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Development of a Versatile Method for the Detection of Nicotine in Air

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Nicotine, a rapid-acting poison, is present in environmental tobacco smoke and has been used as a greenhouse insecticide. Due to its toxicity, several health hazard evaluations (HHE) have resulted from potential nicotine exposures to casino workers, airline flight attendants, and greenhouse employees. Exposure to nicotine can occur by inhalation, skin adsorption, and ingestion, resulting in such adverse health effects as nausea, vomiting, headache, dizziness, tachycardia, hypertension, convulsions, and cardiac arrhythmia. The development of an improved sampling and analytical methodology for nicotine was required to accommodate the broad concentration of nicotine levels and varying sampling scenarios presented by the differing HHE requests. A XAD-4 sorbent tube was selected for the collection of airborne nicotine. Analytical methodology for the separation, identification, and quantitation of nicotine by both gas chromatography-flame ionization detection and gas chromatography-nitrogen/phosphorous detection is described. The limit of detection for nicotine was 0.013 μg/sample. The desorption efficiency for nicotine was determined over the range of study and ranged from 90.9% (0.096 μg) to 93.7% (24.0 μg). Nicotine exhibited storage stability for 30 days at 5°C and for 14 days at ambient temperature. Based on the results of this research study, the new method for nicotine was published in the *NIOSH Manual of Analytical Methods* (NMAM® 2551).
Keywords: environmental tobacco smoke, gas chromatography, nicotine

Increased awareness about the potential adverse health effects of environmental tobacco smoke (ETS) have prompted several recent National Institute for Occupational Safety and Health (NIOSH) health hazard evaluation (HHE) requests from casino employees and has provided the impetus for studying the effects of ETS on flight attendants.[1] Nicotine, a major component of ETS, is used as a marker compound in health effects studies for several reasons. Nicotine is specific to tobacco and the combustion of tobacco, and when compared with the other vapor phase constituents of tobacco, vapor phase nicotine is present in relatively large quantities.

A second unrelated HHE request resulted in a study at a research greenhouse where employees were exposed to nicotine used as a pesticide during a fumigation process. Expected routes of nicotine exposure include air, surface contamination, and hand contact. Replacement of the existing method (*NIOSH Manual of Analytical Methods* [NMAM®] 2544) was necessary because the method could not effectively sample the low nicotine levels encountered in the ETS study. Additionally, the existing method had not been evaluated with the high concentrations of nicotine encountered in the greenhouse study. To meet those requirements, a new NIOSH nicotine method was developed that replaces NMAM 2544. The new sampling and analytical method is described in this article.

Nicotine, 1-methyl-2-(3-pyridyl)pyrrolidine, is a colorless to pale yellow, oily liquid that turns brown when exposed to air or light. Besides its use in the tobacco and leather tanning industries, nicotine is used in agricultural settings as an insecticide and is listed as an Environmental Protection Agency (EPA) toxicity category I insecticide (most acutely toxic; each category [I–IV] is an EPA-established hazard indicator used for labeling pesticide containers by the level of toxicity of the pesticide.[2]) and as a restricted-use pesticide. In addition, nicotine is listed in NIOSH pesticide category group I (most hazardous) because of its potential for posing a significant risk of adverse acute health effects at low concentrations.[3] Exposures to nicotine can occur by inhalation, skin adsorption, and ingestion.

Mention of company names or products does not constitute endorsement by the Centers for Disease Control and Prevention.

It is a potent and rapid-acting poison that is quickly absorbed from all routes of entry, including the skin. Nicotine poisoning resulting from its use as an insecticide was common in the 1920s and 1930s. Small doses of nicotine cause nausea, vomiting, diarrhea, headache, dizziness, and neurological stimulation, resulting in tachycardia, hypertension, sweating, and salivation, whereas exposure to high levels of nicotine may result in convulsions and cardiac arrhythmia.[4,5]

The NIOSH recommended exposure level (REL), Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL), and American Conference of Governmental Industrial Hygienists threshold limit value for nicotine are all 8-hour time-weighted average concentrations of 0.5 mg/m^3, which represents the occupational exposure limit (OEL) for nicotine. Each criterion also carries a "skin" notation, which refers to the potentially significant contribution to the overall exposure by the cutaneous route, including the mucous membranes and eyes, mostly by direct contact with the substance.

MATERIALS AND METHODS

Chemicals and Materials

Nicotine (98%) and quinoline (98%) chromatography grade standards were purchased from Aldrich Chemical Co. (Milwaukee, Wis.). High-performance liquid chromatography-grade ethyl acetate (Burdick and Jackson, Muskegon, Mich.) was used as the desorption solvent. Triethylamine (99+%) was obtained from Aldrich Chemical Co.

Commercially available XAD-4 solid sorbent tubes were selected for this study based on an ASTM reference and an OSHA study indicating successful nicotine recoveries at lower sample concentrations.[6,7] The XAD-4 solid sorbent tubes (SKC #226-93) were obtained from SKC, Inc. (Eighty Four, Pa.).

Analytical Parameters and Calibration

To obtain the required concentration range of calibration standards, it was necessary to prepare primary and secondary stock solutions of both nicotine and quinoline. The nicotine primary stock solution was prepared by diluting 100 mg of nicotine in 100 mL of the modified ethyl acetate solvent (prepared by adding 0.01% triethylamine to ethyl acetate). The nicotine secondary stock solution was prepared by diluting 1.0 mL of the nicotine primary stock solution in 100 mL of the modified ethyl acetate solvent. The quinoline primary stock solution was prepared by diluting 100 mg of quinoline in 100 mL of the modified ethyl acetate solvent. Preparation of the quinoline secondary stock solution was achieved by diluting 10.0 mL of the quinoline primary stock solution in 100 mL of the modified ethyl acetate solvent. Twenty-five microliters of the quinoline secondary stock solution was added to both standards and samples as an internal quantitation standard prior to analysis.

Gas chromatographic analysis was performed using a Hewlett-Packard 6890 GC equipped with either flame-ionization detector or a nitrogen-phosphorous detector and a model 6890 series autosampler (Hewlett-Packard Corp., Avondale, Pa.). Data acquisition was achieved using the AI-450 chromatography system (DIONEX Corp., Sunnyvale, Calif.). Separation of nicotine from the other compounds present in the samples was achieved using a 30-m Rtx®-5 Amine (Restek Corp., Bellefonte, Pa.) fused silica capillary column (0.32 mm ID, 1-μm film thickness) and a temperature program ramped from 60 to 200°C (20°C/min), with the final temperature held at 200°C for 3 min. The injection port temperature was 200°C and the detector temperature was 300°C. The bead power was set at 3.4 volts. The carrier gas was helium (2.4 mL/min) and the injection volume was 1 μL, splitless mode.

Sampling Apparatus

Single XAD-4 solid sorbent tubes attached by flexible tubing to portable sampling pumps were used to collect vapor phase nicotine associated with ETS in personal breathing zone (PBZ) and area samples. PBZ and area samples were collected with GilAir5® portable sampling pumps (Sensidyne, Inc., Clearwater, Fla.) calibrated at a sampling flow rate of 0.1 L/min. After sampling, all samples were capped securely in the field, kept cold during shipment to the analytical laboratory, and then stored in a refrigerator until analyzed.

Due to the unique manner in which nicotine was administered during the greenhouse fumigation process, the sampling approach employed in the ETS study was modified in several ways. To apply the nicotine pesticide as a fumigant throughout the greenhouse, a combustion procedure was employed. The combustion process precipitated the use of a Gelman 13-mm glass fiber filter (Fisher Scientific Co., Pittsburgh, Pa.) in a closed-face, three-piece cassette connected in series with the XAD-4 sorbent tubes to collect any particulate-bound nicotine. Because the nicotine concentrations during the initial period of the fumigation process were expected to be high, two XAD-4 sorbent tubes connected in series were used during sampling. Single XAD-4 sorbent tubes were used to collect samples later in the fumigation process. The XAD-4 sorbent tubes, used to collect PBZ and area air samples, were connected by flexible Tygon® tubing to model 224-PCXR7 programmable universal-flow sampling pumps (SKC, Inc.). A sampling flow rate of 1.0 L/min was used to offset any pressure drop caused by using two XAD-4 sorbent tubes in series.

To determine the levels of nicotine contamination on various greenhouse surfaces, as well as potential skin exposures, wipe samples and 100% cotton glove inserts (both preextracted with hexane) were employed. Surface samples were collected with 3 × 3-inch preextracted cotton gauze moistened with the modified ethyl acetate solution. Approximately 100 cm^2 of each surface was wiped with the gauze pads. After collection the samples and blanks were placed in labeled amber glass vials and immediately extracted in 20 mL of the modified ethyl acetate solvent.

Preextracted 100% cotton gloves, worn under the reusable nitrile protective gloves of the employees involved in the fumigation process, were used to determine potential breakthrough and subsequent skin exposures. After sampling, the cotton glove monitors were removed and placed in labeled amber jars and immediately extracted with 50 mL of the modified ethyl acetate solvent. Each jar was sealed with Teflon®-lined caps and shipped back to the laboratory for analysis.

Desorption Efficiency Recovery

The XAD-4 solid sorbent tubes were spiked with a solution of nicotine in ethyl acetate (containing 0.01% triethylamine) over a range of 0.096 to 24.0 μg/sample (five levels, n=6). The tubes were then allowed to air equilibrate for several minutes before being capped and allowed to stand at room temperature overnight. Prior to sample analysis, the front and back sections of each sorbent tube were placed in separate vials and extracted with 1 mL of the modified ethyl acetate desorption solvent to which 25 μL of quinoline (secondary standard) was added as an internal standard.

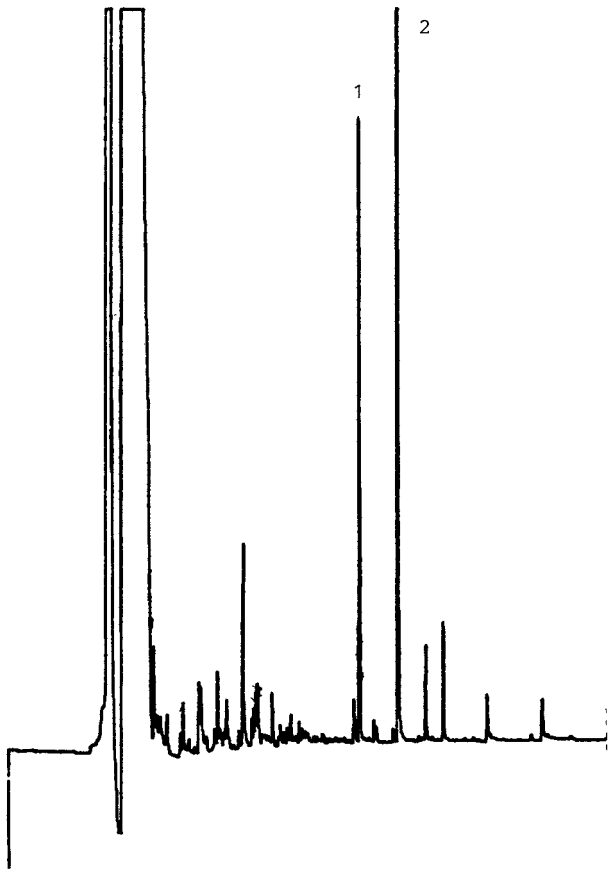

FIGURE 1. Chromatogram of (1) quinoline and (2) nicotine present in ETS exhibiting the overall selectivity of the method

Storage Stability Study

Thirty-six XAD-4 tubes were spiked with 6 μg of nicotine, allowed to air equilibrate for several minutes, and then capped. Eighteen of the sorbent tubes were stored in the dark at ambient temperature while the remaining 18 were stored in the dark at 5°C to determine whether the lower temperature improved sample storage stability. Six tubes from each group were analyzed after 7, 14, and 30 days storage.

RESULTS AND DISCUSSION

The analytical parameters identified in the experimental section allowed the baseline separation of nicotine from quinoline and other nitrogen-containing compounds present in ETS (see Figure 1) and the inert vehicle substrate used in the application of the nicotine pesticide. The elution of both nicotine and quinoline was achieved in less than 10 min. An 11-point calibration curve (in duplicate) ranging from 0.005 to 25.0 μg/sample was used to determine the limit of detection (LOD) and the limit of quantitation (LOQ) and for the quantitation of all nicotine samples. The peak area ratio of quinoline to nicotine depends on variables in calculating the calibration curve. Relative to this method development, the LOD was calculated using the formula, $LOD = 3s_y/m$,[8] where s_y is the standard error of the regression and m is the slope determined from an 11-point calibration curve. The LOD determined for nicotine was 0.013 μg/sample and the LOQ was 0.042 μg/sample.

TABLE I. Nicotine Desorption Efficiency from XAD-4 Solid Sorbent Tubes

Spike Level (μg)	Recovery (%)	RSD (%)
0.096	90.9	2.9
1.2	88.6	2.3
6.0	92.2	2.0
12.0	95.7	1.4
24.0	93.7	2.0
Average	92.2	2.1

Note: N = 6 for each level.

This research effort focused on developing a more sensitive and reliable method for the collection and quantitative determination of nicotine in air. Improvements resulting from this method development (NMAM 2551), when compared with the previous method (NMAM 2544), include improved nicotine recovery at substantially lower levels, lowered LOD/LOQ values, and the inclusion of storage stability results. Method sensitivity was improved by use of capillary column chromatography and an improved nitrogen-phosphorous detector available on the HP6890 gas chromatograph. The improved recovery of nicotine at low levels was twofold. The addition of 0.01% triethylamine to the ethyl acetate solvent improves recovery by preventing nicotine from adhering to the glass walls of the desorption vessel. Although no comparison of XAD-2 and XAD-4 sorbent tubes was performed during this method development, an initial evaluation of this problem had been made in the OSHA laboratory at Salt Lake City, Utah.[7] The use of XAD-4 sorbent tubes allows lower quantitative recovery of nicotine when compared with the results achieved using the XAD-2 sorbent tube where low levels of nicotine were not recovered from the sorbent bed.

Based on favorable results from a preliminary recovery study of nicotine spiked onto XAD-4 sorbent tubes, a full-scale, five-level (n=6) recovery study was conducted ranging from 0.096 to 24.0 μg/sample. Nicotine recovery ranged from 90.9% (RSD = 0.029) at the 0.096 μg level (2 × LOQ) to 93.7% (RSD = 0.020) at the 24.0 μg level (2 × REL/PEL). The average nicotine recovery for all five levels was 92.2% (RSD = 0.021). A complete summary of the results of the desorption efficiency study appears in Table I.

The final phase in this method development was a 30-day storage stability evaluation of nicotine at ambient temperature and at 5°C. Both studies were conducted in the dark because nicotine is a light-sensitive compound. The results for the study at ambient temperature and the results for the study at 5°C are shown in Table II. When stored at ambient temperature for 30 days, the average nicotine recovery was 80.5% (RSD = 0.028), whereas the average nicotine recovery from the XAD-4 sorbent tubes stored for 30 days at 5°C was 97.5% (RSD = 0.035).

The new method was applied to the sampling and quantitation of nicotine in ETS at various entertainment establishments. In one study nicotine was detected in ETS at levels as low as 0.2 μg/sample. The method also was applied to a NIOSH field study of nicotine exposure in the ETS of casinos, where the levels of nicotine detected ranged from 0.3 to 2.0 μg/sample.

The method currently is being used in another NIOSH industrywide study of airline flight attendants' exposure to ETS on international flights.[1] Nicotine is used as a marker compound for exposure to ETS. So far in this study, nicotine exposure levels have

TABLE II. Nicotine Recovery Results from Storage Stability Study at Ambient and at 5°C Temperatures

Storage Temperature	7-Day (%)	14-Day (%)	30-Day (%)
Ambient (25°C)			
(6.0 μg/spike)	94.3	91.1	80.5
RSD	1.0	1.6	2.8
Refrigerated (5°C)			
(5.1 μg/spike)	—	—	97.5
RSD	—	—	3.5

Note: N = 6 for each level. Results corrected for 92.2% desorption efficiency reported in Table I.

ranged from 0.173 to 11.6 μg/sample on international flights that permit smoking.

Additionally, the method was applied with slight modification in response to an HHE request concerning potential exposures of greenhouse employees and university researchers to the insecticide nicotine during maintenance and handling of research plants. The modifications included the addition of a glass fiber filter to trap any particulates in series with two XAD-4 sorbent tubes because of expected high nicotine concentrations and sampling rates of 1 L/min. Although no recovery or storage studies were conducted using the glass fiber filter, the results of the filter analyses showed not detected or levels below the LOD for nicotine. The concern was that the employees were reentering the greenhouse before airborne concentrations of nicotine had fallen below the OEL of 0.5 mg/m³. Analysis of samples taken before, during, and after fumigation indicated that peak nicotine levels (3.3 mg/m³) occurred 10 min into fumigation and fell below the OEL 1 hour after fumigation was initiated.

Also as part of the greenhouse study, the method was applied to the analysis of nicotine collected on 3 × 3-inch gauze wipe samples moistened with the ethyl acetate desorption solvent to ascertain surface contamination before and after the fumigation process. Although no recovery studies were conducted using the gauze wipes, evaluation of the results indicate that wipe samples collected from various common surfaces in the greenhouse after the fumigation process contained levels of nicotine up to 60 times greater than before fumigation.[9] Levels of nicotine detected after the fumigation process ranged from 4.85 to 78.8 μg/100 cm².

Preextracted cotton gloves were worn under the nitrile gloves of the greenhouse workers during the performance of their duties. As with the gauze wipes, no recovery studies were conducted on the cotton glove monitors, as they were used only to provide an estimate of potential dermal contact with the nicotine pesticide. Analysis of the cotton glove monitors worn under the protective nitrile work gloves indicated that there were no detectable concentrations of nicotine collected (LOD was 0.013 μg/sample).

SUMMARY AND CONCLUSIONS

A sampling and analytical method for the determination of low-level concentrations of nicotine in air has been described. Nicotine is effectively recovered from XAD-4 sorbent tubes using ethyl acetate modified with 0.01% triethylamine. The average desorption efficiency recovery for nicotine was acceptable (92.2%). Nicotine was stable when stored on the XAD-4 sorbent tubes for 30 days at 5°C in the dark (89.8% recovery; 97.5% when corrected for desorption efficiency).

The method was successfully applied for the collection and detection of low levels of nicotine in ETS in field studies involving casinos and international airline flights. In addition, with minor modifications, the method was applied to the collection and detection of nicotine used as an insecticide in a greenhouse environment. The analytical parameters of the method allowed for the determination of low levels of nicotine in the presence of other nitrogen-containing compounds present in the inert vehicle substrate of the fumigant canister.

Overall, sampling on XAD-4 tubes, coupled with separation and analysis using gas chromatography-nitrogen/phosphorous detection, provides a comprehensive and flexible method for monitoring low levels of nicotine in ETS and when nicotine is used as a greenhouse insecticide.

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