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CONTENTS

- 503 REVIEW OF THE BIODISTRIBUTION AND TOXICITY OF THE INSECT REPEL-
LENT *N,N*-DIETHYL-*m*-TOLUAMINE (DEET) Philip J. Robbins and Martin G.
Cherniack
- 527 USE OF γ -HEXACHLOROCYCLOHEXANE (LINDANE) TO DETERMINE THE ON-
TOGENY OF METABOLISM IN THE DEVELOPING RAT M. E Copeland, R. W.
Chadwick, N. Cooke, D. A. Whitehouse, and D. M. Hill
- 543 USE OF MULTIPARAMETER ANALYSIS TO QUANTITATE HEMATOLOGICAL
DAMAGE FROM EXPOSURE TO A CHEMICAL (ETHYLENE OXIDE) D. M.
Popp, R. A. Popp, S. Lock, R. C. Mann, and R. E. Hand, Jr.
- 567 BENZENE LEVELS IN AMBIENT AIR AND BREATH OF SMOKERS AND NON-
SMOKERS IN URBAN AND PRISTINE ENVIRONMENTS Ronald C. Wester,
Howard I. Maibach, Larry D. Gruenke, and John C. Craig
- 575 ACUTE HEPATOTOXICITY INDUCED BY HEPATOTOXINS IN *SUNCUS*
MURINUS Song-chow tin, Hiroshi Saito, Takeshi Yohro, and Junji Shiga
- 589 EVALUATION OF CHICKEN EMBRYO, BRINE SHRIMP, AND BACTERIAL BIOAS-
SAYS FOR SAXITOXIN Douglas L. Park, Ivette Aguirre-Flores, William F. Scott,
and Ellen Alterman
- 595 EFFECTS OF ETHANOL ON METHYL MERCURY TOXICITY IN RATS Hidehiko
Tamashiro, Mikio Arakaki, Hirokatsu Akagi, Kouji Murao, Kimiko Hirayama,
and Michael H. Smolensky
- 607 CARCINOGENIC EFFECTS OF ANTIMONY TRIOXIDE AND ANTIMONY ORE
CONCENTRATE IN RATS David H. Groth, Lloyd E. Stettler, JeAnne R. Burg,
William M. Busey, George C. Grant, and Lawrence Wong

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BENZENE LEVELS IN AMBIENT AIR AND BREATH OF SMOKERS AND NONSMOKERS IN URBAN AND PRISTINE ENVIRONMENTS

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Benzene levels in human breath and in ambient air were compared in the urban area of San Francisco (SF) and in a more remote coastal pristine setting of Stinson Beach, Calif. (SB). Benzene analysis was done by gas chromatography-mass spectroscopy (GC-MS). Ambient benzene levels were sevenfold higher in SF (2.6 ± 1.3 ppb, $n = 25$) than SB (0.38 ± 0.39 ppb, $n = 21$). In SF, benzene in smokers' breath (6.8 ± 3.0 ppb) was greater than in nonsmokers' breath (2.5 ± 0.8 ppb) and smokers' ambient air (3.3 ± 0.8 ppb). In SB the same pattern was observed: benzene in smokers' breath was higher than in nonsmokers' breath and ambient air. Benzene in SF nonsmokers' breath was greater than in SB nonsmokers' breath. Marijuana-only smokers had benzene breath levels between those of smokers and nonsmokers. There was little correlation between benzene in breath and number of cigarettes smoked, or with other benzene exposures such as diet. Of special interest was the finding that benzene in breath of SF nonsmokers (2.5 ± 0.8 ppb) was greater than that in nonsmokers ambient air (1.4 ± 0.1 ppb). The same was true in SB, where benzene in nonsmokers breath was greater than ambient air (7.8 ± 0.2 ppb versus 1.0 ± 0.1 ppb on d 1 and 1.3 ± 0.3 ppb versus 0.23 ± 0.78 ppb on d 2). This suggests an additional source of benzene other than outdoor ambient air.

INTRODUCTION

Benzene is one of the most widely used chemicals, with an annual production of about 10 billion pounds (Lee et al., 1983). Concerns about the toxicity and carcinogenicity of benzene have led to continuing pressure to lower allowable occupational exposures (Snyder, 1974; Zenz, 1978; Brief et al., 1980). However, while the major risk of exposure is originally believed to be from the atmosphere in the workplace, new concerns have also been raised about the possible health risk to the general population from atmospheric benzene pollution, thought to arise largely from automobile fuels and exhaust (Lonneman et al., 1968; Pilar and Graydon, 1973). There is, however, little available

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data on the relative importance of various routes of exposure for the nonoccupationally exposed individual.

The objective was to determine background benzene concentrations in the breath of urban smokers and nonsmokers and to compare the levels found to those from subjects who live in a "pristine" environment (where atmospheric benzene levels are much less than those found in typical urban environments).

METHODS

Urban subjects were male volunteers age 18 and above, who were residents of the city of San Francisco and from whom informed consent had been obtained. Most of the subjects studied lived in the Mission District of San Francisco, an inland area somewhat sheltered from the flow of ocean air.

Duplicate breath samples were obtained from 15 smokers and 15 nonsmokers on separate days at a clinic in the city. Each subject was required to fill out a questionnaire detailing his exposure during the previous 24 h and during the previous week to cigarette smoke, solvents (including gasoline), and dietary items known to be high in benzene. The room air at the clinic was also sampled continuously during the collection period on both study days for the determination of benzene levels in the inspired air. Smoking was not allowed in the clinic, and breath samples from smokers were generally obtained 45–85 min after the last cigarette smoked.

Stinson Beach, California, was selected for the pristine environment. Subjects were selected for the pristine environment study from local residents. Breath samples were collected outdoors and were worked up on site within a few hours of collection in a mobile laboratory to avoid contamination of samples on storage in gas sample bags. Otherwise, the collection and analytical procedures used were the same as those used for the urban study. The environmental air was sampled repeatedly on both study days.

Breath samples were collected by asking the subject to exhale normally into a 5-l gas sample bag made of a plastic-metal laminate (Varian 00-996893-02) until the bag was full. The internal standard, 150 ng benzene-1,3,5-*d*3 (98% D, KOR Isotopes) in 25 μ l water, was injected into each bag. Bags were worked up the following day by withdrawing the sample through a silica trap (520/260 mg dual section, SKC Inc. 226-15 or equivalent). The upstream portion of the trap was transferred to a 2-ml vial and water was added to desorb the benzene. Air samples were taken by drawing air directly through a silica trap using a battery-operated pump. Both sections of the trap were saved in separate vials and the internal standard was added to each. Sample vials were heated to about 70°C, and headspace samples were taken for analysis by se-

lected-ion-monitoring gas chromatography-mass spectrometry. Selected-ion records were obtained with Tenax GC or Chemipack C18 columns using an Infotronics model 2468/2600 gas chromatograph coupled to a Kratos/AEI MS-12 mass spectrometer, which had been modified for selected-ion monitoring (Gruenke et al. 1980). Records were obtained at m/z 78 (M^+ of benzene) and at m/z 81 (M^+ of the internal standard). Ion current ratios were compared to those from calibration standards, and a number of quality control samples were run on each day to assess the influence of benzene contamination on the analytical process. The limit of detection was 0.1 ppb (Gruenke et al., 1986). Statistical analyses were done on an Apple computer using ANOVA and Stats Plus programs (Human Systems Dynamics).

RESULTS

Table 1 gives a summary of the atmospheric benzene levels. The average for San Francisco was 2.6 ± 1.3 ppb. The lowest levels were observed near the coast, and the highest levels were observed inland near sources of heavy auto traffic. Benzene in the air at Stinson Beach was found to be significantly lower ($p < 0.001$) than that found in the San Francisco Bay area (Table 1). This is probably not only due to the lack of the usual urban sources of benzene pollution (such as heavy automobile traffic) but also because of the climate, which is dominated by strong winds off the ocean, resulting in low benzene levels at the limit of detection (0.1 ppb). On a day when there was little wind, air

TABLE 1. Summary of Atmospheric Benzene Levels

San Francisco		
Average 2.6 ± 1.3^d ppb benzene in = 25) ^{a,c}		
Range 0.8–5.2 ppb		
Dates of measurement between Nov. 4 and Dec. 6, 1984, at six different urban locations		
Stinson Beach		
Day	n ^a	Atmospheric benzene (ppb)
1 ^b	5	1.02 ± 0.09
2 ^b	6	0.23 ± 0.18
3	6	0.13 ± 0.11
4	4	0.16 ± 0.12
Total	21	0.38 ± 0.39^c

^a Number of samples, n.

^b Days when breath measurements were taken.

^c Significantly different, $p < 0.001$, between benzene in San Francisco and Stinson Beach.
Standard deviation.

TABLE 2. Benzene Concentrations in Breath of Smokers and Nonsmokers and in Respective Ambient Air

Environmental concentration	N ^a	Benzene (ppb) ^b
Urban		
Smokers' breath	15	6.8 ± 3.0
Smokers' ambient air	5	3.3 ± 0.8
Nonsmokers' breath	15	2.5 ± 0.8
Nonsmokers' ambient air	4	1.4 ± 0.1
Pristine (d 1)		
Smokers' breath	8	12.1 ± 9.6
Nonsmokers' breath	6	1.8 ± 0.2
Marijuana-only smokers' breath	3	2.5 ± 1.3
Ambient air	5	1.0 ± 0.1
Pristine (d 2)		
Smokers' breath	6	4.8 ± 2.3
Nonsmokers' breath	3	1.3 ± 0.3
Ambient air	6	0.13 ± 0.18

^a N, Number of samples.^b Mean ± standard deviation

levels reached 1.0 ppb. Overall levels for the site averaged 0.38 ± 0.39 ppb, sevenfold less than the average for the urban site.

Tables 2 summarizes the benzene levels in breath of smokers', nonsmokers', and marijuana-only smokers' breath, and the respective ambient air levels. Table 3 gives the statistical comparisons. This gives a

TABLE 3. Statistical Comparison of Benzene Concentrations

Environment	Comparison		Statistic
	A	B	
Urban	Smokers' breath	> ^a Nonsmokers' breath	$p < 0.001^b$
	Smokers' breath	> Ambient air	$p = 0.02^b$
	Nonsmokers' breath	> Ambient air	$p = 0.02^b$
Pristine (d 1)	Smokers' breath	> Nonsmokers' breath	$p = 0.02^b$
	Smokers' breath	> Ambient air	$p = 0.03^b$
	Nonsmokers' breath	> Ambient air	$p < 0.001^b$
	Smokers' breath	> Marijuana-only smokers' breath	$p = 0.13$ (NS)
	Nonsmokers' breath	> Marijuana-only smokers' breath	$p = 0.18$ (NS)
	Marijuana-only smokers' breath	> Ambient air	$p = 0.03^b$
Pristine (d 2)	Smokers' breath	> Nonsmokers' breath	$p = 0.04^b$
	Smokers' breath	> Ambient air	$p < 0.001^b$
	Nonsmokers' breath	> Ambient air	$p < 0.001^b$

^a >, Greater than; <, less than; >, not greater than.^b Statistically significant difference.^c Not statistically different (NS).

comparison of intake air (ambient) and exhaled air (breath) at a particular time. In the urban environment, benzene levels in breath of both smokers and nonsmokers were higher than the respective ambient air levels ($p = 0.02$), and benzene levels in smokers' breath were almost threefold higher than in nonsmokers ($p < 0.001$). The same patterns held true for the breath levels found in subjects from the pristine environment, where breath levels of benzene were again statistically higher in smokers than in nonsmokers and both were higher than benzene levels in ambient air. Marijuana-only smokers had benzene breath levels between that of smokers and nonsmokers.

In addition to the t-test comparisons (Table 3), a separate analysis of variance of environment (urban, pristine) versus benzene concentrations (smokers, nonsmokers, smokers' ambient air, nonsmokers' ambient air) was significant for benzene concentrations ($F = 12.5$, $p < 0.001$), and was significant for both benzene concentrations ($F = 23.1$, $p < 0.001$) and environment ($F = 14.2$, $p < 0.001$) when smokers were not included. This is because smokers had high benzene breath levels regardless of environment. There was no statistical interaction of benzene and environment (Table 4).

The data show that the concentration of benzene found in the breath of smokers is higher than that found in nonsmokers, and that the concentration for a smoker appears to be independent of environment. Smoking only marijuana adds benzene to the breath, just as with

TABLE 4. Analyses of Variance for Environment and Benzene

Design	Benzene			
	Smokers	Nonsmokers	Smokers' air	Nonsmokers' air
Urban	x	x	x	x
Pristine	x	x	x	x
Results				
Source	Degrees of freedom	Mean square	F	p ^a
I. Including smokers				
Environment	1	5.4	0.4	NS
Benzene	3	167.0	12.8	<0.001
Env × benzene	3	17.7	1.3	NS
Error	76	13.1		
II. Not including smokers				
Environment	1	5.2	14.2	<0.001
Benzene	1	8.5	23.1	<0.001
Env × benzene	1	0.01	0.38	NS
Error	35	0.37		

^a NS, nonsignificant.

any amount of cigarette smoking. Urban smokers averaged 40 ± 12 (SD) cigarettes per day; in the pristine study the average was 23.9 ± 8.4 . There was little correlation between benzene in breath and number of cigarettes smoked ($r = 0.3$ urban; 0.2 pristine). This may be due to differences in individual degree of smoker's inhalation, or to the interval allowed between smoking and taking the breath sample.

DISCUSSION

Although the prevailing flow of air from the ocean gives the San Francisco Bay area a unique climate, we found that atmospheric benzene levels in the city were typical of those reported (Brief et al., 1980; Hanson, 1978) for other urban areas (1–10 ppb). The lowest levels were observed near the coast, and the highest levels were observed inland near sources of heavy auto traffic. Atmospheric benzene may thus determine some of the amount of benzene that gets into the body. This was consistent with our findings that benzene levels in breath of non-smokers was higher in the urban environment than in the pristine environment.

Benzene in the breath of nonsmokers for both urban and pristine environments was higher than the respective ambient air concentrations, suggesting an additional source of benzene exposure other than environmental air. Other possible sources of benzene exposure include cigarette smoke, solvents (including gasoline), and diet (especially the number of eggs eaten). The pharmacokinetics of benzene have been described as a three-compartment model (Sato et al., 1974). It may be that benzene is accumulated in the body in the home or workplace, and the higher concentration seen is body elimination of this concentrated benzene. However, no correlation could be found between breath levels and exposure to any of these sources as indicated on the questionnaires filled out by each subject. Benzene in indoor air may be an important source of exposure. However, it is not known whether the magnitude of exposure to benzene from indoor air is sufficient to explain the elevation in breath levels we have observed in our subjects. Thus, we also suggest the possibility of an in vivo source of benzene production.

REFERENCES

- Brief, R. S., Lynch, J., Bernath, T., and Scala, R. A. 1980. Benzene in the workplace. *Am. Ind. Hyg. Assoc. J.* 41:616–623.
- Gruenke, L. D., Bier, D. M., and Craig, J. C. 1980. An improved selected ion recording system for precise ratio determination. *Biomed. Mass Spectrom.* 7:381–385.
- Gruenke, L. D., Craig, J. C., Wester, R. C., and Maibach, H. I. 1986. Quantitative analysis of benzene by selected ion monitoring gas chromatography-mass spectrometry. *J. Anal. Toxicol.*, in press.

- Hanson, D. A. 1978. Survey of benzene occurrence in ambient air at various locations throughout the United States. American Petroleum Institute Washington, D.C., Publication 4305.
- Lee, S. D., Dourson, M., Mukerjee, D., Stars, J. F., and Kawecki, J. 1983. Assessment of benzene health effects in ambient water. In *Advances in Modern Environmental Toxicology: Carcinogenicity and Toxicity of Benzene*. ed. M. A. Mehlman, vol. IV, pp. 91-125. Princeton, N.J.: Princeton Scientific.
- Lonneman, W. A., Bellar, T. A., and Altshuller, A. P. 1968. Aromatic hydrocarbons in the atmosphere of the Los Angeles basin. *Environ. Sci. Technol.* 2:1017-1020.
- Pilar, S., and Graydon, W. F. 1973. Benzene and toluene distribution in Toronto atmosphere. *Environ. Sci. Technol.* 7:628-631.
- Sato, A., Nakajima, T., Fujiwara, Y., and Hirosawa, K. 1974. Pharmacokinetics of benzene and toluene. *Int. Arch. Arbeitsmed.* 33:169-182.
- Snyder, R. 1974. The benzene problem in historical perspective. *Fundam. Appl. Toxicol.* 4:692-699.
- Tenz, C. 1978. Benzene—Attempts to establish a lower exposure standard in the United States. *Scand. J. Work Environ. Health* 4:103-113.

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