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Development, evaluation and comparison of two independent sampling and analytical methods for *ortho*-phthalaldehyde vapors and condensation aerosols in air† ,‡

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Abstract

Two independent sampling and analytical methods for *ortho*-phthalaldehyde (OPA) in air have been developed, evaluated and compared (1) a reagent-coated solid sorbent HPLC-UV method and (2) an impinger-fluorescence method. In the first method, air sampling is conducted at 1.0 L min^{-1} with a sampler containing 350 mg of silica gel coated with 1 mg of acidified 2,4-dinitrophenylhydrazine (DNPH). After sampling, excess DNPH in ethyl acetate is added to the sampler prior to storage for 68 hours. The OPA-DNPH derivative is eluted with 4.0 mL of dimethyl sulfoxide (DMSO) for measurement by HPLC with a UV detector set at 3S5 nm. The estimated detection limit is 0.016 μg per sample or 0.067 μg m^{-3} (0.012 ppb) for a 240 L air sample. Recoveries of vapor spikes at levels of 1.2 to 6.2 μg were 96 to 101%. Recoveries of spikes as mixtures of vapor and condensation aerosols were 97 to 100%. In the second method, air sampling is conducted at 1.0 L mm^{-1} with a midget impinger containing 10 mL of DMSO solution containing *N*-acetyl-L-cysteine and ethylenediamine. The fluorescence reading is taken 80 min after the completion of air sampling. Since the time of taking the fluorescence reading is critical, the reading is taken with a portable fluorometer. The estimated detection limit is 0.024 μg per sample or 0.1 μg m^{-3} (0.018 ppb) for a 240 L air sample. Recoveries of OPA vapor spikes at levels of 1.4 to 5.0 μg per sample were 97 to 105%. Recoveries of spikes as mixtures of vapors and condensation aerosols were 95 to 99%. The collection efficiency for a mixture of vapor and condensation aerosol was 99.4%. The two methods were compared side-by-side in a generation system constructed for producing controlled atmospheres of OPA vapor in air. Average air concentrations of OPA vapor found by both methods agreed within ±10%.

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‡Mention of company names and products does not constitute endorsement by the Centers for Disease Control and Prevention. The findings and conclusions of this report are those of the author and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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Introduction

ortho-Phthalaldehyde (OPA) (Fig. 1) is replacing glutaraldehyde in healthcare settings as a more effective disinfectant for heat-sensitive medical and dental instruments.[1–7] However, OPA has been found to cause occupational bronchial asthma and contact dermatitis.[8,9] Also, OPA stains proteins and unprotected skin gray.[2] For example, a urology resident has reported facial stains caused by OPA following cystoscopy.[10] OPA-sterilized cystoscopies have been associated with anaphylactic reactions in a small number of patients, namely, bladder cancer patients who have undergone repeated cystoscopy.[11,12] Results of research conducted by NIOSH (the National Institute for Occupational Safety and Health) on the dermal irritancy and allergic potential of OPA raise concern about the intended use of OPA as a safe alternative to glutaraldehyde.[13] Since it is estimated that more than 300000 healthcare workers are exposed to OPA,[7] health effects of workers due to OPA are a major issue. CIDEX OPA is a commercial formulation containing OPA which commonly is used for disinfection in hospitals.[11]

These health effects constitute justification for NIOSH to conduct health hazard evaluations (HHEs) in hospitals in areas where OPA is employed; namely, endoscopy units. In order to conduct HHEs for OPA, NIOSH has needed valid sampling and analytical methods. Products of NIOSH research on developing analytical methods for OPA include two independent sampling and analytical methods for OPA in air plus a method for measuring OPA in surface wipes.[14] Both air methods, which are reported within, are partially validated, and average air concentrations of OPA found by the two methods have agreed within ±10% in the laboratory. Although OPA in air in hospital settings exists only in vapor form, laboratory research has shown that the two methods developed are applicable to both OPA vapors and condensation aerosols in air.

The method employing 2,4-dinitrophenylhydrazine (DNPH) in a solid sorbent tube in the present work is a vast improvement over the DNPH method described in 2008,[14] which, in turn, had incorporated significant improvements over the DNPH HPLC-UV method for OPA in air described by Uchiyama *et al.*[15] The DNPH method for OPA described in 2008 (ref. 14) was plagued with the following problems: (1) the RSDs for recovery of OPA from vapor spikes for two of the four levels studied were unacceptably high (12%).[14] (2) The procedure of eluting OPA species from the sampler soon after air sampling involved a major risk of exposing the two air-sensitive OPA species in the eluent to air, leading to lower recoveries [both OPA and OPA-mono(DNPH) are air-sensitive[14]]. While average recoveries were observed to be as low as 76% in the 2008 method,[14] average recoveries below 75% are considered uncorrectable due to large errors.[16] (3) The use of acetonitrile as the eluting solvent and solvent for standards was unsatisfactory due to the extremely limited solubility of OPA-bis(DNPH) (Fig. 2); this limited solubility severely restricted the working range of OPA-bis(DNPH) in solution. The DNPH-HPLC method for OPA in air described in the present paper is free from the problems just described, (1) The problem of exposing air-sensitive OPA species to air by elution soon after sampling has been solved by maintaining all OPA species inside the sampler with excess DNPH under nitrogen until reactions are complete to form the final product, OPA-bis(DNPH). (2) The problem of limited solubility of OPA-bis(DNPH) in acetonitrile has been solved by replacing acetonitrile with dimethyl

sulfoxide (DMSO) as the solvent for elution and for HPLC standards. Since the solubility of OPA-bis(DNPH) in DMSO at room temperature is about 46 times greater than that in acetonitrile at 30 °C, working ranges of OPA-bis(DNPH) are extended easily. Also, less solvent is required for preparation of a stock solution. (3) The two major changes just described have led to a very reliable sampling and analytical method for OPA in air in which RSDs are acceptable (less than 10%).

A second sampling and analytical method for OPA in air, one which is completely independent of the DNPH-HPLC method just discussed, has been developed, evaluated and compared with the first method. This method employs a midget impinger containing a DMSO solution of *N*-acetyl-L-cysteine and ethylenediamine for air sampling. The basis for this method relates to Roth's fluorimetric method for determining analytes bearing a primary amino group[17,18] and is the same basis for the determination of OPA on surfaces.[14] Under basic conditions, the fluorigenic reagents, OPA, a thiol, and a species containing a primary amino group, undergo reaction to form a thio-*N*-alkyl-substituted isoindole. In this present work, Roth's fluorimetric method has been adapted for the determination of OPA *in lieu* of compounds bearing primary amino groups. The source of the thiol is *N*-acetyl-L-cysteine, a reagent commonly used in Roth's fluorimetric method.[19] While thio-*N*-alkyl-substituted isoindoles are typically unstable in aqueous media,[19] replacement of aqueous media with DMSO as the solvent apparently has improved the stability of the fluorescent product.[14] Another advantage of using DMSO is the virtual nonvolatility of DMSO; impingers need not be replenished during air sampling.

Both the DNPH-HPLC and impinger-fluorescence methods employ DMSO, which is classified as a nonhazardous substance according to Directive 67/548/EC. However, DMSO can transport dissolved chemicals through the skin into the body. Therefore, the use of protective gloves, such as light-weight nitrile gloves, is recommended. Disposal of DMSO into the sewer should be avoided because, otherwise, environmental odor will develop due to bacterial conversion to dimethyl sulfide.

Experimental

Materials

S10 and S10L LpDNPH cartridges (containing single 350 mg beds of silica coated with 1 mg quantities of acidified DNPH) and glass wool, silane treated, were purchased from Supelco. Two fluorometers, a TBS model 380 (plug-in) and a *Pico*fluor™ (battery-powered), were purchased from Turner Biosystems (in 2009, Promega Corporation bought out Turner Biosystems and offered virtually the same fluorometers by the names, QuantiFluor™-ST and QuantiFluor™-P, respectively). A Condensation Particle Counter (CPC), Model 3022a, and a Scanning Mobility Particle Sizer (SMPS), Model 3936L22, using a 3081 long Differential Mobility Analyzer (DMA) were purchased from TSI, Inc. Tubing transporting aerosols to the CPC and SMPS was made from conductive silicone rubber tubing for a 1/4″ nipple and was purchased from Simolex Rubber Corporation. A HEPA-CAP™ 36 Disposable Filter Capsule (with glass microfiber media and polypropylene housing), 600 cm^2 effective filter area, was purchased from Whatman. Disposable cuvettes, 1 cm × 1 cm, made of polystyrene, with polyethylene caps were purchased from Perfector Scientific.

Silica gel, Davisil®, grade 635, pore size 60 Å, 60–100 mesh, was purchased from Sigma-Aldrich. PTFE-coated glass fiber filters, 37 mm in diameter, were purchased from Pall Life Sciences (reorder number 7217). Filter cassettes, 37 mm, 2-piece, polypropylene (solvent-resistant), were purchased from SKC, inc. Glove bags, polyethylene, 94 × 94 cm, model X-37-37, were purchased from Glas-Col. The OPA generation system was constructed from components purchased from a local home improvement store which included Rubbermaid® "clear containers" (made of polypropylene) with capacities of 29.3, 47.0, and 110 L, borosilicate window glass, 3/4″ (1.9 cm) PVC pipe, 3/4″ (1.9 cm) PVC valves, duct tape, silicone cement, and batteries. Minifans, 40 × 40 × 10 mm, 12 VDC, 130 m A^{-1}, 6500 RPM ±10%, air flow of 7.7 cfm (218 L min^{-1}), were purchased from Radioshack. A portable thermometer-hygrometer traceable to NIST calibration was purchased from Fisher Scientific. Compressed gases in gas cylinders, prepurified nitrogen and air of breathing quality, were purchased from Wright Brothers, Inc.

ortho-Phthalaldehyde, minimum of 97% purity, was purchased from Sigma Aldrich, MP Biomedicals, LLC, and Alfa Aesar (three sources due to supply issues). CIDEX OPA was purchased from Advanced Sterilization Products. Dimethyl sulfoxide was purchased from Fisher Scientific (HPLC grade, submicron filtered), Acros Organics (99.7% purity), and Tedia Company, Inc. (HPLC/Spectro grade) (three sources due to supply issues). DNPH (97% and containing about 30% water) and *trans*-1,2-bis(2-pyridyl)ethylene, 97%, were purchased from Aldrich Chemical Company. *N*-Acetyl-L-cysteine, 98%, was purchased from Acros Organics. Ethylenediamine, was purchased from Fisher Scientific (laboratory grade), and Chem Service (99.5%). Acetonitrile, spectrophotometric grade, was purchased from Tedia Company, Inc. Ethyl acetate, spectrophotometric grade, and *N,N*-dimethylformamide, spectrophotometric grade, were purchased from Burdick & Jackson. *N,N*-Dimethylacetamide, Spectro grade, was purchased from Eastman Kodak Company.

Stock solution of OPA-bis(DNPH) in DMSO

OPA-bis(DNPH) was synthesized by addition of a solution of 5.84 g (0.02 mol) of 97% DNPH (containing *ca.* 30% water) in 50 mL of cone, sulfuric acid to a solution of 0.67 g (0.005 mol) of OPA in 320 mL of 78 : 22 ethanol-water.[15] Product was recrystallized from *N,N*-dimethylacetamide and dried at 130 °C, mp = 285.0–285.1 °C. Recrystallized OPA-bis(DNPH) (4.69 mg) was dissolved in 36.4 mL of DMSO. The mixture was heated to 100 °C and allowed to cool to room temperature.

Estimation of solubility of OPA-bis(DNPH) in DMSO

DMSO (10.95 mL) was added to 4.2 mg of pure OPA-bis(DNPH). The mixture was heated to 108 °C. A dark solution formed, but solid material remained. In a second experiment, DMSO (33.98 mL) was added to 5.20 mg of OPA-bis(DNPH), and the mixture was heated to 108 °C. All solid dissolved. The solution was allowed to stand overnight. No particles were observed in the solution.

HPLC

The HPLC was a Waters component HPLC system with a Waters™ 600-MS system controller and a Waters™ 717plus autosampler. The detector was a Shimadzu SPD-10AV

VP UV-VIS detector set at 385 nm [the λ_{max} for OPA-bis(DNPH) in the mobile phase]. The analytical column was an Ascentis RP Amide stainless steel column, 250 × 4.6 mm, containing 5 μm particles, 19.5% carbon loading, 100 Å pore size, from Supelco. The mobile phase was 87 : 13 acetonitrile-water (v/v) with a flow rate of 1.0 mL min^{-1}. The injection volume was 15 μL.

Spectra

UV and visible absorption spectra were obtained with a Hewlett-Packard 8453 UV-VIS spectrometer. A fluorescence spectrum was obtained with a Perkin Elmer LS50B Luminescence Spectrometer at a scan speed of 10 nm min^{-1}. FT-IR spectra were obtained with a Perkin Elmer FT-IR Spectrometer Spectrum 1000.

Preparation of DNPH-ethyl acetate solution

DNPH with *ca.* 30% water (968 mg) was dissolved in 1L of ethyl acetate. Orthophosphoric acid (85%, 40 μL) was added to the solution.

Study of recovery of OPA *versus* storage time of the S10 LpDNPH sampler

PTFE-coated glass fiber filters (PTFE-GFFs, 37 mm) were placed into the outlet pieces of filter cassettes inside a glove bag. The inlets of S10 LpDNPH cartridges were connected directly to the exit ports of the cassette pieces. Each S10 LpDNPH sampler inside the glove bag was connected to an air pump located in the glove bag. The glove bag was sealed, inflated with nitrogen and agitated by hand for mixing of gases. Then the glove bag was deflated, inflated again with nitrogen and agitated at least two more times in order that the composition of the atmosphere inside the glove bag was estimated to be at least 98% nitrogen. (Earth's atmosphere consists of 78% nitrogen, 21% oxygen and 1% other substances including water vapor. The number of deflations and inflations depends, in part, on the numbers and sizes of air pockets next to objects inside the glove bag.) A 500 μL syringe outside of the glove bag was loaded with 100 μL of a freshly prepared acetonitrile solution of OPA (40 μg mL^{-1}). The glove bag was punctured with the needle of the syringe above a selected PTFE-GFF, the tip of the needle was brought within 2 cm of a point above the center of the PTFE-GFF and the OPA solution was applied dropwise to the center of the PTFE-GFF (100 μL of solution was sufficient to wet the entire PTFE-GFF). In two minutes, the pumps were turned on at a flow rate of 1.0 L min^{-1} for 90 min. Then two S10 LpDNPH samplers located outside of the glove bag were fortified with liquid spikes of the same quantities of OPA by the same technique described.[14] By this technique, the inlet frit of the S10 LpDNPH sampler is penetrated by the needle of a 500 μL syringe, and 100 μL of OPA solution in acetonitrile is injected into the center of the silica bed. After the samplers with liquid spikes were allowed to stand 15 min, the OPA-bis(DNPH) was eluted with 4 mL quantities DMSO and measured by HPLC (these liquid spikes served as standards for determination of the actual quantity of OPA applied to each PTFE-GFF). The S10 LpDNPH samplers inside the glove bag were removed and treated with excess DNPH in ethyl acetate solution as described in "Routine procedure for the DNPH-HPLC method" in the Results and discussion, stored for various periods from 1 to 94 h and analyzed by HPLC.

Recoveries of OPA vapor spikes from S10 LpDNPH samplers

S10 LpDNPH samplers were fortified with vapor spikes of OPA inside a glove bag as described above. Concentrations of OPA in acetonitrile solution were 20, 30 and 70 μg mL^{-1}. Three or four samples were prepared at each level. At two min from the time of application of the OPA solutions to the PTFE-GFFs, the pumps were turned on for 60, 75, or 120 min (larger quantities of OPA required longer pumping times for vaporization). Once the pumps were turned off, the S10 LpDNPH samplers were treated with excess DNPH in ethyl acetate solution, stored for 68 h at room temperature in the dark and analyzed by HPLC. Three PTFE-GFFs in the cassette pieces were transferred to 10 mL quantities of DMSO-reagent solution, the mixtures were shaken by hand, and the quantities of OPA were measured by the fluorescence method (the fluorescence method was used to measure residual quantities of OPA on filters due to convenience, expense and time issues).

Recoveries of OPA spikes (as a mixture of vapor and condensation aerosol) from S10 LpDNPH samplers

Each S10 LpDNPH cartridge was butted against the outlet of a glass U-tube partially immersed in a bath of silicone oil heated to 120 °C. While a pump drew laboratory air through the U-tube and S10 LpDNPH cartridge at 1.0 L min^{-1}, the stopcock at the inlet of the U-tube was removed and 100 μL an acetonitrile solution of OPA (5.6, 17.5 or 32.3 μg mL^{-1}) was added by syringe ($n = 4$ for each level). The stopcock was replaced and the pump continued to operate for 45 min. Excess DNPH in ethyl acetate solution was added to the S10 LpDNPH cartridge, the cartridge was sealed and stored for 68 h, and the sample was eluted with DMSO and measured by HPLC.

Recoveries of OPA vapor spikes from S10 LpDNPH samplers following storage

Solutions of OPA in acetonitrile (100 μL; 7.1, 52, and 145 μg mL^{-1}) were applied to PTFE-GFFs as described above. Pumping times for the three concentrations were 75, 90 and 130 min, respectively. Vapor spikes were collected on S10 LpDNPH samplers. Excess DNPH in ethyl acetate was added to the samplers. Samples were eluted with DMSO (4.0 mL) after selected storage times of 3, 6, 10 and 15 days at room temperature (these storage times were selected for statistical analysis). Samples were analyzed by HPLC.

Air draw through S10 LpDNPH samplers containing OPA vapor spikes

Thirty-six S10 LpDNPH samplers were used to collect vapor spikes of OPA in quantities of 1, 6 and 18 μg from PTFE-GFFs inside a glove bag. Laboratory air at 50 to 60% relative humidity (RH) was drawn through each fortified sampler at 1.0 L min^{-1} for 15, 120 or 360 min. An additional set of four S10 LpDNPH samplers was used to collect 0.86 μg quantities of OPA; air was drawn through these samplers for four hours. For each set of four samplers, an additional S10 LpDNPH sampler was used to collect the representative quantity of OPA. The additional S10 LpDNPH samplers served as standards, and no laboratory air was drawn through the standards. DNPH-ethyl acetate solution was added to each sampler, and each sampler was analyzed by the DNPH-HPLC method.

Preparation of DMSO-reagent solution for impinger-fluorescence method

N-Acetyl-L-cysteine (104 mg) was dissolved in 800 μL of distilled-deionized water at 60 °C, and this solution was added to 80 mL of DMSO. Ethylenediamine [42.6 μL (38.3 mg)] was added. This DMSO solution was used immediately. Note: for air sampling at locations away from the laboratory, preparation of the DMSO-reagent solution was modified. The aqueous solution of *N*-acetyl-L-cysteine was added to 40 mL of DMSO in an amber jar equipped with a PTFE-lined lid for shipment. The ethylenediamine was added to 40 mL of DMSO in another amber jar for shipment. At the time of air sampling in the field, the two solutions were combined, and 10 mL aliquots were transferred to midget impingers.

Test of additivity of water to DMSO by volume

A 25 mL graduated cylinder was weighed (58.79 g). DMSO (10.00 mL, 11.00 g) was added to the cylinder (total weight = 69.79 g; total volume reading = 10.00 mL). Distilled-deionized water (0.50 mL, 0.50 g) was added, and the mixture was agitated (total weight = 70.29 g; total volume reading =10.50 mL).

Rates of evaporation of DMSO and water from impingers during air draw

DMSO (10.00 mL) was added to each of four midget impingers. Distilled-deionized water (1.50 mL) was added to one impinger, and 3.00 mL of water was added to a second. The four impingers were weighed. Dry air from a cylinder was used to inflate a glove bag. Pumps inside the inflated glove bag drew air through the impingers at 1.0 L min^{-1} for 120 min. Then the impingers were weighed again. [The RH decreased with incoming dry air from 14.6% to 9.3% for a time-weighted average (TWA) of 11.1% at 23.6 °C. Air pockets next to objects inside the glove bag prevented attainment of an RH of 0%.]

Stability of fluorescence readings

N-Acetyl-L-cysteine (130 mg) was dissolved in 1 mL of water. This solution was dissolved in 100 mL of DMSO. Ethylenediamine (47.9 mg, 53.3 μL) was added. Aliquots (3.1 mL) were added to sixteen cuvettes to serve as blanks. OPA (260 μL of a solution at 960 μg mL^{-1}) was added to the remaining 50 mL of DMSO-reagent solution. Aliquots (3.1 mL) of the OPA mixture were added to sixteen other cuvettes to serve as samples. One sample solution was used for multiple fluorescence readings at intervals from 20 min to 26 h while one-time fluorescence readings were made on the other sample solutions over the same period. Blank solutions were treated similarly.

Recovery of OPA vapor spikes from DMSO-reagent solution

Solutions of OPA in acetonitrile (100 μL, 22, 34 and 58 μg mL^{-1}) were added to PTFE-GFFs in a nitrogen atmosphere inside a glove bag by the technique described previously (three samplers per level). Pumps drew nitrogen through the PTFE-GFFs and impinger solutions for 60, 75 and 75 min, respectively. Fluorescence readings of impinger solutions in cuvettes were taken at 80 min from the end of pump operation. All fluorescence readings of samples were corrected for the blank value. Residual quantities of OPA on the PTFE-GFFs were determined by transfer of the PTFE-GFFs to 5 mL quantities of DMSO-reagent solution, agitation of the mixtures by hand, and analysis by the fluorescence method.

Recoveries of OPA spikes (as a mixture of vapor and aerosol) from midget impingers of DMSO-reagent solution

The inlet of a midget impinger containing 10.0 mL of DMSO-reagent solution was connected to the outlet of a glass U-tube partially immersed in a silicone oil bath at 120 °C. This impinger was followed by another impinger containing charcoal which was followed by a pump; the impinger of charcoal served to protect the pump from splash-over. While the pump drew laboratory air through the system at 1.0 L min^{-1}, the stopcock at the inlet of the U-tube was removed. An acetonitrile solution of OPA (100 μL in volume, 24.6, 121, or 202 μg mL^{-1} in concentration) was added, and the stopcock was returned. The pump operated for 45 min for the lowest level and 75 min for the higher two levels ($n = 4$ for each level). Fluorescence readings were taken 80 min following the completion of pump operation.

Collection efficiency of a midget impinger of DMSO-reagent solution for OPA (as a mixture of vapor and aerosol) in air

Two sampling trains were constructed. In the first train, a glass U-tube partially immersed in a silicone oil bath at 120 °C was connected *via* 19 cm of PTFE tubing (7 mm O.D., 4 mm I.D.) to a midget impinger in the vertical position containing 10 mL of DMSO-reagent solution. The exit port of the impinger was butted directly to an S10 LpDNPH sampler, which, in turn, was connected to a pump. In the second train, another glass U-tube partially immersed in the same oil bath was connected to an impinger containing 10 mL of DMSO-reagent solution *via* 15 cm of PTFE tubing (this impinger was tilted at a 60-degree angle to permit a shorter length of PTFE tubing for connection with the U-tube). The exit port of the impinger was connected *via* 15 cm of PTFE tubing to the inlet of a second impinger containing 10 mL of DMSO-reagent solution (an impinger was the back-up sampler in the second train to determine the utility of an impinger as a back-up). The second impinger was connected to a charcoal trap and a pump. The pumps were turned on at 1.0 L min^{-1}, and 20 μg of OPA in 50 μL of acetonitrile solution was added to each U-tube. Pumping was continued for 60 min. The three impinger solutions and the S10 LpDNPH sampler were analyzed for OPA.

Air draw through midget impingers of DMSO-reagent solution with added OPA

Laboratory air was drawn through four midget impingers at 1.0 L min^{-1} for 120 min at 38.3% RH at a TWA. These impingers were preceded by 0.5 g plugs of silane-treated glass wool which would trap any OPA vapor in the air. Two of the impingers contained 10.00 mL quantities of DMSO-reagent solution with 0.4 μg of OPA, and two impingers contained blank DMSO-reagent solution. Also, 10 mL quantities of DMSO-reagent solution (two with 0.4 μg of OPA) were stored in 20 mL glass vials for comparison.

Construction of generation system for atmospheres of OPA vapor

The dynamic generation system was constructed from polypropylene “clear containers” by Rubbermaid® (see Fig. 4). The major chamber with a total internal volume of 439 L and a total height of 168 cm was built from four containers (110 L each, 64 × 47 cm at opening and 57 × 38 cm at the bottom), bolts and nuts. An opening was cut into one side of three of the 110 L containers to accommodate a glass window plate for access into the generation

system and for viewing. Each window plate was 30.5 × 20.3 cm. Each glass plate was sealed to the chamber with duct tape. Two minifans were placed centrally inside the major chamber (A, Fig. 4). Each of the three PVC pipes shown in Fig. 4 included a PVC valve (not shown). Air lines from a compressed air cylinder were connected to the 47 L polypropylene water chamber containing 12 L of water (B, Fig. 4) and the 59 L polypropylene chamber containing a source of OPA (C, Fig. 4). The manifold (J, Fig. 4) was connected to a glass T. The second port of this glass T was plugged either with either a needle valve or a plastic cap containing multiple needle holes through which OPA atmosphere flowed. The third port of this glass T was connected with Tygon tubing to the laboratory air outside of the OPA generation system and was the source of dilution air. A test tube rack was suspended near the top of the main chamber (A, Fig. 4) for supporting samplers under the manifold (J). The source of OPA in a 250 mL beaker was either CIDEX OPA (125 mL) or crystals of OPA (2 g). This beaker was placed into either the OPA chamber (C in Fig. 4) or the major chamber (A in Fig. 4).

Air sampling inside the generation system

Air samplers were connected to ports of the manifold. Pumps capable of operating at 1.0 L min^{-1} located outside of the generation system were connected to the individual samplers *via* Tygon® and PTFE tubing through ports. Midget impingers containing charcoal backed up the impingers containing DMSO-reagent solution in order to protect the pumps from splash-over.

Attempt to determine capacity of the S10 LpDNPH sampler

Two S10 LpDNPH samplers were connected directly to ports of the manifold inside the generation system. Each sampler was backed up by a second S10 LpDNPH sampler. Pumps drew atmosphere containing OPA vapor at 1728 μg m^{-3} into each sampling train at 1.0 L min^{-1} for 165 min. The RH was 80.8% as a TWA. The DNPH samplers were analyzed by the DNPH-HPLC method.

Preparation of ozone scrubbers

Silica gel (30 g) was coated with 300 mg of recrystallized *trans*-1,2-bis(2-pyridyl)ethylene as described.[20] Ozone scrubbers were constructed by addition of 130 mg quantities of silica gel coated with *trans*-1,2-bis(2-pyridyl)ethylene to cleaned polypropylene tubes obtained from used S10 LpDNPH cartridges. Polyethylene frits from other S10 LpDNPH cartridges held the coated silica gel in place.

Test of constructed ozone scrubbers

Two S10 LpDNPH samplers were connected directly to ports of the manifold inside the OPA generation system. Two other S10 LpDNPH samplers were connected to ozone scrubbers constructed as described, which, in turn, were connected to ports of the manifold. Air sampling for OPA was conducted at 1.0 L min^{-1} for 120 min. The four S10 LpDNPH samplers were analyzed for OPA by the DNPH HPLC method.

Test of multiple polyethylene frits for trapping OPA

Two S10 LpDNPH samplers were connected directly to ports of the manifold inside the OPA generation system. Two other S10 LpDNPH samplers were connected to tubes (used and cleaned S10 LpDNPH cartridges) containing six frits in series each, which, in turn, were connected to ports of the manifold. Air sampling for OPA was conducted at 1.0 L min^{-1} for 60 min. The four S10 LpDNPH samplers were analyzed for OPA.

Measurement of OPA condensation aerosol

A glass U-tube with glass stopcocks was immersed in an oil bath at 118 °C. A HEPA filter (HEPA-CAP™ 36) was connected to the inlet of the U-tube to prevent dust in the laboratory air from entering the U-tube. The glass stopcock at the inlet was replaced with two rubber gloves, powder-free, and the gloves were used to make a tight seal with rubber bands. Vacuum grease was applied to the top layer of rubber glove material to seal any puncture made by a syringe needle during the introduction of OPA solution. While the pump for the Scanning Mobility Particle Sizer drew air through the U-tube and 50 cm of conductive rubber tubing at 1.0 L min^{-1}, 1 mL of an acetonitrile solution of OPA (0.96 mg mL^{-1}) was injected into the U-tube through the rubber gloves *via* the needle of a syringe. Aerosol data were recorded.

Results and discussion

DNPH-HPLC method for OPA in air

Analytical limits in solution—The limit of detection (LOD) and the lower limit of quantitation (LOQ) of OPA in DMSO solution were found to be 0.004 μg mL^{-1} and 0.013 μg mL^{-1}, respectively [calculated from the concentrations of five low-level standards in duplicate of OPA-bis(DNPH) in DMSO plus a blank]. Since the volume of the eluted sample was 4 mL, the LOD and LOQ of OPA were 0.016 and 0.052 μg per 4 mL sample, respectively. For low-level standard solutions of OPA-bis(DNPH), the LOD is defined as three times the standard error of the least squares calibration curve divided by the slope, and the LOQ is defined as ten times the standard error of the least squares calibration curve divided by the slope.[21,22] The calibration curve for five low-level standards in duplicate in the range of 0.092 to 0.55 μg mL^{-1} plus a blank was linear with R^2 = 0.9998 [this range pertained to OPA-bis(DNPH) and was equivalent to 0.025 to 0.15 μg mL^{-1} of OPA]. The slope, intercept and standard error were 12 926, 13 and 17, respectively.

Stability of HPLC standards—A storage study of six standards of OPA-bis(DNPH) in DMSO solution was performed in which the standards were stored at room temperature in the dark and analyzed on a weekly basis. Concentrations ranged from 0.40 to 12.0 μg mL^{-1} in OPA equivalents. Peak areas gradually decreased over time. Average peak areas were found to be 96 to 100% of the original values during the first 73 days of storage and 87.7% of the original values at a storage time of 104 days.

Two crystalline forms of OPA-bis(DNPH)—OPA-bis(DNPH) which had been recrystallized from *N,N*-dimethylacetamide was characterized by fluffy, orange crystals which melted at 285.0 to 285.1 °C with some frothing but no explosion. However,

recrystallization of OPA-bis(DNPH) from *N,N*-dimethylformamide gave rise to dense, red crystals which melted at 301.8 to 301.9 °C with explosive force.[14] The two crystalline forms in KBr pellets were confirmed by FTIR; each crystalline form exhibited five absorption bands with identical wave numbers while other absorption bands were shifted slightly in the range of 1614 to 772 cm^{-1}.

Estimated solubility of OPA-bis(DNPH) in DMSO—The solubility of OPA-bis(DNPH) in DMSO at room temperature is less than 384 μg mL^{-1} (less than 104 μg mL^{-1} in OPA equivalents) and greater than 169 μg mL^{-1} (greater than 45.8 μg mL^{-1} in OPA equivalents). Thus, the estimated solubility of OPA-bis(DNPH) in DMSO is an intermediate value of 276 μg mL^{-1} at room temperature (75 μg mL^{-1} in OPA equivalents). Consequently, OPA-bis(DNPH) is about 46 times more soluble in DMSO at room temperature than in acetonitrile at 30 °C.[15]

Chromatography—A chromatogram of an OPA air sample is shown in Fig. 5. Retention times for OPA-bis(DNPH) are very stable at 7.5 min. The two chromatographic peaks for excess DNPH shown in Fig. 5 are typical. The capacity factor k', = 2.56.

Study of recovery *versus* storage time of OPA inside S10 LpDNPH samplers—Fig. 6 presents a plot of recovery of OPA *versus* storage time of S10 LpDNPH samplers at room temperature with excess DNPH present. Each point in Fig. 6 represents a single sampler. The first six points (from 1 to 9 h) represent a plateau at 70% recovery, a plateau which suggests an induction period. The curve begins to rise to 83% recovery from 9 to 18 h of storage. Finally, a recovery of 100% is realized after a storage period of 68 h. These results are consistent with the hypothesis that recovery increases slowly to 100% after the induction period as DNPH in ethyl acetate solution reacts with OPA to form OPA-mono(DNPH) and with OPA-mono(DNPH) to form OPA-bis(DNPH) in a stepwise process. The apparently slight drop to 94% at 94 h may be within experimental error.

Relative reaction rates of OPA with DNPH in gas and solution phases—At the completion of air sampling, three species are present on the S10 LpDNPH sampler: OPA-bis(DNPH), OPA-mono(DNPH), and OPA itself.[15] The presence of the second and third species is evidence that complete conversion to the desired product, OPA-bis(DNPH), has not taken place at this point. Immediately, DNPH in ethyl acetate solution is added to convert OPA-mono(DNPH) and OPA to OPA-bis(DNPH). The conversion is complete in 68 h according to the previous paragraph. On the other hand, fortification of the DNPH-coated silica gel bed of an S10 LpDNPH sampler with 100 μL of an acetonitrile solution of OPA gives rise to OPA-bis(DNPH) in quantitative conversion in less than 15 min (probably in a matter of seconds). An obvious explanation for the incomplete conversion to OPA-bis(DNPH) during air sampling is the powerful force of hydrogen bonding of OPA with functional groups on the surface of the silica gel in the S10 LpDNPH sampler. It takes a relatively long time to convert OPA-mono(DNPH) and OPA to OPA-bis(DNPH) with excess DNPH in ethyl acetate solution because these two OPA species are still rightly bound to functional groups on the surface of the silica gel. Formation of OPA-bis(DNPH) from addition of OPA in acetonitrile solution to DNPH-coated silica gel is very rapid due to the

facts that the OPA in solution has virtually no opportunity to undergo hydrogen bonding with functional groups on the silica gel and that acid catalyst is present.

Recovery of OPA vapor spikes from S10 LpDNPH samplers—Table 1 presents data for recovery of OPA vapor spikes after the samplers had been stored 68 h at room temperature, eluted with DMSO and measured by HPLC. Average recoveries ranged from 95.8% to 100.9%. Apparently, a residual quantity of OPA (0.8 μg) consistently failed to vaporize from the PTFE-GFFs for collection. Attempts to measure the quantities of OPA remaining on the PTFE-GFFs by soaking the PTFE-GFFs in DMSO-reagent solution for the fluorescence method indicated only 11 to 16% (0.09 to 0.13 μg) of 0.8 μg quantities of OPA which remained the PTFE-GFFs were found by the fluorescence method. Thus, it appears that OPA in quantities of 0.67 to 0.71 μg was irreversibly adsorbed onto the PTFE-GFFs (Table 2).

Virtually complete vaporization of OPA from PTFE-GFFs at relatively high levels of 14 to 19 μg was sometimes unsuccessful (see Tables 3 and 4 for cases of successful vaporization). Spreading the OPA over the area of a 37 mm filter did improve the rate of vaporization in comparison with use of a smaller area. However, the technique of vaporization of OPA from PTFE-GFFs at room temperature is unfavorable at relatively high levels of OPA, such as 10 to 30 μg or more, during a period of pump operation of 1 to 2 h. Therefore, it is recommended that the technique of vaporization of OPA from PTFE-GFFs at room temperature be limited to levels of OPA of 6 μg or less per filter. This range should be acceptable because the typical quantities of OPA collected during industrial hygiene air sampling in a hospital worksite are about 1 μg and smaller,[14]

Recoveries of OPA spikes (as a mixture of vapor and condensation aerosol) from S10 LpDNPH samplers—Table 8 presents average recoveries of OPA from S10 LpDNPH samplers following collection of OPA vapor and condensation aerosol from a U-tube heated to 120 °C. Recoveries are essentially quantitative with RSDs less than 4% at levels of 2.5 to 20.2 μg.

Advantages of preparing OPA spikes from a U-tube include (a) the high bath temperature of 120 °C ensures rapid and complete vaporization of OPA, (b) levels of OPA which can be vaporized rapidly exceed 20 μg, and (c) use of an inert atmosphere, such as nitrogen, is not required.

Procedure employed for making a solution of OPA in acetonitrile—Unopened vials of OPA contain inert gas to protect the OPA crystals from oxidation by air. However, if the OPA in the vial is exposed to minute quantities of air, the OPA will oxidize slowly. The analytical result for purity of a small sample of the OPA may not represent the overall purity because a range of purities may exist due to exposure of only the surfaces of OPA crystals to air. The routine procedure for making a solution of OPA in acetonitrile of known concentration included (a) opening the vial in the air in the balance room, (b) removing 16 to 80 mg of OPA and impurities with a slender spatula 3 mm wide (a slender spatula may minimize the introduction of air into the vial), (c) transferring the OPA and impurities to a tared vial, (d) estimating the purity of the OPA from time of initial opening of the vial, (e)

determining the mass of crystalline material by weight measurement, (f) quickly adding 3 to 16 mL of acetonitrile to the OPA, and (g) determining the actual concentration of OPA in a 100 μL aliquot by either of two techniques: (1) fortifying an S10 LpDNPH sampler with the aliquot and analyzing the eluent by HPLC analysis or (2) adding the aliquot to 10 mL of DMSO-reagent solution and analyzing the solution by fluorescence analysis. When the purity of OPA in the vial was found to be about 60% or lower, the OPA was discarded. Estimating the purity of the OPA depended, in part, on the date the vial from the source had been opened in air for the first time, the frequency of opening the vial in air, and the previous analytical result unless the vial of OPA from the source had never been opened.

This routine procedure was preferable to a conceived procedure for protecting the OPA crystals from air during times of removal of samples from the vial. Since accidental exposure of OPA crystals to oxygen in the conceived procedure would lead to oxidation, one might desire to check the purity of the OPA crystals from time to time if the conceived procedure is employed.

It is recommended that OPA in 1 gram quantities be purchased. Consequently, when the quality of OPA in one vial has deteriorated, other vials will remain which contain OPA in high purity.

Recoveries of OPA from S10 LpDNPH samplers after storage—Table 3 presents recoveries of OPA from S10 LpDNPH samplers after excess DNPH had been added to the samplers and after single samplers had been stored at room temperature. While recoveries tended to remain above 90% at the lowest and intermediate levels, recoveries did decrease with increasing storage time at the highest level. Storage of similar samples at −16 °C failed to make adequate improvement in recovery. Since all recoveries of OPA were above 90% for as much as 6 days of storage, the maximum recommended storage time of OPA samples in S10 LpDNPH samplers treated with excess DNPH is 6 days at room temperature.

Recoveries of vapor spikes following air draw—Recoveries of OPA vapor spikes following air draw are presented in Table 4. This study was performed because two of the three species on the sampler immediately after collection are air-sensitive; namely, OPA and OPA-mono(DNPH).[14,15] Results in Table 4 indicate that recoveries of OPA-bis(DNPH) are decreased due to air oxidation of OPA and OPA-mono(DNPH) and that recoveries of OPA-bis(DNPH) tend to decrease with increasing periods of air draw at the lowest and intermediate levels. However, this trend is not observed at the highest level, where recovery tends to remain somewhat consistent with increasing periods of air draw. No explanation is given for this observation.

Colored bands of OPA derivative on DNPH-coated silica gel—Fig. 7 illustrates colored bands of OPA derivative on four S10 LpDNPH samplers (samplers 2 through 5) which can be observed after the collection of OPA in quantities of 12 to 120 μg. Samplers 2, 3, and 4, which have collected 12, 36 and 120 μg of OPA, respectively, at a relative humidity (RH) of 60% as a mixture of condensation aerosol and vapor, exhibit progressively larger bands orange or red-orange in color. The actual color of a band appears to depend largely on the RH of the air sample. Sampler 5, which has collected 43 μg of OPA at 23% RH as

entirely vapor, exhibits a band which is dark brown. While the DNPH-coated silica gel bed of sampler 1 (a blank through which no air has been drawn) is bright yellow in color, the coated silica gel beds of the other samplers exhibit a darker shade of yellow behind the colored bands (Fig 7)

Capacity of the S10 LpDNPH sampler—Experiments indicated that the capacity of the S10 LpDNPH for OPA before significant breakthrough was more than 273 μg at an average air concentration of 1728 μg m^{-3} for the two front samplers. Although 0.083 μg and 0.045 μg of OPA were found on the two back-up samplers, these quantities were too small to be considered evidence of significant breakthrough. Since a capacity of 273 μg or more is quite sufficient for routine industrial hygiene sampling, no additional work was performed to determine the actual capacity. The stoichiometric quantity of OPA that would react with 1 mg of DNPH on the silica gel bed is 339 μg.

Precision of measurement of the DNPH-HPLC method—The pooled precision (S_r) for the analytical portion of the DNPH-HPLC method for OPA was estimated at 0.01. This value was determined from the five RSDs (0.015, 0.0, 0.0028, 0.011, and 0.012) for analysis of standards in duplicate at the low end of the calibration curve used for estimating the LOD for this method. It is possible to make this estimate of S_r because the RSD of the response values approximates the RSD of the determinations when there is a zero intercept.

Routine procedure for the DNPH-HPLC method—Air sampling is conducted at L min^{-1} for four hours or less. Immediately after sampling, the S10 LpDNPH sampler is held in the vertical position, the outlet cap is removed, and 500 μL of DNPH-ethyl acetate solution in a 500 μL syringe is added from the outlet end (Fig. 3). The inlet cap is removed from the sampler to allow the solution to be absorbed by the silica bed during displacement of air from the silica bed. Then the sampler is placed into the horizontal position, and the airspace in the inlet of the sampler is filled with DNPH-ethyl acetate solution (200 μL). The inlet cap is put into place. The large air space in the outlet of the sampler is flushed with nitrogen, and 200 μL of DNPH-ethyl acetate solution is added (Fig. 3). The outlet cap is put into place. The sampler is sealed tightly with the inlet and outlet caps. The sampler is stored in the dark at room temperature for 68 h to 94 h. DMSO (4.0 mL) is added to the outlet of the S10 LpDNPH sampler to elute the OPA derivative [OPA-bis(DNPH)]. The volume of eluent collected is calculated from the weight of the eluent in a tared vial and the estimated density (1.06 g mL^{-1}) of the eluent. Note: In lieu of the S10 LpDNPH sampler, one may employ the S10L LpDNPH sampler, if desired, for air sampling, treatment with DNPH-ethyl acetate solution, and shipment to the laboratory for analysis. Since the S10L sampler is much smaller, all of the air spaces can be filled with DNPH-ethyl acetate solution, eliminating the need to flush any air space with nitrogen. In addition, the S10L sampler is small enough to fit into a 20 mL vial for shipment (the vial would contain any leaked ethyl acetate solution). Since S10L sampler has a female Luer fitting at the outlet, one may attach the inlet of a cleaned cartridge from a used S10 LpDNPH sampler to the outlet of the S10L sampler for addition of DNPH-ethyl acetate solution and for elution of the air sample with DMSO in the laboratory.

Interferences in the DNPH-HPLC method—Ozone may consume some of the DNPH reagent in the sampler and may degrade OPA-bis(DNPH), OPA-mono(DNPH) and OPA inside the sampler because ozone has been observed to consume DNPH and degrade the DNPH derivative of formaldehyde.[23] Aldehydes and ketones in high concentrations in the air may consume unacceptably large quantities of DNPH reagent in the sampler.

Advantages of the DNPH-HPLC method—(a) The sampler is light-weight and convenient for air sampling in the personal breathing zone of the worker. (b) Air sampling may be conducted for four hours. (c) The identity of OPA can be corroborated by detection of the corresponding derivative, OPA-bis(DNPH), by HPLC at the correct retention time. (d) The method is calibrated against a primary standard, namely, OPA-bis(DNPH). (e) The method is unaffected by humidity. (f) Two different types of samplers are commercially available.

Disadvantages of the DNPH-HPLC method—(a) A storage period of 68 h for the sampler is required prior to analysis. (b) Ozone might degrade three OPA species in the sampler; namely, OPA-bis(DNPH), OPA-mono(DNPH), and OPA itself.[23] However, the typical levels of ozone in hospitals with air conditioning (10 ppb or less) are too low to be of consequence. (c) The total cost of equipment is considerably more than that of the impinger-fluorescence method.

Impinger-fluorescence method for OPA in air

Wavelength requirements—The fluorescent product, a thio-*N*-alkyl-substituted isoindole formed from OPA, *N*-acetyl-L-cysteine, and ethylenediamine, is characterized by an absorption band in the UV which has a λ_{max} at 339 nm (the molecular structure of this fluorescent product has the same basic structure of the fluorescent product produced from OPA, *N*-acetylcysteine and taurine depicted by Vander Heyden *et al.*[18]). Absorption by this band extends from about 315 to 400 nm. Therefore, the excitation wavelengths to induce fluorescence must be within this range. Fig. 8 presents the fluorescence emission spectrum, and the inset is a color photograph of the fluorescent emission, which is predominantly blue. The λ_{max} for the fluorescence is about 448 nm, and emission extends from about 375 to 560 nm. The TBS model 380 and the *Picofluor* fluorometers provided excitation in the range of 365 to 395 nm and were equipped with filters to allow light in the range of 440 to 470 nm to pass through. Photodiodes inside the fluorometers were sensitive to emission in this range of wavelengths.

Analytical limits in solution—The LOD and the LOQ of OPA were found to be 0.024 μg and 0.079 μg per 10 mL of DMSO-reagent solution [calculated from the concentrations of five low-level standard solutions in duplicate plus a blank (concentrations of standards ranged from 0.1 to 0.5 μg of OPA per 10 mL of solution)]. Fluorescence readings were corrected for the blank fluorescence reading, which was near zero (0.297). The calibration curve was linear with $R^2 = 0.9991$. The slope, intercept and standard error were 38, 0.5, and 0.3, respectively.

Rates of evaporation of DMSO and water from impinger during air draw—The two impingers to which only 10.00 mL quantities of DMSO had been added lost 280 mg and 250 mg of weight after air draws of 126 L and 121 L, respectively. The impinger which originally had contained 10.00 mL of DMSO and 1.50 mL of water lost 430 mg of liquid after an air draw of 120 L. The impinger which originally had contained 10.00 mL of DMSO and 3.00 mL of water lost 680 mg of liquid after an air draw of 120 L. The rates of evaporation of liquid from the four impingers were 2.2, 2.1, 3.6 and 5.7 mg per liter of dry air draw, respectively.

Theoretical capacity of the DMSO-reagent solution—Since *N*-acetyl-L-cysteine and ethylenediamine were employed in equimolar quantities, the theoretical capacity of 10 mL of DMSO-reagent solution for OPA was 7.96×10^{-5} mol or 10.7 mg. Breakthrough studies at levels approaching the theoretical capacity were not conducted.

Stability of standards for fluorescence readings—Table 5 presents fluorescence readings of separate samples equivalent to 50 μg of OPA per 10 mL of DMSO-reagent solution. The fluorescence readings of the samples reached 92.6% of the maximum value at 80 min and 100% of the maximum value at 5.0 h. However, the fluorescence reading of the blanks continued to rise with time during the 26 h period.

Recommended techniques for preparation of DMSO-reagent solution which gives rise to low blanks include (a) use of pure reagents and (b) use of an Eppendorf pipet with disposable glass tips for measuring ethylenediamine. On the other hand, use of a syringe which has been rinsed with deionized-distilled water to measure ethylenediamine may lead to contamination, particularly when the syringe is primed with the ethylenediamine. Taking an aliquot of ethylenediamine from a freshly opened vial with a syringe will lead to low blanks; however, the possible contamination may cause high blanks a few days later.

Ethylenediamine which is not contaminated will give rise to low fluorescence blanks with readings of about five fluorescence units or less after a standing time of 4 h following mixing. Table 9 indicates the use of a low blank with a fluorescence reading of 1.118 after a standing time of 2.5 h. Table 5 indicates the use of a high blank with fluorescence readings of 17.31 and 31.00 at 80 min and 3 h of standing, respectively.

It is recommended that multiple vials of ethylenediamine be purchased in the smallest quantities possible, such as 5 mL. Thus, when the reagent from one vial becomes impure due to contamination, other unopened vials of pure reagent will remain available for use.

Recoveries of OPA from impingers of DMSO-reagent solution—Table 6 presents recoveries of OPA following collection of vapor spikes from PTFE-GFFs resting inside the outlet pieces of filter cassettes. Recoveries are essentially quantitative with good precision (97.1% to 105.1%).

Collection efficiency of DMSO-reagent solution—Table 7 presents data to show that the collection efficiency of the impinger for OPA in the first experiment was 99.4% at a flow rate of 1.0 L min^{-1}. Since 20 μg of OPA was added to the U-tube and since a total of 17.91

μg was found (17.8 μg in the impinger and 0.11 μg in the backup S10 LpDNPH sampler), the 19 cm piece of PTFE-tubing trapped 2.09 μg of OPA. Therefore, the first impinger trapped 99.4% (17.8 μg) of the 17.91 μg of OPA which entered the impinger. In the second experiment, the quantity of OPA of 18.4 μg found in the first sampler (impinger), was greater due to a shorter length of PTFE tubing before the first impinger. OPA was not detected in the back-up impinger due to losses in the connecting PTFE tubing.

Effect of air draw through DMSO-reagent solution with added OPA—Table 9 presents the results of 120 L of air draw at 1.0 L min^{-1} through four impingers containing 10 mL quantities of DMSO-reagent solution at an RH of 38.3% as a TWA. OPA (0.4 μg) was present in two of the impingers. The air draw had no significant effect on the samples and blanks according to a comparison with similar samples and blanks through which no air was drawn.

Precision of measurement of the impinger-fluorescence method—The pooled precision (S_r) for the analytical portion of the impinger-fluorescence method for OPA was estimated at 0.06. This value was determined from the five RSDs (0.019, 0.0076, 0.021, 0.0022, and 0.10) for analysis of standards in duplicate at five levels at the low end of the calibration curve. As in the case of the DNPH-HPLC method, it is possible to make this estimate of S_r because the RSD of the response values approximates the RSD of the determinations when there is a zero intercept.

Routine procedure for the impinger-fluorescence method—The midget glass impingers are washed and dried in an oven at 110 °C prior to use. Since weight measurement of the impinger in the field may be impossible, a calibrated scale is attached to the outside wall of the impinger for estimating the volume change of DMSO-reagent solution due to evaporation of solvent or absorption of water from humid air. DMSO-reagent solution (10.00 mL) is added to each impinger and to a 20 mL glass vial to serve as a blank. Air sampling is conducted at 1.0 L min^{-1} for 5.5 h or less. After sampling, the volume change of each impinger solution is estimated from the calibrated scale. Fluorescence readings of samples in 1 cm × 1 cm polystyrene cuvettes are taken 80 min from the time of completion of sampling with a portable fluorometer. The fluorescence reading of each sample is corrected for the blank fluorescence reading and for the volume change. The quantities of OPA are determined from a calibration curve prepared from a freshly opened vial of OPA of known purity.

Interferences in the impinger-fluorescence method—Klenzyme® and Metrizyme® are enzymatic cleaning agents which are used in healthcare settings. In concentrated form, these agents will fluoresce under the same wavelength conditions employed in this method even when *N*-acetyl-L-cysteine and ethylenediamine are absent. However, these agents are used in diluted form for cleaning, and the diluted forms do not fluoresce even when *N*-acetyl-L-cysteine and ethylenediamine are present. Twenty aldehydes and four amino compounds have been tested for interference with a fluorescent method for OPA on surfaces, a method which employs the same chemistry with *N*-acetyl-L-cysteine and ethylenediamine.[14] Of twenty aldehydes and four amino compounds tested for interference,

only 4-(dimethylamino)cinnamaldehyde showed strong, positive interference with the fluorescent method for OPA.[14] All other test compounds caused either no interference or weak to moderate interference.[14] Glutaraldehyde was a moderate negative interference with the method.[14]

Advantages of the impinger-fluorescence method—(a) Analytical results for air sampling are obtained on the same day. (b) The limit of detection of the impinger-fluorescence method is close to that of the DNPH-HPLC method. (c) The reagents required are readily available. (d) The total cost of equipment is considerably less than that of the DNPH-HPLC method. (e) Since DMSO is virtually nonvolatile, the impinger need not be replenished with solvent during air sampling.

Disadvantages of the impinger-fluorescence method—(a) The use of midget impingers is inconvenient for personal sampling. (b) Spillage of sample solution might occur. (c) DMSO is hygroscopic and will absorb water vapor from the air. (d) Since the melting point of DMSO is 18.5 °C, air sampling should not be conducted at temperatures below 20 °C. (e) The portable fluorometer should be calibrated on the day of use. Daily calibration of the fluorometer is possible when an analytical laboratory is available nearby which is equipped with pure OPA, the specified test chemicals, a source of pressurized inert gas and an analytical balance.

Comparison of the two methods—Average concentrations of OPA in air by both sampling and analytical methods agreed with each other within 10% in Tables 10 through 13. The calculated average OPA air concentrations by the DNPH-HPLC method were higher man those by the impinger-fluorescence method by 9.3% and 5.5% in Tables 10 and 12, respectively. The reverse was true in Tables 11 and 13 with average concentrations by the impinger-fluorescence method higher by 6.3% and 3.7%, respectively. While the RH was low (15.3 to 24.6%) in Tables 10, 12, and 13, an increase to a very high RH (82.4% as a TWA) did not cause any disparity in found concentrations of OPA by the two methods and did not cause any uncorrectable problems (see Table 11). Weights of the two impingers for Table 11 increased by 1.058 g and 1.055 g at 82.4% RH as a TWA during two hours of air sampling. However, the weights of the impingers for Tables 10, 12, and 13 decreased by 0.05 to 0.26 g during air sampling for periods of 1.5 to 4.0 h at low humidity due to evaporation of DMSO. Data in Table 13 indicate that both the DNPH-HPLC and impinger-fluorescence methods can be employed for air sampling periods of at least four hours. Data in Table 4 indicate that the impinger-fluorescence method may be employed for sampling periods as long as 5.5 hours due to the fact that significant decay of the fluorescent product does not take place during the sampling period plus 80 min of standing. In addition, the effect of air draw through impingers containing the fluorescent product is insignificant with respect to fluorescence (see Table 7).

Test of ozone scrubbers—While two S10 LpDNPH samplers connected directly to ports of the manifold inside the generation system gave rise to OPA air concentrations of 502 and 503 μg m^3, two S10 LpDNPH samplers which were preceded by ozone scrubbers failed to collect any detectable OPA. Therefore, these scrubbers trapped OPA from the air

very effectively. Ozone scrubbers were tested because industrial hygienists often are concerned about possible interference by ozone.

Test of multiple polyethylene frits—The two S10 LpDNPH samplers which were connected directly to ports of the manifold inside the OPA generation system gave rise to air concentrations of OPA of 474 and 486 μg m^{-3}. However, the two S10 LpDNPH samplers which were preceded by tubes containing six polyethylene frits in series during the same sampling period gave rise to apparent OPA air concentrations of 156 and 196 μg m^{-3}. Therefore, each tube containing six frits trapped a significant quantity of OPA. Six frits in series were used in order to magnify the effect of a single frit.

Adsorption of OPA by polypropylene surfaces—The interior polypropylene surfaces of the OPA generation system reversibly adsorbed OPA vapors during generation of atmospheres. Upon removal of all intentional sources of OPA inside the generation system, OPA vapors persisted inside the system. Initial air concentrations of OPA without an intentional source of OPA were as high as about 1300 μg m^{-3} Concentrations of OPA vapor decreased to about 1% of the maximum value during a period of two weeks during which time air sampling experiments were conducted. Polypropylene had been selected as the construction material for the generation system because polypropylene had been the construction material of the cartridges for the S10 LpDNPH samplers.

Measurement of condensation aerosols—Measurement during the first two minutes of a run plus the second two minutes of the same run (following a 30 s gap in data collection) are presented in Table 14 following injection of an OPA solution into a glass U-tube at 118 °C. The scanning mobility particle sizer was capable of recording data for the first two minutes of a run and additional two-min segments. However, there was a 30 s delay with no data recording prior to the next two-min segment. Since the lower limit of measurement was 7 nm, it is possible that particles smaller than 7 nm were not detected. However, the data clearly show that condensation aerosol of OPA did form.

Applicability of the two methods—Both the DNPH-HPLC and the impinger-fluorescence methods are useful for measuring OPA vapors in air over a wide range of concentrations and humidities. The limit of detection (LOD) of OPA by the DNPH-HPLC method is 0.016 μg per 4 mL sample or 0.067 μg m^{-3} (0.012 ppb or 12 ppt) for a 240 L air sample collected over a 4 h period. The lower limit of quantitation (LOQ) for OPA by the DNPH-HPLC method is 0.052 μg per 4 mL sample or 0.22 μg m^{-3} (0.040 ppb or 40 ppt) for a 240 L air sample.

The LOD of OPA by the impinger-fluorescence method is 0.024 μg per 10 mL impinger sample or 0.1 μg m^{-3} (0.02 ppb or 20 ppt) for a 240 L air sample collected over a 4.0 h period. The LOQ for OPA by the impinger-fluorescence method is 0.079 μg per 10 ml sample or 0.33 μg m^{-3} (0.060 ppb or 60 ppt) for a 240 L air sample collected over a 4.0 h period.

Both methods are useful for measuring OPA vapors in air at concentrations which exceed 2000 μg m^{-3} (364 ppb or 0.364 ppm) for 2 h sampling periods. Since the vapor pressure of

OPA is 0.0052 mm Hg at 21 °C,[7] the maximum possible vapor concentration of OPA at that temperature is 38 mg m^{-3}, Generally, a closed system would be required to achieve this concentration at 21 °C. Both methods are capable of measuring OPA in atmospheres containing a mixture of both OPA condensation aerosol and OPA vapors. Both the S10 LpDNPH sampler and the midget impinger containing DMSO-reagent solution can collect OPA condensation aerosols without significant breakthrough (note the colored bands near the inlets of the S10 LpDNPH samplers for samples 2, 3 and 4 in Fig. 7).

Recommended sampling rates—When the OPA in air exists entirely as vapor, the range of recommended sampling rates for the DNPH-HPLC method is 0.1 to 1.2 L min^{-1}. However, when there is presence of a mixture of OPA condensation aerosols and vapors, the recommended sampling rate is 1.0 L min^{-1}. In all cases, the recommended sampling rate for the impinger-fluorescence method is 1.0 L min^{-1}

Justification for status of "partially validated" for methods—Both methods, the DNPH-HPLC method and the impinger-fluorescence method, have a status of "partially validated" due to the successfully evaluated analytical portions of the methods, However, each method does not have a status of "fully validated" due to the fact that there was no independent method for determining the "true" concentrations of OPA in the generation system. Consequently, the bias of each method during air sampling was not calculated. The DNPH-HPLC and impinger-fluorescence methods for OPA in air will appear in the *NIOSH Manual of Analytical Methods* as methods 2025 and 3519, respectively.

Choice of which method for OPA in air to use—The first choice of a method for OPA in air is the DNPH-HPLC method because (1) the standard employed, OPA-bis(DNPH), is a primary standard, (2) the air sampler is adaptable to air sampling in the personal breathing zone, and (3) HPLC analysis of the sample can be conducted in the laboratory after 68 hours of storage. A primary standard in an analytical method is desirable because the standard is very pure, is stable during storage, and is weighed easily.

The impinger-fluorescence method is a second choice for sampling in the vicinity of the analytical laboratory. Also, it can be used for comparison with the DNPH-HPLC method in the analytical laboratory. However, OPA is not a primary standard, and daily calibration of the fluorometer away from the laboratory is a definite problem.

Principle of any sampling method for OPA in air—No materials should precede any sampler for OPA in air unless testing has shown that a particular material of interest will not collect OPA. The ozone scrubber described in this work will trap 100% of the OPA entering the scrubber. A small quantity of silane-treated glass wool (3 mg) will trap a portion of the OPA in an airstream. Polyethylene and polypropylene surfaces will adsorb OPA vapors reversibly.

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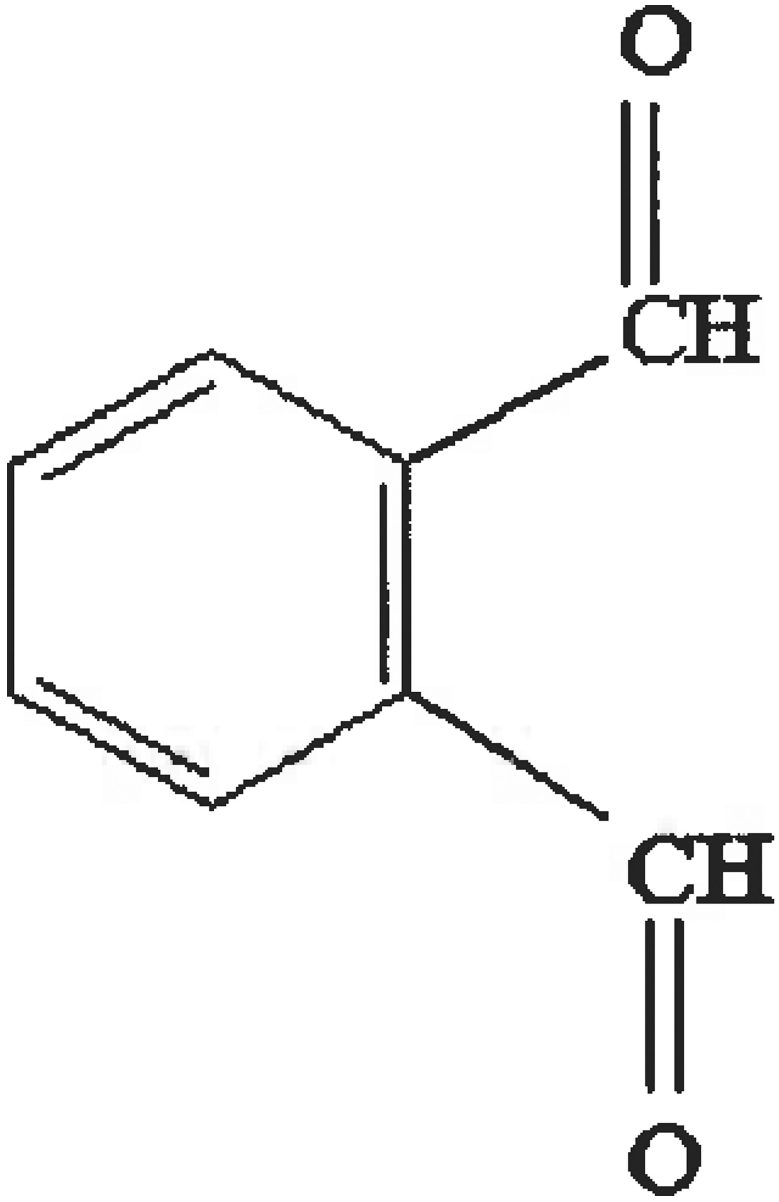

Fig. 1.
Molecular structure of *ortho*-phthalaldehyde with two aldehyde groups (MW = 134.13; CAS RN = 643-79-8).

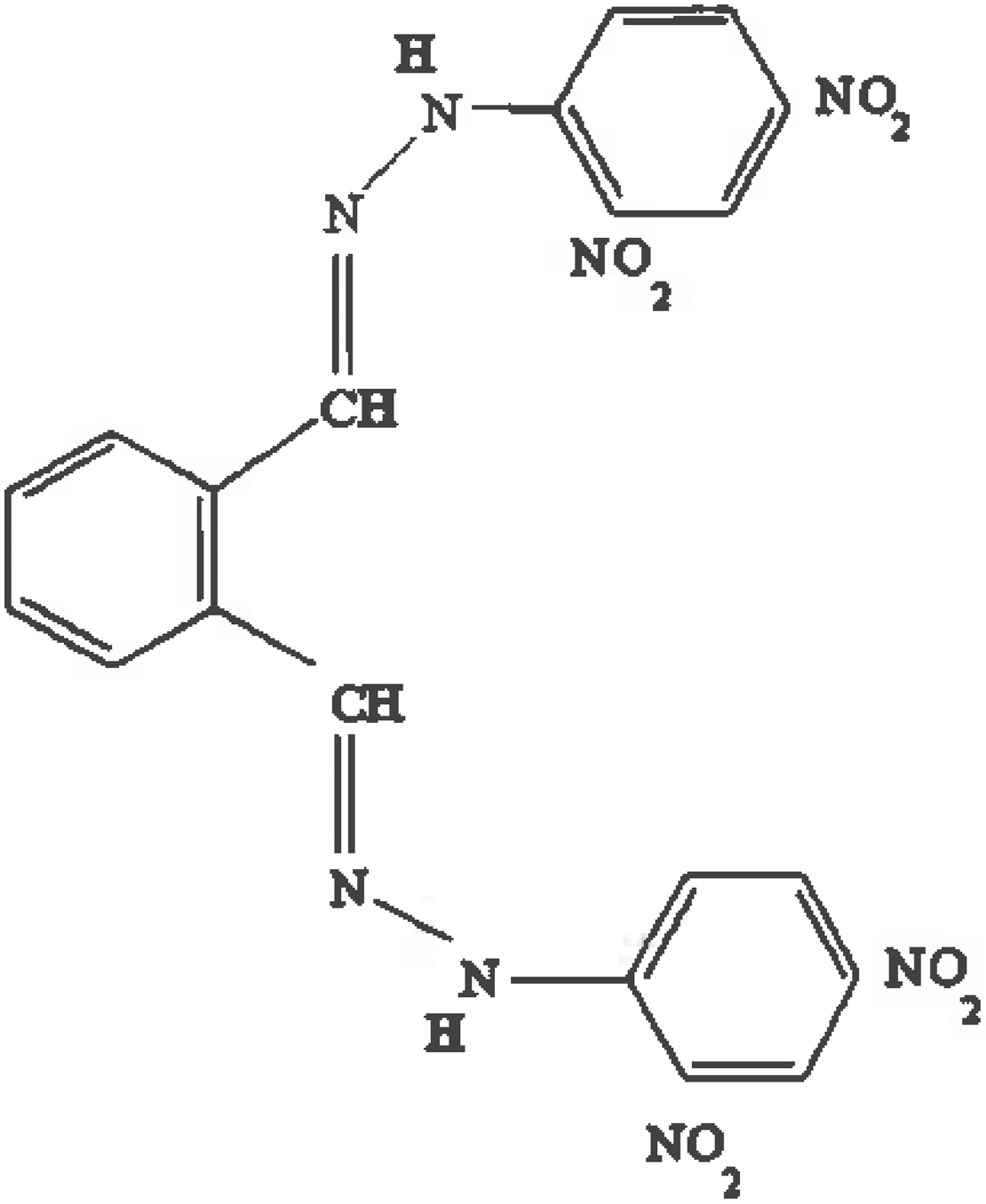

Fig. 2.
Molecular structure of OPA-bis(DNPH) (MW = 494.37).

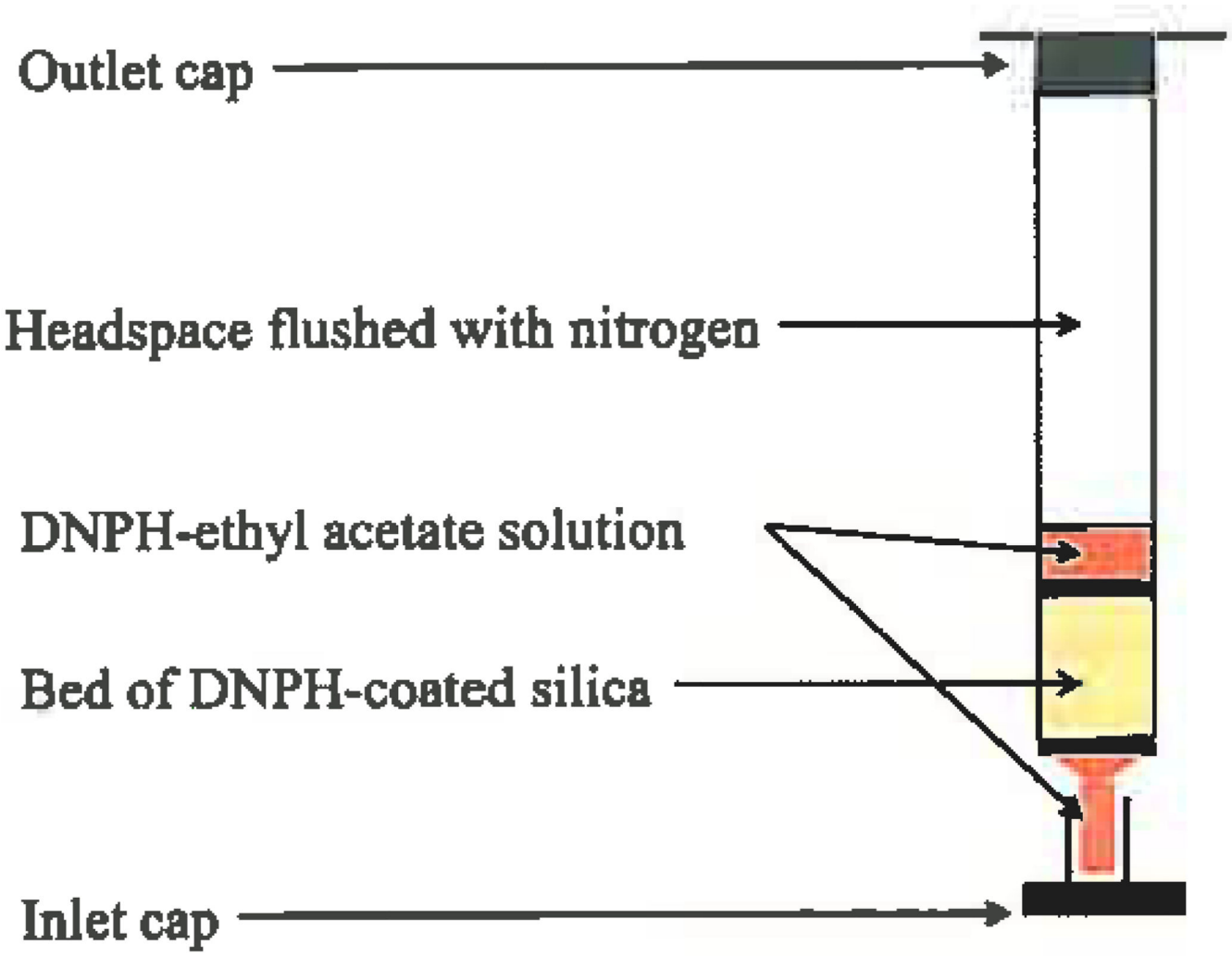

Fig. 3.
Diagram of S10 LpDNPH sampler to which excess DNPH in ethyl acetate solution has been added.

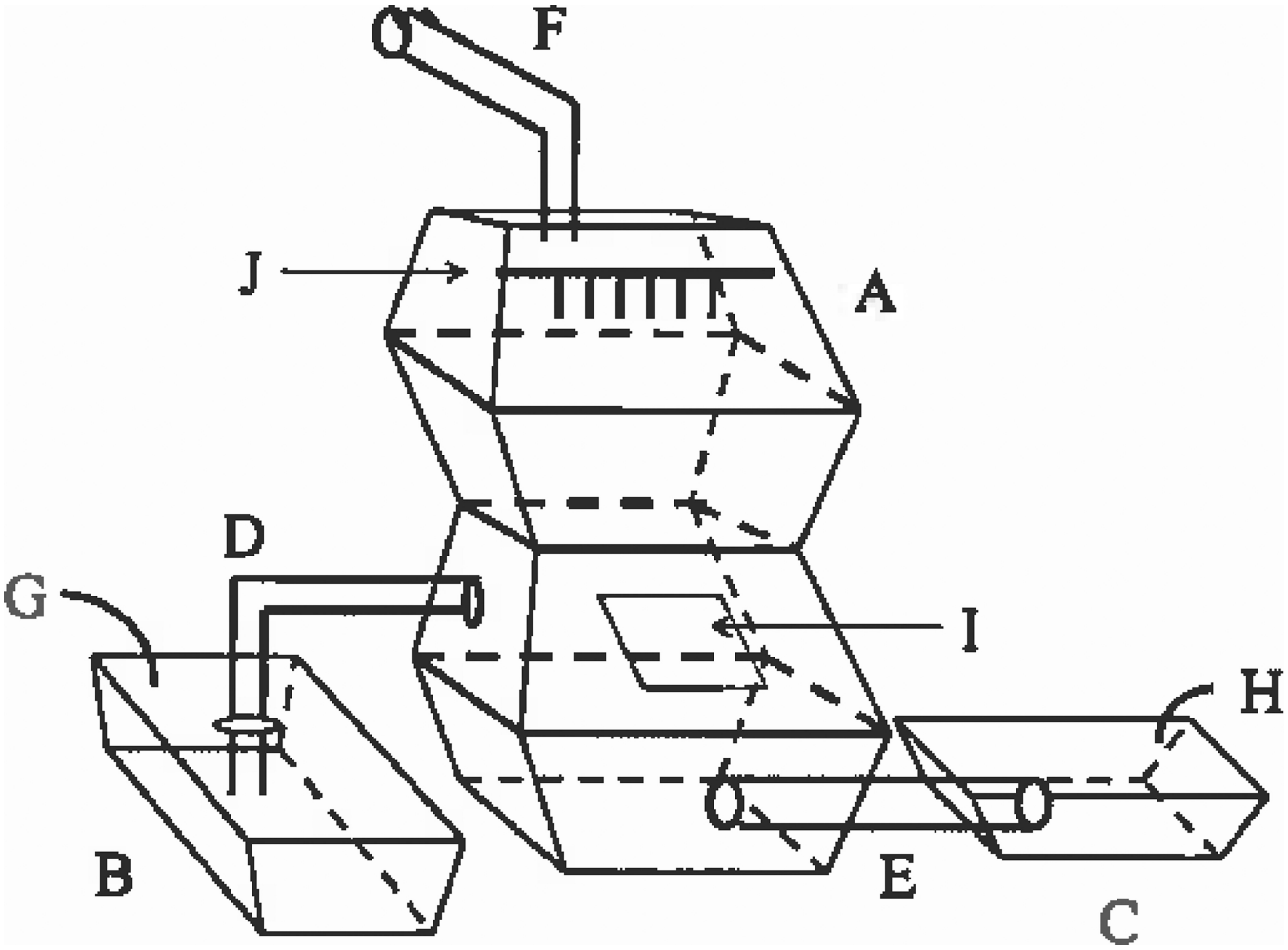

Fig. 4.

Diagram of simplified OPA generation system. (A) Main chamber with volume of 439 L. (B) 47 L water chamber for 12 L of water. (C) 59 L chamber for source of OPA. (D) PVC pipe for leading humidified air into the main chamber. (E) PVC pipe for connecting the PVC chamber (C) to the main chamber. (F) PVC pipe for leading exhaust vapors into a fume hood. (G) Tygon tubing through which compressed air flows into the water chamber. (H) Tygon tubing through which compressed air flows into the PVC chamber. (I) Removable glass window plate. (J) Glass manifold inside the main chamber. Parts not shown include minifans, flowmeters. test tube rack, glass T. Tygon tubing, source of OPA, water in chamber B. PVC valves, additional windows, and duct tape.

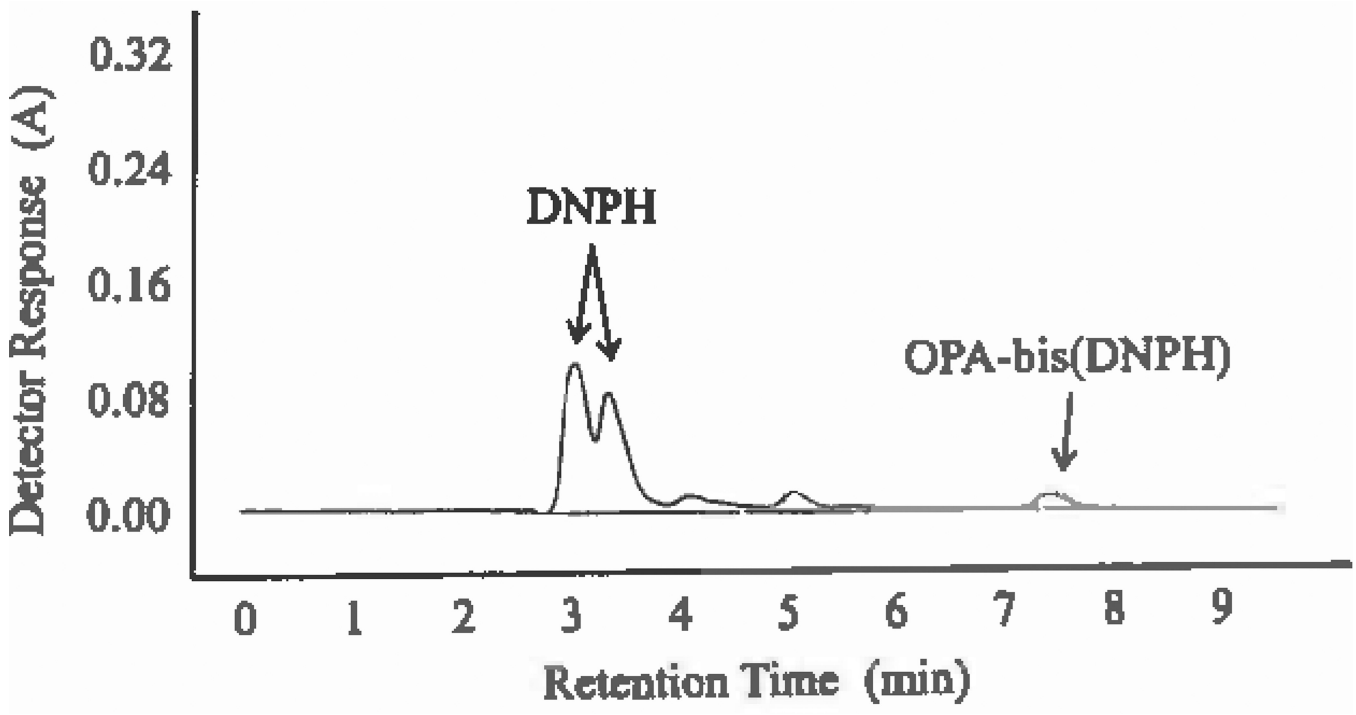

Fig. 5.

Chromatogram showing peak for OPA-bis(DNPH) from OPA air sample in DMSO solution at a concentration of 1.47 μg mL^{-1} (expressed in OPA equivalents).

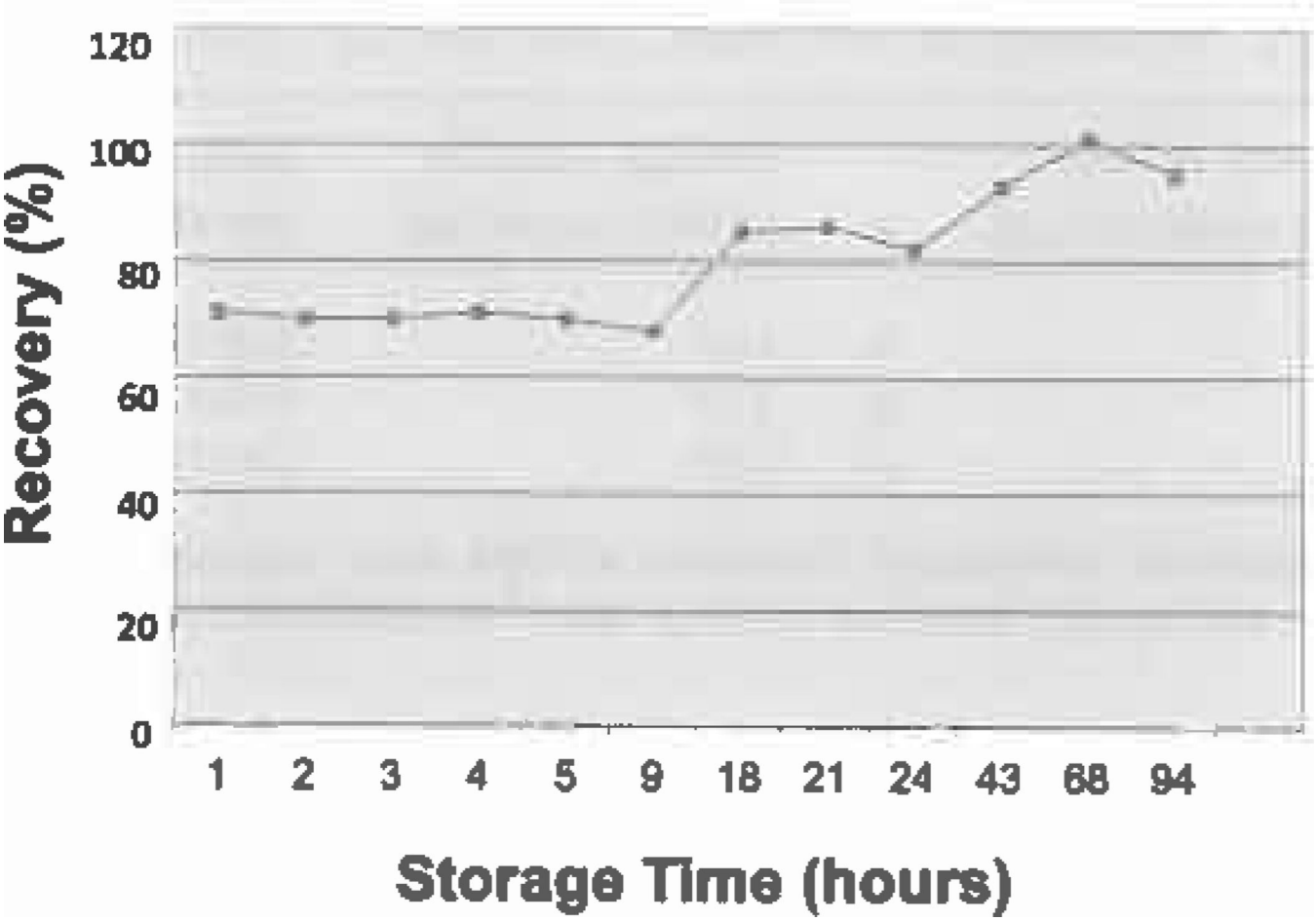

Fig. 6.

Plot of recovery of OPA from the S10 LpDNPH sampler *versus* storage time at room temperature. Excess DNPH in ethyl acetate solution is present during storage.

Fig. 7.

Colored bands of OPA derivative on S10 LpDNPH samplers. Sampler 1 is a blank sampler and exhibits DNPH-coated silica in a bright yellow color. Samplers 2, 3, and 4 exhibit orange or red-orange bands after the collection of 12, 36 and 120 μg of OPA. respectively, in mixtures of condensation aerosol and vapor at 60% relative humidity from a glass U-tube partially immersed in an oil bath at 120 °C. Sampler 5 exhibits a brown band after collection of 43 μg of OPA vapor at 23% relative humidity from the OPA generation system.

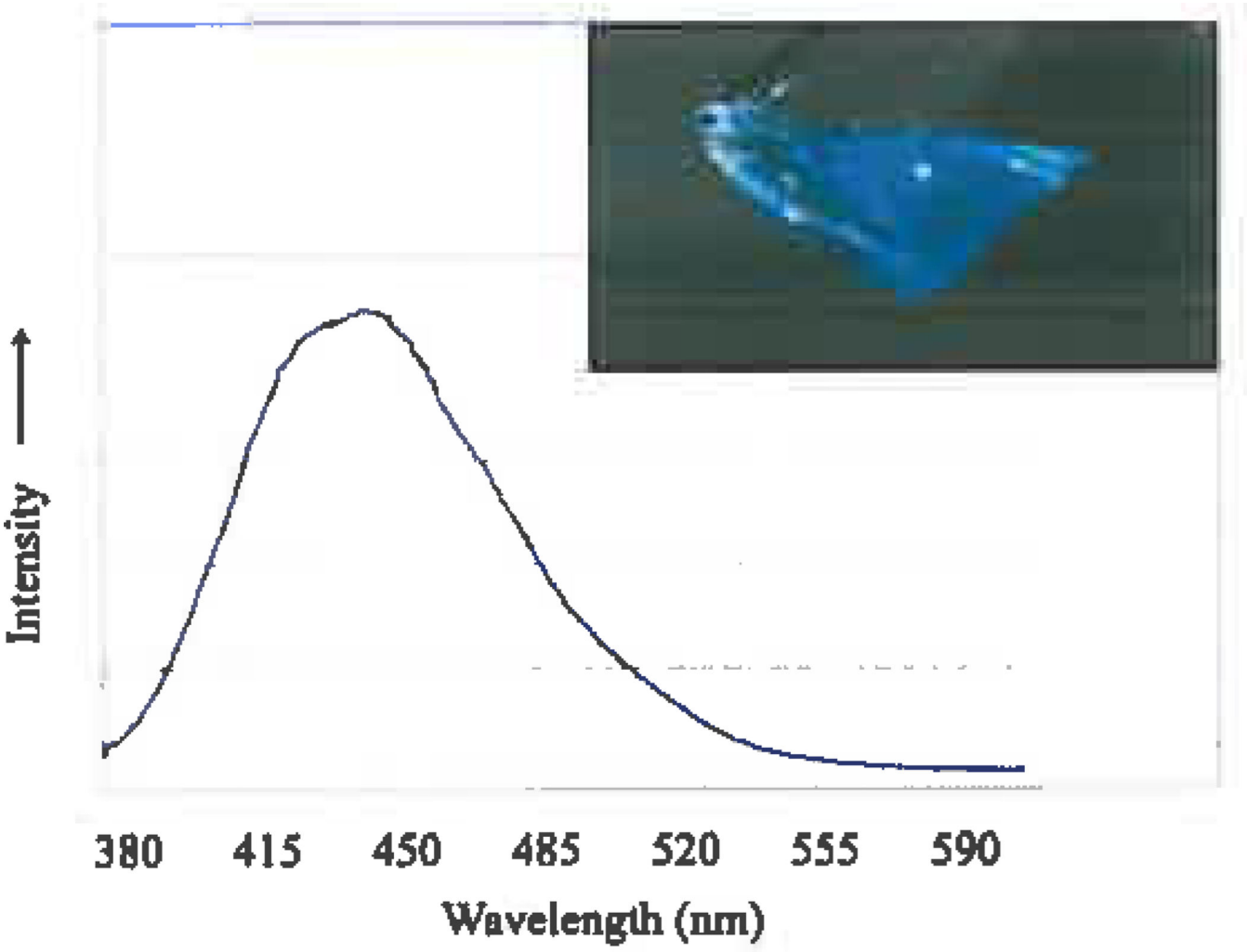

Fig. 8.

Fluorescence emission spectrum of the thio-*N*-alkyl-substituted isoindole formed from OPA. *N*-acetyl-L-cysteine, and ethylenediamine in DMSO solution in a 1 × 1 cm polystyrene cuvette. Excitation wavelength = 366 nm. OPA fluorescence from a glass vial during irradiation with light at 366 nm from a UV lamp.

Table 1

Recoveries of OPA vapor spikes from S10 LpDNPH samplers[a]

Quantity of OPA vapor collected (μg)	*n*	Average quantity of OPA found (μg)	Average recovery	RSD
1.19	3	1.20	1.009	0.0430
2.22	4	2.13	0.958	0.0352
6.21	4	5.97	0.961	0.0477

[a]Aliquots of acetonitrile solutions of OPA were deposited on PTFE-GFFs, and pumps operated at 1.0 L min^{-1} in a nitrogen atmosphere.

Table 2

Recoveries of OPA spikes (as mixture of vapor and condensation aerosol) from S10 LpDNPH samplers[a]

Quantity of OPA applied to U-tube (μg)	*n*	Average quantity of OPA found (μg)	Average recovery	RSD
0.56	4	0.54	0.967	0.0332
1.75	4	1.75	0.998	0.0470
3.23	4	3.13	0.989	0.0800

[a]The glass U-tube was partially immersed in an oil bath at 120 °C. Each pump operated for 45 min at 1.0 L min^{-1} and drew laboratory air through the U-tube. OPA in acetonitrile solution (100 μL) was applied to the U-tube for each spike.

Table 3

Recoveries of OPA vapor spikes from S10 LpDNPH samplers after storage at room temperature[a]

Quantity of OPA vapor collected (μg)	Period of storage (days)	Quantity of OPA found (μg)	Recovery
0.71	3	0.68	95.8%
0.71	6	0.66	93.0%
0.71	10	0.61	85.9%
0.71	15	0.69	96.8%
5.17	3	5.15	99.6%
5.17	6	4.99	97.1%
5.17	10	5.24	101.3%
5.17	15	4.85	93.8%
14.5	3	14.4	99.3%
14.5	6	13.5	93.1%
14.5	10	12.2	84.1%
14.5	15	10.9	75.2%

[a]Aliquots of acetonitrile solutions of OPA were deposited on PTFE-GFFs, and pumps operated at 1.0 L min^{-1} in a nitrogen atmosphere.

Table 4

Recoveries of OPA vapor following air draw through S10 LpDNPH samplers[a]

Quantity of OPA vapor collected (μg)	Period of air draw through DNPH sampler @ 1.0 L min^{-1} (min)	Average quantity of OPA found (μg)	Average recovery[b]	RSD
0.86	240	0.731	0.850	0.060
1.16	15	1.21	1.039	0.0284
1.32	120	1.12	0.850	0.0319
1.25	369	0.785	0.628	0.157
6.49	15	6.53	1.003	0.0382
6.16	120	5.80	0.942	0.0322
6.24	363	4.18	0.670	0.364
19.2	15	16.1	0.836	0.179
18.0	120	15.9	0.881	0.0407
18.9	360	17.1	0.904	0.0830

[a]Aliquots of acetonitrile solutions of OPA were deposited onto PTFE-GFFs, and pumps operated at 1.0 L min^{-1} in a nitrogen atmosphere.

[b]Each average recovery was based on four samples. Thus, Table 4 represents 40 samples.

Table 5

Fluorescent readings of separate samples of OPA in DMSO-reagent solution and blanks

Time elapsed after addition of OPA	Fluorescence reading of sample[a]	Fraction of maximum reading	Fluorescence reading of blank	Fraction of maximum reading[b]
20 min	1747	84.1%	4.951	7.94%
40 min	1781	85.7%	8.860	14.2%
60 min	1891	91.0%	13.89	22.3%
80 min	1924	92.6%	17.31	27.8%
100 min	1965	94.6%	20.46	32.8%
2 h	1974	95.0%	23.91	38.4%
3 h	2059	99.1%	31.00	49.7%
4 h	2056	98.8%	32.61	52.3%
5 h	2078	100.0%	38.46	61.7%
6 h	2022	97.3%	41.56	66.7%
7 h	1994	96.0%	43.42	69.7%
23 h	1270	61.1%	60.60	97.2%
26 h	1046	50.3%	62.32	100.0%

[a]Fluorescence readings of samples were not corrected for blank readings.

[b]Maximum reading of blank for this 26 h period.

Table 6

Recoveries of OPA vapor spikes from impingers of DMSO-reagent solution

Quantity of OPA which entered each impinger (μg)	*n*	Average quantity of OPA found (μg)	Average recovery	RSD
1.40	3	1.49	1.053	0.0350
2.80	3	2.88	1.027	0.0315
5.01	3	4.81	0.971	0.0233

Table 7

Study of collection efficiency of DMSO-reagent solution in impingers

Quantity of OPA applied to U-tube	Length of PTFE tubing before first impinger	Quantity of OPA found in first impinger	Length of PTFE tubing after first impinger	Quantity of OPA found in second sampler
20 μg	19 cm	17.8 μg	0 cm	0.11 μg (S10 LpDNPH sampler)
20 μg	15 cm	18.4 μg	15 cm	(Not detected) (impinger)

Table 8

Recoveries of OPA spikes (as a mixture of vapor and aerosol) from impingers of DMSO-reagent solution[a]

Quantity of OPA applied to U-tube (μg)	*n*	Average quantity of OPA found (μg)	Average recovery	RSD
2.46	4	2.35	0.954	0.0345
12.1	4	11.9	0.985	0.0261
20.2	4	19.8	0.981	0.0221

[a]The glass U-tube was partially immersed in an oil bath at 120 °C. Each pump operated for 75 min at 1.0 L min^{-1} (except for 45 min at the 2.46 μg level) and drew laboratory air through the U-tube. OPA in acetonitrile solution (100 μL) was applied to the U-tube for each spike.

Table 9

Air draw though DMSO-reagent solutions with and without OPA

Type of solution	Quantity of OPA added (μg)	Volume of air draw @ 1.0 L min^{-1} (L)	Volume of water absorbed (mL)	Fluorescence reading corrected for static blank[a] and water
Standard	0.4	120	0.24	16.08
Standard	0.4	120	0.22	16.16
Standard	0.4	0	—	16.05
Standard	0.4	0	—	15.91
Blank	0.0	120	0.23	0.03
Blank	0.0	120	0.23	0.01
Blank	0.0	0	—	0.00

[a]The static blank is the blank solution through which no air has been drawn. Fluorescence reading of static blank was 1.118.

Table 10

Air sampling for 90 mm at 23.6 °C and 24.6% relative humidity (TWA[a])

Method	Volume of air sampled (L)	Quantity of OPA found (μg)	Concentration of OPA in air (μg m^3)	Average concentration (μg m^3)
DNPH-HPLC	90.7	22.2	245	246
DNPH-HPLC	90.2	22.4	248	
Impinger-Fo[b]	91.7	21.5	234	225
Impinger-Fo[b]	93.7	20.1	215	

[a]"TWA" means "time weighted average".

[b]Water (110 mg and 100 mg) was found in the first and second impingers, respectively. "Fo" is an accepted abbreviation for "fluorescence".

Table 11

Air sampling for 125 min at 22.8 °C and 82.4% relative humidity (TWA)

Method	Volume of air sampled (L)	Quantity of OPA found (μg)	Concentration of OPA in air (μg m^{-3})	Average concentration (μg m^{-3})
DNPH-HPLC	126	75.8	602	600
DNPH-HPLC	125	74.7	598	
Impinger-Fo[a]	127	86.4	680	638
Impinger-Fo[a]	130	77.3	595	

[a]Water (1058 mg and 1055 mg) was found in the first and second impingers, respectively.

Table 12

Air sampling for 150 min at 22.6 °C and 15.3% relative humidity (TWA)

Method	Volume of air sampled (L)	Quantity of OPA found (μg)	Concentration of OPA in air (μg m^3)	Average concentration (μg m^3)
DNPH-HPLC	156	176	1128	1154
DNPH-HPLC	151	178	1179	
Impinger-Fo[a]	135	148	1096	1094
Impinger-Fo[a]	153	167	1092	

[a]The first and second impingers lost 220 mg and 260 mg, respectively.

Table 13

Air sampling for 240 min at 22.3 °C and 20.9% relative humidity (TWA)

Method	Volume of air sampled (L)	Quantity of OPA found (μg)	Concentration of OPA in air (μg m^{-3})	Average concentration (μg m^{-3})
DNPH-HPLC	250	1.24	4.96	4.88
DNPH-HPLC	242	1.16	4.79	
Impinger-Fo[a]	240	1.23	5.13	5.06
Impinger-Fo[a]	245	1.22	4.98	

[a]The first and second impingers lost 130 mg and 50 mg in weight, respectively.

Table 14

Measurement of condensation aerosol of OPA

Parameter	Measurement data during first two min	Measurement data during second two min (following a 30 s gap)
Mass median diameter	8.96 nm	42.4 nm
Number median diameter	7.75 nm	15.6 nm
Geometric std Dev. by number	1.18	1.49
Geometric std Dev. by mass	1.52	1.43