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Passive Monitoring Method for 3-Ethenylpyridine: A Marker for Environmental Tobacco Smoke

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A new method was developed to assess environmental tobacco smoke in air. The method is based on passive sampling and subsequent measurement of the concentration of 3-ethenylpyridine, a vapor-phase compound specific to tobacco smoke. Air samples were collected using a 3M organic vapor monitor. Tests were carried out in a dynamic chamber to determine the sampling rate (25.7 cm^3/min). 3-Ethenylpyridine was desorbed from the sampler with 1 mL of pyridine/toluene mixture. 3-Ethenylpyridine was quantified by gas chromatography/mass spectrometry. The limit of detection was 0.01 μg/sample, corresponding to a concentration of 0.27 $\mu g/m^3$ air calculated for a sampling period of 24 h. Field measurements were carried out to test the performance of the method. Mean concentrations ranging from 1.3 to 5.3 $\mu g/m^3$ were measured for 3-ethenylpyridine in smoking environments, but no 3-ethenylpyridine was detected in nonsmoking environments. Active sampling using charcoal tubes was used as a reference method in the chamber tests and field measurements. Individual exposures can be easily and accurately measured by means of the passive sampler. Because of simple sample treatment, the method is also well-suited for large-scale monitoring of environmental tobacco smoke.

Introduction

3-Ethenylpyridine (3-EP) is formed during pyrolytic decomposition of nicotine and is present in the vapor phase of tobacco smoke (*1*). Many researchers have proposed 3-EP as a suitable indicator of environmental tobacco smoke (ETS). 3-EP meets the criterion set for an ETS tracer in having a similar emission rate across different tobacco products (*2*). Three additional criteria have been set by the U.S. National Research Council (*3*): uniqueness to tobacco smoke, easy detection at low smoking rates, and consistent proportions to other ETS compounds for different environments and tobacco products (*4*, *5*).

Although nicotine is widely used as a marker for vapor-phase ETS, the recent literature lists several advantages of the use of 3-EP: it is present solely in the vapor phase and has greater stability than nicotine under ultraviolet irradiation (*6*). Furthermore, it decays following nearly first-order kinetics (*7*), whereby its concentration increases linearly with the number of cigarettes smoked (*4*). A good correlation with carbon monoxide and other gas- or vapor-phase components of ETS has been shown (*8*).

There is much more nicotine than 3-EP in mainstream smoke, but the difference is much smaller in sidestream smoke (*9*). As ETS is composed mainly of aged and diluted sidestream smoke, the concentrations of 3-EP in ETS are only slightly lower than those of nicotine. Therefore, 3-EP can be easily measured in ETS with modern analytic instruments (*2*, *8*, *10*, *11*). In experimental conditions, an emission factor of 660 ± 155 μg/cigarette has been measured (*2*, *5*).

Various methods of sampling and analysis have been used to monitor 3-EP. ETS has been collected both by passive and active sampling. Passive collection has been achieved with glass fiber filters impregnated with 4% sodium bisulfate (*12*). The active samplers are usually sorbent traps, such as XAD-4, Tenax/Carbotrap, or Tenax TA (*12*−*14*). The analytic methods are based on thermal desorption or liquid extraction with subsequent gas chromatographic (GC) analysis using mass spectrometric (*13*, *14*) or nitrogen-specific detection (*12*). The limits of quantification of these methods are typically around 0.01 μg/sample.

In this paper, a new method for sampling and analysis of 3-EP is introduced, and the method is also applied to field samples. The passive sampling using a commercial device together with simple sample treatment and specific detection of 3-EP could open up a way to routine and inexpensive monitoring of ETS. The easy-to-use personal sampler is well-suited to the assessment of individual ETS exposures at population level.

Experimental Section

Testing of the Sampling Method. Test samples were collected on 3M organic vapor monitors (type 3500) (3M OH&ESD, 3M Center, St. Paul, MN) containing a single charcoal adsorbent pad. The sampling rate was determined in a dynamic test chamber (1 m^3; steel/glass), using 4-ethenylpyridine (4-EP; experiment I) or a mixture of 3-EP and 4-EP (experiment II) as test substances.

4-EP (Aldrich Chemical Co, St. Louis, MO) or a mixture of 3-EP and 4-EP (1:2) was vaporized under an air flow of 0.02 L/min and fed into the chamber. In each experiment, two sampling periods (240 and 480 min) were used, and six samples were collected during each period. The temperature and relative humidity (% RH) of the chamber air were monitored during the experiments. The air velocity in the chamber was also measured. In each experiment, 12 reference samples (six during each period) were collected in charcoal tubes (SKC 226-01; SKC Inc., Eighty Four, PA) with sampling pumps (SKC-222; SKC Inc.) at an air flow rate of 0.1 L/min (240 min) or 0.05 L/min (480 min). The reference samples were analyzed as the test samples.

The stability of samples collected with the test samplers was investigated by spiking the sampler pads with 3 μg of 3-EP and 4-EP calibration solution. A total of 10 μL of calibration solution in toluene was injected on each pad. The pads were placed in Kimax tubes, and the tubes were kept at room temperature for 2 d or in a freezer for 2 weeks. The stability of the sample solutions (sampler pads in desorption solution in vials; 3.7 μg of 4-EP/sample) during

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TABLE 1. Sampling Rate in Chamber Tests[a]

	sampling period (min)	concn of 3-EP in air (μg/m^3), mean $\pm$ SD	concn of 4-EP in air (μg/m^3), mean $\pm$ SD	sampling rate of 3-EP (cm^3/min)	sampling rate of 4-EP (cm^3/min)
expt I	240		150 $\pm$ 7		26.2
	480		300 $\pm$ 13		27.1
expt II	240	120 $\pm$ 3	230 $\pm$ 5	24.7	24.8
	480	160 $\pm$ 5	320 $\pm$ 8	25.4	26.0
overall mean $\pm$ 2 SD (cm^3/min)				25.7 $\pm$ 1.8	

[a] SD, standard deviation.

storage was tested by keeping the vials at −20 °C for at least 3 weeks.

Analysis. The samples were extracted in glass vials (volume, 2 mL; height, 32 mm; diameter, 12 mm) with 1 mL of toluene (Merck, Darmstadt, Germany) containing 10% (v/v) pyridine (Pierce, Rockford, IL), shaken with a Vortex mixer, and allowed to stand overnight in a refrigerator. A total of 1 μL of the eluate was injected with an automatic injector (HP 7673; Hewlett-Packard, Palo Alto, CA) into a gas chromatograph (HP 5890) equipped with a quadrupole mass-selective detector (HP 5970 A). The separation was carried out in an HP-INNOWax fused silica capillary column (30 m × 0.32 mm × 0.25 μm phase thickness). Helium was used as a carrier gas at an inlet pressure of 45 kPa. The following oven temperature program was used: 60 °C for 1 min, increase to 120 °C at 6 °C/min. The injector port was set to a temperature of 225 °C, and a splitless injection mode was used (valve time, 0.5 min).

The mass-selective detection was based on the electron impact ionization mode (EI), and the ions (m/z) 105, 79, 78, and 51 were monitored. The area of the base peak (105) was used for quantification. The retention time of 3-EP was 8.33 min, whereas 4-EP eluted at 8.54 min. No blank value was detected for either 3-EP or 4-EP; therefore, the limits of detection and quantification were determined using low-level calibration samples.

Calibration standards were made by the phase equilibrium method by adding a charcoal pad to a glass vial containing 1 mL of calibration solution prepared in a mixture of pyridine and toluene. Subsequently, the calibration standards were treated as samples. The stock and calibration solutions, the latter containing both 3-EP and 4-EP, were prepared weekly. The calibration standards were prepared daily. The external standards method was applied: 3-EP was calibrated using 3-EP and 4-EP correspondingly using 4-EP.

GC using flame ionization detection (FID) presented an alternative analytic system, but due to lack of specificity it was not used for field samples. The equipment comprised an HP 5890 gas chromatograph and a fused silica HP-INNOWax column (30 m × 0.32 mm × 0.5 μm phase thickness). The oven temperature program was as follows: 50 °C for 1 min, increase to 140 °C at 3 °C/min. Splitless injection was used (valve time, 0.5 min), and the flow rate of the carrier (helium) was 1.7 mL/min. The retention time of 3-EP was 25.4 min, and that of 4-EP was 25.9 min.

Synthesis of 3-EP. Triphenylmethyl phosphoniumbromide (0.06 mol), sodium amide (0.07 mol), and 100 mL of dry tetrahydrofuran were refluxed under an argon atmosphere overnight. The orange−yellow reaction mixture was cooled on an ice bath, and 3-pyridinecarboxaldehyde (0.04 mol) was added dropwise. The reaction mixture was allowed to warm at room temperature and then was stirred for 4 h. A total of 40 mL of diethyl ether was added, and the reaction mixture was filtered. A 50-mL sample of water was added to the filtered solution, which was then extracted with diethyl ether. The water phase was saturated with NaCl. The organic layer was dried with $MgSO_4$ overnight, and the solvent was evaporated. The crude product was soaked in silica and purified by flash chromatography using hexane:EtOAc (4:1) as eluent (yield 20%). ^{1}H nuclear magnetic resonance (NMR) (200 MHz): δ 5.37 (d, J = 11 Hz, 1H), 5.83 (d, J = 17.8 Hz, 1H), 6.71 (dd, J = 11, 17.8 Hz, 1H), 7.23 (ddd, J = 7.8, J_o = 4.8, J_p = 0.4 Hz, 1H), 7.71 (ddd, J = 7.8, 2.8, J_m = 1.8, J_p = 0.4 Hz, 1H), 8.49 (dd, J = 1.8, 4.8 Hz, 1H), 8.62 (d, J = 2 Hz, 1H). ^{13}C NMR (50 MHz): δ 116.0, 123.2, 132.4, 132.9, 133.3, 148.2, 148.8.

The purity of the product was determined by GC-FID. The result (77%) was obtained by comparing the intensity of the FID response to 3-EP with that to 4-EP (known to be 95%) and by assuming equal molar responses for both compounds.

Field Studies. Measurements were conducted in smoking and nonsmoking environments to evaluate and verify the utility of the method in field conditions. Parallel stationary samples were collected for different sampling periods (4.5 h−5 d) in a home, in an office room, and in a restaurant. On all occasions, the sampling was continuous without breaks. Charcoal tube sampling (at 0.05 or 0.1 L/min) was used as a reference method for sampling periods of 2 d or less.

Results

Sampling. The overall sampling rate of the passive test sampler was 25.7 $\pm$ 1.8 cm^3/min at 22 °C and 48% RH. The air velocity in the chamber was on average 0.1 m/s (range 0.09−0.2 m/s). The overall sampling rate was calculated as an arithmetic mean of the six arithmetic mean sampling rates obtained in the chamber experiments (Table 1). The concentrations of 3-EP and 4-EP measured by the reference method (charcoal tube) are also shown in Table 1. No adsorption of the test compound occurred on the plastic surfaces of the sampler housing (data not shown).

No breakthrough occurred in the charcoal tubes during the chamber tests at 320 μg/m^3 of 4-EP and 160 μg/m^3 of 3-EP (air volume 24 L) or during field sampling at 4.9 μg/m^3 of 3-EP (air volume 143 L).

For the test samplers, no change in recovery was observed after 2 d at room temperature. In the freezer, there was no loss after 1 week of storage, but a slight loss in recovery was seen after 2 weeks of storage. For the sample solutions, there was no change in recovery after 2 weeks, whereas a slight recovery loss (8%) was noted after 3 weeks of storage.

Analysis. Desorption efficiencies are shown in Table 2. For the test samplers, the recoveries were 83% (3-EP) and 79% (4-EP) by the phase equilibrium method and 81% (3-EP) and 76% (4-EP) by the spiking method. Compared with the test samplers, the charcoal tubes yielded slightly higher desorption efficiencies by both preparation methods (86% and 89%).

The mass spectra of 3-EP and 4-EP, derived using the EI mode, are shown in Figure 1. The spectra of the two isomers were similar in basic fragmentation. The ions m/z 105 and 51 were equally abundant in the two isomers, but a difference could be seen in the abundance of m/z 78.

TABLE 2. Validation Parameters Calculated for Diffusive Test Samplers and Charcoal Tubes

	3-EP	4-EP	test concn (μg/sample)
Detection Limit (μg/sample)			
test sampler	0.01	0.01	
charcoal tube	0.01	0.01	
Desorption Efficiency by Phase Equilibrium Method (%), Mean ± SD (No. of Samples)			
test sampler	83 ± 3 (10)	79 ± 3 (14)	3.1
charcoal tube	89 ± 4 (8)	89 ± 4 (8)	3.1
Desorption Efficiency by Spiking Method (%), Mean ± SD (No. of Samples)			
test sampler	81 ± 1 (5)	76 ± 4 (7)	3.1
charcoal tube	86 ± 3 (6)	87 ± 4 (6)	3.1
Precision (RSD, %)			
test sampler, 12 samples	4.5		0.7−2
test sampler, 24 samples		4.1−4.8	0.9−3.9
charcoal tube, 12 samples	2.1−2.9		2.8−3.7
charcoal tube, 22[b] samples		2.2−5.8	3.6−7.2

[a] SD, standard deviation. RSD, relative standard deviation. [b] Two outliers excluded.

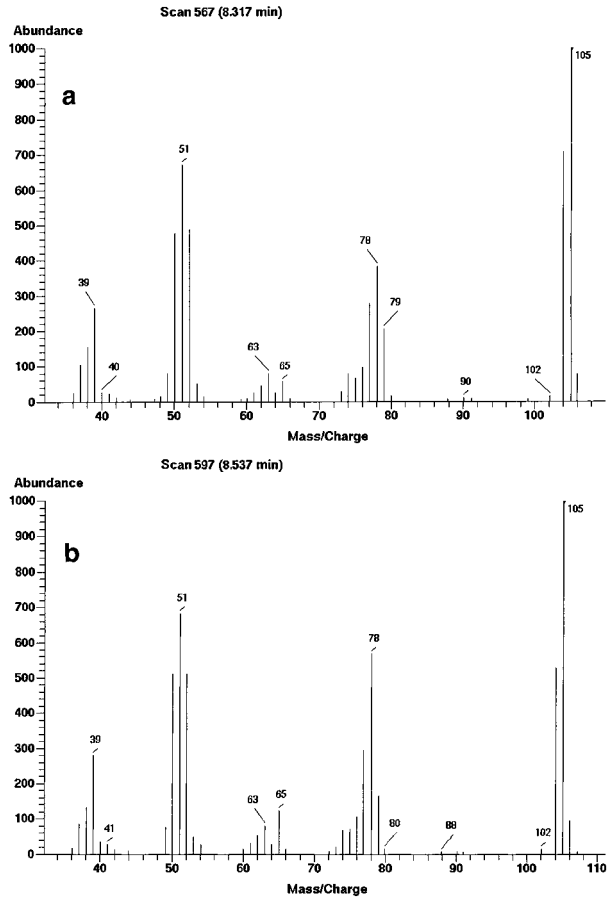

FIGURE 1. EI mass spectra of 3-EP (a) and 4-EP (b).

The calibration curves of 3-EP and 4-EP were linear in the range of 0−10 μg/sample. The slopes for the two isomers were very similar, the difference being on average 1.2% with both the test samplers and the charcoal tubes. The calibration curves were somewhat steeper with the charcoal tube method than with the tested sampling method (difference in slope on average 7%). No average slope value was calculated because the slope values were significantly changed during the study due to servicing of the mass detector. A good correlation coefficient (r > 0.999) was obtained for the regression line. The detection limit was 0.01 μg/sample. The limit of quantification (based on the monitoring and detection of two ions) was 0.017 μg/sample, corresponding to 0.46 μg/m^3 calculated for an air sample of 37 L (24 h).

Overall precision values (RSDs), covering sampling and analysis, were calculated for the test samplers and charcoal tubes on the basis of the chamber tests. These RSD values are shown in Table 2. The precision of the tested method varied only slightly (4.1−4.8%) between the different series. The charcoal tube method yielded higher RSD values in experiment I (4.7% and 5.8%) than in experiment II (2.1% and 2.9%).

Field Studies. The results of the field measurements are shown in Table 3. Sampling in nonsmoking environments (a home and an office room; n = 6) for about 3 d yielded no 3-EP (<0.1 μg/m^3) or interfering substances, which produce the specific ion used, at the retention time of 3-EP.

The mean concentration of 3-EP in a smoker's office room ranged from 3.8 to 4.9 μg/m^3 (n = 27), measured using both active and passive sampling methods and for different sampling periods (4.5 h−5 d). The room temperature averaged 24 °C, and the air humidity was 24% RH. The RSD for parallel test sampler samples ranged from 2.6% to 7.8% (average 5.8%). The mean RSD for the charcoal tube method was 5.1%. Although the ventilation rate was not determined, the room (50 m^3) was poorly ventilated judged by the slow air flow in the room during sampling (<0.05 m/s). In the same room, a background 3-EP concentration of 1.3 μg/m^3 was measured during a 24-h period when no cigarettes were smoked. In a restaurant, concentrations ranging from 2.5 to 10.2 μg/m^3 (n = 3) were measured during a 3-d sampling period.

Discussion

3-EP is a volatile organic compound (VOC) with a boiling point of 162 °C (*15*). VOCs are generally defined as compounds with vapor pressures in the range of 0.01−10 kPa. Airborne compounds in this group occur in the vapor phase at room temperature, whereas semivolatile organic compounds occur in both particle and vapor phases. As a vapor-phase substance of ETS, 3-EP is well-suited to monitoring using diffusive sampling. Nicotine, although a semivolatile substance, is known to occur almost exclusively in the vapor phase of ETS and has also been monitored using diffusive sampling. Unfortunately, its strong adsorption and re-emission can cause discrepancies in the results. Compared with ethenylpyridine, nicotine has been found to adsorb much more strongly on various surfaces (*16*).

The present experiments indicate that the tested method meets the criteria set for diffusive monitoring and also the analytic requirements set for monitoring ETS (*3*). The sampling method showed good precision, and the specificity and sensitivity of the GC/MS analysis were high enough for reliable ETS measurements.

Comparison of 3-EP and 4-EP. The chamber test was first carried out using 4-EP (experiment I), and the results were confirmed in a subsequent test with a mixture of 3-EP and 4-EP (experiment II). 4-EP was first used instead of 3-EP because 3-EP is not commercially available, and a rather high amount of a test chemical is needed to provide a dynamic standard atmosphere. The physicochemical properties of these isomers are reported to be similar (*16*). The characteristics of 3-EP and 4-EP investigated in this study showed close similarity. The responses of ion m/z 105 on GC/MS and the slopes of the two isomers were very alike. However, the chromatographic properties were not identical, thus allowing separation of the isomers in the polar column used. As no 4-EP is formed in tobacco smoke, it seems to be an ideal candidate for an internal standard in 3-EP quantification.

TABLE 3. Concentrations of 3-EP in Field Measurements

measurement site	measurement method	concn of 3-EP, mean ± SD (μg/m^3)	no. of samples	sampling period	no. of cigarettes smoked
restaurant	test sampler	5.3 ± 4.2	3	3 d	not known
office (smoker)	test sampler	3.8 ± 0.3	3	5 d	not known
office (smoker)	test sampler	4.1 ± 0.2	4	2 d	60
	charcoal tube	4.9 ± 0.2	4	2 d	
office (smoker)	test sampler	4.7 ± 0.3	4	4.5 h	7
	charcoal tube	4.9 ± 0.04	4	4.5 h	
	test sampler	3.8 ± 0.1	4	8.5 h	12
	charcoal tube	3.9 ± 0.3	4	8.5 h	
office (smoker)	test sampler	1.4 ± 0.1	3	24 h	0
	charcoal tube	1.3 ± 0.1	3	24 h	
office (nonsmoker)	test sampler	<0.1	3	3 d	0
home (nonsmoker)	test sampler	<0.1	3	3 d	0

Evaluation of the Test Sampler. The sampling rates of 3-EP and 4-EP were very similar. The overall sampling rate of 25.7 cm^3/min was well within the uptake range (20−48 cm^3/min) published for 3M organic vapor monitors (*17*). The sampling rate obtained here is not inconsistent with the uptake reported for styrene (28.9 cm^3/min), a compound resembling ethenylpyridine in molecular structure (*17*). The theoretical sampling rate was calculated according to Fick's law, using the following parameters: diffusion coefficient 0.076 cm^2/s (*12*); cross-sectional area/diffusion length (A/L) 8.54 cm (*18*). The theoretical value obtained (38.9 cm^3/min) was higher than the experimental value. This is consistent with a previous study reporting 27−61% higher values for theoretical sampling rates than for measured rates (*18*). Using a recalculated value (5.86 cm) reported to be the "effective" A/L for 3M samplers (*18*), the calculated sampling rate 26.7 cm^3/min is in good agreement with the experimental rate.

Previous publications have presented passive samplers based on impregnated glass fiber filters with sampling rates of 23.7 (nicotine; *19*), 31.5 (nicotine; *12*), and 27.8 cm^3/min (3-EP; *12*). These sampling rates do not differ essentially from those found in the present study. As the detection limits of the previous methods appear to be adequate, the major advantages of our method are a very simple sample treatment and a commercially available sampling device with no in-house preparation of the sampler. The application is suitable for routine surveys and also allows large-scale assessment of individual ETS exposures. According to recent data (*20*), personal monitoring is needed for accurate determination of individual ETS exposures.

Charcoal Tube Method. The charcoal tube method and the passive sampling method were not identical in desorption efficiency: a higher desorption efficiency was obtained for the charcoal tube. The charcoal tube method can be used as a reference method for the diffusive method. It is also suitable for short-term monitoring of 3-EP when the detection limit cannot be reached by the diffusive method.

Evaluation of the Field Results. The field measurements with parallel sampling confirmed that the tested passive method can be used with good precision (RSD 5.8%) for monitoring low amounts of 3-EP (0.03−0.7 μg/sample). The difference in results between the charcoal tube method and the passive sampler method was generally small. The largest difference between the methods was noted for a rather long sampling period (2 d). In all, a comparison of passive and active field samples indicates that the actual sampling rate of the office samples was slightly lower than the calculated value. This may be explained by the air velocity (<0.05 m/s) being below the values recommended for badge-type passive monitors (*21*).

In this study, the average 3-EP concentration in the office room during smoking was 4.3 μg/m^3. This result is consistent with previous studies. For instance, mean concentrations ranging from <1.5 to 13.3 μg/m^3 have been measured in smoking areas (53−219 m^3) with 21−103 smoked cigarettes/5 h and air exchange rates of 3.7−21/h (*5*). Although the number of cigarettes smoked/h was clearly higher than in our study, poor ventilation explains the relatively high concentration of 3-EP in the office room in our study.

We found no 3-EP in nonsmoking environments (office, home), whereas low levels of 3-EP (mean 0.08 μg/m^3) in nonsmoking homes have been reported by previous studies (*4*, *10*). However, in our study, there was a clearly detectable background concentration of 3-EP in the office room of a smoker. Therefore, it is useful to carry out separate measurements in smoking areas during nonsmoking periods.

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