

Measurement of Volatile Organic Compounds in Human Blood

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Volatile organic compounds (VOCs) are an important public health problem throughout the developed world. Many important questions remain to be addressed in assessing exposure to these compounds. Because they are ubiquitous and highly volatile, special techniques must be applied in the analytical determination of VOCs. The analytical methodology chosen to measure toxicants in biological materials must be well validated and carefully carried out; poor quality assurance can lead to invalid results that can have a direct bearing on treating exposed persons. The pharmacokinetics of VOCs show that most of the internal dose of these compounds is quickly eliminated, but there is a fraction that is only slowly removed, and these compounds may bioaccumulate. VOCs are found in the general population at the high parts-per-trillion range, but some people with much higher levels have apparently been exposed to VOC sources away from the workplace. Smoking is the most significant confounder to internal dose levels of VOCs and must be considered when evaluating suspected cases of exposure. — *Environ Health Perspect* 104(Suppl 5):871–877 (1996)

Key words: volatile organic compounds, blood, methods, pharmacokinetics, reference range, smoking

Introduction

The field of risk assessment is becoming better defined as researchers continue to investigate the sources of exposure to toxic chemicals and their health effects. Risk assessment includes four components: hazard identification (determining which hazards are present), dose–response assessment (determining how biological systems respond to contaminants), exposure assessment (determining the extent of exposure), and risk characterization (determining whether this exposure constitutes a threat to the health of exposed populations) (1). Trace analytical techniques are essential in assessing exposure, internal dose (the level of a particular agent within the body), and

biologically effective dose (the level of an active agent at the sight of action). Internal dose measurements of toxicants, their metabolites, or protein and DNA adducts provide an assessment of exposure that can be related to resulting health outcomes and thus provide important information upon which risk assessment is based.

Volatile organic compounds (VOCs) are a class of chemicals that are commonly encountered by people as they go about their daily routines. Exposure to chloroform and other chlorinated hydrocarbons occurs when people shower or bathe with chlorinated water (2). Air levels of many VOCs are increased when air fresheners or other consumer products are used (3). Smoking causes an increase in blood benzene and toluene levels (4). Trihalomethane levels in blood are increased in swimmers and bath attendants swimming or working in indoor swimming pools (5). Tetrachloroethene is found in the blood of workers in dry-cleaning establishments and in the blood of the people living directly above these businesses (6). Commuters are exposed to methyl-*tert*-butyl ether when this compound is used as a gasoline oxygenate (7). Thus, people can be exposed to VOCs through avenues other than what

are usually considered traditional sources of exposure (occupation, water pollution, ambient air pollution, industrial accidents).

Exposure to VOCs is associated with a wide variety of symptoms, but most of the acute health effects associated with these substances are the result of occupational exposure, controlled laboratory experiments on animals and people, or substance abuse and not of low-level exposure. The symptoms of VOC exposure range from slight respiratory irritation to death. For example, upper respiratory irritation has occurred in workers exposed to styrene (8). Liver damage has been manifested as toxic jaundice among factory workers exposed to chloroform (9), and cases of carbon tetrachloride hepatotoxicity have been reported in humans (10). Central nervous system depression has been associated with exposure to carbon tetrachloride (11), methylene chloride (12), tetrachloroethene (13), and toluene (14). National agencies, including the U.S. Environmental Protection Agency, the U.S. Occupational Safety and Health Administration, and international agencies such as the World Health Organization and the International Agency for Research on Cancer have declared benzene to be a human carcinogen. Kidney damage has resulted from exposure to carbon tetrachloride (15). VOCs have been reported as the likely cause of death in cases of intentional inhalation of 1,1,1-trichloroethane (16) and exposure to carbon tetrachloride (15). Because of these documented health effects and others, there is ample reason for concern about the toxicity of VOCs in spite of the lack of understanding about morbidity and mortality resulting from low-level exposure.

Method Considerations

Measuring low levels of VOCs in human biological media accurately and reproducibly presents a complex analytical problem that requires special techniques and exceptional care. Volatile organic compounds by definition are highly evaporative, and analytes can easily be lost while samples or standards are being manipulated. Loss of analytes from the unknown samples is of particular concern, since there are no analytical checks that will indicate this loss, and inaccurate results can lead to normal blood levels being reported when the internal dose is actually elevated. On the other hand, because many VOCs are commonly found in various consumer products and are popular laboratory solvents, it is reasonable

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Abbreviations used: VOCs, volatile organic compounds; NIOSH, National Institute for Occupational Safety and Health; NHANES III, Third National Health and Nutrition Examination Survey; amu, atomic mass unit.

that contamination might occur during sample collection or analysis. Such contamination would have the opposite effect of volatility by causing normal blood levels of VOCs to be measured as elevated. Blood is a highly complex matrix with numerous compounds present that are separated along with VOCs when purge-and-trap or headspace sampling is used. These additional compounds can interfere with the analytical response of the VOCs of interest and prevent accurate characterization. A proper quality-assurance program considers all aspects of the analysis process from sample collection to data reporting and minimizes and/or characterizes all possible sources of error in measurement. A complete discussion of each of these aspects is beyond the scope of this review, but some of the more common sources of error in VOC measurement and ways of reducing or eliminating their effects can be described.

Sample collection can be a major source of error in measurements of VOCs in blood. A VOC sample collection system should only use materials that have been proven to not introduce VOCs into blood samples and should not allow the blood samples to be exposed to the atmosphere. Because isopropyl alcohol or other cleaning materials can be introduced into the collection needle when a vein is punctured, these substances should not be used, or their volatile components should be removed before a sample is collected. Samples have been collected into either commercial vacutainers or glass syringes. Brugnone et al. (17) and Angerer et al. (18) have collected blood into glass syringes and then introduced this sample into glass tubes containing EDTA. This procedure allows for careful cleaning of the sample collection materials to remove contamination, but it does necessitate the use of an additional sample manipulation step. Gill et al. (19) have shown that significant VOC loss can occur with sample handling, and thus sample manipulation steps should be minimized. DeLeon and Antoine (20) collected blood into unprocessed commercial vacutainers, but the baseline VOC levels they reported for 198 specimens showed a pattern of blood VOC concentrations that was similar to the later reported pattern of contamination from the vacutainers themselves (21). Ashley et al. (22) collected blood samples in commercial vacutainers that had been processed to remove VOC contaminants. This method simplifies the sample collection step while preventing contamination. Ashley et al. collected

blood into vacutainers containing sodium fluoride and potassium oxalate. These chemicals were previously described as important in VOC measurement because they inhibit esterase activity (23).

Figure 1 shows the levels of bromoform, *m*-*p*-xylene, and 1,4-dichlorobenzene found in blood collected in processed and unprocessed vacutainers along with detection limits and the typical blood levels in subjects with no known occupational exposure. The measurements of these three compounds are variably affected by vacutainer decontamination. Bromoform is found in the normal population at concentrations less than the detection limit of 20 ppt, and processed vacutainers do not add significant contamination. Bromoform levels in blood samples collected in unprocessed vacutainers, however, are significantly higher and increase as samples are stored for up to 1 week. Blood levels of *m*-*p*-xylene are typically higher than the analytical detection limit and greater than background vacutainer levels determined by measuring the concentration of blank water added to processed vacutainers (less than the detection limit). As with bromoform, processed vacutainers do not contribute any measurable contamination of *m*-*p*-xylene, and unprocessed vacutainers contribute a substantial contamination that increases upon storage. The mean blood level of 1,4-dichlorobenzene is well above the detection limit of 13 ppt and the background vacutainer level, but the concentrations of this compound in samples taken in processed vacutainers are the same as concentrations in samples in unprocessed vacutainers. This indicates that, under normal conditions, vacutainers do not contribute significantly to the blood levels of 1,4-dichlorobenzene, as unprocessed vacutainers do to the blood levels of bromoform and *m*-*p*-xylene. These results demonstrate that for some VOCs, decontaminating the sample collection materials is critical if blood concentrations are to be determined accurately.

Angerer et al. (18) suggested that samples can be frozen at liquid nitrogen temperatures, but in most cases, samples have been stored at 4°C (17,22,24). Freezing the samples will lyse the red blood cells and may change the equilibrium within the matrix. Wang et al. (25) stated that samples can be successfully stored at 4°C for up to 40 days, and Ashley et al. (26) confirmed this by demonstrating that whole blood samples can be stored at 4°C in decontaminated vacutainers for at least 50 days.

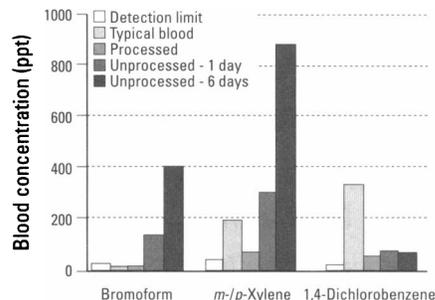


Figure 1. Detection limits, typical blood levels, and measured blood levels of bromoform, *m*-*p*-xylene, and 1,4-dichlorobenzene collected in processed and unprocessed vacutainers. Separate results are given for blood collected in unprocessed vacutainers and stored for either 1 or 6 days.

Both headspace analysis and purge-and-trap techniques have been used to remove the volatile constituents from blood. In contrast to headspace analysis, the purge-and-trap method is a dynamic process that is capable of removing a higher percentage of the VOCs into the gas stream, where they are available for later analysis. The use of an antifoam agent is critical in purge-and-trap analysis of VOCs, and the antifoam must be present at a level high enough to prevent blood from foaming. Cailleux et al. (27), however, described the contamination that resulted from their use of antifoam, and Michael et al. (28) suggested that the contamination resulting from the use of antifoaming agents makes purge-and-trap analysis of blood VOCs impractical. Erickson et al. (29) helped solve this problem by showing that by heating antifoam agents under vacuum, the volatile contaminants could be removed, enabling the subsequent incorporation of purge-and-trap techniques into blood VOC analysis (22).

The choice of analytical instrumentation is based on three variables: sensitivity, selectivity, and cost. Depending on the particular application, these variables carry different weights in the decision-making process. For recent measurements of VOCs in blood from subjects with low-level exposure, three analytical instruments have been used. All of these instruments include the use of gas chromatography for analyte separation, but they use different detection systems. These detection systems are, in order of increasing cost per sample, flame ionization detection (18), low-resolution mass spectrometry (4,24), and magnetic-sector mass spectrometry (22). These systems have detection limits typically in

the low parts-per-trillion range that are necessary to measure blood levels resulting from low-level exposure, but they differ in analyte selectivity. As shown by Angerer et al. (18), many volatile compounds are present in blood. Most of these are not of interest in a particular application, but they can interfere with the analytes that are of interest. In some cases, gas chromatography temperature control can remove these interferences, but when a large number of VOCs are being measured, these interferences can be prohibitive. Low-resolution mass spectrometry improves this problem significantly because choosing a particular mass of interest can remove most interferences. For some analytes, interferences are still present, and either a nonoptimal mass must be chosen for quantitation or a higher-resolution mass spectrometric detection method must be used (30). The differences between these techniques are shown in Figure 2, which includes the retention time region in which chloroform is found at scan 606. Figure 2A is a total ion chromatogram trace (no mass discrimination) and shows that the complexity of the trace prevents the quantitation of this analyte. In Figure 2B, a nominal mass window is applied, which eliminates some of the complexity, but chloroform is still a shoulder on another interfering peak. Figure 2C shows a medium-resolution mass window applied to these same data. The improvement in selectivity due to higher resolution is shown by complete separation from interfering peaks. Thus, when using purge-and-trap methods and gas chromatography, researchers can accurately quantitate chloroform in blood only with accurate-mass mass spectrometry. Using medium-resolution mass spectrometry as a detector for gas chromatography has enabled researchers to simultaneously measure 32 VOCs at the low parts-per-trillion level in 10 ml of human blood (22).

Pharmacokinetics

Exposure is not a static process. When an individual contacts a contaminant, his or her internal dose levels change with time depending on many physical, chemical, and metabolic processes. Pharmacokinetics is the field of investigation that describes these processes, and studies in this field can range from the simple measuring of a contaminant's half-life in the body to complex modeling of concentration changes in multiple organ systems during uptake and elimination. Understanding the pharmacokinetics

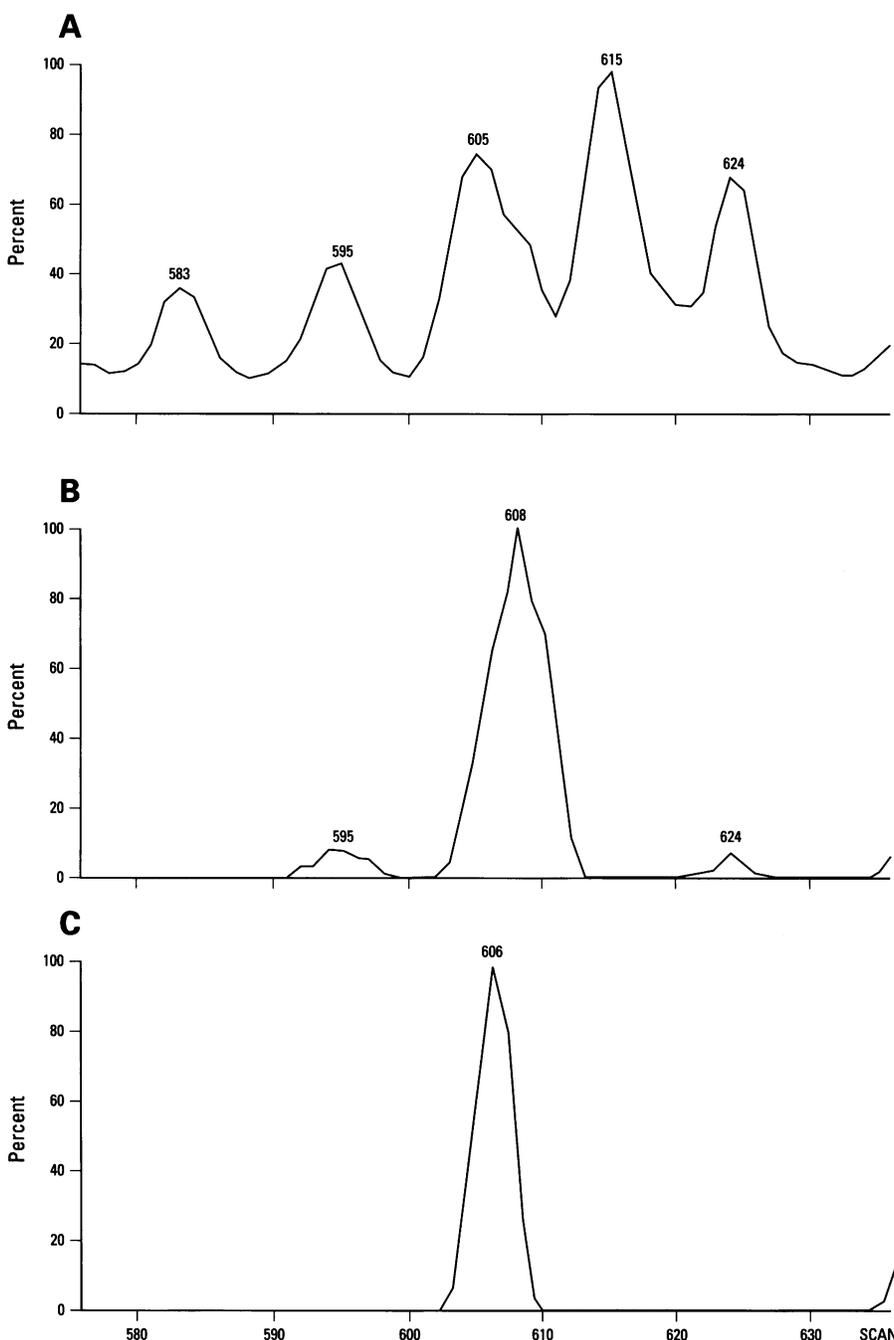


Figure 2. Gas chromatographic traces of blood examined by using purge-and-trap isolation and mass spectrometric detection with varying mass windows: (A) total ion chromatogram (no mass differentiation); (B) nominal mass (1 amu); and (C) accurate mass (0.03 amu).

of VOCs is critical in relating internal dose levels to exposure and in understanding the dynamics of distributed dose that can lead to health effects. Studies of the pharmacokinetics of VOCs have been carried out for many years, and even though these processes are far from completely understood, some

parts of the processes have been described by investigators.

For example, the rate that VOCs enter and leave the body through the lungs is a function of the partitioning of the individual compounds between lipid and aqueous sites in the body. The compounds with a

greater lipid solubility will deposit in fat and be eliminated more slowly than those compounds that are lipophobic. Vapor pressure can also influence the pharmacokinetics of these compounds because the lungs are an important route of elimination. Researchers evaluating the kinetics of internal dose levels of VOCs have found that uptake and elimination follow similar patterns. Astrand (31) has shown that during the exposure phase, blood levels of VOCs increase rapidly and then level off. Monster et al. (32) found this same effect during the elimination phase of tetrachloroethene (half-life 12–55 hr), during which the levels of tetrachloroethene dropped rapidly at first and then much more slowly. A similar result has been estimated for tetrachloroethene levels in breath (33). Brugnone et al. have determined shorter half-lives of 4.5 hr for toluene (34), 8 hr for benzene (35), and 3.9 hr for styrene (36). These disparities in the half-lives of VOCs may be a function of different lipid/water partition coefficients for the substances or of variations in exposure scenarios. Overall, the data consistently indicate that the extent and length of exposure will affect the elimination kinetics because a longer, more intense or repeated exposure will allow the compounds to more readily deposit into adipose tissue.

The uptake and elimination of VOCs from the body is controlled by a series of dynamic mechanisms controlling the movement of compounds through various body stores and metabolizing these compounds into more water-soluble entities. The elimination phase of VOCs after exposure gives insight into the various body stores from which VOCs are removed. Investigators have shown that the VOC elimination phase is a multiexponential process. The results of some of these investigations are shown in Table 1, where the results have been organized in an attempt to reconcile the reported half-lives. It is clear from these reports that at least two exponentials with half-lives of 10 to 60 min and 2 to 4 hr are required to successfully describe the elimination of benzene and toluene. Two other exponential components have also been reported, one with a very short half-life of 1.6 min and the other with a very long half-life of 20 to 90 hr. Measuring these last two exponential components requires that the sample collection be specifically designed to observe them. Because of the extreme requirements for measuring these exponential components, samples in some studies

Table 1. Half-lives of benzene and toluene internal dose with multiexponential regression fits.

Compound	Exponentials	First half-life (min)	Second half-life (min)	Third half-life (min)	Fourth half-life (hr)	Reference
Benzene	2			156	24	(37)
Benzene	3	1.6	30	222		(38)
Benzene	3		55	192	19.7	(39)
Benzene (breath)	2		28	248		(40)
Toluene	3		9	120	90	(41)
Toluene (breath)	2		19	124		(40)

may not have been collected at the times necessary to detect the shortest and longest exponential components. It is also quite conceivable that the shortest and longest exponential components may result from anomalies in the data.

Experimentally determining multiexponential elimination of VOCs after short-term exposure has suggested that the different exponential components derive from different body stores (41,42). Quick elimination from the blood, an intermediate half-life in muscle, and a much longer half-life in adipose tissue have been suggested as a means of explaining the various exponential components. Thus, the lipid solubility of the compound of interest and the length of exposure time will be important factors in the fraction of deposition that occurs in the separate sites.

Accumulation of chemicals in the body occurs whenever uptake exceeds elimination. The studies done to determine VOC pharmacokinetics also suggest that with repeat exposure of long enough duration, bioaccumulation may occur. Some measurements have been performed on workers repeatedly exposed to VOCs over a matter of weeks. Berlin et al. (37) exposed volunteers to low levels of benzene over 5 days for 6 hr/day. These workers showed accumulation during the exposure period and continued to release benzene for more than a week after the exposure ended. Brugnone et al. (36) found bioaccumulation of styrene in workers exposed repeatedly over a week. Nise and Orbeck (43) found this same result in workers who were repeatedly exposed to toluene. Preshift levels of these VOCs in workers increased during the week they were exposed because their internal dose levels had not returned to baseline between exposures. Bioaccumulation in VOC exposure is important because most exposures to these compounds occur repeatedly and are usually not one-time events. Thus, although short-term exposure experiments give insight into the pharmacokinetics of VOCs, they

are of limited value in most exposure scenarios. In repeat exposure cases, the exponential component with the longest half-life will have the greatest influence on internal dose levels, and in many cases bioaccumulation can occur. The extent of bioaccumulation will depend on the level of exposure, the length of time during which exposure occurs, and the time period between exposure events.

Reference Range

Studies have been performed to determine the background blood concentrations of VOCs in individuals with no known occupational exposure. A summary of the studies that have examined more than 50 subjects is given in Table 2. Significant background levels of six nonchlorinated aromatic hydrocarbons, two chlorinated aliphatic compounds, and one chlorinated aromatic compound have been found. The levels are found generally in the parts-per-trillion to low parts-per-billion range, with styrene and ethylbenzene at the lowest levels and 1,4-dichlorobenzene at the highest. These compounds have common household applications, so it is not surprising that measurable levels are found in people without any known occupational exposure to VOCs. Their presence in gasoline and tobacco smoke and their use as deodorizing, degreasing, and dry-cleaning agents present many opportunities for people to be exposed to them.

Reference ranges for VOCs have been determined by two research groups, one located in Italy and the other in the United States. For all analytes that have been evaluated by both groups, the levels reported in the United States (44) are lower than the levels reported in Italy (17,25,35,36). This may result from differences in sample collection procedures, analytical methodology, or the exposure of the populations examined. Samples from the United States were collected into specially prepared vacutainers with sodium fluoride and potassium oxalate as the anticoagulant and then

Table 2. Background reference range levels of VOCs in blood from subjects with no known occupational exposure.^a

Compound	Subject group	No. of subjects	Mean level (ppt)	Reference
1,1,1-Trichloroethane	All	574	340	(44)
1,4-Dichlorobenzene	All	1037	1900	(44)
Benzene	Nonsmokers	293	200	(35)
Benzene	Smokers	138	380	(35)
Benzene	All	883	130	(44)
Ethylbenzene	All	631	110	(44)
<i>m</i> - <i>p</i> -Xylene	All	649	370	(44)
<i>o</i> -Xylene	All	711	140	(44)
Styrene	All	81	220	(36)
Styrene	All	657	74	(44)
Tetrachloroethene	All	590	190	(36)
Toluene	All	269	1100	(17)
Toluene	Nonsmokers	179	810	(25)
Toluene	Smokers	53	900	(25)
Toluene	All	604	520	(44)

^aStudies with more than 50 subjects.

stored at 4°C. Samples from Italy were collected into a heparinized syringe and injected into a glass vial before being stored at 5°C. Differences in background levels may result from low-level contamination of the anticoagulant or differences in the methods of sample handling. The analytical methodology was similar for the two methods used except that nominal-mass mass spectrometry without isotope dilution was used in studies done in Italy. Our experiments suggest that there is no interference when nominal-mass mass spectrometry is used to measure benzene, toluene, or styrene in human blood, but differences in sample workup procedures may alter this finding. The subjects of the study in the United States were a subset of those participating in the Third National Health and Nutrition Examination Survey (NHANES III), an investigation of individuals across the United States. Although the VOC measurements were not probability-based to predict a larger population, subjects came from urban and rural environments, all races, both sexes, and all regions of the country. The subjects in the Italian studies were police officers, white-collar workers, blue-collar workers, and chemical workers (25,35) or hospital staff and blood donors (36). Thus, these populations may have different background exposures to VOCs.

The distribution of detectable 1,4-dichlorobenzene levels in the blood of NHANES III participants is shown in Figure 3. Of the members of this population, 96% had blood levels that were above the detection limit of 73 ppt (ng/l), and 75% of those with detectable concentrations had blood levels of 1000 ppt or less. The remaining 25% had significantly

higher levels, up to as high as 50,000 ppt. Thus, most people in the United States have 1,4-dichlorobenzene blood levels less than 1 ppb, although some with no known occupational exposure have significantly elevated blood levels. Some individuals with elevated levels were found in the NHANES III population for all of the compounds listed in Table 2, confirming the wide extent of exposure to VOCs apart from occupational sources.

Smoking

The blood levels of benzene and toluene reported by Brugnone et al. (35) and Wang et al. (25) are given in Table 2, which lists separate blood levels for smokers and nonsmokers. Studies by many researchers of both blood and breath have shown that internal dose levels of certain VOCs are significantly different in smokers than in nonsmokers. Levels of benzene and toluene have also been determined in breath by Wallace et al. (45) and in blood by Ashley et al. (in preparation). These two studies differ from the work by Brugnone

et al. (35) and Wang et al. (25) in that they also report elevations in internal dose levels of styrene, ethylbenzene, *m*-*p*-xylene, and *o*-xylene in smokers and thus indicate that smoking is an important source of exposure to aromatic VOCs. Wallace et al. (45) have even suggested that smoking is the most significant source of exposure to benzene. Passive exposure to tobacco smoke has not yet been shown to have an effect on blood VOC concentrations, but such an effect may also be significant.

To accurately evaluate the environmental effects on internal dose levels of VOCs, one must account for smoking as a confounding factor. In some cases, low-level exposure to benzene, toluene, or styrene might not be detected because the effect of smoking may confound the analysis designed to detect elevations in blood levels. Therefore, steps must be taken to either remove smokers from the subject population or correct for the confounding effect of smoking on VOC levels. Ashley et al. (in preparation) have suggested using 2,5-dimethylfuran as a marker for smoking and a method for adjusting the data collected from smokers to eliminate the contribution of smoking to blood VOC levels. This technique has yet to be completely assessed, but it shows that identifying a volatile compound that is unique to a particular activity can be a useful technique in separating the effect of environmental exposure from the effect of exposure to other known sources of that compound.

Conclusions

Investigators have recently performed studies to understand the link between exposure to VOCs and health effects. Methods have been developed that can accurately and precisely measure these compounds in blood through the use of techniques that are becoming more commonly

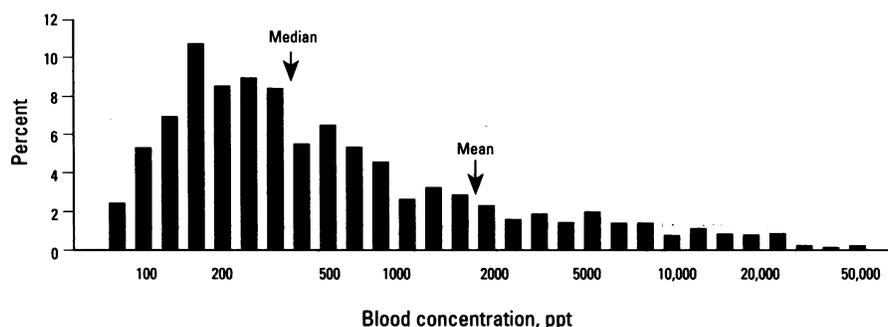


Figure 3. Frequency distribution (percent of total) of the concentration of 1,4-dichlorobenzene in blood. Subjects are a subset of the population enrolled in the Third National Health and Nutrition Examination Survey. The blood concentrations are plotted using a log scale.

used throughout the analytical community. Care must be exercised when using these methods, because contamination and loss of analyte are still significant concerns, but these methods are feasible and have been performed by various research groups. The levels of VOCs in the body change rapidly upon exposure and following cessation of exposure. Internal dose levels of most VOCs decrease rapidly after exposure ceases, with most having a half-life of a few

hours, but the actual decrease depends on the exposure scenario. A fraction of a particular VOC may have a longer half-life that can result in bioaccumulation with repeated exposure. The pharmacokinetics of VOCs in the body must be taken into account when evaluating possible exposures. Reference range studies indicate that most VOCs occur in the parts-per-trillion range in the blood of people with no known occupational exposure, but there are some

individuals with significant exposure even among this group. The largest confounding factor in evaluating exposure to VOCs is whether a person smokes. Smoking can lead to elevated levels of many aromatic VOCs and can obscure the effects of environmental exposure to these compounds. The effects of smoking on VOC levels must always be taken into account whenever exposure evaluations are performed.

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