EFFECTS OF RELATIVE HUMIDITY ON NONSMOKER RESPONSE TO ENVIRONMENTAL TOBACCO SMOKE

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Respiratory, eye blink and sensory ballot responses to environmental tobacco smoke (ETS) levels, 13 to 65 times greater than average concentrations found in field studies, were measured at three relative humidity (RH) levels, 18, 50 and 82%. These responses were measured to determine the influence of RH on nonsmokers' responses to ETS. Nonsmokers rated air quality several times during each session; their breathing patterns and eye blinks were recorded continuously. All sessions were carried out in an 18-m<sup>3</sup> environmental test chamber equipped to measure target ETS constituents. #4406

RH and ETS levels independently affected nonsmokers' eye blink rates and ballot ratings of nasal irritation and annoyance. Breathing patterns, responses to odor strength, eye irritation and throat irritation were related to ETS level, but not to RH.

Concentrations of CO,  $NO_x$ , NO,  $NO_2$ , ammonia and nicotine varied directly with ETS level and indirectly with RH. RSP (respirable suspended particle) concentrations increased directly with both ETS and RH levels. Volatile organic compounds, as measured by FID (Flame Ionization Detector) response varied directly with ETS level, but were unaffected by RH.

## INTRODUCTION

The objective of this study was to explore the effects of relative humidity on nonsmokers' physiological and perceptual responses to ETS. Concurrently, the effect of RH on ETS chemistry was evaluated. Exorbitantly high ETS levels, 13 to 65 times greater than the approximately 5 to 12  $\mu$ g/m<sup>3</sup> nicotine levels found in field studies (1, 2), were selected for this study so that quantifiable and significant differences in subject responses could be obtained. Preliminary results from our lab had indicated that nicotine and RSP levels greater than 100 and 680  $\mu$ g/m<sup>3</sup>, respectively, generated from smoking more than 1 1R4F cigarette in a static chamber, were required to obtain eye blink rates which are significantly different than those from clean air (Figure 1). Unlike ETS, RH conditions selected for this study were forty minutes; exposures to ETS were limited to 28 minutes.



## MATERIALS AND METHODS

### Chamber Facility and Chemical Measurements

All experiments were performed in an  $18 \text{-m}^3$ , stainless steel environmental chamber equipped with temperature and humidity control and equipment for monitoring ETS constituents (6). Chamber conditions were  $75 \pm 1^\circ$  F and  $18 \pm 4$ ,  $50 \pm 1$  or  $82 \pm 2^\circ$  RH. The air exchange rate was  $6.0 \pm 0.2$  room volumes per hour. Chemical analyses, with the exception of ammonia, were performed according to methods described elsewhere (6 - 9). Ammonia, trapped in an impinger containing 0.01 M HCl, was measured by cation exchange chromatography on a DIONEX ion chromatograph system with conductivity detection.

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### Subjects

Six male and six female nonsmokers were recruited from the local community by an independent marketing firm. Subjects wore no eye makeup, scented toiletries, glasses or contact lenses during sessions. Two subjects, one male and one female, participated in each session.

### ETS Production

Three male and three female R.J. Reynolds employees were recruited to smoke University of Kentucky Reference 1R4F cigarettes. Three of these smokers participated in each session. Twelve minutes after a session started, two smokers together smoked 0, 2 or 4 cigarettes, depending on whether the total number of cigarettes required for the particular session was 0, 4 or 8, respectively. At 19.5 minutes, the two smokers were replaced by a single smoker who, depending on session type, consecutively smoked two sets of cigarettes, each set consisting of 0, 1 or 2 cigarettes. Eight puffs were taken on each cigarette \* at one-minute intervals. When smokers were required to smoke two cigarettes simultaneously, they puffed alternate cigarettes at 30 second intervals. This smoking regime was selected to generate uniform and consistent ETS levels and at the same time minimize effects that the smokers exerted on chamber RH.

### Experimental Design

The experiment consisted of nine session types, 3 ETS levels (ETS generated by smokers smoking 0, 4 or 8 cigarettes) at each of 3 RH levels 18, 50 and 82%. All subjects experienced all session types. The order in which subjects were exposed to session types varied. Sensory responses were recorded three times during each session: ballot 1 during the first two minutes of the experiment, ballot 2 after generation of the peak smoke level (minutes 24 to 25) and ballot 3 at the conclusion of the session (minutes 39 to 40). Respiration and eye blinks were continuously monitored. For data analysis purposes, the forty-minute sessions were divided into pre-smoke (minutes 0 to 12) and smoke periods (minutes 12 to 40).

### Response Measurements

Ballots consisted of 6-inch, horizontal, unstructured line scales on which subjects indicated their perceptions of odor strength, nasal irritation, eye irritation, throat irritation and annoyance by making vertical pencil strokes. Scales range from "slight" to "extreme". Responses were quantified by measuring the distance to the strokes from the left end of the scales. Maximum score was defined as 60. A Respitrace<sup>TM</sup> system (NIMS, Miami, FL) equipped with a Respicomp<sup>TM</sup> analyzer continuously measured subjects' breathing patterns. Videotape recordings of the subjects' faces were used to quantify eye blink frequency.

## Statistical Analyses

Statistical analyses were performed using analyses of variance (ANOVA) with post hoc paired t-tests. Criteria for significance were based on  $p \leq 0.05$ . The criteria were adjusted if repeated tests were performed by dividing 0.05 by the number of tests (Bonferroni adjustment).

# RESULTS AND DISCUSSION

### Sensory Responses

Responses to ballot 1 (ballot completed before smoke generation) were not related to ETS level or RH. Significant RH effects during this initial pre-smoke period were difficult to detect due to low ballot scores and high subject response variability.

Subjects' responses to ballot 2 attributes were related to ETS level (p=0.0001), but not to RH (0.18  $\leq$  p  $\leq$  0.86). Responses to ballot 3 indicated that perceptions of all five sensory attributes increased directly with increased ETS level (p=0.0001, Figure 2). Subjects' responses to ballot 3 indicated that perceptions of nasal irritation and annoyance varied indirectly with RH (p=0.0031 and p=0.016, respectively) and perceptions of odor strength, eye irritation and throat irritation were unrelated to RH (p=0.22, p=0.25, p=0.08, respectively, Figure 3). On all ballots, interactions between RH and ETS level for the individual attributes were not significant.

## Respiratory Responses

Generally, breathing behavior was affected by exorbitantly high levels of ETS but not by RH (p=0.87 and p=0.48 for the effect of RH on breathing frequency and tidal volume, respectively). Subjects' breathing patterns were the same when exposed to ETS from either 0 or 4 cigarettes (p=0.69 and p=0.73 for the significance of the differences between the two ETS levels for breathing frequency and tidal volume, respectively). Subjects' breathing frequencies were less (p=0.001) and their tidal volumes were more (p=0.021) when exposed to ETS from 0 versus 8 cigarettes.

Although subjects' breathing patterns, as indicated by breathing frequency and tidal volume were related to ETS, their minute volumes were unaffected (p=0.66).

## Eye Blink Frequency

Eye blink frequency was significantly affected by both RH and ETS levels (p=0.0001 for both main effects). Interactions between these stimuli were not significant (p=0.31). Average eye blink frequency,  $26 \pm 14$  blinks/minute, for the 8 cigarette ETS level, was significantly greater than those for the 0 and 4 cigarette ETS levels,  $16 \pm 13$  (p=0.0052) and  $18 \pm 12$  (p=0.0006) blinks/minute, respectively. Average eye blink frequency of subjects exposed to 4 cigarettes did not differ significantly from that for 0 cigarettes (p=0.46). Subjects' eye blink frequencies for the 18 ( $24 \pm 17$  blinks/minute) versus 82% ( $17 \pm 9$  blinks/minute) RH levels were significantly different (p=0.0068). Blink frequencies ( $20 \pm 13$  blinks/minute) for the 50% RH level did not differ from those for the 18 or 82% RH levels (p=0.09 and p=0.27).

### ETS Composition

Real-time analyte concentrations (nicotine, CO, NO<sub>x</sub>, NO, NO<sub>2</sub>, and FID response) varied directly with ETS level. Average nicotine concentrations were 160 and 332  $\mu g/m^3$  during the smoke period for the 4 and 8 cigarette ETS levels, respectively. Average RSP concentrations were 692 and 1375  $\mu$ g/m<sup>3</sup> during the smoke period for the 4 and 8 cigarette ETS levels, respectively. FID responses (estimates of vapor phase organic compound concentration) were independent of RH. RSP levels increased by approximately 20% as RH increased from 18 to 82%. Increased RSP concentration with increased RH was probably due to more water in particles at 82% versus 18% RH. Concentrations of CO (Figure 4),  $NO_x$ , and NO were 10% to 30% greater for 18% versus 82% RH sessions. Smokers observed that under drier conditions slightly more tobacco was burned. Increased tobacco combustion may have produced higher CO,  $NO_x$ , and NO concentrations at 18% versus 82% RH. The  $NO_2$  and nicotine (Figure 5) concentrations during the smoke period were approximately three and two times higher, respectively, at 18% RH versus 82% RH. Reduced oxidation of NO to NO2 with increased RH is consistent with published results (10). The change in nicotine concentration with RH may have been due to increased deposition of nicotine onto chamber walls, floors and other available surfaces in high versus low RH conditions.

Time-weighted average concentrations of ammonia increased with increased ETS level and decreased by approximately 10% with increased RH. Acetaldehyde and acrolein concentrations increased with ETS level, but were not affected by RH. Formaldehyde and acetone concentrations were not related to either ETS or RH based on our significance criteria.

#### CONCLUSIONS

Keeping in mind that ETS levels, as indicated by real-time chemical analyses, were 13 to 65 times greater than average concentrations encountered in the field, that our relative humidity levels ranged from 18 to 82% RH and that our exposure period was limited to 28 minutes, the following conclusions can be drawn:

- 0 Eye blink frequency and perceptions of annoyance and nasal irritation are significantly affected by RH.
- 0 At exorbitantly high ETS levels (8 cigarettes or approximately 332  $\mu$ g/m<sup>3</sup> nicotine), subjects' eye blink frequencies are significantly greater than those for clean air.
- 0 Perceptions of odor strength, nasal irritation, eye irritation, throat irritation and annoyance significantly increase with increased ETS.
- O ETS affects subjects' tidal volumes and breathing frequencies, but not their minute volumes. The effect of ETS on subjects' tidal volumes and breathing frequencies were observed only at exorbitantly high ETS levels (8 cigarettes or  $332 \ \mu g/m^3$  nicotine). Subjects' respiratory functions are not affected by RH.
- 0 Subjects' perceptions of eye irritation did not significantly relate to relative humidity. Longer exposure times or more subjects might be necessary to observe this documented (11) relationship.

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