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Blood Concentrations of Volatile Organic Compounds in a Nonoccupationally Exposed US Population and in Groups with Suspected Exposure

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Exposure to certain volatile organic compounds (VOCs) commonly occurs in industrialized countries. We developed a method for measuring 32 VOCs in 10 mL of whole blood at low concentration. We used this method to determine the internal dose of these compounds in 600 or more people in the US who participated in the Third National Health and Nutrition Examination Survey. From our study results, we established a reference range for these VOCs in the general population of the US. We found detectable concentrations of 1,1,1-trichloroethane, 1,4-dichlorobenzene, 2-butanone, acetone, benzene, chloroform, ethylbenzene, *m,p*-xylene, styrene, tetrachloroethene, and toluene in most of the blood samples of nonoccupationally exposed persons. The accuracy of VOC evaluations depends on the ability of investigators to make sensitive and reproducible measurements of low concentrations of VOCs and to eliminate all sources of interference and contamination.

Indexing Terms: *toxicology/reference range/gas chromatography–mass spectrometry*

Tobacco smoke is a common source of volatile organic compounds (VOCs), but VOCs are also present in many synthetic products in daily use.[2] Building materials, home furnishings, clothing, and other consumer products contribute to increased VOC concentrations in people. Humans are exposed regularly to these compounds, but since the half-lives of the compounds in humans are very short (*1, 2*), they are generally considered not to bioaccumulate. Previous efforts to measure VOCs in humans have been limited by the lack of sensitivity of the methods used, by failure to adequately account for sources of contamination, or by a reference population that was too small. To measure the internal dose of VOCs from low-level exposures, we developed and applied an analytical method (*3*) for measuring VOCs in human blood in the low ppt range. Our first objective was to characterize reference ranges of VOCs in blood for nonoccupationally exposed persons by applying this method to 600 or more people in the US who participated in the Third National Health and Nutrition Examination Survey (NHANES III). Participants were selected on the basis of their age, race, gender, and region of residence, and each participant also completed a self-administered questionnaire regarding recent possible exposures. By comparing this reference range with the VOC concentrations of groups with suspected low levels of exposure, we were able to distinguish exposed persons from nonexposed persons.

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[2] Nonstandard abbreviations: VOCs, volatile organic compounds; NHANES IIII, Third National Health and Nutrition Examination Survey; and amu, atomic mass units.

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Materials and Methods

The method used for measuring VOCs in a large sample population has been described elsewhere (*3*) and only a summary of the key details is given here.

Apparatus. The purge and trap apparatus consisted of a Tekmar (Cincinnati, OH) LSC 2000 purge and trap concentrator with an attached ALS 2016 automated sampler. The helium flow rate was maintained at 30 mL/min at 20 psi. Chromatography was carried out with a Hewlett-Packard (Avondale, PA) Model 5890 gas chromatograph that was specially modified with a heated interface to allow effluent to pass into the mass spectrometer ion source. The chromatograph was equipped with a J & W (Folsom, CA) 30-m DB-624 column with a 1.8-μm film thickness. A 70E high-resolution mass spectrometer (VG Analytical, Manchester, UK) was used, operating at 3000 resolving power. Mass values were referenced to perfluorokerosene. The instrument was operated in full-scan mode [40–200 atomic mass units (amu)] with a scan rate of 1.0 s/decade and a settling time of 0.2 s.

Reagents. Unlabeled compounds were obtained from Aldrich Chemical Co. (Milwaukee, WI) or Burdick and Jackson (Muskegon, MI). Deuterium- or carbon-13-labeled analogs were obtained as the neat compounds from either Cambridge Isotope Lab. (Woburn, MA) or Merck, Sharpe, and Dohme/Isotopes (St. Louis, MO). Purge-and-trap-grade methanol, which was used for dilution of standards, was also obtained from Burdick and Jackson.

Procedures. Samples were collected as part of a regimen of health assessment and clinical measurements performed on people throughout the US; all sample collection and medical evaluation were performed in mobile vans used exclusively for this purpose. Before the study was begun, all cleaning solutions, pesticides, air fresheners, and similar materials were chosen on the basis of their chemical composition so as to exclude sources of VOC contamination. Study subjects typically spent 3.5–4 h inside the mobile examination vans. During this period, functional testing, allergy testing, spirometry, bone densitometry, and a complete physical examination were carried out, and questionnaires were administered. Finally, blood samples were drawn for various tests, including VOC measurement. Whole-blood samples were collected by venipuncture into Vacutainer Tubes (Becton Dickinson, Rutherford, NJ) containing a mixture of potassium oxalate and sodium fluoride. These tubes had been previously treated to remove VOC contaminants

and examined to verify adequate removal of these contaminants. Measurements were completed within 2 weeks of collection to minimize any changes in blood concentration that might occur during refrigerator storage. After an antifoaming agent was introduced, all sample spargers were decontaminated overnight by heating and helium purging. We introduced 10-mL blood samples into the sampling spargers after directly puncturing the Vacutainer caps with an airtight syringe and adding 20 μL of a stable isotope analog solution to the syringe contents. The blood samples were directly purged to remove the VOCs. Quantitation was achieved by monitoring specific ions and by using the isotope dilution technique. Analyte identities were verified by examining retention times and full-scan spectra and then comparing these times and spectra to those of standard compounds. Blanks, standards, and quality-control materials were included in each analytical run to ensure analytical stability, the maintenance of sensitivity, and the absence of contamination. Detection limits for the analytes reported in this study were generally in the low parts-per-trillion range (*3*).

Results

Table 1 presents summary results of the analysis of blood samples from NHANES III for 11 of the 32 VOCs examined. The analytes in this table were all found at detectable concentrations in 75% or more of the samples examined. Some analytes were measured in the ppb range or higher, but most had a mean concentration of 0.1–0.5 ppb. The medians in most cases were substantially lower than the means, indicating that the data were not normally distributed. The large differences between the means and medians for 1,4-dichlorobenzene and tetrachloroethene were caused by some samples with very high relative concentrations. For instance, half of the 1,4-dichlorobenzene results fell below 0.35 ppb, but some blood samples had concentrations as high as 12 ppb. The wide range of blood concentrations both with individual analytes and between different analytes illustrates the importance of wide analytical linearity in measuring blood VOCs.

Table 2 gives the percentage of blood samples found to have concentrations above the detection limits for those analytes that were detectable in 10%–75% of the samples analyzed. We also measured several other analytes, but found detectable concentrations in fewer than 10% of the samples examined. These analytes (with their detection limits in ppb) were 1,1,2,2-tetrachloroethane (0.008), 1,1,2-trichloroethane (0.016), 1,1-dichloroethane (0.009), 1,1-dichloroethene (0.018), 1,2-dichlorobenzene (0.044), 1,2-dichloroethane (0.012), 1,2-dichloropropane (0.008), 1,3-dichlorobenzene (0.019), bromoform (0.027), carbon tetrachloride (0.019), *cis*-1,2-dichloroethene (0.013), dibromomethane (0.044), hexachloroethane (0.079), methylene chloride (0.089), and *trans*-1,2-dichloroethene (0.014).

Because VOCs have very short half-lives in humans (*1, 2*), the presence of these compounds in the air people breathe immediately before samples are collected can have a significant impact on the concentration of VOCs in the blood. We tested this possible confounder by comparing blood VOC concentrations in a group of nonsmoking volunteers before they entered the NHANES III vans with their blood VOC concentrations after they had been in the vans for 3 h. The results of this investigation are given in Table 3. The last column in this table shows the probability that the mean of the difference between the paired measurements is significantly different from zero. Only for benzene are the results significant at the 0.05 level; the benzene concentrations were higher before people entered the van than they were afterward.

Table 4 shows the blood VOC concentrations of occupants of a building that had documented indoor air problems, including detectable concentrations of 1,1,1-trichloroethane, *m,p*-xylene, *o*-xylene, toluene, and trichloroethene. These increased air concentrations were reflected in increased blood concentrations of 1,1,1-trichloroethane and trichloroethene. Trichloroethene is not usually detected in the blood of people who are not occupationally exposed. By contrast, compared with the reference ranges given in Table 1, the concentrations of toluene, *m,p*-xylene, and *o*-xylene in the blood of the building's occupants were not increased.

Table 1. Blood concentrations of selected volatile organic compounds in a reference group of a nonoccupationally exposed US population.[a]

Analyte[b]	Detection limit, ppb	No. of samples	Mean, ppb	Median, ppb	5th percentile	95th percentile
1,1,1-Trichloroethane	0.086	574	0.34	0.13	ND	0.80
1,4-Dichlorobenzene	0.073	1037	1.9	0.33	ND	9.2
2-Butanone	0.50	1101	7.1	5.4	1.9	16.9
Acetone	200	1062	3100	1800	640	>6000
Benzene	0.030	883	0.13	0.061	ND	0.48
Ethylbenzene	0.020	631	0.11	0.060	ND	0.25
m,p-Xylene	0.033	649	0.37	0.19	0.074	0.78
o-Xylene	0.040	711	0.14	0.11	0.044	0.30
Styrene	0.019	657	0.074	0.041	ND	0.18
Tetrachloroethene	0.030	590	0.19	0.063	ND	0.62
Toluene	0.092	604	0.52	0.28	0.11	1.5

[a] Subset of NHANES III.
[b] The analytes were detectable in ≥75% of the samples examined.
ND, result below detection limit.

Table 2. Percentage of blood samples with concentrations above the detection limit for selected VOCs in a reference group of a nonoccupationally exposed US population.[a]

Analyte[b]	Detection limit, ppb	No. of samples	Percent above detection limit
Bromodichloromethane	0.009	1072	14
Chlorobenzene	0.007	1024	21
Chloroform	0.021	979	54
Dibromochloromethane	0.013	1035	12
Trichloroethene	0.010	677	13

[a] Subset of NHANES III.
[b] The analytes were detectable in 10–75% of the samples examined.

Table 4. Blood concentrations (ppb) of selected VOCs in occupants of a building with increased indoor air concentrations of some VOCs (marked*).

	Building occupants (n = 12)	
Analyte	Mean	Range (5–95%)
1,1,1-Trichloroethane*	1.1	0.52–4.0
1,4-Dichlorobenzene	1.1	ND–11
Benzene	0.068	ND–0.19
m,p-Xylene*	0.17	0.044–0.45
o-Xylene	0.11	ND–0.16
Styrene	0.061	ND–0.20
Tetrachloroethene	0.14	ND–0.46
Toluene*	0.53	0.17–1.2
Trichloroethene*	0.039	0.016–0.061

ND, result below detection limit.

Discussion

In this study we measured blood concentrations of VOCs in a large nonoccupationally exposed population, using an analytical method designed primarily to measure extremely low concentrations of VOCs. Researchers have previously attempted to establish a nonoccupationally exposed reference range of VOCs in blood, but these studies have been limited for various reasons.

In 1986, Antoine et al. (*4*) reported a method for measuring a wide range of VOCs in human blood by using gas chromatography–mass spectrometry. They examined the blood VOC concentrations of 250 people. For most analytes, the results of that study were significantly higher than those found in the present work; moreover, some of the analytes that Antoine et al. reported they had found at measurable concentrations in human blood were not detectable in our study. However, the concentrations that they reported are similar to the concentrations that we obtained in preliminary studies with unprocessed commercial Vacutainer Tubes. We measured significant concentrations of methylene chloride (dichloromethane) and bromoform in blood collected in unprocessed Vacutainer Tubes, but these analytes were no longer detectable after the tubes were decontaminated. The lack of Vacutainer cleanup is a likely cause of differences in VOC concentrations between the study of Antoine et al. and our study.

In 1986, Hajimiragha et al. (*5*) reported measuring blood concentrations of chloroform, 1,1,1-trichloroethane, trichloroethene, and tetrachloroethene in the blood of 39 normal subjects without known occupational exposure to VOCs. They were able to quantify these compounds in 60%–95% of the subjects examined, a percentage in good agreement with our results, even though their detection limits were generally an order of magnitude higher than ours. The mean blood concentrations they found were higher than those we found and may have resulted from differences in the sample collection procedure. Theirs was a limited sample population that did not provide enough information to establish a reference range.

In a series of studies (*6–9*), Brugnone et al. examined the concentration of benzene, and in some cases of toluene and styrene, in a nonoccupationally exposed population. Results of their latest study (*9*) showed that the mean blood benzene concentration of 431 subjects was 0.262 ppb. This result is double the mean concentration found in our study. The mean blood concentrations of styrene and toluene reported by these researchers are

Table 3. Comparison of blood concentrations (ppb) of selected VOCs in study subjects before they entered examination vans and after 3 h in the vans.

	Before entry (n = 9)		After 3 h (n = 9)		
Analyte	Mean	Range	Mean	Range	Prob >|T|[a]
1,1,1-Trichloroethane (n = 7)	0.083	ND–0.17	0.12	ND–0.16	0.30
1,4-Dichlorobenzene	1.0	ND–6.2	0.85	ND–4.6	0.36
2-Butanone	4.5	1.9–6.6	4.8	3.1–7.6	0.61
Acetone	1900	1000–3600	2000	930–5000	0.71
Benzene (n = 7)	0.046	ND–0.061	0.033	ND–0.055	0.02
Ethylbenzene	0.036	ND–0.058	0.042	ND–0.058	0.46
m,p-Xylene	0.092	0.044–0.18	0.11	0.065–0.14	0.38
o-Xylene	0.061	ND–0.072	0.072	ND–0.12	0.37
Styrene	ND	ND–0.027	0.020	ND–0.036	0.30
Tetrachloroethene	0.41	0.045–2.5	0.35	0.061–1.7	0.55
Toluene	0.25	0.14–0.40	0.20	0.12–0.31	0.06

[a] Probability that the mean of the differences between the pairs = 0.
ND, result below detection limit.

also substantially higher than those determined in our study. It is not clear whether these discrepancies are due to differences in populations examined, sample collection procedures, or some unknown effects.

Angerer et al. (*10*) measured benzene concentrations in the blood of eight nonsmokers and two smokers by using dynamic head space chromatography with flame ionization detection. They gave particular attention to preventing contamination and reported mean blood benzene concentrations in these small populations of 0.176 ppb for nonsmokers and 0.211 ppb for smokers. These results agree well with the mean of 0.13 ppb found in the population we studied, especially considering the differences between the two populations.

The results given in Table 3 confirm the effectiveness of the precautions that we took to prevent results from being biased by exposing study subjects to the microenvironment of the van. Benzene was the only analyte for which we found a significant difference between samples taken before and after people entered the van; that difference showed lower blood benzene concentrations in the samples taken after people had been in the van for 3 h. In this investigation, the volunteers were transported by automobile to the study site before the initial sampling was performed. Their exposure to benzene from automobile exhaust during this step could have transiently raised their blood benzene concentrations, thus accounting for the differences seen here. Further investigation is required to test this hypothesis.

The results of the exposure investigation reported in Table 4 illustrate the use of the VOC reference range in investigating low concentration exposure. In this study of indoor air exposure, only some of the compounds that were present at measurable concentrations in the air were also found at increased concentrations in the blood of study participants. Differences in the nature of the exposure and in typical background exposure to these compounds account for the dissimilarity in individual VOC blood concentrations. In this case, the air concentrations of 1,1,1-trichloroethane and trichloroethene varied widely between sampling sites within the building, indicating the presence of specific point sources of these compounds. Thus, exposure of these building occupants to 1,1,1-trichloroethane and trichloroethene was atypical of indoor air exposure and resulted in blood concentrations higher than those seen in the reference US population. In contrast, air concentrations of toluene, *m,p*-xylene, and *o*-xylene did not show any spatial differences. This finding suggests that there was a more widespread background exposure for toluene, *m,p*-xylene, and *o*-xylene, which are all commonly found in commercial products. Thus, regular exposure to these compounds, through the air results in residual blood concentrations. Only in cases in which the environmental exposure is substantially greater than typical background exposure can detectable changes be identified and related to exposure.

In a separate study (Etzel RA, manuscript in preparation) we report measurements of blood concentrations of VOCs from persons residing in Kuwait during the oil fire crisis in 1991. Separate studies were performed on residents of Kuwait City and on firefighters. The concentrations of some of these analytes (1,1,1-trichloroethane, 1,4-dichlorobenzene, 2-butanone, and acetone) were not significantly different from the reference ranges given in Table 1 for either study group. The blood VOC concentrations for the remaining analytes listed in this table were all significantly increased in the firefighter group. Taken as a group, Kuwait City residents did not have significantly increased blood concentrations of VOCs, but one person had blood ethylbenzene and *o*-xylene concentrations that were higher than the expected ranges.

In summary, by using an analytical method specifically designed to measure background concentrations of VOCs in blood, we have been able to determine a reference range for 11 of these compounds in a nonoccupationally exposed US population. We then applied these results to cases of exposure to VOCs, thus illustrating their usefulness in identifying exposure. Careful attention to sensitivity and prevention of contamination are required to identify low-level VOC exposure.

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