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Project Summary

Method Validation for Measurement of Selected Semivolatile Phenols in Dust and Soil

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The objectives of this study were to evaluate and validate analytical methods for analysis of persistent organic pollutants (POP) in house dust and soil and to obtain concentration profiles for the target POP in house dust and soil samples from the homes of 13 low-income families.

The analytical method for determining p-pentylphenol, poctylphenol, nonylphenols, and bisphenol-A consisted of sequential extraction of the dust/soil with 5% acetic acid in methanol (MeOH), 100% dichloromethane (DCM), and 5% acetic acid in water; liquid-liquid partitioning the resulting extract with water; and analyzing the concentrated DCM extract by gas chromatography/mass spectrometry (GC/MS). With this method, quantitative recoveries (>80%) of the target phenols were obtained from the spiked soil samples. Estimated detection limits for the target phenols are 0.001 ppm.

The analytical method for 2-acetylaminofluorene (2AF) and 3-amino-9-ethylcarbazole (AEC) consisted of extracting dust/soil with 30% water in MeOH at pH 10, and analyzing the extract by liquid chromatography with tandem mass spectrometry (LC/MS/MS). Recoveries for 2AF and AEC from the spiked soil samples ranged from 98% to 110% and from 39% to 110%, respectively. Estimated detection limits are 0.001 ppm for 2AF and 0.005 ppm for AEC.

The sums of concentrations of target phenols ranged from 1.94 to 14.8 ppm in house dust samples, from 0.047 to 1.51 ppm in entryway dust samples, and from 0.021 to 0.265 ppm in pathway soil samples. The observed concentration trend was house dust > entryway dust > pathway soil. There were no detectable amounts of 2AF and AEC in any dust or soil samples. Other compound classes found in dust and soil samples from one household were alkanes, aliphatic alcohols, fatty acids, fatty acid esters, and phthalates.

This Project Summary was developed by EPA's National Exposure Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Persistent organic pollutants (POP), including polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and other semivolatile organic compounds (SVOC), nonvolatile organic compounds (NVOC) and some metals (M) are found in air, house dust, soil, food, and water. Many of these compounds are putative endocrine disrupters and are known mutagens or probable human carcinogens. Humans can be exposed to these pollutants through inhalation, dietary and non-dietary ingestion, and dermal adsorption, and adverse health effects have been linked to such exposures. The non-dietary pathway resulting from ingestion of soil and dust may be more important for young children because of their play activities.

Children of low-income families or families living in urban environments may have increased exposure to POP and M. This

may arise because of their proximity to areas of high traffic, industrial activities, or lifestyle aspects. Under Cooperative Agreement CR822073, a preliminary study to develop and evaluate field methods to estimate children's exposure to PAH was conducted. The results from the first two years of this study indicated that the loadings of house dust in several urban low-income households are more than one order of magnitude higher than those of middle-income families. Such high dust loadings can increase children's exposure to POP and M through the non-dietary pathway.

Many POP were not included in the Cooperative Agreement study. It is desirable to include these pollutants in the evaluation of the field exposure methods targeted at low-income families. Two analytical techniques, gas chromatography/ mass spectrometry (GC/MS) and liquid chromatography with tandem mass spectrometry (LC/MS/MS) were evaluated for analysis of target POP that include putative endocrine disrupters. The GC/MS method was evaluated and validated for analysis of target phenols. The LC/MS/MS method was evaluated for analysis of all target POP, but only validated for the analysis of 2-acetylaminofluorene (2AF) and 3-amino-9-ethylcarbazole (AEC). House dust, entryway dust, and pathway soil samples collected from 13 homes in other studies were analyzed for target phenols, 2AF, and AEC using the validated analytical methods.

The objective of this study was to validate analytical methods for analysis of target POP in dust and soil, and to determine target POP in 39 dust/soil samples collected from the homes of 13 low-income families using the validated analytical methods.

The following tasks were carried out in this study:

1. Conduct GC/MS method evaluation/ validation for analysis of p-pentylphenol, p-octylphenol, nonylphenols, and bisphenol-A.

2. Conduct LC/MS/MS method valuation/ validation for 2AF, AEC, 2,4-dinitrotoluene (DNT), anthraquinone, vinclozolin, and phenols.

3. Analyze 39 samples and one method blank for target POP using the appropriate validated methods.

Procedure

Analytical Method for Phenols

Two extraction methods were evaluated for removing phenols from the dust and soil sample matrices. Initially, the soil samples were spiked with known amounts of target phenols and extracted with dichloromethane (DCM) in a sonication bath. This approach did not provide satisfactory recoveries. Another extraction method was then evaluated. For spike recovery, known amounts of target phenols were spiked into each aliquot of the soil samples. The spiked sample was extracted sequentially with 10 mL of 5% acetic acid in methanol, 10 mL of DCM, and 10 mL of 5% acetic acid in distilled water, in a sonication bath for 15 min with each type of solvent. The resulting extracts were combined and transferred to a separatory funnel. The DCM extract was transferred to another separatory funnel and washed with 20 mL of distilled water. The DCM extract was dried with sodium sulfate and concentrated to 2 mL for GC/MS analysis.

Thirty-nine dust and soil samples collected in previous studies from thirteen low-income households were analyzed for target phenols. The house dust samples were collected using the High Volume Small Surface Sampler (HVS3, Cascade Stack Sampling Systems, Bend, OR) in designated areas where the child's greatest play activity occurred. The entryway dust samples were collected from a doormat at the primary entrance of the house. The walkway soil samples were collected from a primary walkway into the home. Aliquots of the 39 dust/soil samples and one method blank were prepared by the above method except that the target phenols were not spiked into the samples prior to extraction. Known amounts of internal standard, phenanthrene-d_{10}, were added to each concentrated DCM extract prior to GC/MS analysis. An aliquot of each DCM extract was also removed for residue weight measurement.

The extracts were analyzed by 70 eV electron impact (EI) GC/MS. A Finnigan TSQ-45 GC/MS/MS instrument, operated in the GC/MS mode, was used. Data acquisition and processing were performed with an INCOS 2300 data system. The GC column was a DB-5 fused silica capillary column (60 m x 0.25 mm, 0.25 μm film thickness, J&W), and the column outlet is located in the MS ion source. Helium was used as the GC carrier gas. Following injection, the GC column was held at 70°C for 2 min and temperature programmed to 120°C at 20°C/min and then to 300°C at 8°C/min. The MS was operated in the selected ion monitoring (SIM) mode. Masses monitored were the molecular ions and their associated characteristic fragment ions. Identification of target compounds was based on their GC retention times relative to those of the internal standard phenanthrene-d_{10}. Quantification of target compounds was based on comparisons of the respective integrated ion current responses of the target ions to those of the corresponding internal standards using average response factors of the target compounds generated from standard calibrations. The dust/soil sample extracts from Household A were analyzed by GC/MS in full mass scan mode to identify major compounds tentatively. The MS was set to scan from m/e 45 to 450 amu at 1 sec/scan. Tentative identification of the compounds was accomplished by manual interpretation of background-corrected spectra together with an on-line computerized library search. The on-line library was the most currently available EPA/NIH mass spectral data base, containing 42,197 unique reference spectra.

LC/MS/MS Method Evaluation

The following compounds were evaluated for analysis by LC/MS/MS using the Sciex TAGA 6000E with an atmospheric pressure chemical ionization (APCI) source: 2AF, AEC, DNT, anthraquinone, vinclozoline, p-pentylphenol, p-octylphenol, nonylphenols, and bisphenol-A.

Each compound was analyzed in the single MS mode to identify the precursor ion formed by the APCI process. Once the precursor ion was identified, a fragment ion spectrum (MS/MS) was obtained by introducing energy to the collision cell. Standards of the above chemicals were introduced into the TAGA ion source as either vapors or liquids. Standards with sufficient vapor pressure were introduced by placing an open vial of the standard at the inlet of the TAGA sampling stream. For nonvolatile standards, solutions were prepared at known concentration levels. Aliquots of the standard solutions were introduced into the ion source through a Battelle-developed vapor jet system. Characteristic fragment ions for each standard were selected from the MS/MS spectrum, for use in the SIM mode. A series of standard solutions was analyzed by LC/

MS/MS to establish calibration curves and to estimate detection limits. The initial evaluation results showed that the LC/MS/MS technique can provide adequate detection sensitivity for two of the above standards, namely 2AF and AEC. These two compounds were selected for further analysis in dust/soil samples.

Analytical Method for 2-Acetylaminofluorene and 3-Amino-9-ethylcarbazole

Extraction recovery experiments were conducted for 2AF and AEC. Two extraction methods were evaluated for removal of the AEC and 2AF from the dust/soil samples. The first method, sonication with methanol (MeOH), did not provide satisfactory recoveries for AEC. The extraction solvent was then changed to 30% water in MeOH at pH 10. A spike recovery study was conducted, where known amounts of the two target compounds were spiked into aliquots of selected soil samples. The spiked sample was extracted with 5 mL aliquots of 30% water in MeOH at pH 10 in a sonication bath for 15 min. This step was repeated four times. The resulting extracts were combined, filtered, and concentrated to 3 mL for LC/MS/MS analysis.

The LC gradient elution conditions for the analysis of the standards and sample extracts are:

Column:	Supelco LC-304 Guard Column
Flow Rate:	1.2 mL/min
Sample Loop:	50 µL
Gradient Elution Scheme:	
0 - 2 min	100% H_2O
2 - 8 min	100% H_2O/0% MeOH → 25% H_2O/75% MeOH
8 - 10 min	25% H_2O/75% MeOH
10 - 15 min	25% H_2O/75% MeOH → 100% H_2O

The mass spectrometer was operated in the MS/MS (SIM) mode. Vaporized eluent from the LC was introduced into the APCI ion source, where the samples were ionized using a corona discharge. Protonated precursor ions were selected with the first quadrupole mass analyzer (thus eliminating all other possible interference ions). The precursor ions were then focused into the collision cell where they were fragmented at a collision energy of 35 volts (E_{lab}) with argon as the collision gas with a target thickness of approximately 350 x 10^{12} molecules/cm^2. Selected fragment ions from the isolated precursor ions were passed through the second MS and were detected by an electron multiplier. For 2AF, two precursor/fragment ion transitions were monitored, namely m/z 224/182 and 224/43. For AEC, three precursor/fragment ion transitions were monitored: m/z 211/182, 211/194, and 211/179. Identification of the target compounds was based on their correct LC retention times and their correct relative responses for each of the precursor/fragment ion transitions when compared with those from the standards calibrations. Quantification of the target compounds was based on comparisons of the respective integrated ion current responses of the target compounds in the sample extract to those in the standard solutions.

Results

GC/MS Analysis of Dust and Soil Samples

The analytical method for analyzing target phenols consisted of sequentially extracting the samples by sonication with 5% acetic acid in methanol, DCM, and 5% acetic acid in water, followed by liquid-liquid partitioning, and analyzing the concentrated DCM by GC/MS. Quantitative recoveries (>80%) of the spiked phenols were obtained. The recoveries ranged from 90% to 104% at 5 ppm spiked levels, from 84% to 101% at 0.2 ppm spiked levels and from 84% to 110% at 0.1 ppm spiked levels. The precision for the phenols for the triplicate spiked samples was within 13% (relative standard deviation).

Among the measured phenols, the most abundant were nonylphenols. The least abundant phenols were in general, p-pentylphenol and its isomer. The concentrations of phenols ranged from 0.043 ppm of p-pentylphenol to 11.1 ppm of p-octylphenol in house dust samples. Relatively lower concentrations were found in entryway dust samples and ranged from < 0.001 ppm of p-pentylphenol to 0.974 ppm of nonylphenols. The concentrations of phenols in pathway soil samples were from < 0.001 ppm of p-octylphenol to 0.204 ppm of nonylphenols. The relative concentration trend within individual households was house dust > entryway soil > pathway soil.

Levels of nonylphenols found in house dust samples were greater than 1 ppm, while those levels found in entryway dust and pathway soil samples were less than 1 ppm. The concentrations of nonylphenols ranged from 1.24 to 3.56 ppm in house dust, from 0.024 to 0.974 ppm in entryway dust, and from 0.015 to 0.204 ppm in pathway soil. The concentrations of bisphenol-A were lower than those of nonylphenols in the dust/soil samples. The levels of bisphenol-A ranged from 0.322 to 3.50 ppm in house dust, from 0.019 to 0.335 ppm in entryway dust, and from <0.001 to 0.036 ppm in pathway soil.

The sample extracts of dust/soil samples from Household A were analyzed by GC/MS in the full mass scan mode to determine the major components present in the samples. The major components found in house dust were alkanes, fatty acids, fatty acid esters, phthalates, and aliphatic alcohols. Similar components including alkanes, fatty acid esters and phthalates were found in entryway dust, but at lower levels. The pathway soil samples showed the fewest chromatographic peaks of the three samples. The major components found in pathway soil samples were aliphatic alcohols, alkanes, fatty acid esters, and phthalates.

LC/MS/MS Analysis of Dust and Soil Samples

In order for a compound to be ionized by APCI, its gas phase basicity (for positive ion mode) or gas phase acidity (for negative ion mode) should be greater than the gas phase basicity/acidity of water. For this reason, the MS/MS spectra of anthraquinone, phenols, and vinclozolin could not be obtained. Of all the compounds evaluated, MS/MS spectra were obtained for only three compounds, namely 2AF, AEC, and DNT. The 2AF and AEC spectra were obtained under positive ion APCI conditions and the DNT spectrum was obtained by negative ion APCI. Standard solutions of 2AF, AEC and DNT were prepared in the range from 1 ng/mL to 1200 ng/mL (1 ppb to 12 ppm) and analyzed using LC/MS/MS. The estimated detection limits for 2AF, AEC and DNT were 1 ng/mL, 5 ng/mL, and 500 ng/mL, respectively. Since an adequate overall method detection limit for DNT could not be obtained, only 2AF and AEC were selected as target analytes.

The analytical method for 2AF and AEC consisted of extracting the sample with 30% water in MeOH at pH 10, concentrating the extract, and analyzing the concentrated extract by LC/MS/MS. Quantitative recoveries were obtained for 2AF.

Conclusions

An analytical method for determining phenols in dust and soil samples was validated. This method consisted of (1) sequential sonication of the dust/soil sample with 5% acetic acid in methanol, 100% DCM, and 5% acetic acid in water, (2) liquid-liquid partitioning the resulting extract with water, and (3) GC/MS analysis of the concentrated DCM extract. With this method, quantitative recoveries (>80%)

of the phenols were obtained from the spiked soil samples and the estimated detection limits are 0.001 ppm of target phenols in dust/soil.

An analytical method consisting of extracting the sample with 30% water in methanol at pH 10 and analyzing the extract by LC/MS/MS was validated for the determination of 2AF and AEC in dust/soil. The recoveries of spiked 2AF ranged from 98% to 110% in the soil samples. The recoveries of spiked AEC ranged from 39% to 110% and showed more variations than the recoveries of 2AF. The estimated detection limits for this method were 0.001 ppm for 2AF and 0.005 ppm for AEC. The LC/MS/MS method was evaluated but not validated for the analysis of other target POP either because appropriate MS/MS conditions could not be established or because detection limits were inadequate.

The most abundant target phenols were nonylphenols and the least abundant one was, in general, p-pentylphenol in the dust and soil samples. The concentrations of target phenols ranged from 0.043 to 3.56 ppm in house dust, from < 0.001 to 0.974 ppm in entryway dust, and from < 0.001 to 0.204 ppm in pathway soil. There were no detectible levels of 2AF and AEC in these dust and soil samples. Other compound classes found in the dust/soil samples from one household were alkanes, aliphatic alcohols, fatty acids, fatty acid esters, and phthalates.

The general concentration trend observed for phenols in these samples was house dust > entryway dust > pathway soil. Therefore, human exposure to these compounds, especially exposures of young children, through non-dietary ingestion or dermal contact of house dust should not be overlooked.

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The complete report, entitled "Method Validation for Measurement of Selected Semivolatile Phenols in Dust and Soil," (Order No. PB97-143150; Cost: $21.50, subject to change) will be available only from:
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