative derived from BQ and DNPH (Fig. 6). Ozone reacts with HQ completely to form BQ, however, partial subsequent reaction with DNPH also occurs. Ozone concentration can be determined by summing BQ-D and underivatized BQ concentrations.

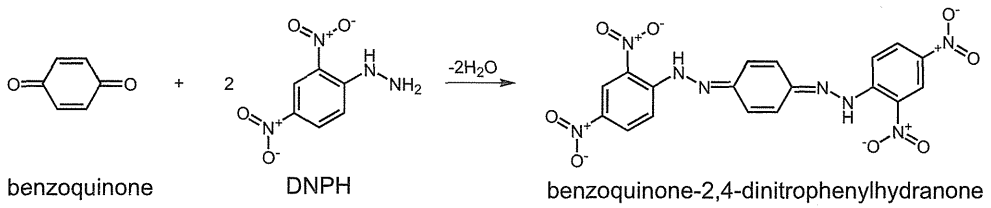

Fig. 6. Reaction of benzoquinone with DNPH.

4. Conclusions

For measuring carbonyl compounds using DNPH-cartridge, a potassium iodide has been widely used as ozone scrubber. However, when air sampling is performed under conditions of high humidity, moist potassium iodide in the KI-scrubber traps carbonyls before they reach the DNPH-cartridge. Moreover, wet KI reacts with I_2 to form KI_3 and this oxidative reagent moves to the DNPH cartridge and destroys the DNPH and DNPhydrazone derivatives. BPE-DNPH and HQ-DNPH methods have the advantage of air sampling under high humidity conditions without the problems associated with the hygroscopic nature of potassium iodide, and besides, these methods allow the simultaneous measurement of ozone and carbonyl compounds.

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Technical note

A diffusive sampling device for measurement of ammonia in air

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ABSTRACT

A diffusive sampling device, the DSD-NH_3, has been developed for measurement of ammonia in air. The DSD-NH_3 comprises silica gel impregnated with phosphoric acid as the absorbent, a porous sintered polyethylene tube that acts as a diffusive membrane, and a small polypropylene syringe that is used for the elution of analytes from the absorbent. Silica gel impregnated with phosphoric acid is used as absorbent for the DSD-NH_3; basic gases in ambient air, including ammonia, are trapped in the DSD-NH_3 device by their reaction with phosphoric acid in the sampler to form their corresponding phosphoric acid salts. After collection, the DSD-NH_3 samplers are eluted by water. Cations in the eluate, including ammonium ions, are analyzed by ion chromatography. A side-by-side comparison was made with active samplers, demonstrating good correlation ($r^2 = 0.996$). The sampling rate (94.5 ml min^{-1}) was determined from comparison with an active sampling method and sampling rates. The sampling rate is also calculated from the respective molecular weights according to a rule based on Graham's law. The theoretical sampling rate with the DSD-NH_3 is 95.4 ml min^{-1} and agrees with the experimental value (94.5 ml min^{-1}). Little influence of wind velocity on the sampler was observed. The relative standard deviations for ammonia concentrations were 4.7% with face velocity ranging 0–5.0 m/s.

1. Introduction

Atmospheric ammonia is the most prevalent base gas and its role in the neutralisation of atmospheric acidic compounds such as hydrochloric acid, nitric acid, sulphuric acid and ammonium hydrogen sulphate is of primary relevance. Ammonia is used to make household cleaners, refrigeration units, fertilizers, explosives, fuels, and other chemicals. Humans and animals produce ammonia in their intestinal tracts and release ammonia in urine. Ammonia is considered a high health hazard because it is corrosive to the skin, eyes, and lungs. Ammonia levels below 1 ppm are not expected to cause health problems. Exposure to household ammonia gas above 1 ppm can cause irritation of the eyes, nose, and throat in some people. Most people can begin to detect ammonia odors when it is at least 1 ppm. Exposure to more concentrated levels, above 25 ppm, can cause headaches, nausea, and intense burning of the eyes, nose, throat, and skin. Consequently, reliable methods for monitoring ammonia in air are required.

Measuring gaseous ammonia in air is not an easy task due to the existing equilibrium between gaseous ammonia and particle components such as ammonium nitrate and ammonium chloride, which are appreciably volatile at ambient temperature. Good results in determining gaseous ammonia and particulate ammonium have been obtained by the diffusion denuder technique, which allows the selective removal of the gaseous phase on the denuders and the subsequent collection of the particles on a downstream filter pack (Perrino and Gherardi, 1999). Similar to the diffusion denuder, a diffusive sampler using permeable media such as a porous polytetrafluoroethylene tube (Uchiyama et al., 1999) or a porous sintered polyethylene tube (Uchiyama et al., 2004) can collect gaseous compounds through the gas and particle mixture.

A diffusive sampler is generally preferable to an active sampler for measurement of indoor air concentrations and personal exposure because diffusive samplers are small, lightweight, and work without power. Moreover, diffusive samplers can be deployed in large numbers over wide areas for monitoring and modeling dispersion and the fate of gases released from various sources (Roadman et al., 2003). The efficacy of diffusive samplers in measuring atmospheric ammonia has been shown in previous

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research (Carmichael et al., 2003; Perrino and Catrambone, 2004; Thoni et al., 2003). In our previous study, we developed diffusive sampling device (DSD) methods: DSD-voc for measurement of volatile organic compounds (Uchiyama et al., 1999), DSD-DNPH for measurement of carbonyl compounds (Uchiyama et al., 2004) and DSD-BPE/DNPH for simultaneous measurement of ozone and carbonyl compounds (Uchiyama et al., 2011), which have a porous extended polytetrafluoroethylene tube or a porous sintered polyethylene tube as diffusion-permeable media. These samplers offer many advantages over traditional samplers, including a rapid sampling rate, simplicity of operation, and omnidirectionality; moreover, these samplers can be connected to a pump and used for active sampling applications. The sampling rates when using DSD samplers are calculated according to a rule based on Graham's law, according to which the rate of diffusion of a gas is inversely proportional to the square root of the density of the gas; therefore, the sampling rate of the target compound can be calculated without using any standard gas. In this study, we extended the DSD method to the diffusive sampling device (DSD-NH_3) for the measurement of ammonia in air.

2. Experimental

2.1. Reagents

The water used in ion chromatography (IC) and sample preparation was deionized and further purified using a Milli-Q Water System (Millipore, Bedford, MA, USA). Acetonitrile (HPLC grade, >99.9%), methanol (HPLC grade, >99.9%), and methanesulfonic acid (≥99.5% solution in water) were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Phosphoric acid (85% w/v solution in water, pure grade) and the standard solution of ammonium ion were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was obtained from AGC Si-Tech Co. Ltd. (Fukuoka, Japan).

2.2. Apparatus and IC analysis

The Dionex DX-120 IC system was used with an IonPac CS16 analytical column (250 mm × 5 mm i.d.) and a self-regenerating suppressor (CSRS 300, 4 mm) for the measurement of ammonium ion. The mobile phase was 30 mmol l^{-1} methanesulfonic acid, the flow rate was 0.85 ml min^{-1}, and the injection volume was 25 μl.

An environmental test chamber supplied by Ohnishi Netsugaku Co. Ltd. (Tokyo, Japan) was used for the sampler exposure tests. The test chamber had a volume of 34.8 m^3 (4.2 × 3.6 × 2.3 m) and was equipped with an adjustable constant-temperature and humidity controller. Wind velocity in the test chamber is about 1 m s^{-1}. Air pumps (MP-Σ30N, Sibata Co., Saitama, Japan) and a wet gas meter (WS-1A, Shinagawa Co., Tokyo) were used for collecting air samples.

2.3. Preparation of silica gel impregnated with phosphoric acid

Silica gel (50 g) was washed three times with 500 ml water, two times with 500 ml methanol, and lastly two times with 500 ml acetonitrile. Phosphoric acid (1.2 ml) was dissolved in 200 ml acetonitrile. This solution was added to washed silica gel. The mixture was stirred, and then the solvent was evaporated to dryness at 40 °C under vacuum on a rotary evaporator. Phosphoric acid–coated silica (250 mg) particles were packed into a reservoir of the DSD-NH_3.

2.4. Structure and sampling procedures of the DSD-NH_3

The DSD-NH_3 device comprises three sections: an exposure part, made of porous sintered polyethylene (diffusion filter); an analysis part, made of polypropylene tubing (reservoir); and an absorbent part; see Fig. 1. The DSD-NH_3 device contains 250 mg of silica gel impregnated with phosphoric acid as absorbent. An outline of the procedure for measuring ammonia using the DSD-NH_3 device is shown in Fig. 1. In the case of diffusive sampling, the shelter tube was removed and the absorbent was transferred from the reservoir to the diffusion filter by orienting the DSD-NH_3 device to an upright, vertical position. Exposure to the sample began at this point. Basic gases in ambient air, including ammonia, were trapped in the DSD-NH_3 device by their reaction with phosphoric acid in the sampler to form their corresponding phosphoric acid salts. After a fixed period of time, sampling was stopped by inverting the DSD-NH_3 device to return the absorbent from the diffusion filter to the reservoir, and replacing the shelter tube. In the case of active sampling, a shelter tube and the end cap of the DSD-NH_3 were removed and connected to the sampling pump equipped with a wet gas meter. Air was drawn through the DSD-NH_3 using a pumping system with a wet gas meter (Fig. 1). After collection, the DSD-NH_3 samplers were analyzed by IC. For IC analysis, the reservoir of the DSD-NH_3 device was removed from the diffusion filter and connected to a clean 5 ml syringe. Basic ions, including ammonium ion, are eluted from the reservoir absorbent by passing the solution through pure water via the syringe to a graduated test tube; this was done over a 1 min period, and 5 ml of eluate was collected. After elution, the eluate was analyzed by IC.

3. Results and discussion

3.1. Influence of phosphoric acid content of H_3PO_4–silica on collection of ammonia

Ammonia gas was emitted using the diffusion tubes (5 cm × 3 cm i.d.) filled with 1% ammonia solution in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 50%. The ammonia concentration reached a constant value of 88 μg m^{-3} after 24 h. H_3PO_4–silica particles containing various concentrations of phosphoric acid were packed into the DSD-NH_3 samplers and placed in the environmental test chamber for 24 h. After air sampling, ammonium ion is analyzed by IC. Fig. 2 shows the change in the ammonia amount collected by DSD-NH_3 with the phosphoric acid content of silica. As can be seen in Fig. 2, the amount of ammonia collected

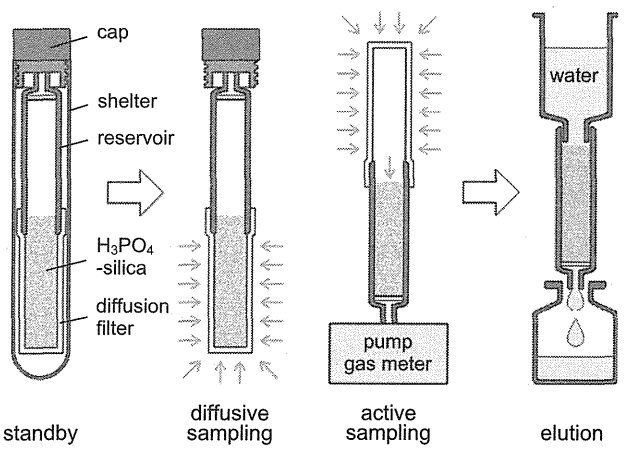

Fig. 1. Measurement of ammonia using the DSD-NH_3 device. The collection and elution steps are conducted by transferring the absorbent to the PSP-diffusion filter and PP-reservoir, respectively.

increases with phosphoric acid content of silica until 0.5%, and is constant in the range 0.5–10%. Fig. 2 suggests a phosphoric acid concentration higher than 1% is effective for the collection of ammonia in air.

3.2. Comparison of diffusive and active sampling methods

In order to determine the sampling rate of ammonia for the DSD-NH_3 sampler, parallel measurements by diffusive and active sampling were performed in the environmental test chamber. Active sampling was set to 100 ml min^{-1}. It is possible to obtain the sampling rate of the diffusive sampler from comparison with the known sampling rate of active sampling. Amounts collected (μg) by each sampling method were measured by IC. Fig. 3 shows a comparison of the DSD-NH_3 method with the active sampling method for ammonia. Data from the DSD-NH_3 sampler showed a good correlation with that from the active sampling method. The experimental sampling rate (94.5 ml min^{-1}) of ammonia for the DSD-NH_3 can be calculated from the slope ratio of DSD-NH_3 diffusive sampling to the active sampling (ml min^{-1}) in Fig. 3.

Additionally, the theoretical sampling rate of the DSD-NH_3 can be calculated from Graham's law of diffusion (Uchiyama et al., 2004). According to this law, the diffusion coefficient (D_{gr}) is inversely proportional to the square root of the density (Z) or molecular weight (M) of the gas:

$$D_{gr} \propto \frac{1}{\sqrt{Z}} \propto \frac{1}{\sqrt{M}} \tag{1}$$

When the diffusion coefficient (D_f) of formaldehyde is given, the diffusion coefficients of various other compounds can be calculated from equation (2):

$$D_{gr} = D_f \sqrt{\frac{M_f}{M_d}}, \tag{2}$$

where M_f is the molecular weight of formaldehyde, and M_d is the molecular weight of the desired compound. The sampling rates of the compounds can be calculated from equation (3):

$$R = \frac{D_d}{D_f} R_f, \tag{3}$$

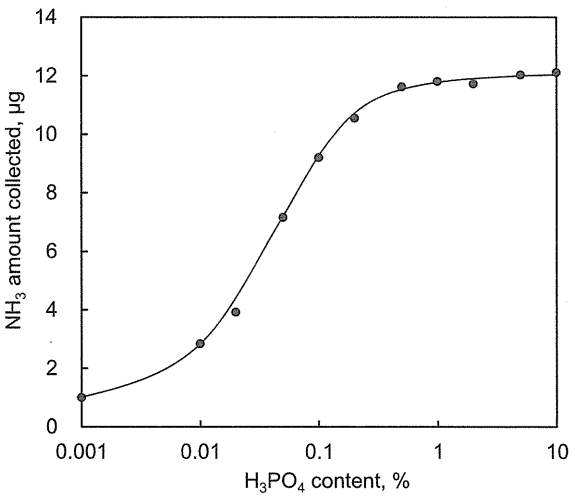

Fig. 2. Change in the amount of ammonia collected by the DSD-NH_3 with the phosphoric acid content of silica.

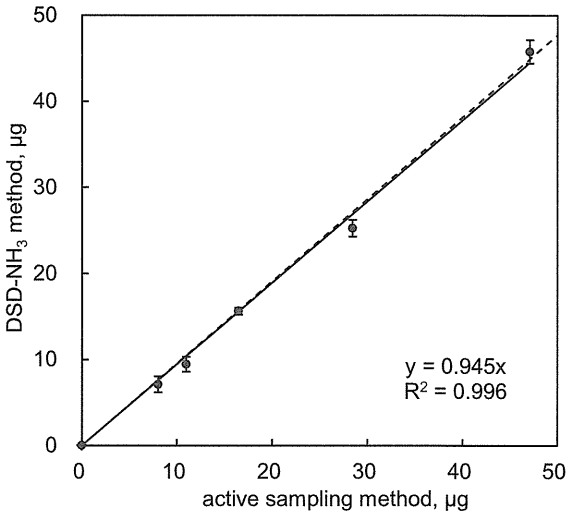

Fig. 3. Comparison of the DSD-NH_3 method with the active sampling method for ammonia. Active sampling was performed at a flow rate of 100 ml min^{-1}. The dashed line represents theoretical values calculated from Graham's law of effusion. Bars show twice the standard deviations of measurements of triplicate samples.

where R is the sampling rate of the desired compound; D_d, the diffusion coefficient of the desired compound; and R_f (71.9 ml min^{-1}), the sampling rate of formaldehyde by the DSD-DNPH (Uchiyama et al., 2004). The theoretical sampling rate of ammonia can be calculated from equation (3) because the diffusion filter is the same as in the DSD-DNPH. For ammonia, the theoretical sampling rate with the DSD-NH_3 is 95.4 ml min^{-1} and agrees with the experimental value of 94.5 ml min^{-1}. The dashed line in Fig. 3 represents theoretical values calculated using Graham's law.

3.3. Influence of temperature and humidity on ammonia collection

The effect of temperature and humidity on the sampling rate for the DSD-NH_3 samplers was investigated in the environmental test chamber. DSD-NH_3 diffusive sampling and active sampling were performed for 2 h in the environmental test chamber. As a temperature test, the environmental test chamber was set at temperatures of 5, 20, and 35 °C and constant relative humidity of 50%. As a humidity test, the environmental test chamber was set at humidity of 7, 46, and 93% and constant temperature of 25 °C. Changes in the sampling rate with temperature are shown in Fig. 4. The sampling rates were calculated by comparison with DSD-NH_3 diffusive and active sampling (described above). The sampling rate of the DSD-NH_3 diffusive sampler increased slightly depending on the temperature. On the other hand, the sampling rate decreased slightly depending on the humidity.

The kinetic theory of gases (Gilliland, 1934) indicates that the diffusion coefficient is a function of absolute temperature and pressure by equation (4).

$$D = \frac{0.0043T^{3/2}(1/M_A + 1/M_B)^{1/2}}{P\left(V_A^{1/3} + V_B^{1/3}\right)^2} \tag{4}$$

where D is the diffusion coefficient of analyte in air in units of $cm^2\ s^{-1}$, P is the total pressure in units of atmosphere, and T is the absolute temperature in units of K. M_A and M_B are molecular weights of components A and B. V_A and V_B are molal volumes of components A and B at the normal boiling point. From equation (4),

the diffusion coefficient can be shown to be independent of pressure and only dependent on temperature.

$$D = kT^{3/2} \quad (5)$$

From equation (5), the ratio of the diffusion coefficients at 35 °C and 5 °C is 1.17 : 1. From Fig. 4, the ratio of the sampling rates of the DSD-NH_3 diffusive sampler at 35 °C and 5 °C is 1.16 : 1. Close agreement was obtained between the theoretical and experimental values for the temperature dependence of the sampling rate.

3.4. Influence of wind velocity

The effect of air velocity on the sampling rate was studied by moving the DSD-NH_3 samplers in indoor air according to the method described in the previous report (Uchiyama et al., 2004). Twenty DSD-NH_3 samplers were fixed at intervals of 10 cm on a 2 m rod, and then rotated by an electric motor at 45 rpm for 2 h. A sampler fixed 1 m from the central pivot of the rod corresponded to a face velocity of 5.0 m s^{-1}. Fig. 5 shows the relation between face velocity and the sampling rate of ammonia. The sampling rates of ammonia increased slightly depending on the face velocity. The relative standard deviation (RSD) values for ammonia concentrations were 4.7%, with face velocity of 0–5.0 m s^{-1}. Wind velocity showed little influence on the DSD-NH_3 method; results were within 5 m s^{-1}. The DSD-NH_3 sampler indicated little susceptibility to face velocity because of the rounded tube structure, which is omnidirectional.

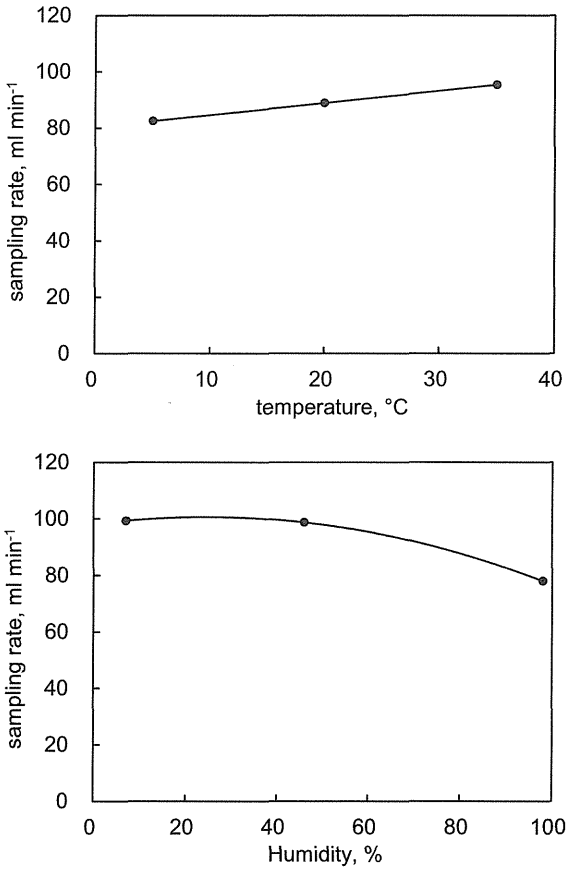

Fig. 4. Effect of temperature and humidity on the sampling rate of the DSD-NH_3 sampler.

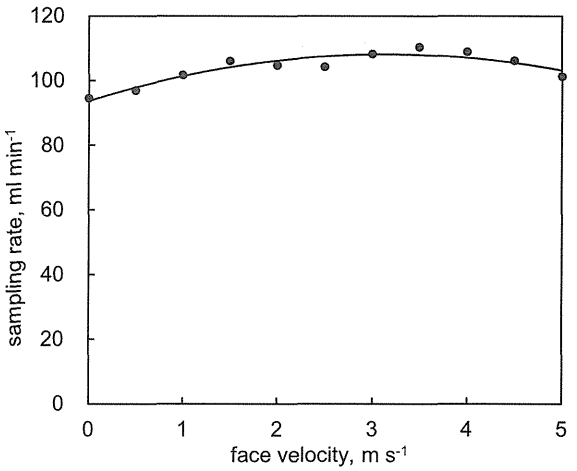

Fig. 5. Effect of face velocity on the sampling rate of the DSD-NH_3 sampler.

3.5. Limit of detection (LOD) and comparison with the OSHA method

The LOD calculated on the basis of three times the standard deviation of the blank (Ministry of the Environment, 1997) was about 0.14 μg m^{-3} for ammonia when exposure time was 24 h and elution volume was 5 ml. The repeatability (RSD) of this proposed method was about 4.0% for ammonia.

In order to compare DSD-NH_3 with a different device, parallel measurements were made with an established active sampling method (OSHA, 2002), which is widely used as a conventional method for active sampling of ammonia in workplace air. Parallel measurements were performed for 3h in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 50%. Active sampling (OSHA method) was set to 100 ml min^{-1}. Fig. 6 shows a comparison of the DSD-NH_3 method with the OSHA method for ammonia. The Ammonia concentrations measured using the DSD-NH_3 samplers agreed reasonably well with the concentrations measured by the OSHA method.

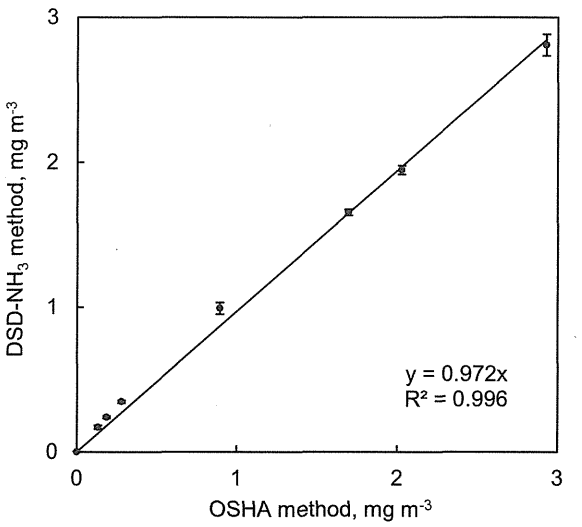

Fig. 6. Comparison of the DSD-NH_3 method with the OSHA method for ammonia. Bars show twice the standard deviations of measurements of triplicate samples.

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REGULAR ARTICLE

Factors in genetic susceptibility in a chemical sensitive population using QEESI

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Abstract
Objectives Inherited impairment of xenobiotic metabolism is a postulated mechanism underlying environmentally associated pathogeneses such as multiple chemical sensitivity (MCS). Using the Quick Environmental Exposure and Sensitivity Inventory (QEESI), we defined people who have a strong response to chemical substances as "chemical sensitive populations (CSP)." The aim of this study is to evaluate the condition of subjects sensitive to chemicals and to analyze their genotypes in order to identify susceptibility factors in CSPs in Japanese populations.
Methods A total of 1,084 employees of Japanese companies were surveyed using the QEESI, history of MCS, and sick house syndrome. The common genotypes of the participants were analyzed for *glutathione S-transferase* (*GST*) *M1*, *GSTT1*, *aldehyde dehydrogenase2* (*ALDH2*), and *paraoxonase1* (*PON1*) in order to identify factors in the susceptibility to sensitivity to chemicals.
Results Four subjects had history of diagnosis of MCS; no subjects had diagnosis of sick house syndrome. The subjects were divided into four levels according to scores of 0, 1–19, 20–39, and 40 or more on three of the QEESI subscales. In addition, we used the MCS criteria by Hojo to differentiate between cases (CSP) and controls. No significant differences in the allelic distribution of genetic polymorphisms in the *GSTM1*, *GSTT1*, *ALDH2* or *PON1* genes were found among the four levels of each subscale, or between cases and controls.
Conclusions Our findings suggest that the common genotypes of *GSTM1*, *GSTT1*, *ALDH2*, and *PON1* are of little importance to CSP in a Japanese population.

Keywords Genetic susceptibility · Multiple chemical sensitivity (MCS) · Idiopathic environmental intolerance (IEI) · Quick Environmental Exposure and Sensitivity Inventory (QEESI) · Logistic regression analysis

Introduction

Multiple chemical sensitivity (MCS), also known as idiopathic environmental intolerance [1], has been described as disabling multi-organ symptoms triggered by multiple exposures to chemicals. A number of hypotheses concerning the etiology and pathophysiology of MCS have been proposed [2], including impaired ability to metabolize toxic chemicals [3] and psychological mechanisms [4].

There is no widely accepted instrument to measure general chemical intolerance, and no objective criteria for identification of chemicals contributing to MCS, but Miller and Prihoda [5, 6] developed the Quick Environmental Exposure and Sensitivity Inventory (QEESI), which has its origins in the Environment Exposure and Sensitivity Inventory [7]. The QEESI is a reliable and valid screening instrument for chemical intolerance that consists of five subscales: chemical sensitivity, other chemical sensitivity, symptom severity, life impact, and masking index. The Japanese version of the QEESI was translated by Ishikawa and Miyata in 1999 [8].

The present study was designed to determine whether the results of the QEESI reveal a genetic difference for

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specific enzymes, primarily those possibly associated with chemical detoxification: glutathione *S*-transferase (GST) M1, GSTT1, aldehyde dehydrogenase 2 (ALDH2), and paraoxonase 1 (PON1).

The GSTs are a family of multifunctional enzymes and play a central role in detoxification of toxic and carcinogenic electrophiles. The polymorphic GSTs catalyze conjugation of glutathione to a variety of electrophilic compounds, including formaldehyde. Absence of activity of GSTM1, a μ class enzyme which detoxifies the reactive metabolites of benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons, is due to homozygous deletion of the gene [9]. A similar polymorphism of the *GSTT1* gene, encoding the θ class enzymes, has been described [10]. GSTT1 metabolizes various potential carcinogens, such as monohalomethanes, which are widely used as methylating agents, pesticides, and solvents [9].

Although GSTs are presumed to be involved in the first step of formaldehyde metabolism, it is still not clear which GST molecular species is responsible for formaldehyde metabolism. In addition, GST cytosolic activity in olfactory epithelium, the highest among extrahepatic tissues [11], is of particular interest in MCS, where the role of odorous triggers is important.

Acetaldehyde is one of the important chemicals that induce sick house syndrome and MCS [12]. Approximately half of the Japanese population lack ALDH2 activity because of a structural point mutation in the *ALDH2* gene. This genetic polymorphism, which is seen in Asians, including Japanese, but not in Caucasians, results in catalytic deficiency of aldehyde metabolism [13]. However, there are few studies regarding MCS and genetic polymorphism among Asians.

PON1 is known to be polymorphic in humans, with two isoforms displaying distinct hydrolyzing activities. The Arg192 isoform hydrolyzes paraoxon rapidly, whereas the Gln192 isoform acts slowly [14]. *PON1* genes were associated with Gulf War Syndrome [15], and PON1 reacts with toxic organophosphorus compounds [16].

Methods

Subjects

The present study was conducted from August to October 2003 at two companies (company A, an integrated circuit manufacturing company; company B, a paper pulp producing company) in Kyushu, in the south of Japan. The participants numbered 1,310 people from company A (males 936, females 374) and 891 from company B (males 778, females 113). Those who replied to the questionnaires (90.2%) and furthermore agreed to give genetic samples (52.2%) were included. Finally, a total of 1,084 subjects (49.3%) who had purified DNA in good condition, including 502 subjects from company A (males 390, females 112) and 582 from company B (males 579, females 3), were eligible for this study (Table 1).

Table 1 Demographic characteristics of the subjects

	All subjects
Sex	
Male	969 (89.4%)
Female	115 (10.6%)
Total	1,084
Average age (range), years	
Male	42.2 ± 8.9 (19–63)
Female	32.3 ± 6.3 (23–67)
Total	41.2 ± 9.1 (19–67)
Smoking (>once/week)	
Male	572 (59.0%)
Female	31 (27.0%)
Total	603 (55.6%)
Drinking (>once/week)	
Male	742 (76.6%)
Female	43 (37.4%)
Total	785 (72.4%)
History of diagnosis	
Multiple chemical sensitivity	
Male	2 (0.2%)
Female	2 (0.2%)
Total	4[a] (0.3%)
Sick house syndrome	
Male	0 (0%)
Female	0 (0%)
Total	0 (0%)
Allergic disease	
Male	212 (21.9%)
Female	47 (40.9%)
Total	259[a] (23.9%)
Chemical sensitivity ≥40	
Male	60 (6.2%)
Female	8 (7.0%)
Total	77 (7.1%)
Other chemical sensitivity ≥25	
Male	29 (3.0%)
Female	5 (4.3%)
Total	34 (3.1%)
Symptom severity ≥40	
Male	68 (7.0%)
Female	6 (5.2%)
Total	74 (6.8%)

[a] Three subjects had both multiple chemical sensitivity and allergy disease

Instruments

We used the QEESI (Japanese version) [8] for the survey described above. The QEESI consists of five subscales: the chemical sensitivity subscale measures the extent to which certain odors or exposures make one sick, the other chemical sensitivity subscale measures the extent to which a variety of other exposures make one sick, the symptom severity subscale refers to the extent to which one experiences certain symptoms, the life impact subscale measures the extent to which the sensitivity affects certain aspects of life, and the masking index measures whether there are ongoing exposures from routinely used products. Unlike other studies in which the subjects were patients, the participants in our study were collected from the general population. We selected 3 of the subscales, eliminating life impact and masking index. Each subscale has ten questions, and each question has a possible score of 0–10. Therefore, the total possible score was 0–100. All study subjects completed a self-reporting questionnaire which covered history of MCS, sick house syndrome, and allergic disease, drinking history, and smoking history.

As defined by Miller and Prihoda [5, 6], scores on the QEESI reveal three levels of symptom: low, medium, and high. The criteria for chemical sensitivity and symptom severity are low = 0–19, medium = 20–39, and high = 40–100. The criteria for other chemical sensitivity are low = 0–11, medium = 12–24, and high = 25–100. It has been reported that these three subscales can distinguish individuals with high susceptibility and a control group using cutoff values [5].

Hojo et al. [17] designed a study to establish a new cutoff value for Japanese using the QEESI for screening of MCS patients. According to that study, one difference from patients in America was that in Japanese patients the other chemical sensitivity subscale had low sensitivity and low specificity. They concluded that the other chemical sensitivity subscale should be excluded when applying the QEESI to evaluate subjective symptoms in Japan. The new cutoff values for Japanese subjects were determined to be $\geq$40 for the chemical sensitivity subscale, $\geq$20 for the symptom severity subscale, and $\geq$10 for the life impact subscale. Using their criteria, we divided our subjects into two groups according to the score achieved (Table 2). Individuals with chemical sensitivity score $\geq$40 and symptom severity score $\geq$20 were defined as chemical sensitive population (CSP) (cases), while individuals with moderate or no symptoms were classified as nonsensitive (controls, chemical sensitivity score <39 or symptom severity score <19).

Table 2 Demographic characteristics of cases and controls defined by QEESI score

	Cases (CSP)[a] (n = 47)	Controls (n = 1,037)	P value
Sex			
Male	41 (87.2%)	928 (89.5%)	
Female	6 (12.8%)	109 (10.5%)	0.62[b]
Average age, years			
Mean ± SD	44.2 ± 8.8	41.1 ± 9.1	0.69[c]
Median (range)	44 (30–59)	41 (19–67)	
Smoking status (>once/week)	18 (38.3%)	585 (56.4%)	0.01[b]
Drinking status (>once/week)	32 (68.1%)	753(72.6%)	0.50[b]

Cases (chemical sensitive population, CSP): chemical sensitivity score $\geq$40 and symptom severity score $\geq$20. Controls: chemical sensitivity score $\leq$39 or symptom severity score $\leq$19

[a] Classification in cases and controls according to Hojo et al. [17]

[b] P value, chi-square test. $P < 0.05$, difference significant

[c] P value, Student's t-test. $P < 0.05$, difference significant

Genotyping

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation. A multiplex polymerase chain reaction (PCR) method was used to detect the presence or absence of *GSTM1*and *GSTT1* [18].

Absence of *GSTM1* and *GSTT1* is due to homozygous deletion of these hereditary genes, termed the null genotype. The genotypes of *ALDH2* (rs671) were identified by the method of Harada and Zhang [13] as the homozygous genotype of normal *ALDH2* (**1/*1*), the homozygous genotype of inactive *ALDH2* (*2/*2), and the heterozygous genotype of normal and inactive *ALDH2* (**1/*2*). The genotype of the Gln192Arg (rs662) polymorphism of the *PON1* gene was determined essentially as described previously [19].

Statistical analysis

Relative associations between the CSP and controls were assessed by calculating crude odds ratios (ORs) from contingency tables. Corresponding chi-square tests were carried out on the cases and controls. In logistic regression analysis, ORs with corresponding 95% confidence intervals (CI) were calculated. P values smaller than 0.05 were considered significant. Statistical analysis was carried out using SPSS version 19.

Statistical power calculations were performed using Epistat (Finnish Institute of Occupational Health). This study sample size had at least 80% power (two-sided test significant, α of 0.05) to detect an OR of at least 2.5, following the calculations used in previous studies [18–20]. We used the dominant model for *GSTM1* and *GSTT1* and the recessive model for *ALDH2* and *PON1* in the test analysis.

Ethical considerations

The Ethics Review Board of Miyazaki University (no. 82, April 9, 2003) and Kumamoto University (no. 168, May 11, 2011) approved this study, following the ethical guidelines for human genome research. All participants were given full explanations of informed consent and the full protection of their personal data in written form.

Results

Table 1 presents the frequency of MCS, sick house syndrome, and allergic diseases such as asthma, allergic coryza, and atopic dermatitis. Four subjects reported history of diagnosis of MCS, three of whom also had history of allergy. The QEESI score and genotypes of these 4 subjects were as follows: (1) Q1 (27), Q2 (15), Q3 (36), *GSTM1 null*, *GSTT1 null*, *ALDH2*1/*1* and *PON1 Arg/Arg*; (2) Q1 (38), Q2 (5), Q3 (14), *GSTM1 null*, *GSTT1 null*, *ALDH2*1/*1* and *PON1 Arg/Arg*; (3) Q1 (27), Q2 (30), Q3 (78), *GSTM1 non-null*, *GSTT1 non-null*, *ALDH2*1/*1* and *PON1 Arg/Arg*; and (4) Q1 (9), Q2 (12), Q3 (23), *GSTM1 non-null*, *GSTT1 null*, *ALDH2*1/*2* and *PON1 Arg/Arg*. No subjects in this study population had diagnosis of sick house syndrome.

Chemical sensitivity was estimated by high cutoff values in the three subscales of chemical sensitivity ($\geq$40), other chemical sensitivity ($\geq$25), and symptom severity ($\geq$40). The percentage of subjects to whom high cutoff values were applied for the subscales of chemical sensitivity, other chemical sensitivity, and symptom severity were 7.1%, 3.1%, and 6.8%, respectively. The risks for chemical sensitivity as defined by Miller and Prihoda, estimated by high cutoff values on two subscales or three subscales, were 2.9% ($n = 31$) and 0.7% ($n = 8$), respectively.

When Hojo et al. [17] confirmed that the QEESI is effective for screening Japanese MCS patients, they suggested that the cutoff values for Japanese subjects should be chemical sensitivity score $\geq$40 and symptom severity score $\geq$20. In our study population, 4.3% ($n = 47$) of the subjects met that criteria and were defined as the cases (Table 2). There was a significant difference between the cases and controls in smoking status. However, no significant differences between the two groups in drinking status were observed.

Next we examined the association between genetic variants in *GSTM1*, *GSTT1*, *ALDH2*, and *PON1* and chemical sensitivity in the total population and the case–control population. The frequencies of genotypes for all examined gene variants by chemical sensitivity subscale score are presented in Table 3. On the chemical sensitivity subscale, 32.7% scored 0, 42.7% scored from 1 to 19, 17.5% scored from 20 to 39, and 7.1% scored 40 or higher. On the other chemical sensitivity subscale, 26.6% scored 0, 67.9% scored from 1 to 19, 5.0% scored from 20 to 39, and 0.5% scored 40 or higher (Electronic Supplementary Table S1). On the symptom severity subscale, 15.5% scored 0, 54.9% scored from 1 to 19, 22.8% scored from 20 to 39, and 6.8% scored 40 or higher (Electronic Supplementary Table S2). No significant difference in frequency was found for any gene variant between any levels in the total population or on any subscale of the QEESI. Similarly, there were no significant differences between the QEESI scores of genetic variants in *GSTM1*, *GSTT1*, *ALDH2*, and *PON1* (Table 3, Electronic Supplementary Tables S1 and S2).

The genotype data were also analyzed in the case–control design using logistic regression analyses with chemical sensitive status (cases: chemical sensitivity score $\geq$40 and symptom severity score $\geq$20) as outcome variables and genotype as the predictor variable, as presented in Table 4. No categorical predictor variable reached statistical significance. To check the effect of the genes in combination with smoking status, we calculated the OR for data classified by smoking status and by gene genotypes. The summarized data and ORs are presented in Table 5 together with 95% CIs. None of the distributions of genotypes showed any significant differences from the controls.

Discussion

This study focused on gene polymorphisms as a factor of chemical sensitivity, and analyzed *GSTM1*, *GSTT1*, *ALDH2*, and *PON1*.

Schnakenberg et al. [21] observed that the *GSTM1* and *GSTT1* gene deletion genotype occurred significantly more often in those individuals in German populations who reported chemical-related hypersensitivity. On the other hand, the allele and genotype frequencies of *GSTM1* and *GSTT1* were similar in Italian MCS patients and control populations [22]. In our study, no significant differences between the genotype frequency of *GSTT1* and *GSTM1* were found for any severity of three QEESI subscales or in the case–control study. The contradictory results are

Table 3 Association of QEESI chemical sensitivity subscale score with variants of *GSTM1*, *GSTT1*, *ALDH2*, and *PON1*

Gene	Genotype	QEESI score					
		0 *n* (%) 354 (32.7)	1–19 *n* (%) 463 (42.7)	20–39 *n* (%) 190 (17.5)	40–100 *n* (%) 77 (7.1)	Total *n* (%) 1,084 (100)	*P* value[a]
GSTM1	*Non-null*	152 (42.9)	213 (46.0)	95 (50.0)	36 (46.8)	496 (45.8)	
	Homozygous-null	202 (57.1)	250 (54.0)	95 (50.0)	41 (53.2)	588 (54.2)	0.47
GSTT1	*Non-null*	199 (56.2)	257 (55.5)	99 (52.1)	48 (62.3)	603 (55.6)	
	Homozygous-null	155 (43.8)	206 (44.5)	91 (47.9)	29 (37.7)	481 (44.4)	0.49
ALDH2	**1/*1*	222 (62.7)	276 (59.6)	113 (59.5)	54 (70.1)	665 (61.3)	
	**1/*2*	109 (30.8)	166 (35.9)	66 (34.7)	20 (26.0)	361 (33.3)	
	**2/*2*	23 (6.5)	21(4.5)	11 (5.8)	3 (3.9)	58 (5.4)	
	**1/*2* or **2/*2*	131 (37.3)	187 (40.4)	77 (40.5)	23 (29.9)	419 (38.7)	0.30[b]
PON1	*Arg/Arg*	147 (41.5)	178 (38.5)	81 (42.6)	33 (42.8)	439 (40.5)	
	Arg/Gln	185 (52.3)	253 (54.6)	91(47.9)	38 (49.4)	567 (52.3)	
	Gln/Gln	22 (6.2)	32 (6.9)	18 (9.5)	6 (7.8)	78 (7.2)	
	Arg/Gln or *Gln/Gln*	207 (58.5)	285 (61.5)	109 (57.4)	44 (57.2)	645 (59.5)	0.68[c]

[a] *P* value, chi-square test. $P < 0.05$, difference significant

[b] **1/*2* or **2/*2* against **1/*1*

[c] *Arg/Gln* or *Gln/Gln* against *Arg/Arg*

Table 4 Association of cases and controls defined by QEESI score with the variants of *GSTM1*, *GSTT1*, *ALDH2*, and *PON1*

Gene	Genotype	Cases (CSP) $n = 47$ (%)	Controls $n = 1037$ (%)	Crude OR[a]	*P* value[b]	Adjusted OR[b,c]
GSTM1	*Non-null*	20 (42.6)	476 (45.9)	1		1
	Homozygous-null	27 (57.4)	561 (54.1)	1.15 (0.61–2.15)	0.62	1.16 (0.64–2.10)
GSTT1	*Non-null*	31 (66.0)	572 (55.2)	1		1
	Homozygous-null	16 (34.0)	465 (44.8)	0.63 (0.33–1.22)	0.12	0.61 (0.33–1.13)
ALDH2	**1/*1*	32 (68.1)	633 (61.0)	1		1
	**1/*2*	13 (27.7)	348 (33.6)			
	**2/*2*	2 (4.2)	56 (5.4)			
	**1/*2* or **2/*2*	15 (31.9)	404 (39.0)	0.73 (0.37–1.42)	0.18[d]	0.63 (0.32–1.24)[d]
PON1	*Arg/Arg*	19 (40.4)	420 (40.5)	1		1
	Arg/Gln	25 (53.2)	542 (52.3)			
	Gln/Gln	3 (6.4)	75 (7.2)			
	Arg/Gln or *Gln/Gln*	28 (59.6)	617 (59.5)	1.00 (0.53–1.88)	0.85[e]	1.06 (0.58–1.94)[e]

[a] Odds ratio (OR) and 95% confidence interval (95% CI)

[b] *P* value, chi-square test. $P < 0.05$, difference significant

[c] ORs were adjusted for age (continuous), gender, smoking, and drinking

[d] **1/*2* or **2/*2* against **1/*1*

[e] *Arg/Gln* or *Gln/Gln* against *Arg/Arg*

mainly due to the inclusion criteria adopted by the different studies.

Our study is the first to analyze the association between *ALDH2* variants and chemical sensitivity, and no significant association was observed in our Japanese population. This result suggests that the *ALDH2* variants may not be involved in CSP.

PON1 plays a major role in biodegradation of various organophosphates that can function as potent cholinesterase inhibitors. Previous studies suggested that the polymorphic site in *PON1* was related to an increased risk of MCS [23] and Gulf War Syndrome, which is an MCS-related syndrome [15]. However, no significant association between the Gln192Arg polymorphism and *PON1* was

Table 5 Odds ratio for genotypes related to CSP by smoking status

Gene	Genotype	Nonsmokers, OR (95% CI)[a] $n = 481$ (44.6%)	Smokers (>once/week), OR (95% CI)[a] $n = 603$ (55.6%)
GSTM1	*Homozygous-null* versus *non-null* genotype	1.49 (0.69–3.23)	0.78 (0.30–2.01)
GSTT1	*Homozygous-null* versus *non-null* genotype	1.18 (0.36–3.92)	0.85 (0.32–2.23)
ALDH2	**1/*2* or **2/*2* versus **1/*1*	1.21 (0.37–4.00)	0.50 (0.15–1.67)
PON1	*Arg/Gln* or *Gln/Gln* versus *Arg/Arg*	1.24 (0.38–4.09)	1.08 (0.41–2.85)

[a] ORs were adjusted for age (continuous), gender, and drinking

obtained in our study. This trend in the MCS case–control design was reversed in the general population samples, perhaps reflecting that the *PON1* polymorphism played a minor or no role in the development of MCS in our population. In support of our study, Wiesmuller et al. [24] failed to detect an association between *PON1* polymorphism and self-reported MCS in a population sample.

On the other hand, we tried to define chemical sensitivity cases by Miller and Prihoda and estimated the risk for the genetic variants of *GSTM1*, *GSTT1*, *ALDH2*, and *PON1*, respectively. However, no case–control differences were observed in each genotype of the 4 genes.

Smoking status was significantly lower in the cases than in the controls. Several reports suggest that MCS patients who are aware of their chemical intolerance avoid exogenous chemicals such as those from smoking [23, 24]. Tobacco smoke contains many kinds of chemicals, including formaldehyde and acetaldehyde [25]. For these reasons, we hypothesize that the genotypes of *GSTM1*, *GSTT1*, *ALDH2*, and *PON1* might contribute to development of CSP in smokers. However, among the smokers there were no significant differences between CSP and controls in the *GSTM1*, *GSTT1*, *ALDH2*, and *PON1* genotypes.

In the present study, no significant differences between sequence variations of *GSTM1*, *GSTT1*, *ALDH2*, and *PON1* were found between the CSPs and controls. One possible weakness of our study design is a lack of assessments of environmental exposure to chemicals metabolized by the examined enzymes. It is plausible that gene–environment interactions exist, and the genetic variations of metabolic enzymes may either confer protection against or increase risk from harmful effects of chemical exposure. A second problem is the possibility that the subject sample we defined, following the QEESI protocols, was not a correct sampling of MCS. Our survey included the other chemical sensitivity subscale, but not life impact. Extrapolating from the results of Hojo et al., our defined cases whose scores exceeded the two cutoff values of chemical sensitivity ≥40 and symptom severity ≥20 were considered to be equivalent to 65% of the patients suspected to have MCS and 7% of the healthy controls. This screening criteria means that detecting the condition has sensitivity of 65% and specificity of 93%. It is unlikely that sensitivity, specificity, positive predictive value, and negative predictive value are influenced by the prevalence of the disease. As a result of that, we might not have been able to find the effect of important genotypes. A future strategy could be to subgroup patients according to symptoms, which may be genetically more homogeneous that a patient population as whole.

In conclusion, an association between risks for CSP-related MCS and genetic variation in biologically plausible candidate genes was not observed. Additionally, our results suggest that an exact case criterion is required to determine the actual importance to MCS of genetic variants in genes that encode metabolic enzymes.

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Conflict of interest No conflicts of interest.

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