Title

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Journal

Tobacco Control, 27(6)

ISSN

0964-4563

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Publication Date

2018-11-01

DOI

10.1136/tobaccocontrol-2017-054052

ONLINE MATERIAL

A Casino Goes Smoke-Free: A Longitudinal Study of Secondhand and Thirdhand Smoke Pollution and Exposure

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Methods

Casino Locations Sampled

The Appendix Figure 1 shows the casino schematics and the locations where measures were collected.

Measures

Air Particulate Matter. The following instruments were placed onto a wheeled cart which was moved around to sample different areas of the casino: Sidepak AM510 real-time aerosol monitor (TSI, Inc., Shoreview, MN, USA), DustTrak DRX aerosol monitor (Model 8533, TSI, Inc., Shoreview, MN, USA), Condensation Particle Counter (CPC) (Model 3007, TSI, Inc., Shoreview, MN, USA), and HOBO U12 Temp/RH data logger (Onset, Bourne, MA, USA). The target sampling duration for each location was approximately 30 minutes. Both the Sidepak and DustTrak DRX were used to monitor PM2.5 mass concentrations, the CPC was used to monitor PM1 number concentrations (reported elsewhere), and the HOBO was used to record temperature and relative humidity. All data were measured in real-time and logged every 10-seconds. The mass concentrations for all PM measurements were reported in $\mu g/m^3$.

The reported mass concentrations of PM2.5 are the average of the values for Sidepak and DustTrak DRX data in the same time-frame. A calibration factor of 0.29 (dimensionless) was used for the Sidepak when there was smoking activity.[1-3], and a factor of 0.31 after the smoking ban.[4] In addition, a humidity correction factor was applied to all PM2.5 data with simultaneous relative humidity (RH) over 45%.[5]

Air nicotine. Air samples were collected overnight with a sorbent tube (SKC West 226-93) connected to a sampling pump (SKC Airchek Model XR5000). Sampling time

ranged from 12-14 hours. Pumps were calibrated to 1.5 lpm before and after use, and samples for which pump flow rates changed by more than 10% were discarded. Our methods for samples handling and analysis have been previously described. [6]

Surface nicotine. Surfaces were sampled for nicotine through the method described in Quintana et al.[7] Briefly, prescreened cotton rounds (100% organic cotton facial wipes) were wetted in 0.1% ascorbic acid, wiped inside rigid paper 10 x 10 cm templates taped into place, placed into glass vials with Teflon lids, stored in a cooler during transport, then stored in the laboratory at -20° C until analysis. Two different surfaces in each casino area were sampled for nicotine at each visit. One field blank (a cotton round handled in the casino but not wiped on a surface) was collected in the main smoking area, one in the nonsmoking slots area, and one in the Human Resources area.

Dust. Floor dust samples were collected using the cyclone vacuum High-Volume-Small Surface-Sampler (HVS4, CS3 Inc., Venice, FL). Samples were collected from a 3 m^2 area and if the collected dust content did not exceed 1/2 inch in the dust collection bottle, additional area as needed (Mean=5 m^2). Dust samples were pre-weighed, sieved through a 150 µm mesh sieve to remove artifacts such as large debris and hair, and weighed again. Sieved dust samples were stored at -20° C until extraction.

Laboratory Analysis. For surface nicotine analysis, deuterated nicotine (d4) was added as an internal standard because it can be distinguished from nicotine contained in the sample matrix, allowing for accurate quantitation and assessment of extraction efficiency. Nicotine-d4 as an internal standard (Cambridge Isotope Laboratories, Tewksbury, MA) was spiked directly onto the surface wipe in an amber glass vial. 0.1%

Formic acid solution was added to the vial, and then the vial was vortexed. Addition of 1M potassium hydroxide (KOH) was followed and the vial was vortexed again. Acetonitrile was added to the solution and the vial was placed in a shaker for 30 minutes. The extract was transferred to a centrifuge tube containing a pouch of MgSO4/NaCl (UCT ENVIRO MgSO4/NaCL mylar pouch, UCT, Bristol, PA), then vortexed and centrifuged. Afterwards, 1 mL of each extract was transferred from each centrifuge tube into 2mL micro-centrifuge tubes containing UCT ENVIRO CLEAN 150mg MgSO4/50mg CEC18. The 2mL micro-centrifuge tubes were then vortexed and centrifuged. In the last step, the clear part of each sample extract was transferred to a pre-labeled LC vial for LC/MS/MS analysis.

For the air nicotine analysis, glass wool and beads in each air tube were transferred to a pre-cleaned centrifuge tube and the samples were prepared following the same steps used for the surface wipes. The preparation of dust samples for nicotine and TSNAs and the instrumental conditions of an Agilent 1200 series liquid chromatograph with Agilent 6460 triple quadrupole mass spectrometer (LC/MS/MS) have been described elsewhere.[8]

Finger nicotine was measured through collection of wipe samples of nonsmoking confederates before and after their 4-hour visit to casino. A baseline sample was obtained in a hotel room prior to the confederate leaving for the casino. The research assistant wiped the confederate's dominant hand index finger with a pre-wetted cotton round as detailed for surface wipes in our companion paper.[7] A second sample from the same finger was obtained after the visit to the casino. One field blank (i.e., a cotton round handled in the hotel room but not wiped on a finger) was collected for the pre-

exposure and one for the post-exposure samples each evening. The finger wipe samples and field blanks were placed into glass vials with Teflon lids, stored in a cooler during transport, and stored in the laboratory at -20° C until analysis.

Urinary cotinine. Cotinine is a major metabolite of nicotine and a sensitive and specific biomarker of SHS and THS exposure in nonsmokers.[9-11] Confederates collected a urine sample in a casino hotel restroom immediately before and after spending 4 h on the casino floor, and at each urination through the night until approximately 11 am the following day. The number of post exposure samples varied from 2 to 6 (Median=4). Post-exposure samples were combined in equal amounts to yield a single post-exposure sample for cotinine analysis by highly sensitive LC-MS/MS previously described.[6]

Urinary NNAL. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) is a metabolite of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). In nonsmokers, urinary NNAL is a carcinogen biomarker of exposure to SHS or THS.[12 13] Post-exposure urine samples were analyzed individually for NNAL using liquid chromatography – tandem mass spectrometry (LC-MS/MS). The lower limit of quantitation (LOQ) of the method is 0.25 pg/mL.

RESULTS

Figures 2 and 3 show concentrations of PM 2.5 and dust TSNAs before, during, and after the smoking ban.

Before the Smoking Ban: SHS and THS Pollution

PM 2.5 concentration. Measures taken at baseline across six public casino locations (A1-A6; see Figure 1) showed elevated levels similar to those reported by Klepeis et al. for the same casino (24-87 $\mu g/m^3$)[14] and in previous studies of other casinos where patrons actively smoked (e.g., Achutan et al.: 23-86 $\mu g/m^3$)[15]. The median PM 2.5 level was 39 $\mu g/m^3$ with an interquartile range of (IQR) of 63 $\mu g/m^3$ (Q1=5.2; Q3=68.4).

Dust TSNAs concentration. Baseline measures showed a similar pattern as dust nicotine with highly elevated median levels compared to previously examined smoking environments and more restricted variability across locations than we observed for surface nicotine. Figure 2 shows data for the sum of NNK NNN, NAT, and NAB concentrations. The median total TSNA concentration was 95 ng/g, approximately 5 to 8 times higher, respectively, than found in homes of active smokers.[8] The IQR was 66 ng/g (Q1=81; Q3=147), about 70% of the median levels, indicating that dust TSNA concentrations were more evenly distributed across the casino than surface nicotine loadings. The total TSNA concentration in the nonpublic nonsmoking area A7 was 42 ng/g (not shown in Figure 2), or 44% of the median levels found in the public nonsmoking casino areas.

During the Ban: Changes in THS Pollution

In Figures 2 and 3, measures taken from W1 through M6 reflect the time period when smoking was prohibited in the casino. During this time, tobacco smoke specific markers found in the air, on surfaces, and in dust reflect the persistent THS reservoir that accumulated over 27 years when smoking was permitted.

PM 2.5 concentration. Figure 2 shows that the smoking ban was well implemented, achieving an immediate 94% decline from 39 $\mu g/m^3$ to 2.3 $\mu g/m^3$ in W1. Concentrations stayed at levels <5 $\mu g/m^3$ from M1 to M6 before they rebounded to 22.4 $\mu g/m^3$ after smoking resumed in M12. Statistical tests comparing the geometric mean at baseline to each of the six measures taken during the smoking ban ($Chi2(7)=72.0$; $p<0.001$) revealed significant reductions at W1 through M6 (all $p<0.001$).

Dust TSNA concentration. Similar to changes in dust nicotine, dust TSNAs showed no or small declines from high baseline levels during the earlier months of the smoking ban (see Figure 3). The total dust TSNA concentration remained at levels between 48 and 84 ng/g until 2 months into the smoking ban before declining to lower levels in M3 and M6 (13 ng/g and 22 ng/g). Statistical tests comparing the geometric mean at baseline to each of the six measures taken during the smoking ban ($Chi2=34.4$; $p<0.001$) revealed no significant reductions through M2 ($p>0.15$) followed by significant reductions in M3 and M6 (all $p<0.001$). Total TSNA concentrations in the nonpublic nonsmoking areas (A7, A10) declined to 27 ng/g during the first two months of the ban and reached levels below 9 ng/g at M3 and M6 (not shown in Figure 3).

After the Reversal of the Smoking Ban

The smoking ban was reversed 11 months after its initiation, and the M12 measures show an overall rebound in THS pollution levels.

The median PM 2.5 level increased to 22.4 $\mu g/m^3$ from levels below 3.5 $\mu g/m^3$ throughout the smoking ban (see Appendix Figure 2). Statistical tests showed that the M12 level was significantly higher than all measures during the smoking ban (all $p<0.004$). It was, however, not significantly different from the baseline level before the smoking ban ($p=0.12$).

Levels of TSNAs measured in dust increased at M12 to 42 ng/g from 23 ng/g six months earlier (see Appendix Figure 3). This M12 level was significantly higher than those at M6 ($p=0.019$) and M3 ($p=0.006$) and was not significantly different from any of the earlier measures during the ban and before the ban (all $p>0.13$).

In the nonpublic nonsmoking areas (A7, A10; not shown in Figures 1 and 2), THS levels did not rebound at 12 months but remained at or near the lowest levels observed during the smoking ban (surface nicotine: 0.1 $\mu g/m^2$; dust nicotine: 3.7 $\mu g/g$; dust NNK: 1.9 ng/g; air nicotine: 0.05 $\mu g/m^3$).

Figure 1: Floor schematic of sampling locations in casino.

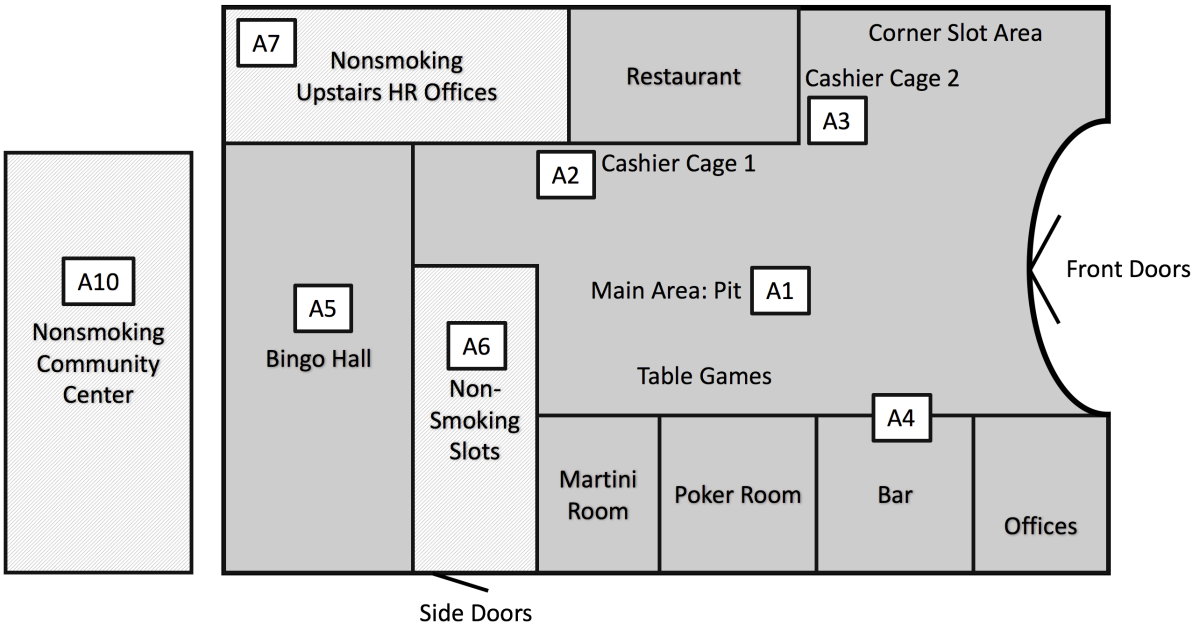
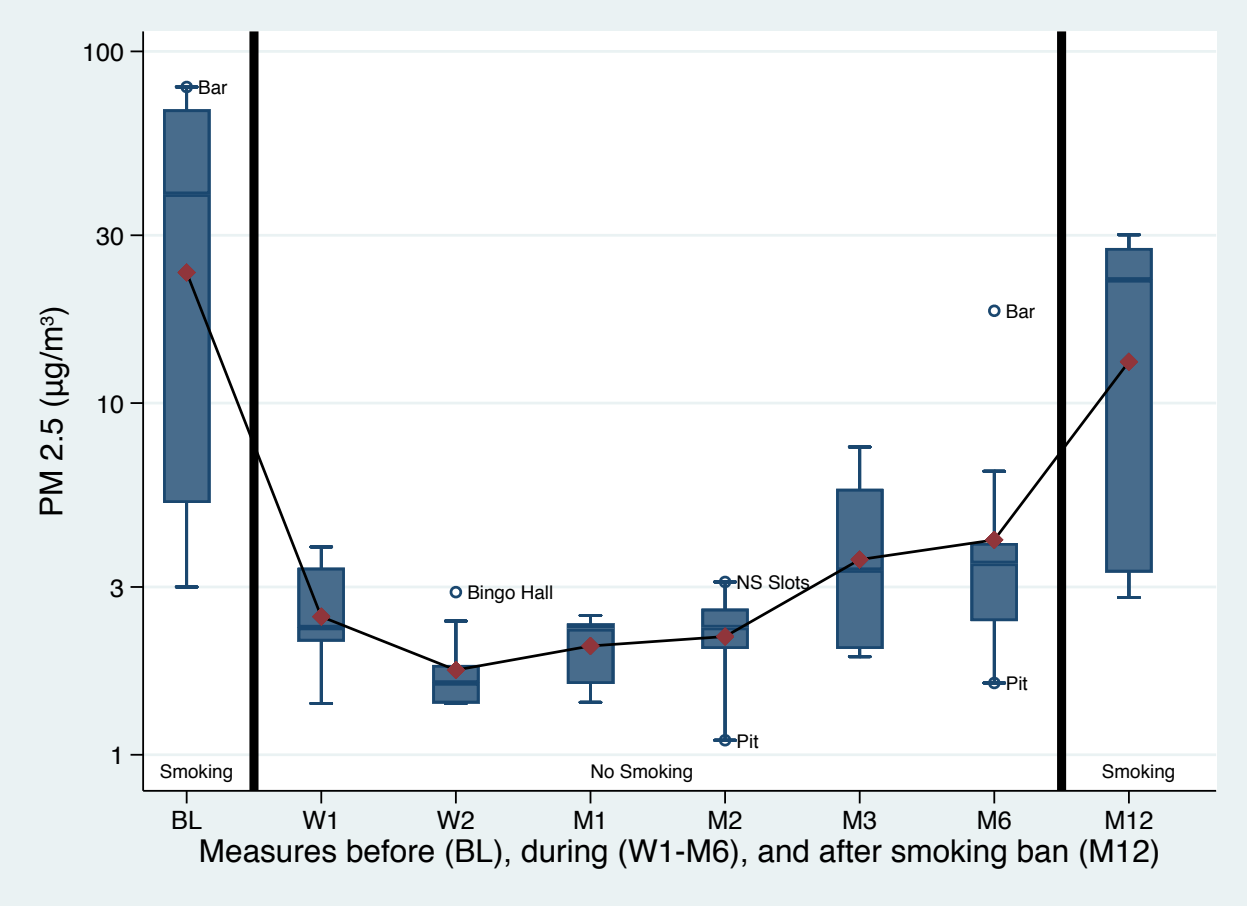
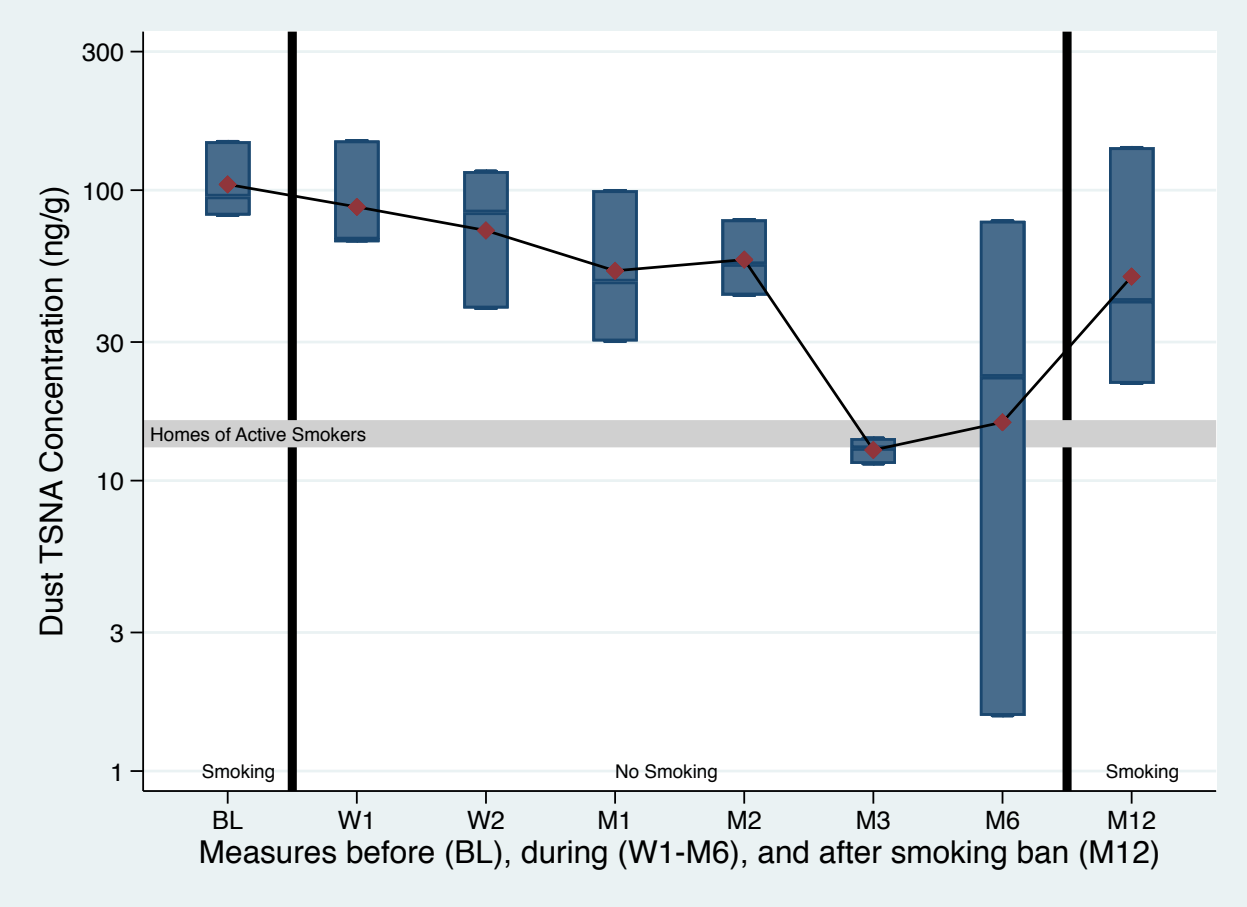


Figure 2: Boxplots of PM 2.5 concentrations across casino locations open to the public.



Note. Areas Sampled: 1 – Pit; 2 – Cashier's Cage 1; 3- Cashier's Cage 2; 4 – Bar; 5 – Bingo Hall; 6 – Nonsmoking Slots

Figure 3: Boxplots of Tobacco Specific Nitrosamine concentrations (sum of NNK, NNN, NAB, NAT) across three casino locations open to the public with reference level from previous studies of private homes of smokers.[8]



Note. Areas Sampled: 1 – Pit; 5 – Bingo Hall, 6 – Nonsmoking Slots

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