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Environmental Exposure to Nitrated Polycyclic Aromatic Hydrocarbons in the Taiwanese Temple

H.-W. Chen

Department of Environmental Engineering and Health, Yuanpei University of Science and Technology, 306 Yuanpei Street, Hsinchu 300, Taiwan, Republic of China

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Nitrated polycyclic aromatic hydrocarbons (NPAHs) are a group of highly carcinogenic and mutagenic compounds and pose a significantly higher human health (carcinogenic) risk than polycyclic aromatic hydrocarbons (Busby et al 1994; Nitta et al 1993; Ohnishi et al 2001; Tokiwa et al 1993). NPAHs can form as a result of either combustion of petrochemical fuel, preparation of food with grilling, cigarette smoking, activities associated with occupational settings such as the coke-oven industry, or photochemical reactions of polycyclic aromatic hydrocarbons (PAHs) with ambient or atmospheric hydroxyl radicals and NO_2 (Atkinson et al 1994; Cecinato et al 2000; Brian et al 1997). Burning incense on a daily basis during worship is an important religious rite for most Chinese. About 80% of families and nearly all temples in Taiwan engage in this practice (Lin et al 2001). Incense smoke has been proved to contain polycyclic aromatic hydrocarbons (PAHs), aliphatic aldehydes, and potential photochemical mutagens such as NPAHs (Schoenotol et al 1967; Lin et al 1994). The exposure to burning incense is believed to be an important factor in risk of developing nasopharyngeal and lung cancer in Taiwan (Chen et al 1990; Lin et al 2003; Yu et al 1990). The mutagenic effect of Chinese incense smoke and NAPH products have been identified by the Ames or Salmonella test (Rasmussen 1987; Busby et al 1994). Because NPAHs exhibit the strongest mutagenic effects, they also are classified as possible human carcinogens by the International Agency for Research on Cancer (IARC) Monographs (IARC 1989). Past studies of environmental exposure to incense smoke has focused on PAH or other organic compounds (Lin et al 1994; Kao et al 2000; Lin et al 2001). However, the health effect of environmental exposure to nitrated polycyclic aromatic hydrocarbons is a more important topic. Study of nitrated polycyclic aromatic hydrocarbons emission has been insufficient. The present study is the first to describe and monitor indoor emission of NPAH compounds stemming from incense burning.

MATERIALS AND METHODS

This study reports on indoor and outdoor exposure to NPAHs in and outside of one Taiwanese temple. The significance of differences in NPAH concentrations between different sampling sites was determined by statistical analysis. Variation in NPAH concentration in this temple was assessed during a 30-day period. The concentration of contaminants obtained in this investigation was compared with values published in other studies (Kuo et al 2000; Lee 1994; Hayakawa 1995b).

Our investigation took place in a celebrated Taiwanese temple located in Taichung County of central Taiwan. The procedure for the collection and analysis of each sample was based on that of a previous study (Kuo et al 2000 and 2003). Ambient air samples of particulate-phase NPAHs were collected with high volume PM_{10} samplers (pore size <10 μm in diameter, PM_{10}, Kimoto Denshi, Osaka, Japan). Samplers were placed in both indoor and outdoor sampling positions. To study the characteristics of NPAHs emission, we selected three types of sampling sites (including inside the temple, outside the temple, and one atmospheric source that served as a control). The control atmospheric site was located in a high school (far away from traffic and incense-burning pollution). The flow rate of all samplers were set to approximately 1 m^3/min and calibrated approximately once per week. Each sample (at each of the sampling points) was continuously collected during a 12-hr period from 8:00 AM to 8:00 PM. Sampling took place every day for 30 days from July 1 to July 31, 2003. The PM_{10} air samplers were mounted with glass fiber filters (10 x 8 in., Gelman Science, Ann Arbor, MI, USA). Prior to use, glass fiber filters were cleaned with distilled-deionized water and heated to 450℃. Glass fiber filters were weighed before and after sampling to determine the amount of NPAHs and the particle-bound NPAH concentration (which was the mass of NPAHs divided by the mass of particles). Finally, each filter was covered with tinfoil and then stored in a freezer (– 70℃) until use.

Four representative NPAHs, 1-nitropyrene (1-NP), 1,3-dinitropyrene (1,3-DNP), 1,6-dinitropyrene (1,6-DNP), and 1,8-dinitropyrene (1,8-DNP) were identified and quantified for all samples as follows: After the final weighing, the particulate NPAHs were extracted ultrasonically. The filter was cut into halves; one half was divided into smaller pieces (7 x 1 cm^2) and then put in a centrifuge tube (Pyrex, 50 ml) containing 50-ml of cyclohexane. The tube was placed in an ultrasonic ice-bath (4℃) for 40 min and then centrifuged for 10 min (3000 rpm). A top 40 ml of solution in the tube was removed and then rotary-evaporated to 2 ml. These concentrated extracts were dried under a nitrogen stream at ambient temperature. The residue was extracted with cyclohexane (3 ml), washed with sodium hydroxide (5%, 4 ml), and then partitioned between an organic and aqueous phase solution. About 2.5 ml of cyclohexane phase was transferred to a 10-ml tube and dried under a nitrogen stream. Acetonitrile solution (0.5 ml) was used to dissolve the residue. Finally, the samples were analyzed using a column-switching liquid chromatography (Model LC-10AD, Shimadzu, Kyoto, Japan) system equipped with a 6-port switching valve (FCV-2AH, Shimadzu, Kyoto, Japan), a pre-separation column (150 x 4.6 mm I.D., Tosh ODS-80TM, Tokyo, Japan), and a reversed-phase analytical column (250 x 4.6 mm I.D. Kaseisorb LC ODS 60-5; Tokyo, Japan). A BAS-100A amperometric controller system with a glassy carbon working-electrode (Bioanalytical Systems, West Lafayette, IN, USA), in a reducible mode at -1.5 V (vs. Ag/AgCl) was used to achieve reduction prior to fluorescence detection. A programmable fluorescence detector (RF-10 AXL; Shimadzu, Kyoto, Japan) was used to detect signals of NPAH compounds. The elution was performed using 70% (v/v) acetonitrile and 30% 0.15-M sodium monochloroacetate buffer solution with pH 4.7. In addition, the temperature was controlled at 40℃. The flow-rate and switching programs were similar to those used in a previous study (Kuo, 2003).

Analytical-reagent grade cyclohexane and acetonitrile (Fisher, Springfield, MO, USA) were used as the solvents. NPAH standards containing 1,3-DNP, 1,6-DNP and 1,8-DNP were purchased from Aldrich (Milwaukee, WI, USA). 1-NP was obtained from TCI (Tokyo, Japan). Stock solutions of NPAH compounds were prepared 1 – 5 mg /ml in acetonitrile. Calibration curves were plotted from concentrations (0.013 to 3.24 μg/ml) of NPAH standards. The recovery yields of NPAHs were 83%, 83%, 84%, and 88% for 1,3-DNP, 1,6-DNP, 1,8-DNP, and 1-NP, respectively. The method detection limits of NPAH compounds were ca. 2 pg.

To test for significant differences between group means including mean concentrations inside the temple, outside the temple, and at a remote atmospheric site, analysis of variance (F) and Kruskal-Wallis test were performed. Statistical analyses were conducted using SPSS/PC+ (SPSS, Inc., Chicago, IL, USA). All tests were regarded as significant when $p < 0.05$.

RESULTS AND DISCUSSION

Our results could provide a database with which to gauge potential health risk or develop environmental strategy for controlling indoor NPAH pollutants generated by incense burning inside Taiwanese temples.

The concentrations of four different NPAHs in the ambient air inside and outside the temple and the ambient air from a control atmospheric source are given in Table 1. The average emission levels of 1-NP, 1,3-DNP, 1,6-DNP and 1,8-DNP inside the temples were 1244.6, 86.8, 52.1 and 180.5 pg/m^3, respectively. The trend analysis of the four NPAHs revealed in decreasing order of concentration the presence of: 1-nitropyrene > 1,8-dinitropyrene > 1,3-dinitropyrene > 1,6-dinitropyrene. The concentration of 1-NP was about 6–24 times the concentration of the other dinitropyrenes. A similar decreasing order was apparent outside the temples and at the control atmospheric site. These results demonstrated the 1-nitropyrene is the major NPAH pollutant produced during incense burning or other combustion processes. Total mean of NPAH concentrations inside the temple (site 1, 386.0 pg/m^3) was approximately 20.2 times higher than that found outside the temple (site 2, 19.1 pg/m^3) and 30.9 times higher than at the control atmospheric site (site 3, 12.5 pg/m^3). A statistically significant difference in total mean of NPAHs was shown between sites inside and outside the temples ($p < 0.05$), as well between the temple sites and the control atmospheric site ($p < 0.05$). Accordingly, the high NPAH concentrations monitored inside the temple deserve more attention.

Table 1. PAH concentrations in the ambient air of various sampling sites duration one month.

Concentration (pg/m^3)	Sampling sites Inside(1)	 Outside(2)	 Atmospheric source(3)
1-NP			
Range	265.4-2869	21.8-105.0	18.9-80.2
Mean	1224.6	58.7	40.1
1,3-DNP			
Range	23.8-219.6	0.1-15.3	0.1-3.9
Mean	86.8	4.5	1.9
1,6-DNP			
Range	14.8-96.0	0.06-9.9	0.3-5.2
Mean	52.1	2.9	1.8
1,8-DNP			
Range	58.3-293.0	3.2-22.5	2.8-12.0
Mean	180.5	10.3	6.0
Total NPAHs	1544.0	76.4	49.8
Total Mean	386.0	19.1	12.5
P value	(1)(2); (1)(3) $p<0.05$		(2)(3) $p>0.05$

Particle-bound NPAH (PBN) concentrations (*n*g/g) were defined as NPAH mass (*n*g) adsorbed to air particulates divided by the particle mass (g) in the standardization procedure. These concentrations in three sampling sites are given in Table 2. Mean concentrations of NPAH compounds inside the temple were 5059.9, 547.2, 109.4 and 451.0 for 1-NP, 1,3-DNP, 1,6-DNP, 1,8-DNP, respectively. The 1-NP concentration was higher than the concentration of DNPs, as already mentioned (Table 1). However, the ratio of 1-NP/DNPs was also apparently increased 1.5 times from inside to outside the temple. A probable explanation is that the dry-deposition efficiency and particle size of 1-NP were lower than those of the DNPs. A study by Hayakawa et al (1995a) confirmed that the particle size of 1-NP is less than 1.1 μm and easily emitted from diesel-engine vehicles or other uses of combustion such as incense burning. The total mean PBN concentration of the inside site was approximately 17.4 times that of the outside site, and 26.8 times that of the control atmospheric site, and the differences between inside and outside and between inside and atmospheric source were statistically significant ($p<0.05$). Since NPAH yields between the outside and control atmospheric source were not significant, we conclude that NPAH pollutants do not travel from inside the temple to the ambient region.

Figures 1 and 2 show chromatograms indicating the concentration distributions

Table 2. Particle-bound four NPAH compositions in the ambient air of various sampling sites duration one month.

Concentration (*n*g/g)	Sampling sites		
	Inside(1)	Outside(2)	Atmospheric source(3)
1-NP			
Range	1725.1-18649.8	113.3-546.0	96.4-410.0
Mean	5059.9	305.2	205.5
1,3-DNP			
Range	95.2-879.4	0.3-50.0	0.3-14.1
Mean	547.2	15.6	6.9
1,6-DNP			
Range	31.1-201.6	0.1-18.8	0.5-9.3
Mean	109.4	5.5	3.3
1,8-DNP			
Range	145.7-732.5	7.2-49.5	6.5-27.6
Mean	451.0	22.6	10.8
Total NPAHs	6167.5	348.9	226.5
Total Mean	1514.8	87.2	56.6
P value	(1)(2); (1)(3) $p<0.05$		(2)(3) $p>0.05$

of NPAH compounds in the indoor air of the investigated temples. A consistent increase in concentration over the period of investigation can be seen for 1-NP and DNPs. The cyclic variations occurred on a weekly basis. The highest peak concentration of 1-NP and DNPs often occurred on weekends. Attendance at religious rites increases tenfold and therefore incense burning increases inside temple during special holidays including Chinese New Year, and on weekends. Results clearly indicate that peak NPAH concentrations are related to religious holidays and weekends.

Comparison of the present results to those of other published studies (Kuo et al 2000; Lee 1994; Hayakawa 1995b) revealed that NPAH concentrations are significantly higher inside than outside temples and significantly higher inside temples than in ambient air at a distant location. The major contributor to NPAHs levels is incense burning. The levels in air outside temple and air from other atmospheric sources are similar to those in other areas of Taiwan such as Chierding and Kaohsiung. However, the levels of nitropyrenes are higher than those reported by Japanese groups. In general, the higher concentration of NPAHs present in Taiwan infers that Taiwan has more air pollution and that more photochemical nitration occurs because of its tropical climate to yield more

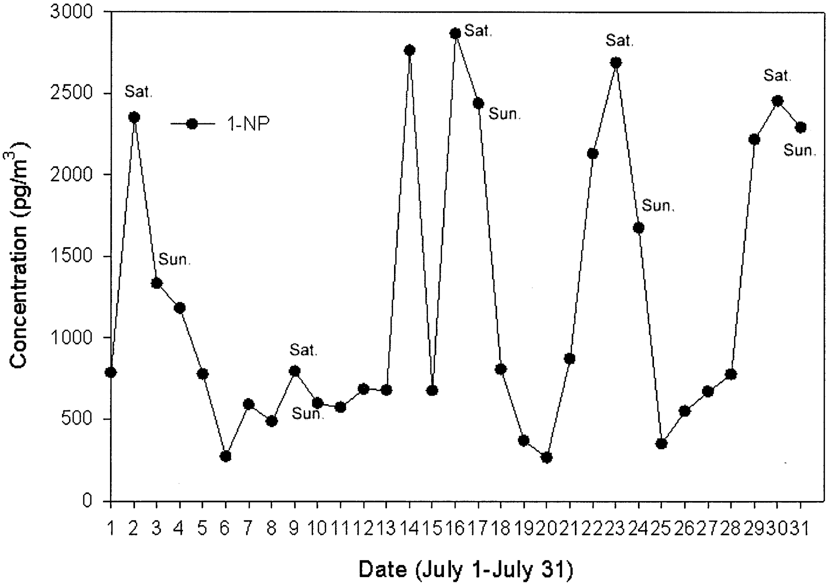

Figure 1. Concentration distribution and weekly trend of 1-nitropyrene in the indoor air of the investigated temple.

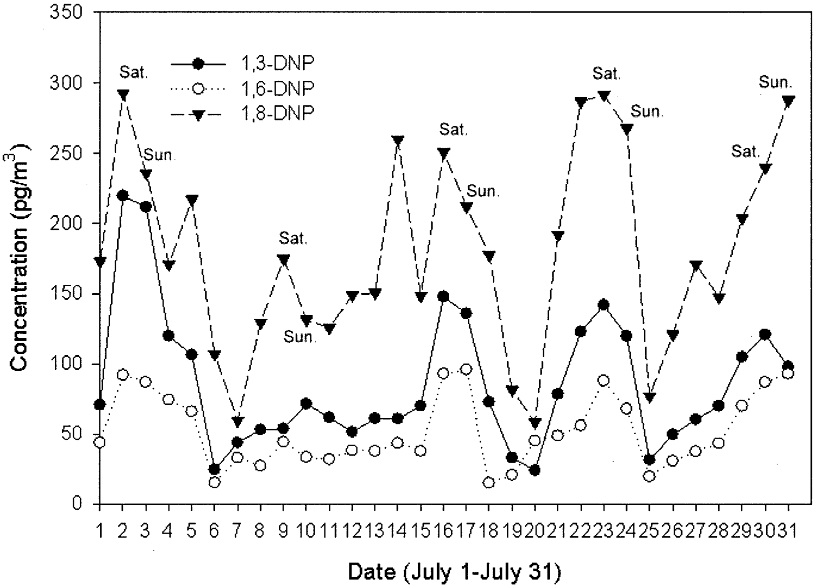

Figure 2. Concentration distribution and weekly trend of dinitropyrenes in the indoor air of the investigated temple.

NPAHs.

Results in present study indicate that the high levels of NPAH pollutants found inside temple were due to incense burning. The level was significantly higher inside than outside the temple or at a control atmospheric site. The pattern of incense burning should be noted and a complete epidemiological investigation (including biological monitoring, follow-up study and risk assessment of environmental exposure inside Taiwanese temples) should be undertaken. However, incense burning is not ineffectual, in that the religious rite may provide some spiritual benefit or comfort for most Chinese. We suggest improving the ventilation of Taiwanese temples to reduce the inside levels of NPAHs. Finally, the presence and levels of NPAHs determined in this study can be used to assess potential health risk or develop an environmental strategy to protect the public health.

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