Personal Reflections on 50 Years of Study of Benzene Toxicology

Dennis V. Parke

School of Biological Sciences, University of Surrey, Guildford, Surrey, England

Abstract

The metabolism of benzene is reviewed, and the objectives of a quantitative balance study begun in 1945 are outlined; problems of toxicology and metabolism research of some 50 years ago are considered. The quantitative metabolism of ^{14}C-benzene in the rabbit is annotated and compared with that of unlabeled benzene quantified by nonisotopic methods. The anomalies of phenylmercapturic acid and trans-trans-muconic acid as metabolites of benzene are examined in detail by isotopic and nonisotopic methods; these compounds are true but minor metabolites of benzene. Oxygen radicals are involved in both the metabolism of benzene and its toxicity; the roles of CYP2E1, the redox cycling of quinone metabolites, glutathione oxidation, and oxidative stress in the unique radiomimetic, hematopoietic toxicity of benzene are discussed. Differences between the toxicity of benzene and the halobenzenes are related to fundamental differences in their electronic structures and to the consequent pathways of metabolic activation and detoxication.
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Key words: benzene, myelotoxicity, oxygen radicals, CYP2E1, metabolism, historical perspectives

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Address correspondence to Dr. D. V. Parke, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, England. Telephone: 01483-300800. Fax: 01483-576978/300374.

Abbreviations used: GM, Geiger-Muller; GSH, glutathione; **ROS,** reactive oxygen species; UV, ultraviolet.

Introduction

My first insight into benzene toxicity came in the autumn of 1939 when, as a young first-year medical student at University College, London, I was sent to work on a research project at Glaxo Laboratories, Greenford, U.K. Glaxo had been given the wartime task of manufacturing penicillin and a host of other drugs and vitamins needed for the war effort; in return they had been given the services of numerous science graduates who otherwise would have been conscripted into the armed services. In those days of World War 11, full-time university students were exempt fkom conscriptionz but were encouraged to undertake part-time war work in addition to their studies; hence my casual employment at Glaxo, assisting the works' medical officer to ascertain why so many of the research workers were showing clinical signs of scurvy. Ascorbate excretion and loading studies confirmed the vitamin C deficiency; and it quickly became apparent that this was associated with high exposure to benzene, which was the major solvent used in the various production lines. Open vats containing 100 litres or more of benzene solutions were moved around on trolleys. Often the solvent was spilled, so the concrete floors were awash with benzene and the workers' clothes were soaked in the solvent. In addition, benzene was continuously being distilled in open systems to recover the solvent for further use, and serious conflagrations were all too frequent. Strangely, although it was well documented at that time that

exposure to benzene was associated with aplastic anemia, none of the chemists employed considered that there was any health hazard in their negligent use of this solvent.

When the exposure problems were brought out, the Glaxo management acted most expeditiously. Henceforth, benzene solutions were contained in closed systems, all exposed workers were given orange juice and vitamin-reinforced milk drinks twice a day; blood counts and urinary ascorbate excretions were taken weekly; and, most important of all, alcohol, acetone, and other solvents were used as alternatives to benzene wherever possible. This was probably one of the earliest uses of positive measures in industrial hygiene in the chemical industry, occurring as it did more than 55 years ago. The study of oxygen radicals and oxidative stress were very much in vogue at the time, and the role of ascorbic acid as an antioxidant was well recognized. Furthermore, the radiomimetic character of benzene toxicity indicated that, as with ionizing radiation, oxygen radicals or reactive oxygen species (ROS) were ultimately involved, and that vitamin C was probably a vital component of the biological defense against ROS and benzene toxicity.

Nearly 10 years later, after graduating in medicine and chemistry, and spending 4 years in the armed services, I made my second contact with benzene, this time as a research student with the late R.T. Williams at St. Mary's Hospital Medical School, London. Williams agreed that I should study all the known pathways of benzene metabolism, quantitatively and simultaneously, so as to obtain a balance-sheet of the metabolic fate of the chemical. Benzene was chosen, not only because of its known toxicity, but also because this was to be the parent compound and a model for more extensive programs of metabolism of a variety of aromatic chemicals. Furthermore, since benzene was known to be a radiomimetic toxin, it was considered that knowledge of its metabolism and mechanism of toxicity might reveal information concerning the mechanism(s) of radiation toxicity, a subject of great interest at that time, only 3 years after Hiroshima. Our research objectives included the following: a) to determine if benzene forms a mercapturic acid; b) to learn if benzene yields cis-cis and cis-trans isomers of muconic acid; c) to discover if benzene forms an epoxide or a dihydrodiol; d) to draw up a balance sheet for benzene metabolism; e) to synthesize ^{14}C-benzene and confirm the metabolism balance sheet; f) to determine if ^{14}C-benzene completely oxidized to $^{14}CO_2$; and g) to learn if its radiomimetic toxicity makes benzene a suitable model compound for studying biological radiation damage.

Initial Problems and Deficiencies

It is interesting today to reflect on how we financed this research program, since in the late 1940s almost no research grants were available on either side of the Atlantic. Consequently, there were almost no research assistants and all academic staff, including clinicians doing research, worked at the bench and fed and cleaned their own experimental animals. The work week was around 100 hr, with few free weekends and only 2 weeks vacation a year; little wonder that very few academics chose to undertake research. Money for animals and supplies came largely from commercial ventures, such as the marketing of 1%-labeled chemicals synthesized by us but surplus to our requirements, marketing of 100% pure chemicals made "in house," e.g., cysteine, phenylglucuronide, and phenyl sulphate, and initially--while the cost was high--the recovery of penicillin from patients' urine and purifying this for reuse. Instruments, such as an ultraviolet (UV) spectrophotometer, a scintillation spectrometer for quantification of radioactive isotopes, and chromatography equipment, were not commercially available and had to be designed and fabricated in our own laboratory; in several instances this resulted in the establishment of new scientific instrument companies and a diversity of new products. Thus, these endeavors to meet research needs by pioneering scientific innovation resulted in successful commercial exploitation and development of several new industries.

A general problem that concerned us from the outset of this research was how to achieve and quantify chemical purity. The only methods then available were fractional recrystallization and distillation, and column chromatography on silica gel, alumina, or partially activated charcoal. Few purified solvents or chemical reagents were available commercially; indeed, as subsequent work showed, most commercially available chemicals were very crude mixtures. Benzene was purified by shaking with sulfuric acid to remove thiophene, then fractionally distilled and fractionally crystallized but was still far fkom pure as judged from melting-point studies and from later studies with ^{14}C-benzene when that was finally

prepared. Other chemicals, especially the potential metabolites of benzene, namely, phenol, catechol, quinol (hydroquinone), and hydroxyquinol (1,2,4-trihydroxybenzene), were rigorously purified before use by preparation of derivatives, recrystallization to constant melting points, then hydrolyzed to regenerate the original compounds, which were further recrystallized to purity. In many cases the physical constants of the highly purified metabolites and their derivatives were significantly different from those previously recorded in the literature; this was particularly true where ^{14}C-labeled compounds had been synthesized and where constant specific radioactivity was the accepted criterion of purity. This gave rise to the view, often expressed by British and American chemists at that time, that the physical constants of aromatic compounds characterized before 1950 were probably incorrect and should be redetermined using specific radioactivity as the criterion of purity--an invitation that we firmly resisted *so* that we could concentrate our research efforts on the metabolism problems. Many years later, when we had adopted gas-liquid chromatography as the means of purification, with a symmetrical peak in two systems as a criterion of purity, we were amazed to receive a sample of ^{14}C-cyclohexane from Amersham (Amersham, U.K.) that contained not only ^{14}C-cyclohexene, ^{14}C-cyclohexadiene, and ^{14}C-benzene, but also ^{36}Cl-chlorobenzene as impurities. This discovery led to the realization that coelution of chemically similar compounds could prejudice the use of gas chromatography in purification procedures. Initial studies at Oxford with gas chromatography in the early 1960s showed that commercially recrystallized phenol, as used as a solvent for paper chromatography, was only some 60% pure, thus explaining our earlier need for rigorous purification of this benzene metabolite in isotope dilution studies. This great difficulty in obtaining pure compounds, i.e., 100% pure, greatly affected this research program, both in its inception and in the methodology we chose. Radioisotope labeling now was considered essential for the benzene metabolism study, and synthesis of ^{14}C-benzene was given high priority. Unlabeled benzene, for nonisotopic metabolism studies, was purified by fractional distillation, removal of thiophene, followed by fractional crystallization, and finally purification as the clathrate complex, as used in the purification of ^{14}C-benzene.

Synthesis of ^{14}C-Benzene

The only ^{14}C-labeled material available in the United Kingdom in 1948 was $Ba^{14}C_2$. We planned to use $Ba^{14}C_2$ to generate $^{14}C_2H_2$, then to pyrolyze this to $^{14}C_6H_6$ by passing through a red-hot tube, but the very poor yields led us to consider alternative synthetic approaches. Eventually, we concentrated our efforts on studying the metabolism of benzene using unlabeled material and left the radiosynthesis of ^{14}C-benzene to our chemistry collaborators.

^{14}C-Benzene with a single atom of ^{14}C per molecule was prepared eventually by ring closure of cyclopentane-^{14}C-carboxylic acid to ^{14}C-cyclohexane and dehydrogenation of the latter with Pt/C (1). ^{14}C-Benzene was quantified by end-window counting of the solid benzene-clathrate complex and checked by end-window counting of solid m-dinitrobenzene. The benzene-clathrate complex was formed by shaking benzene with a solution of nickel ammonium cyanide cooled in ice; the solid complex was filtered off, washed with water, ethanol, and diethyl ether, and dried in air (2). Dry distillation of the clathrate complex gave pure benzene.

Determination of the specific radioactivities of 1%-metabolites was carried out by end-window counting, as liquid scintillation spectrometry had not been developed at that time. Indeed, researchers in the field decided to abandon scintillation counting because of the high background and lack of specificity. However, the potential advantages over end-window counting appeared *so* substantial that we decided to continue this line of research and were fortunate to find collaborators who, by the invention of coincidence circuitry and voltage discriminators, finally constructed a prototype Packard scintillation spectrometer. In subsequent metabolism studies with ^{14}C-labeled compounds, we were able to use the more specific, and more sensitive, scintillation spectrometry in place of end-window Geiger-Muller (GM) tube counting.

Benzene Eliminated Unchanged

Benzene was quantified by a colorimetric method based on trapping the exhaled benzene in a nitrating mixture, extracting the m-dinitrobenzene with methyl ethyl ketone, shaking with alkali to develop a purple color, and measurement of the light absorption in a Spekker absorptiometer using suitable light filters. The original method of Pearce et al. (3) was modified to optimize for sensitivity (4), which was 1 μg benzene with an accuracy of +/- 5%.

Subsequently, when a variable wavelength UV spectrophotometer had been built, the benzene was trapped in ethanol, then quantified spectrophotometrically in the prototype Unicam SP500 (Cambridge Instruments, Cambridge, U.K.) by measurement of the absorption at 255.0 nm with a slit width of 0.4 mm and an ε_{max} of 240 (5). Also, when ^{14}C-benzene had been synthesized, a radiometric method was used; the benzene was trapped in ethanol, converted to the clathrate complex, then counted by the end-window GM tube method.

The experimental animals were chinchilla rabbits (23 kg bw); they were dosed with benzene administered orally by gastric intubation or by ip injection. The animals were then placed in a Perspex chamber connected to the appropriate absorption train, and air was drawn through the system at 20 liters/hr for 20 to 30 hr. The absorption train was changed every 2 hr.

Agreement among the three methods of determination (colorimetric, spectrophotometric, and radiometric) of unchanged benzene was good (+/-10%). Elimination unchanged by rabbits was maximal during the first 12 hr after dosage and amounted to about 40% per dose at dose levels of 0.25 and 0.5 g/kg and 64% at 1.0 g/kg (4). The conclusions drawn from these studies were that the rabbit can metabolize benzene up to a limit of about 400 mg/kg/day and that with doses exceeding this, the excess benzene is exhaled in the expired air by a process similar to steam distillation (4,6). Negligible amounts of unchanged benzene (<0.01% dose) were found in rabbit urine.

Metabolism to 1-Phenylmercapturic Acid

Phenylmercapturic acid, equivalent to 0.4% dose, had been isolated from the urine of rats dosed with benzene (7), but attempts to repeat this in other laboratories had failed. Furthermore, attempts to repeat the synthesis of 1-phenylmercapturic acid **(8)** also failed. As a result, in the late 1940s the metabolism of benzene to phenylmercapturic acid was very much in doubt; indeed, even the role of mercapturic acids in the metabolism of other organic compounds was in question. One of the reasons for this was the failure of other laboratories to synthesize the phenylmercapturic acids as described by Zbarsky and Young **(8).** ***An*** investigation into the cause of this failure showed that the definitive paper had been erroneously altered by the journal editor, for throughout the paper, HCl had been changed to H_2SO_4 merely for the sake of uniformity, yet the chloride anion had been shown to be an essential catalyst for the synthesis. Moreover, our commercial cystine was grossly impure. As soon as sodium chloride was added to the reaction mixture and pure cystine was used, the various phenylmercapturic acids were synthesized in good yield; the validity of Zbarsky and Young's synthesis of the mercapturic acids and of their occurrence as metabolites of aromatic compounds were confirmed.

The excretion of phenylmercapturic acid in rabbit urine was quantified by the iodometric method of Stekol (9) and by a turbidimetric determination of the phenylmercapturic mercaptide (10). The two methods showed excellent agreement (+/-2%) in the determination of standard amounts of synthetic 1-phenylmercapturic acid added to rabbit urine, but the turbidimetric method was the more specific and was preferred over the iodometric titration, which gave high blank values with rabbit urine because of the excretion of dietary thiols. After dosing rabbits with benzene, the iodometric method gave 1.8+/-0.2% dose as phenylmercapturic acid at 0.5 g benzene/kg bw and 1.2+/-0.1% at 1.0 g/kg; the turbidimetric method gave 1.0+/-0.1% and 0.8+/-0.1% as phenylmercapturic acid at doses of benzene of 0.5 and 1.0 g/kg, respectively. Many attempts to isolate phenylmercapturic acid from the urine of rabbits dosed with benzene were unsuccessful, and the only crystalline material so obtained was benzoic acid.

Unequivocal proof of the formation of phenylmercapturic acid in the metabolism of benzene was obtained by oral administration of ^{14}C-benzene to rabbits. Reverse isotope dilution studies with synthetic 1-phenylmercapturic acid gave material which, after repeated recrystallization to constant

specific radioactivity, was equivalent to 0.73% of the dose of ^{14}C-benzene. Conversion of this material to phenylmercapturic mercaptide gave specific radioactivity equivalent to 0.70% dose and conversion to thiophenol p-nitrobenzoate gave activity equivalent to 0.74% dose. This figure of 0.7% dose from the radiobenzene study is in close agreement with the figures of 0.8 and 1.0% dose from the turbidimetric determination of phenylmercapturic acid formed from unlabeled benzene.

Metabolism to Muconic Acid

Muconic acid was first isolated as a metabolite of benzene by Jaffé (11) fiom the urine of dogs and rabbits. Although it was thought that if muconic acid were formed by opening of the benzene ring in vivo the cis-cis isomer should have been the initial product--as is obtained by the in vitro oxidative ring scission of catechol or phenol with peracetic acid--only the trans-trans isomer was isolated from the urine of animals dosed with benzene (12). The third geometric isomer of muconic acid, namely, the cis-trans isomer, was first characterized by Elvidge et al. (13) and was formed merely by recrystallization of the cis-cis isomer fiom water; in contrast, the trans-trans acid was formed only after UV irradiation of a solution of the cis-cis isomer in the presence of iodine as catalyst.

The objectives of the 1945 to 1950 study were therefore a) to develop a quantitative methodology for determination of each of the three isomers of muconic acid in urine; b) to isolate and characterize each isomer that was shown to be a metabolite of benzene; c) to determine the in vitro and in vivo stabilities of the three isomers to obtain an insight into the mechanism of formation of muconic acid from benzene; and d) to quantify the muconic acid isomers excreted after administration of ^{14}C-benzene to rabbits.

A colorimetric method, based on condensation of the muconic acid with phenol in the presence of H_2SO_4 to give a red pigment and soluble in ethanol, was developed to quantify each of the three isomers; cis-cis and cis-trans isomers give maximal red color when heated at 100°C for 6 hr; the trans-trans isomer, however, requires heating at 160°C for 20 min. Hence, the cis acids can be quantified when present in mixtures with the trans-trans acid; recoveries of all three isomers from urine were 100+/-10% (5,12). cis-cis-Muconic acid added to rabbit urine was recovered quantitatively as a mixture of the cis acids; no trans-trans acid was formed. After ip injection of each of the three isomers of muconic acid into rabbits and quantification of the urinary excretion, the trans-trans acid was excreted unchanged equivalent to 52+/-5% dose, the cis-cis acid was excreted as cis acids, equivalent to 66+/-5% dose, and the cis-trans acid as cis acids equivalent to 55+/-5% dose; no trans-trans acid was detected after injection of either of the cis acids. Hence, no evidence was found for isomerization of the cis acids to the trans-trans acid in vivo; furthermore, no evidence was found for selective loss of the cis acids added to rabbit urine in vitro. The quantitative method therefore indicates that trans-trans-muconic acid is a true metabolite of benzene in the rabbit, equivalent to 0.5% dose (0.15-1.0%) (12). Furthermore, dosing rabbits with phenol or catechol also resulted in the urinary excretion of trans-trans-muconic acid equivalent to 0.5 and 1.4% dose, respectively (5). The oxidative ring opening of benzene first gives rise to cis-cis-muconaldehyde,which then isomerizes to cis-trans- and trans-trans-muconaldehyde;the latter is oxidized in vivo to trans-trans-muconic acid, and this may be the actual route of formation of this paradoxical metabolite (14).

After oral dosing of benzene (7 g) to four rabbits, continuous ether extraction of the acidified urine gave 120 mg of crude crystalline material equivalent to 1.2% dose, which was shown not to contain any cis-cis- or cis-trans-muconic acid. Recrystallization from ethanol gave the pure trans-trans-muconic acid equivalent to 0.1% dose of benzene, and this was further characterized as the benzhydryl ester. Finally, after administration of ^{14}C-benzene to rabbits, reverse isotope dilution studies showed that trans-trans-muconic acid was excreted in the urine equivalent to 1.3% of the dose.

cis-cis-Muconic acid had originally been prepared in the 1930s by the oxidation of phenol and catechol with dilute peracetic acid using 100 vol hydrogen peroxide. Twenty years later, because of the development of rocket fuel technology, 98% peroxide became available; therefore this was used in the synthesis of cis-cis-muconic acid from phenol to obtain increased yields. To decrease the risk of explosion, small amounts of reaction mixture (2 ml peracetic acid and 0.2 g phenol in 5 ml conical flasks) kept cool at 0°C were used with apparent safety. This was laborious and, to accelerate

production, the individual volumes of peracetic acid were increased to 5 ml, with a total of 250 ml. However, temperature control was lost and a series of explosions ensued that wrecked the entire laboratory, so we reverted to the smaller individual volumes of reactant with complete safety.

Metabolism to Phenols

Phenol, catechol, and quinol (hydroquinone) had long been recognized as metabolites of benzene, but the oxidation of benzene to resorcinol, hydroxyquinol (1,2,4-trihydroxybenzene) and other trihydric phenols was uncertain. Attempts to devise specific colorimetric or spectrophotometric assays for the individual phenols all failed because of the interference of other phenols. Quantification of the individual phenolic metabolites of benzene was therefore dependent on the administration of ^{14}C-benzene, followed by reverse isotope dilution of the urines with a number of phenols and purification of crystalline derivatives. Phenol (23% dose) quinol (5%), catechol (3%), and hydroxyquinol (0.3%) were the major phenols present in the urines of rabbits dosed with ^{14}C-benzene; resorcinol, if present, was formed only in trace amounts (<0.3% dose), and phloroglucinol and pyrogallol were absent. The dihydrodiol of chlorobenzene had been isolated from rabbit urine at that time (15), so a similar isolation procedure was carried out on the urine of rabbits given ^{14}C-benzene. No dihydrodiol was ever detected. Nevertheless, it was presumed that the phenols had been formed by the dehydration of the corresponding diols, so that resorcinol and phloroglucinol were considered unlikely metabolites. Pyrogallol could have been a trace metabolite but was not detected, possibly because of polymerization of the quinone oxidation products, a process that may have led to low values for catechol, quinol, and hydroxyquinol despite the various precautions.

Determination of total conjugates in the urine of rabbits dosed with unlabeled benzene was the only approximation that could be made of the total phenols formed fiom unlabeled benzene. The total glucuronides were equivalent to 11% dose, and the ethereal sulfates were equivalent to *25%* dose, a total of 36% (Table 1). This compares with a total of 31% being excreted as phenol, quinol, catechol, and hydroxyquinol in ^{14}C-benzene urine. This lower total might be due to the formation of bis conjugates of quinol, catechol, or hydroxyquinol.

Table 1. Metabolism of ^{14}C-benzene in rabbit.

Source	^{14}C-Benzene	Unlabeled benzene
Expired air		
Benzene	46.5	48.4
$^{14}CO_2$	1.0	–
Total in expired air	47 5%	48.4%
Urine		
Phenol	22.9[a]	
Catechol	2.9[a]	
Quinol	4.8[a]	
Resorcinol	<0.3[a]	
Hydroxyquinol	0.3[a]	
L-Phenylmercapturic acid	0.4	1.0
trans-trans-Muconic acid	13	1.0
Total in urine	32.9%	38%
Feces	0.5%	–
Tissues	ca 5%	–
Total accounted for	*86%*	*86%*

[a] 25 as ethereal sulfates; 11 as glucuronides. Data from Parke and William (*4,10,12,16*) and Parke (*5*).

Metabolism of ^{14}C-Benzene

As seen (Table 1), the quantitative balance sheet for the metabolism of unlabeled benzene is in good agreement with the more detailed picture obtained by dosing with ^{14}C-benzene (16). The totals in the

expired air (47.5, 48.4%) and totals excreted in the urines (33, 38%) for the labeled and unlabeled benzenes are in excellent agreement.

Evidence was obtained that ^{14}C-benzene could be oxidized completely to $^{14}CO_2$ (1% dose), and consequently ^{14}C was incorporated into the body tissues to the extent of at least 5% dose. Indeed, it is most likely that the shortfall of some 15% dose in the overall balance sheet could be due to incorporation of the isotope ^{14}C into aliphatic carboxylic acids, formed as the result of oxidative ring scission, and the subsequent biosynthesis of these fatty acids into tissue components. In one rabbit experiment when ^{14}C-benzene was administered, the total residual radioactivity remaining in all of the tissues 48 hr after dosing was equivalent to some 16% dose *(5)*.

Benzene and Oxygen Radicals

The known radiomimetic toxicity of benzene suggested, even some 50 years ago, that this solvent gives rise to the production of oxygen radicals ROS. Diethyl ether administered to rats also results in ROS production, glutathione depletion, and oxidative stress (17,18), for both diethyl ether and benzene activate the ROS-generating cytochrome P450, CYP2E1, (19) and can thus result in oxidative stress in vivo. However, benzene also generates ROS by the redox cycling of its quinone metabolites, which may explain the unique radiomimetic, hematopoietic toxicity of benzene, as CYP2E1 is not dominant in the bone marrow whereas myeloperoxidase is. Sources of ROS are as follows: a) ionizing radiation; b) iron and other redox metals; c) inflammation: activated leukocytes, infections, interleukins; d) eicosanoid biosynthesis (prostaglandin H synthase activity); e) redox cycling of quinones; f) futile cycling of cytochromes P450: P450-activated O_2 is partly inserted as ROS into the substrate and partly released as superoxide, etc.; and g) activation of CYP2E1: O_2 activation to ROS is preferred to the direct insertion of oxygen into the substrate. Bone-marrow phagocytes play a major role in benzene-induced hernotoxicity, and exposure of mice to benzene results in the activation of the phagocytes with increased ROS production, changed bone marrow progenitor-cell development, and increased production of interleukins (IL-1) and tumor necrosis factor (TNFα) (20,21).

Induction of CYP2E1 by benzene, ether, ethanol, acetone, and a variety of other small molecules, or by fasting, increases the metabolism of benzene and phenol to quinol and other myelotoxic metabolites (22) and also increases ROS production. ROS, from whatever origin, are the means of the metabolic activation of benzene and are the source of its toxicity, causing depletion of glutathione (GSH), oxidative stress, DNA damage, activation of protein kinase c, tissue necrosis, and malignancy (23). The metabolic activation of benzene and the generation of ROS by CYP2E1 might be genetically dependent and polymorphic, as is the metabolism of ethanol by this cytochrome (24), *so* that benzene toxicity would show individual variation. Prostaglandin H synthase activity similarly metabolically activates benzene **and** phenol to toxic reactive intermediates, and consequently indomethacin inhibits benzene-induced bone-marrow toxicity (25). Myeloperoxidase in bone marrow also hydroxylates benzene to quinol and other reactive metabolites by generation of singlet oxygen (26).

The synergism of catechol and quinol in benzene-induced toxicity and leukemia (27) could involve a concerted action of redox cycling of quinol, generating ROS and thereby hydroxylating catechol to hydroxyquinol (benzene 1,2,4-triol). This would be followed by further redox cycling of hydroxyquinol, further ROS generation, interaction with GSH to form 2,5-dihydroxyphenylmercapturic acid (28), with consequent damage to hemopoietic tissues (29), and malignancy.

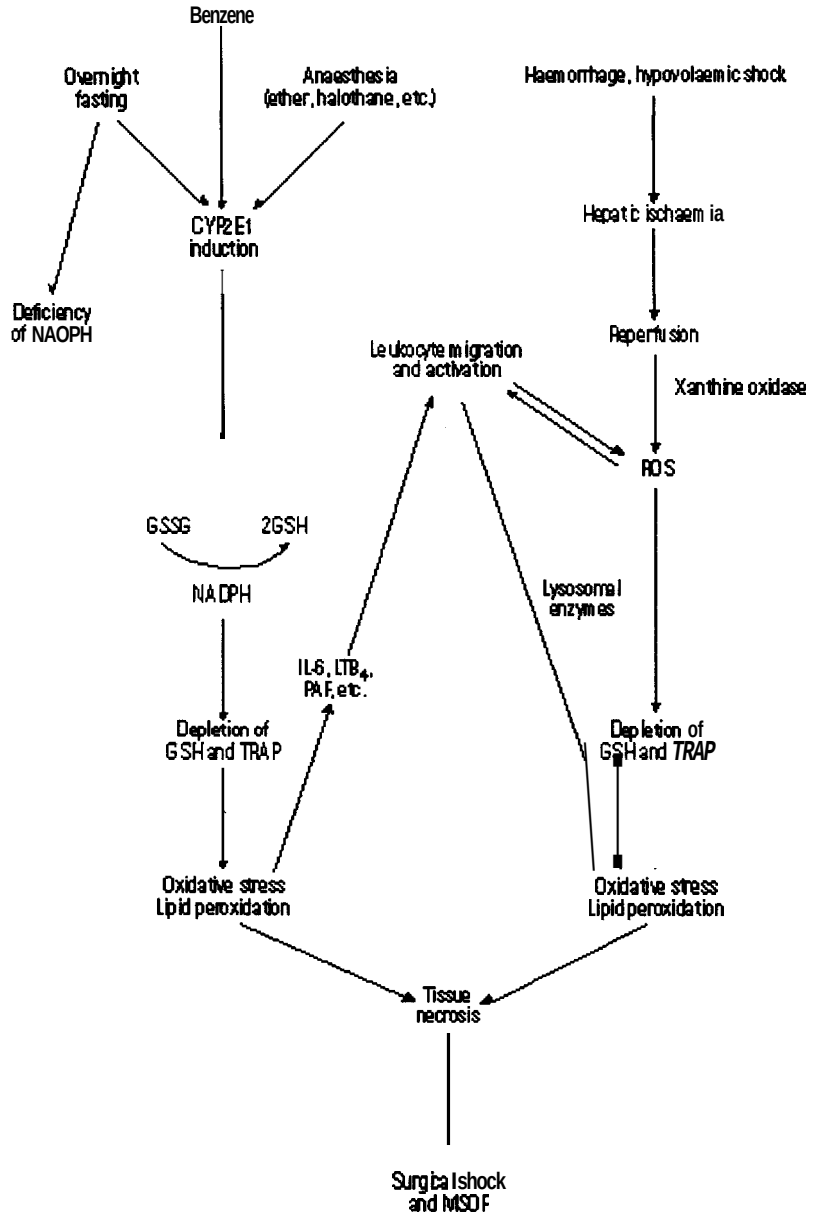

Figure 1. Mechanisms of oxidative stress and tissue inflammation. ROS are generated from cytochrome P4502E1 (CYP2E1), stimulated by exposure to benzene, ether, and other small molecules, and by fasting. The ROS oxidize intracellular GSH to GSSG, which is lost from the cell unless GSH is regenerated by glutathione reductase plus NADPH. **Loss** of GSH and other antioxidants (TRAP = total radical antioxidant parameter = tocopherols, ascorbic acid, retinoids, etc.) results in oxidative stress, lipid peroxidation, release of interleukins **(IL-6),** leukotrienes **(LTB_4),** platelet-activating factor (PAF), etc. This, in turn, leads to leukocyte activation and migration into tissues, with generation of further ROS, depletion of GSH and TRAP, resulting in surgical shock and multiple system organ failure (MSOF). Hence, exposure to benzene might result in conditions associated with chronic systemic inflammation.

This generation of **ROS** from CYP2E1, redox cycling of the quinones, and other mechanisms have been shown in the case of ether anesthesia (17,18) to result in loss of GSH, oxidative stress, tissue inflammation and tissue necrosis (Figure 1), which may lead to chronic inflammatory disease such **as** rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, multiple system organ failure, and malignancy (30,31). Many chemicals, particularly substrates of CYP2E1 such as halothane and ether, initiate immune or inflammatory responses associated with **ROS** production and **GSH** depletion and are associated with many syndromes of chronic inflammation **(30).** Thus it is possible that benzene exposure may likewise be associated with various manifestations of systemic inflammation in addition to the known myelotoxicity and malignancy. Protection against ROS and oxidative stress is given by intracellular GSH and by dietary ascorbic acid, tocopherol, and other radical-trapping antioxidants (30).

Conclusions

It has long been known that the unique radiomimetic myelotoxicity of benzene is associated with its metabolism and that the difference in toxicity between benzene and the halobenzenes is associated with fundamental differences in their metabolic fates (32). Whereas the hepatotoxic chlorobenzene and bromobenzene are oxidized by various cytochromes P450 to form a relatively stable 3,4-epoxide that yields the corresponding dihydrodiol, catechol, and mercapturic acid as the major metabolites (15), the myelotoxic, leukemogenic benzene is oxidized by ROS from CYP2E1 to yield a metastable radical that rearranges to form phenol as the major metabolite, with only traces of catechol (3%) and phenylmercapturic acid (1%). Deactivation of the aromatic ring by the halogen substituent thus facilitates the insertion of an activated oxygen from a cytochrome P450, and this allows epoxidation and consequent metabolic detoxication. By contrast, benzene appears to be metabolized with difficulty; and as with ethanol, diethyl ether, carbon tetrachloride, acetone, and many other small molecules that are resistant to oxidative metabolism by the cytochromes P450, it is metabolized by CYP2E1 by generating ROS. The ROS also mediate malignancy and form quinones that undergo redox cycling to generate ROS in bone marrow, mediating myelotoxicity (19,25). The identification of other CYP2E1 substrates that are likely to manifest myelotoxicity and malignancy is now well advanced and depends on determination of the molecular diameter of the chemical and of the energies of the molecular orbitals (23,33). Thus, our knowledge of benzene metabolism and toxicity has continued to evolve over more than 50 years and is now enabling the prediction of chemicals with similar toxicity.

References

1. Turner HS. A convenient sythesis of carbon-labelled benzene. Nature 168:73-74 (1951).

2. Evans RF, Ormrod I, Goalby BB, Staveley LAK. Purification of benzene. J Chem Soc 3346 (1950).

3. Pearce SJ, Schrenk HH, Yant WP. Microcolorimetric determination of benzene in blood and urine. Rep Investig No 3302 Chem Abstr 305600 (1936).

4. Parke DV, Williams RT. Studies in detoxication. 30: The metabolism of benzene. a) The determination of benzene. b) The elimination of benzene unchanged by rabbits. Biochem J 46:236-243 (1950).

5. Parke DV. The Metabolism of Aromatic Compounds. PhD Thesis, London: University of London, 1952.

6. Azouz WM, Parke DV, Williams RT. Studies in detoxication. 42: Fluorobenzene. Spectrophotometric determination of the elimination of unchanged halobenzene by rabbits. A comparison of the oxidation in vivo of fluorobenzene and of benzene. Biochem J 50:702-706 (1952).

7. Zbarsky SH, Young L. Mercapturic acids. 111: The conversion of benzene to phenylmercapturic acid in the rat. J Biol Chem 151:487-492 (1943).

8. Zbarsky SH, Young L. Mercapturic acids. I: Synthesis of phenyl-1-cysteine and 1-phenylmercapturic acid. J Biol Chem 151:211-215 (1943).

9. Stekol JA. The determination of p-bromophenylmercapturic acid in the urine of dog. J Biol Chem 113:279-288 (1936).

10. Parke DV, Williams RT. Studies in detoxication. 38: The metabolism of benzene. a) The determination of phenylmercapturic acid in urine. b) The mercapturic acid excretion by rabbits receiving benzene. Biochem J 48:624-628 (1951).

11. Jaffé M. Cleavage of the benzene ring in the organism. 1. The excretion of muconic acid in the urine after ingestion of benzene. Z Physiol Chem 62:58-67 (1909).

12. Parke DV, Williams RT. Studies in detoxication 44. The metabolism of benzene. The muconic acid excreted by rabbits receiving benzene. Determination of the isomeric muconic acids. Biochem J 51:339-348 (1952).

13. Elvidge JA, Linstead RP, Sims P, Orkin BA. Third isomeric (cis-trans) muconic acid. J Chem Soc 2235-2241 (1950).

14. Latriano L, Goldstein BD, Witz G. Formation of muconaldehyde, an open-ring metabolite of benzene in mouse liver microsomes: an additional pathway for toxic metabolites. Proc Natl Acad Sci USA 83:8356-8360 (1986).

15, Smith JN, Spencer B, Williams RT. Detoxication. XXXIV. Metabolism of chlorobenzene in the rabbit. Isolation of dihydrodihydroxychlorobenzene, p-chlorophenylglucuronide, 4-chlorocatechol glucuronide and p-chlorophenylmercapturic acid. Biochem J 47:284-293 (1950).

16. Parke DV, Williams, RT. The metabolism of benzene containing ^{14}C-benzene. Biochem J 54:231-238 (1953).

17. Liu PT, Ioannides C, Symons AM, Parke DV. Role of tissue glutathione in the prevention of surgical trauma. Xenobiotica 23:899-911 (1993).

18, Liu PT, Ioannides C, Shavila J, Symons AM, Parke DV. Effects of ether anaesthesia and fasting on various cytochromes P450 of rat liver and kidney. Biochem Pharmacol 45:871-877 (1993).

19, Johansson I, Ingelman-Sundberg M. Hydroxyl radical-mediated cytochrome P450-dependent metabolic activation of benzene in microsomes and reconstituted enzyme systems from rabbit liver. J Biol Chem 258:7311-7316 (1983).

20. MacEachern L, Laskin DL. Bone marrow phagocytes, inflammatory mediators and benzene toxicity. In: Xenobiotics and Inflammation (Schook LB, Laskin DL, eds). San Diego:Academic Press, 1994;149-171.

21. MacEachern L, Snyder R, Laskin DL. Alterations in the morphology and functional activity of bone-marrow phagocytes following benzene treatment of mice. Toxicol Appl Pharmacol 117:147-154 (1992).

22. Schrenk D, Ingelman-Sundberg M, Bock KW. Influence of P4502E1 induction on benzene metabolism in rat hepatocytes and on biliary excretion. Drug Metab Dispos Biol Fate Chem 20:137-141 (1992).

23. Parke DV. The cytochromes P450 and mechanisms of chemical carcinogenesis. Environ Health Perspect 102:852-853 (1994).

24. Tsutsumi M, Wang J-S, Takase S, Takada A. Hepatic messenger RNA contents of cytochrome P4502E1 in patients with different P4502E1 genotypes. Alcohol Alcohol 29(Suppl 1):29-32 (1994).

25. Kalf GH, Schlosser MJ, Renz JF, Pirozzi SJ. Prevention of benzene-induced myelotoxicity by nonsteroidal antiinflammatory drugs. Environ Health Perspect 82:57-64 (1989).

26. Subrahmanyam V, Kolachana P, Smith MT. Hydroxylation of phenol to hydroquinone catalysed by a human myeloperoxidase-superoxide complex: possible implications in benzene-induced myelotoxicity. Free Radic Res Commun 15:285-296 (1991).

27. Robertson ML, Eastmond DA, Smith MT. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. Mutat Res 249:201-209 (1991).

28. Nerland DE, Pierce M Jr. Identification of N-acetyl-S-(2,5-dihydroxyphenyl)-l-cysteine as a urinary metabolite of benzene, phenol and hydroquinone. Drug Metab Dispos Biol Fate Chem 18:958-961(1990)

29. Zhang L, Robertson ML, Kalachana P, Davis AJ, Smith MT. Benzene metabolite, 1,2,4-benzenetriol, induces micronuclei and oxidative damage in human lymphocytes and HL60 cells. Environ Mol Mutagen 21:339-348 (1993).

30. Parke AL, Parke DV. The pathogenesis of inflammatory disease: surgical shock and multiple system organ failure. Inflammopharmacology 3:1079-1099 (1995).

31. Parke AL, Ioannides C, Lewis DFV, Parke DV. Molecular pathology of drug-disease interactions in chronic autoimmune inflammatory diseases. Inflammopharmacology 1:1-36 (1991).

32. Parke DV, ed. The Biochemistry of Foreign Compounds. Oxford:Pergamon Press, 1968;216-217.

33. Lewis DFV, Ioannides C, Parke DV. A retrospective evaluation of COMPACT predictions of the outcome of NTP rodent carcinogenicity testing. Environ Health Perspect 103:178-184 (1995).

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Environmental Exposure to Benzene: An Update

Lance Wallace

U.S. Environmental Protection Agency, Reston, Virginia

Abstract

During the 1990s, several large-scale studies of benzene concentrations in air, food, and blood have added to our knowledge of its environmental occurrence. In general, the new studies have confirmed the earlier findings of the **U.S.** Environmental Protection Agency Total Exposure Assessment Methodology (TEAM) studies and other large-scale studies in Germany and the Netherlands concerning the levels of exposure and major sources. For example, the new studies found that personal exposures exceeded indoor concentrations of benzene, which in turn exceeded outdoor concentrations. The new studies of food concentrations have confirmed earlier indications that food is not an important pathway for benzene exposure. The results of the National Health and Nutrition Examination Survey on blood levels in a nationwide sample of 883 persons are in good agreement with the concentrations in exhaled breath measured in about 800 persons a decade earlier in the TEAM studies. Major sources of exposure continue to be active and passive smoking, auto exhaust, and driving or riding in automobiles. New methods in breath and blood sampling and analysis offer opportunities to investigate short-term peak exposures and resulting body burden under almost any conceivable field conditions. --Environ Health Perspect 104(Suppl 6): 1129-1136 (1996)

Key words: benzene, exposure, indoor air, outdoor air, personal monitors, TEAM study, body burden, breath, blood

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Address correspondence to L. Wallace, U.S. EPA, 11568 Woodhollow Court, Reston, VA 20191.

Telephone: (703) 341-7509. Fax: (703) 341-7575. E-mail: wallace.lance@epamail.epa.gov

Abbreviations used: NHANES, National Health and Nutrition Examination Survey; TEAM, Total Exposure Assessment Methodology.

Introduction

Much of our knowledge of nonoccupational exposure to benzene was supplied throughout the 1980s by the U.S. Environmental Protection Agency (U.S. EPA) Total Exposure Assessment Methodology (TEAM) studies of volatile organic compounds (VOCs) (1). These studies employed personal air quality monitors to measure direct personal exposures of approximately 800 persons in about eight areas in the United States between 1980 and 1987 (2-13). The participants were selected on a strict probability sampling basis to represent about 800,000 persons in these areas. Measurements of indoor and outdoor air, drinking water, and exhaled breath were made to supplement the personal air measurements. In a pilot study (2,14), measurements were also made in food and beverages; since few VOCs and no benzene was detected, those measurements were not repeated in the main study.

The basic results of the TEAM study as they apply to benzene may be summarized as follows (15-20):

- Benzene was not found, or was found in insignificant amounts, in water, food, and beverages. More than 99% of the total personal exposure was through air.
- Mean personal air exposures exceeded indoor air concentrations, which in turn exceeded outdoor air concentrations. A global average personal exposure was about 15 $\mu g/m^3$ (range 7-29 $\mu g/m^3$). Indoor concentrations were measured only in the 1987 TEAM studies in Los Angeles, CA, Baltimore, MD, and Bayonne, NJ, and appeared to be on the order of 10 $\mu g/m^3$. Outdoor concentrations had a global average of 6 $\mu g/m^3$ (range 2-19 $\mu g/m^3$).
- No effect on personal exposure of living close to major fixed sources of benzene (oil refineries, storage tanks, chemical plants) could be detected in Beaumont, TX (2,3); Bayonne and Elizabeth, NJ (5-8); or Los Angeles, Antioch, and Pittsburg, CA (9-11).

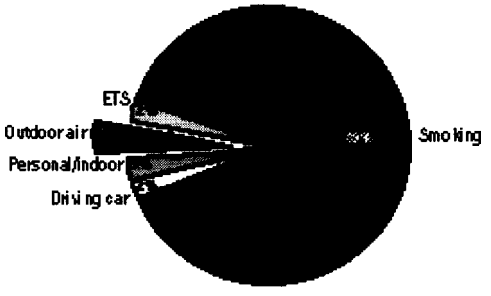

Figure 1. Sources of benzene exposure: smokers. A typical smoker takes in roughly 2 mg benzene/day; about 1.8 mg is delivered by mainstream smoke (55 μg/cigarette X 32 cigarettes per day). Source: U.S. EPA TEAM studies.

- The overwhelming source of benzene exposure for smokers was mainstream cigarette smoke (15). Smokers had an average benzene body burden about 6 to 10 times that of nonsmokers, and received about 90% of their benzene exposure fiom smoking (Figure 1). Roughly half the total benzene exposure in the United States was borne by smokers.

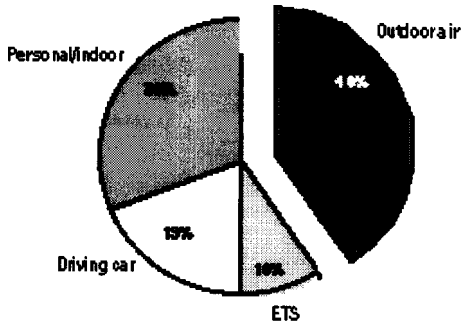

Figure 2. Sources of **benzene exposure: nonsmokers. A typical nonsmoker inhales about 0.2 mg benzene/day, assuming an average exposure of 15 μg/m³ and an alveolar respiration rate** of **14 m³/day. Outdoor air contributes about 40%** of **that amount, assuming an average outdoor level of *6* μg/m³. The remaining 9 μg/m³ are split between driving** (1 **00 min at** 30-40 **μg/m³), indoor sources such aS automobile vapor emissions in attached garages or storage** of **gasoline or kerosene in the garage or the basement, and environmental tobacco-smoke exposures at home or at work. Source: U.S. EPA TEAM studies.**

- For nonsmokers, most benzene exposure ultimately is derived from auto exhaust or gasoline vapor emissions. This includes most of the benzene exposure due to outdoor air, indoor exposures due to intrusion of evaporative gasoline fumes from attached garages **(21),** and personal activities such as driving (Figure **2).** A portion of the exposure is due to environmental tobacco smoke **(15).** A small portion (about 6%) of the exposure is due to major point sources of benzene, such as petrochemical plants or refineries.

Two large-scale European studies (22,23) confirmed the TEAM study results for indoor and outdoor benzene concentrations. The study in Germany **(23)** also confirmed the effect of environmental tobacco smoke, finding an increase of **4.5** μg/m³ in homes with smokers compared to the TEAM study finding of an increase of 3.5 μg/m³. Both results were based on about **200** homes with smokers and **300** homes without smokers.

Recent Studies

About half-a-dozen large-scale studies of personal or indoor air levels of benzene have been conducted since **1990.** They are briefly described below.

Personal Exposure Studies

A 1991 study **(24)** took place in **128** homes in Woodland, California, a community in a largely agricultural region. Personal, indoor, and outdoor benzene concentrations were measured using both Tenax (Enka Research Institute, Arnhem, the Netherlands) and evacuated canister samplers. Good agreement was noted between the side-by-side Tenax and canisters. Mean concentrations were 5.0, 4.0, and **1.2** μg/m³ for the personal, indoor, and outdoor samples.

Day and night **12-hr** average concentrations of benzene were measured for **58** residents of Valdez, Alaska **(25).** The mean benzene concentrations in the personal, indoor, and outdoor samples were **20,** 16, and 5 μg/m³ during the summer, and 28, 25, and 11 μg/m³ during the winter, respectively.

Table 1. Personal air concentrations (µg/m³) of benzene measured in the TEAM, Valdez, and Woodland studies.

Site	Household. estimated no.	Year, season	Time	n	Mean	SE	Geom mean	Percentile 25	50	75	90	95
NJ1	130.000	1981, fall	Day	340	26.2	2	11	7	17	32	65	81
			Night	347	297	5	13	7	15	32	54	73
NJ3	Unweightej data	1983, winter	Day	47	21.0	2	16	9	16	26	46	62
			Night	49	16B	1	13	9	14	24	29	32
GNC	130.000	1932. spring	Day	24	7 9	2	8	–	8	13	–	–
			Night	24	10.2	2	12	–	12	16	–	–
A-P	91,000	1984, spring	Day	67	8 5	1	7	5	6	11	17	21
			Night	69	6.5	1	5	2	4	8	16	18
LA1	360.000	1984, winter		112	19.1	2	15	10	15	23	35	51
			Night	112	165	1	14	11	15	21	30	34
LA2	330,000	1984, summer	[By	50	105	2	7	3	7	12	2 5	3 4
			Night	50	78	1	5	2	4	9	2 5	2 9
LA3	Unweghted data	1987, winter	Day	33	21.8	6	13	7	13	221	40	139
			Night	32	138	2	10	6	12	19	22	32
IA4	Unweighted data	1987, summer	b y	40	13.7	3	9	5	7	13	26	84
			Night	40	7.1	1	5	4	5	8	16	22
BAL	70.000	1987, spring	Day	70	16.4	2	9	–	11	22	32	45
			Night	70	20.0	3	12	–	14	24	42	62
VAL	Unweighted data	1990, summer	Day	55	25.4	5	14	7	13	23	70	130
			Night	58	155	3	9	5	9	20	30	70
VAL	Unweighted data	1991. winter	Day	56	34.4	6	21	13	20	37	90	110
			Night	58	238	4	13	7	12	E	65	100
WDL	30.000	1990, spring	24 hr	93	5.0	1	3	2	3	5	9	–

Abbreviations: n, number, SE. standard error, geom mean, geometric mean. NJ1, Bayonne–Elizabeth, NJ; NJ3, Bayonne–Elizabeth, NJ; GNC. Greensboro. I Antioch–Pittsburg, CA; LA1, Los Angeles, CA; LA2, Los Angelas. CA; LA3, Las Angelas. CA; LA4, Los Angeles, CA; BAL Baltimore. MD (Dundalk); VAL, Valdez, A Woodland. CA.

Personal exposures to benzene were measured over a 3-hr period in the evening for 49 nonsmoking females in Columbus, Ohio (26). The median value in 25 homes with a smoker was 4.0 $\mu g/m^3$ compared to 2.4 $\mu g/m^3$ in 24 homes without smokers. The difference was statistically significant.

Personal exposures to benzene as measured in the TEAM studies and in the Valdez and Woodland studies are summarized in Table 1. Outdoor and indoor benzene values in the TEAM, Valdez, and Woodland studies are summarized in Tables 2 and 3.

Table 2. Household-weighted outdoor air concentrations (µg/m³).

Site	Households, estimated no.	Year, season	Time	n	Mean	SE	Geom mean	Percentile 25	50	75	90	95
NJ1	40.000	1981, fall	Day	88	9 5	0.9	38	1.2	7 8	16	20	27
			Night	84	8 8	1	4.1	22	6.7	11	15	24
NJ3	Unweighted data	1983, winter		8	3.9	0.8	–	2	4	5.4	73	–
			Night	9	4 3	07	–	28	45	55	79	–
A-P	25,000	1984, spring	Day	10	2	08	15	09	13	18	63	–
			Night	10	18	03	18	1.4	13	19	3.2	–
LA1	120.000	1984, winter	Day	24	13	13	11	8.1	14	18	21	22
			Night	24	19	19	16	11	19	25	32	33
LA2	110.000	1984, summer	Day	24	4.2	0.8	3.2	2	3.1	48	8.7	12
			Night	23	3.1	0.4	28	1.7	25	4.4	58	6.i
IA3	Unweighted data	1987, winter	Day	41	4.7	05	38	28	38	6.2	8.7	12
			Night	46	98	1	6.7	38	79	15	19	24
LA4	Unweighted data	1987, summer		38	3.4	0.4	28	19	28	48	68	87
			Night	40	4	05	3.2	2	33	45	9	11
VAL	Unweightej data	1990, summer	Day	30	48	04	4.1	3	5	7	8	9
			Night	28	5	1	38	2	5	7	10	11
VAL	Unweighted data	1991, winter	Day	29	15	34	10	7	11	15	27	29
			Night	28	8.4	09	7	5	8	12	15	16
WDL	10.000	1990, spring	24hr	48	1.2	0.9	1.1	0.8	1.1	1.4	1.9	–

Abbreviations: n, number, SE, standard error, geom mean, geometric mean. NJ1, Bayonne–Elizabeth, NJ; NJ3, Bayonne–Elizabeth.NJ; A-P, Antioch–Pittsburg, CA; Angeles, CA; LA2, Los Angeles, CA; LA3, Los Angeles, CA; LA4, Los Angeles, CA; VAL Valdez, AK; WDL, Woodland, CA.

Table 3. Indoor air concentrations (µg/m³).

Site	Households. no.	Year, season	Time	Room	n	Mean	SE	Geom mean	Percentile 5	50	75	90	95
IA3	Unweighted data	1987, winter	[By	LR	36	99	1.4	7 3	4.2	7.1	13	19	32
			Day	Ki	38	11	2 8	6.5	3.7	7.5	12	21	30
			Night	Kit	36	15	2.2	9.7	5	11	18	40	46
IA4	Unweighted data	1987, summer	[By	LR	40	6.5	0 9	4 9	2.8	4 8	9.4	15	20
			Day	Kit	38	5.5	0B	4.4	2 5	4 7	6.7	99	14
			Night	K i	37	65	1.2	4.4	2 3	4 5	7.4	13	23
VAL	Unweighted data	1990, summer	Day	LR	30	13	4	8.1	4	8	14	19	41
			Night	LR	30	18	5.2	7.6	4	8	22	29	120
VAL	Unweighted data	1991, winter	[By	LR	29	26	4.4	17	7	16	34	62	81
			Night	LR	27	24	4 8	14	7	16	28	74	79
WOL	10,000	1990, spring	24 hr	LR	104	4.7	1.1	2 5	1 3	2.2	5.1	83	–

Abbreviations: n, number; SE, standard error; geom man. geometric mean; LR, living room; kit, kitchen. LA, Los Angeles. CA; VAL Valdez, AK; WDL. Woodland, CA.

Indoor Air Studies

A nationwide Canadian study (27) measured 24-hr indoor air concentrations of benzene in 754 randomly selected homes. Benzene mean indoor concentrations were 6.39, 5.60, 2.72, and 6.98 µg/m³ in the winter, spring, summer, and fall seasons, respectively.

Indoor and outdoor 48-hr average concentrations of benzene were measured at 161 homes throughout much of California (28). The Pro-Tek charcoal badges formerly manufactured by E.I. duPont (Newark, DE) were used. Indoor mean concentrations were 8.3 µg/m³ compared to 6.1 µg/m³ outdoors.

Seventeen volunteers in Windsor, Canada, wore 3-stage adsorbent tubes with pumps in three microenvironments: at home, at work, and during commuting (29). Benzene concentrations were 3.5, 4.7, and 15.7 µg/m³ in these three locations during summer 1991 and 2.7, 2.7, and 15.1 µg/m³ during winter 1992. Outdoor levels near homes were 3.8 and 2.0 µg/m³ during summer and winter, respectively. A later study (summer 1992) considered various microenvironments. Benzene levels averaged 2.2 µg/m³ in homes of 26 asthmatics, 4.6 µg/m³ in 13 samples fkom hotel rooms, 6.0 µg/m³ in 17 samples collected during commuting, 20.8 µg/m³ in 39 samples from four bingo halls, and 34.5 µg/m³ in two taverns.

Brown and Crump **(30)** reported on a study of 173 homes in Avon, England. Passive Tenax tubes (Perkin-Elmer) collected 28-day samples in the living room and main bedroom of the home for 1 year. Thirteen sets of 12-month outdoor samples were also collected over the course of the study (November 1990-February 1993). The mean indoor concentration was 8 µg/m³ (n=3000 samples) compared to an outdoor mean of 5 µg/m³ (n=125).

Ambient Concentrations

Benzene concentrations were reported for 586 ambient air samples collected from 10 Canadian cities (T Dann, unpublished data). The overall mean was 4.4 µg/m³, with Ottawa and Montreal ranging between 5.1 and 7.6 µg/m³. A more recent survey (T Dann and D Wang, unpublished data) found similar levels, with three rural sites ranging from 0.6 to 1.2 µg/m³.

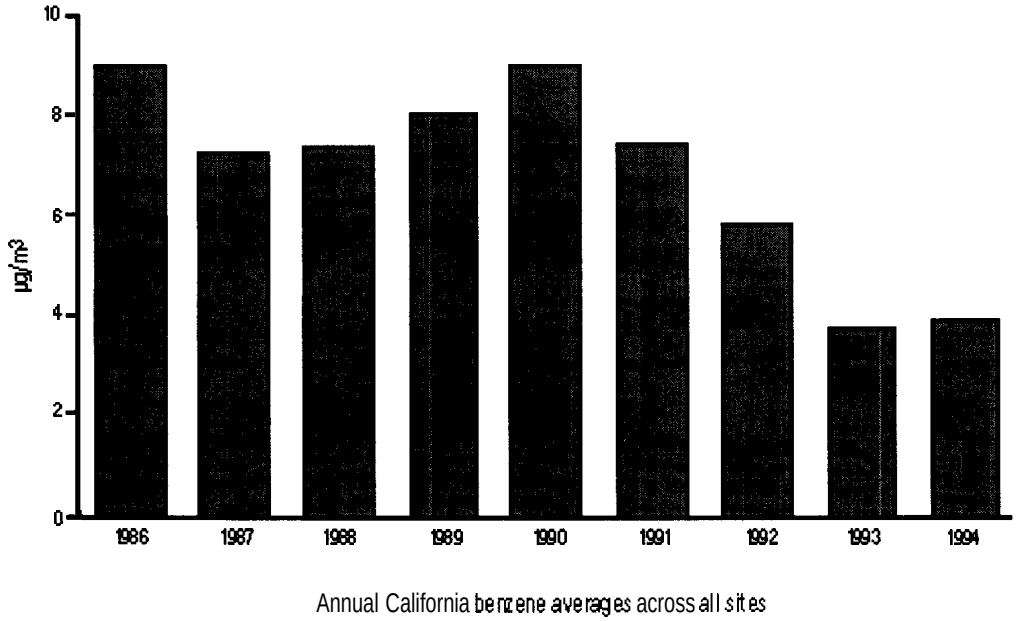

Figure 3. Ambient benzene in California, annual averages across all sites 1986-1994. Annual average outdoor benzene concentrations at about 20 sites in California. At each site, a 24-hr average is taken every 12 days. The decline in 1993 to 1994 may be due to reduced emissions from automobiles. Source: California Air Resources Board, data from 20+ cities.

Twenty-four-hour average benzene levels have been measured every 12th day at about 20 sites throughout California since 1986 (31). Statewide average annual values fluctuated between 5 and 7 $\mu g/m^3$ until 1993 and 1994, when they dropped to about 4 $\mu g/m^3$ (Figure 3). This decline appears to be real and may be due to one or more of several factors: a) the 50% reduction in hydrocarbon emissions mandated for new cars; b) the Stage II vapor recovery controls recently in effect; c) a reduction in benzene content in gasoline down to the 1% mandated in the 1990 Clean Air Act Amendments.

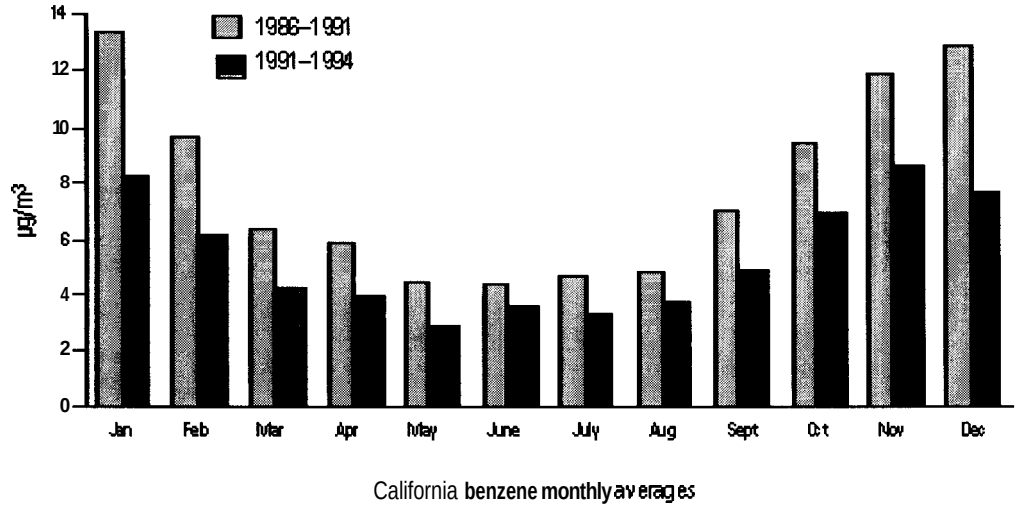

Figure 4. California benzene monthly averages. Seasonal variation in outdoor benzene at about 20 sites in California. Higher values in winter may be due to seasonally varying gasoline formulations, and perhaps to increased frequency of atmospheric inversions. The decline in benzene concentrations over the past few years is consistent over all seasons. Source: California Air Resources Board, data from all (about 20) sites.

The California database also allows analysis of seasonal variation. A clear sinusoidal curve is apparent, with winter values about twice summer values (Figure 4). This may be due to changes in the blend of the gasoline toward greater volatility in the winter or to increased likelihood of inversions during the winter.

The mean personal, indoor, and outdoor values of benzene measured in these more recent studies are

compared in Table 4.

Table 4. Mean benzene concentrations ($\mu g/m^3$) reported in recent studies.

Reference	Location	n	Personal	Indoor	Outdoor
Goldstein et al., 1992 (25)	Alaska	112	24	20	a
Sheldon et al., 1991 (24)	California	120	5.0	4.0	1
Heavner et al., 1994 (26)	Ohio	49	3.2		
Brown and Crump 1996 (30)	England	173		a	5
Wilson et al., 1993 (28)	California	161		8.3	6
Fellin and Otson, 1993 (27)	Canada	754		5.4	
Dann (unpublished data)	Canada	586			4
CARB 1989–1992 (31)	California	3000			7
CARB 1993–1994 (31)	California	1000			4

In-Vehicle Studies

The largest study of in-vehicle benzene exposure continues to be the 200-trip study (32) of Los Angeles commuters carried out in the summer and winter seasons. This study found an average benzene exposure of 13 ppb (40 $\mu g/m^3$) for commuters during rush hour, on the order of 5 times the concentration measured at a fixed outdoor site.

A small study in North Carolina (33) also showed in-vehicle concentrations 3 to 8 times background ambient levels. **A** second small study in Boston (34) resulted in passenger levels 1.5 times roadway levels on an interstate highway.

More recently, a study (35) of benzene levels in two-passenger vehicles during typical commutes in the New Jersey-New York area resulted in measured exposures of 9 to 12 $\mu g/m^3$ in suburban and turnpike conditions, and 26 $\mu g/m^3$ in the Lincoln Tunnel. The author stated that the concentrations during the commutes to New York City were about 10 times the ambient background concentration measured the same day in suburban New Jersey.

Unfortunately, none of the studies measured the benzene concentration in the gasoline used, so it is not possible to determine whether the lower concentrations in the later studies might be due to lower amounts of benzene in gasoline.

Gasoline Spill Study

A study **of** exposure to benzene while showering with gasoline-contaminated groundwater (36) was carried out in a home in North Carolina. The ground water had a measured benzene concentration of 292 μg/liter, well above the U. **S.** EPA's Maximum Contaminant Level of 5 μg/liter. Three 20-min showers on consecutive days resulted in peak shower-stall concentrations of 800 to 1670 $\mu g/m^3$. Bathroom concentrations reached 370 to 500 $\mu g/m^3$, and concentrations in the remainder of the house peaked (0.5-1 hr later) at 40 to 140 $\mu g/m^3$. The inhalation dose during the 20-min shower ranged from **80** to 100 μg. **A** dermal dose of 160 μg was also calculated, using measured breath concentrations. The combined dose of about 250 μg from the 20-min shower is roughly equal to the mean total daily inhalation dose of about 200 μg for all nonsmokers in the TEAM study (assuming 15 $\mu g/m^3$ X 14 m^3/day alveolar inspiration).

Body Burden

Benzene in the blood of 883 persons was measured (37) as part of the National Health and Nutrition Examination Survey (NHANES 111). These blood concentrations were compared with the breath concentrations measured in about 800 persons in the TEAM studies of the 1980s. Since the TEAM study measurements were made using mixed breath, the breath values were multiplied by 10/7 to account for a dead space estimated at 30% of the volume of an inhaled breath. Theoretically, one might expect that if

the two populations are comparable, the ratio of blood to alveolar air concentrations for corresponding percentiles should remain constant at the magnitude of the blood/air partition coefficient for benzene, for which several estimates ranging between 7 and 10 have been made. However, the actual observed ratio of blood to alveolar air concentrations appears to decrease with increasing concentrations, from **24** and **33** at the 16th and 25th percentiles through a range of 11 to **8** at progressively higher percentiles (Table 5). This is similar to the observation **(38)** of a blood/breath ratio of about **20/38** for an unexposed population of nonsmoking nurses, while the ratio for an occupationally exposed cohort **of** smokers was about **7.7.** Both these findings may be explained by the possibility suggested by Travis and Bowers **(39)** that at low concentrations, a saturable blood component (e.g., proteins) binds a limited amount of benzene, making it unavailable for distribution throughout the body or elimination in breath. Travis and Bowers estimated the capacity of the blood proteins to be **90** ng/liter, based on the observations of Perbellini et al. **(38).** They also estimated the plasma partition coefficient to be **9.0.** Adding the NHANES/TEAM data to those of Perbellini et al. and adjusting the Travis/Bowers model to fit all the data, one arrives at a lower estimate of blood capacity of **30** ng/liter, and a slightly lower plasma partition coefficient of **8** (Figure 5).

Table 5. Breath and blood concentrations and blood/breath ratios at selected percentiles from all TEAM study sites (n = 800) and from the NHANES III population (n = 883).

Percentile	Breath, ng/liter	Blood, ng/liter	Blood/breath ratio
16th	0.63	15	24
25th	1.1	38	35
75th	4.58	166	10.6
90th	33.7	324	9.6
95th	58.4	477	8.2
99th	101	807	8.0
Median	5.8	61	11
Mean	13.1	131	10
Max	330	1880	5.7

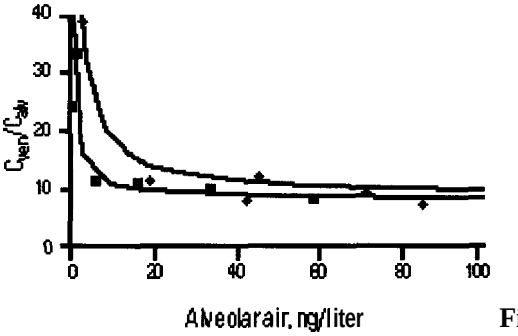

Figure 5. The upper curve is a model by Travis and Bowers (39) fitted to venous blood/alveolar air (C_{ven}/C_{alv}) ratios observed by Perbellini et al. (38). The model assumes that some benzene is bound by proteins in the blood, with a maximum capacity of 90 ng/liter. The lower curve is an adjusted model to fit both Perbellini's observations (◆) and the blood/breath ratios (■) calculated from corresponding percentiles of the NHANES blood measurements on 883 persons and the TEAM study breath measurements on about 800 persons. The adjusted model employs a somewhat smaller maximum capacity of 30 ng/liter and a slightly lower estimate of the plasma partition coefficient [8 compared to the value of 9 estimated by Travis and Bowers (37)].

Concentrations in Food

There were reports in the 1970s of benzene being found at ppm levels in some foods such as eggs (40). However, in a special study that was part of the TEAM pilot study in **1980,** breath measurements before and after eating eggs showed no increase in benzene. Also, no effect on benzene levels in breath from eating eggs or any other food item could be discerned from regressions on all participants in the main TEAM study, using the participants' responses to a detailed questionnaire on food intake. It is possible that minor levels of benzene in foodstuffs could still have been present and not detected in breath due to efficient metabolization by the liver, which receives materials from the gut directly before they enter the blood stream. However, it was thought that major concentrations in food would be detectable in breath; since they were not, it was concluded that food and beverages were an unimportant pathway for benzene exposure.

Two recent studies of benzene levels in foods have confirmed that conclusion by finding negligible quantities in nearly all foods measured. In one study by the **U.S.** Food and Drug Administration (FDA), more than 50 foods were analyzed for benzene (41). Most of these were under **2** ng/g ppbw (parts per billion by weight) benzene. Exceptions included strawberry preserves **(38** ng/g), taco sauce **(9** and 22 ng/g), duck sauce (7 ng/g), and barbecue sauce (5 ng/g). The authors speculated that the added benzoates and ascorbates in these foods might react to form benzene; thus, if either one or the other were removed, the benzene might no longer be formed. In a second study (42), 57 foods were measured, with only

shelled peanuts and fried eggs giving positive results, each at **30** ng/g, again far below the parts per million (ppm) levels previously reported. A recent Canadian review of benzene exposures **(43)** concluded that food and drinking water each contributed only about 0.02 µg/kg benzene per day compared to a total intake of **2.4** µg/kg per day from airborne exposures **(3.3** µg/kg/day if exposed to cigarette smoke). Thus, airborne exposure accounts for **98** to **99%** of total benzene intake for Canadian nonsmokers.

Discussion

The general finding from previous studies that personal exposures to benzene exceed indoor air concentrations, which in turn exceed outdoor air concentrations, has been confirmed by the more recent studies.

Two of the three personal monitoring studies mentioned above had somewhat lower mean personal exposures to benzene than had previously been reported. One such study **(24)** was in a small rural community in California, which also had a lower mean outdoor benzene value **(1.2** µg/m^3) than has been previously reported. The second study **(26)** included only **3-hr** exposures in the evening at home; to the extent that all other personal exposure studies included time spent in vehicles, where benzene exposures have been shown to range up to **40** µg/m^3, such a study limited to the home microenvironment might be expected to produce smaller personal exposures. Therefore, both of these studies would be expected to be at the low end of benzene exposures.

On the other hand, the outdoor concentrations of about 5 µg/m^3 in Valdez were similar to outdoor concentrations in the various TEAM study sites, but the indoor and personal concentrations (20 and **24** µg/m^3) were considerably greater than in all TEAM study sites except for Los Angeles in the winter. It may be speculated that persons in frontier-type situations make more use of gasoline-powered instruments such as chain saws, snow blowers, and snowmobiles than persons in urban communities. It may also be that the requirements for warming up automobiles for extended periods, and the larger amounts of benzene that are found in Alaskan and Canadian gasoline blends, led to higher exposures from attached garages and driving.

Considering that the TEAM Studies showed a range of benzene exposures from **7** to **29** µg/m^3 **(16),** the range observed since **1990** of **3.2** to **24** µg/m^3 provides no firm evidence as yet for a downward trend in benzene exposures.

A second finding from previous studies, that benzene levels were increased in homes with smokers, was also replicated. The new study **(26)** found a significant increase of **1.6** µg/m^3, which is less than the increases of **3.5** and **4.5** µ ALT="micro" g/m^3 found in the TEAM and West German studies (15,23) but represents about the same percentage increase of 50 to **67%** compared to nonsmoking homes. The Windsor study **(29)** that found increased benzene concentrations in bingo halls and taverns, where smoking is prevalent, might also be viewed as confirming the effect of smoking on indoor benzene concentrations.

Several small studies replicated the findings of an earlier major study in Los Angeles that showed increased benzene exposures while driving. The later studies appeared to involve much smaller exposures but also had much smaller outdoor concentrations, so the ratio of personal exposure to outdoor concentration continued to be in the neighborhood of 5 to 10. The smaller concentrations could be due to differences in location (Los Angeles vs North Carolina and New Jersey-New York) but could also reflect reductions in the amount of benzene in the gasoline. The results of the national fuel survey carried out by the American Automobile Manufacturers Association **(44)** indicate that the goal of 1% benzene in gasoline set by the **1990** Clean Air Act Amendments has been very nearly met, with the average for premium, intermediate, and regular gasoline for the winter of **1994** to **1995** being 0.9, 0.9, and 1.1% by volume, respectively. This is a considerable reduction compared to the **2** to **3%** levels that were probably common during the large California in-vehicle study. However, it should also be noted that the amount of benzene in the exhaust may be related only weakly to the amount of benzene in the

gasoline. One study (45) indicated that exhaust benzene remained unchanged at about 5% of total hydrocarbon emissions whether the gasoline burned contained 1 or 3% benzene by volume.

A large number of food groups were tested but found to contain negligible amounts of benzene. This corroborated the conclusions of the TEAM studies, which found no evidence of food contributions to body burden of participants.

Although nearly all the studies reviewed here have been more in the nature of confirmatory studies rather than breaking new ground, the study of benzene exposures while showering in gasoline-contaminated water presented new data of considerable value. The 20-min exposure from this source was the same order of magnitude as a full day's exposure to benzene for a typical nonsmoker. However, a smoker (of more than five cigarettes a day) using the same gasoline-contaminated water would still get most of his or her exposure through smoking--an indication of the extensive exposure encountered by some 43 million U.S. citizens. Since the number of persons affected by such spills is very small, the effect on the national exposure budget for benzene is also very small.

Apart from these presumably very rare gasoline spill situations, there may be a larger number of cases where well water is contaminated by benzene at low concentrations. A number of studies have reported finding benzene at levels on the order of 5 ng/liter (ppb) in surface and well waters. However, these levels correspond to a daily intake of <10 ng benzene, assuming 2 liters of water drunk daily. This amount is only 0.5% of the average daily intake for nonsmokers of 200 ng from air. Thus, it is concluded that the effect of contaminated water on total benzene intake is negligible.

It may fairly be asked whether any of the differences observed in various studies at different locations and times are dependent on the different methods employed. The TEAM studies employed Tenax-GC with active pumping, as did the later Woodland and Valdez studies; thus, all the studies using personal monitors used very similar or identical methods.

The indoor air studies in England and Canada employed passive (diffusive) samplers with extended monitoring periods. The English investigators performed a number of tests on the effect of extended sampling on the net uptake of different VOCs by the Perkin-Elmer sorbent tubes containing Tenax-TA. They found that the more volatile VOCs such as benzene and toluene had net diffusive uptakes that declined over time, probably because of back diffusion off the tubes. For sorbent tubes exposed to a concentration of 2500 $\mu g/m^3$ toluene, the diffusive uptake rate declined to 71% of the ideal after 7 days and 54% after 28 days. For benzene, the net diffusive uptake was 30% after 28 days. For the less volatile compounds such as xylenes, decane, and trimethylbenzenes, the sampling rate stayed nearly constant over the 28-day period. Therefore, given a month-long sampling period, an average uptake rate can be chosen for any given chemical; however, for chemicals more volatile than the xylenes, this rate will only be an average value fkom a declining curve. This means that the early part of the sampling period for these volatile compounds may be underrepresented because of back diffusion losses from the substrate. However, if the average sampling rate is correctly chosen, this would not cause a bias, only greater variability than exists in fact.

The Canadian investigators used commercial samplers with a charcoal sorbent (the 3M organic vapor badge), followed by solvent desorption using carbon disulfide (CS_2). Carbon disulfide is well known to have a contamination problem with benzene; however, the Canadian investigators developed their own methods for cleaning the CS_2, and report no serious problems with contamination. It is not clear whether they used the ideal sampling rate for benzene or determined an effective sampling rate for the 1-week sampling period.

Both Tenax and charcoal have problems with nonzero background benzene concentrations. The Tenax must be carefully cleaned to avoid such problems. The early TEAM studies had high and variable backgrounds of benzene equivalent to about 5+/-3 $\mu g/m^3$ on the Tenax cartridges. This would lead to decreased precision, although since average backgrounds were subtracted from each raw datum, it is not clear whether any bias remained. The later TEAM studies reduced backgrounds to the equivalent of about 1+/-0.5 $\mu g/m^3$, reducing the uncertainty in the estimated exposures considerably. The charcoal

badges also have high backgrounds of benzene; however, the extended sampling period should have provided sufficient benzene to reduce the background effect. Therefore it is unlikely that a significant bias or lack of precision has affected the personal, indoor, or outdoor air concentrations of benzene.

The initial breath measurements in the TEAM New Jersey study of 1981 employed Tedlar (Nutech Corp., Durham, NC) bags stored in a van. Because of the possibility that exhaust vapors had penetrated the Tedlar bags, future studies passed pure helium over the bags at positive pressure to remove this possible source of contamination. Both higher exposures and higher breath concentrations were noted in this period, but about 45% of the New Jersey participants were smokers compared to 22% of the California participants; thus the higher breath concentrations observed in the New Jersey participants may well have been due to the higher smoking rate.

References

1. Wallace LA. The TEAM Study: Summary and Analysis. Vol 1. EPA 600/6-87/002a. NTIS PB 88-100060. Washington:U.S. Environmental Protection Agency, 1987.

2. Zweidinger R, Erickson M, Cooper S, Whitaker D, Pellizzari ED, Wallace LA. Direct Measurement of Volatile Organic Compounds in Breathing-zone Air, Drinking Water, Breath, Blood, and Urine. NTIS PB-82-186-545. Washington:U.S. Environmental Protection Agency, 1982.

3. Wallace LA, Zweidinger R, Erickson M, Cooper S, Whitaker D, Pellizzari ED. Monitoring individual exposure: measurement of volatile organic compounds in breathing-zone air, drinking water, and exhaled breath. Environ Int 8:269-282 (1982).

4. Wallace LA, Pellizzari ED, Hartwell T, Rosenzweig R, Erickson M, Sparacino C, Zelon H. Personal exposure to volatile organic compounds. I: Direct measurement in breathing-zone air drinking water, food, and exhaled breath. Environ Res 35:293-319 (1984).

5. Wallace LA. Pellizzari ED. Hartwell T, Sparacino C. Sheldon L. Zelon H. Personal exposures, indoor-outdoor relationships and breath levels of toxic air pollutants measured for 355 persons in New Jersey. Atmos Environ 19:1651-1661 (1985).

6. Wallace LA, Pellizzari E, Hartwell T, Whitmore R, Sparacino C, Zelon H. Total Exposure Assessment Methodology (TEAM) Study: personal exposures, indoor-outdoor relationships, and breath levels of volatile organic compounds in New Jersey. Environ Int 12:369-387 (1986).

7. Wallace LA, Pellizzari ED, Hartwell TD, Sparacino C, Whitmore R, Sheldon L, Zelon H, Perritt R. The TEAM study: personal exposures to toxic substances in air, drinking water, and breath of 400 residents of New Jersey, North Carolina, and North Dakota. Environ Res 43:290-307 (1987).

8. Pellizzari ED, Perritt K, Hartwell TD, Michael LC, Whitmore R, Handy RW, Smith D, Zelon H. Total Exposure Assessment Methodology (TEAM) Study: Elizabeth and Bayonne, New Jersey; Devils Lake, North Dakota; and Greensboro, North Carolina. Vol II. Washington:U.S. Environmental Protection Agency, 1987.

9. Pellizzari ED, Perritt K, Hartwell TD, Michael LC, Whitmore R, Handy RW, Smith D, Zelon H. Total Exposure Assessment Methodology (TEAM) Study: selected communities in northern and southern California. Vol III. Washington:U.S. Environmental Protection Agency, 1987.

10. Wallace LA, Pellizzari ED, Hartwell TD, Whitmore R, Perritt R, Sheldon LS. The California TEAM study: breath concentrations and personal exposures to 26 volatile compounds in air and drinking water of 188 residents of Los Angeles, Antioch, and Pittsburg, CA. Atmos Environ 22:2141-2163 (1988).

11. Pellizzari ED, Michael LC, Perritt R, Smith DJ, Hartwell TD, Sebestik J. Comparison of Indoor and Outdoor Toxic Air Pollutant Levels in Several Southern California Communities. Final Report, Contract 68-02-4544. Research Triangle Park, NC:U.S. Environmental Protection Agency, 1988.

12. Wallace LA, Nelson WC, Ziegenfus R, Pellizzari E. The Los Angeles TEAM study: personal exposures indoor-outdoor air concentrations, and breath concentrations of 25 volatile organic compounds. J Expos Anal Environ Epidemiol 1(2):37-72 (1991).

13. Lioy PJ, Wallace LA. Pellizzari ED. Indoor/outdoor and personal monitor and breath analysis relationships for selected volatile organic compounds measured at three homes during 'New Jersey TEAM--1987. J Expos Anal Environ Epidemiol 1(1):45-61 (1991).

14. Entz R, Thomas K, Diachenko G. Residues of volatile halocarbons in food using headspace gas chromatography. J Agric Food Chem 30:846-849 (1982).

15. Wallace LA, Pellizzari ED, Hartwell T, Perritt K, Ziegenfus R. Exposures to benzene and other volatile organic compounds from active and passive smoking. Arch Environ Health 42:272-279 (1987).

16. Wallace LA. The exposure of the general population to benzene. Cell Biol Toxicol 5:297-314 (1989).

17. Wallace LA. The Total Exposure Assessment Methodology (TEAM) Study: an analysis of exposures, sources, and risks associated with four chemicals. J Am Coll Toxicol 8:883-895 (1989).

18. Wallace LA, Pellizzari ED, Hartwell TD, Davis V, Michael LC, Whitmore RW. The influence of personal activities on exposure to volatile organic compounds. Environ Res 50:37-55 (1989).

19. Wallace LA. Major sources of benzene exposure. Environ Health Perspect 82:165-169 (1989).

20. Wallace LA. Major sources of exposure to benzene and other volatile organic compounds. Risk Anal 10:59-64 (1990).

21. Thomas KW, Pellizzari ED, Clayton CA, Perritt RL, Dietz RN, Goodrich RW, Nelson WC, Wallace LA. Temporal variability of benzene exposure for residents in several New Jersey homes with attached garages or tobacco smoke. J Expos Anal Environ Epidemiol 3:49-73 (1993).

22. Lebret E, Van de Weil HJ, Noij D, Boleij JSM. Volatile hydrocarbons in Dutch homes. Environ Int 12(1-4):323-332 (1986).

23. Krause C, Mailahn W, Nagel R, Schulz C, Seifert B, Ullrich D. Occurrence of volatile organic compounds in the air of 500 homes in the Federal Republic of Germany. In: Proceedings of the 4th International Conference on Indoor Air Quality and Climate, Vol 1 (Seifert B, Esdorn H, Fischer M, Ruden H, Wegner J, eds). Berlin:Institute for Soil, Water, and Air Hygiene, 1987; 102-106.

24. Sheldon LS, Clayton A, Jones B, Keever J, Perritt R, Smith D, Whitaker D, Whitmore R. Indoor Pollutant Concentrations and Exposures. Final Report. Sacramento:California Air Resources Board, 1991.

25. Goldstein BD, Tardiff RG, Baker SR, Hoffnagle GF, Murray DR, Catizone PA, Kester RA, Caniparoli DG. Valdez Air Health Study. Anchorage, AL:Alyeska Pipeline Service Co., 1992.

26. Heavner DL, Morgan WT, Ogden MW. Determination of volatile organic compounds and ETS apportionment in 49 homes. Environ Int 21:3-21 (1996).

27. Fellin P, Otson R. Seasonal trends of volatile organic compounds (VOCs) in Canadian homes. In: Indoor Air '93: Proceedings of the 6th International Conference on Indoor Air Quality and Climate, Vol 1 (Jaakola JJK, Ilmarinen R, Seppänen O, eds). Espoo, Finland:Helsinki University of Technology, 1993;339-343.

28. Wilson AL, Colome SD, Tian Y. California Residential Indoor Air Quality Study. Vol 1: Methodology and Descriptive Statistics. Irvine, CA:Integrated Environmental Services, 1993.

29. Bell RW, Chapman RE, Kruschel BD, Spencer MJ. Windsor Air Quality Study: Personal Exposure Survey Results ISBN 0-7778-3492-8. Toronto:Ministry of Environment and Energy, 1994.

30. Brown VM, Crump DR. Volatile organic compounds. In: Indoor Air Quality in Homes: Part I. The Building Research Establishment Indoor Environment Study (Berry RW, Brown VM, Coward SKD, Crump DR, Gavin M, Grimes CP, Higham DF, Hull AV, Hunter CA, Jeffery IG, Lea RG, Llewellyn JW, Raw GJ, eds). London:Construction Research Communications, 1996.

31. California Air Resources Board. Ambient Air Quality Data Base. Technical Support Division. Sacramento, CA:California Air Resources Board.

32. SCAQMD. In-vehicle Characterization Study in the South Coast Air Basin. Los Angeles:South Coast Air Quality Management District, 1989.

33. Chan C-C. Commuter exposure to volatile organic compounds. PhD dissertation. Boston:Harvard University, 1990.

34. Chan C-C, Ozkaynak H, Spengler JD, Sheldon L. Driver exposure to volatile organic compounds, CO, ozone, NO_2 under different driving conditions. Environ Sci Technol 25:964-972 (1991).

35. Weisel C, Lawryk NJ, Lioy PJ. Exposure to emissions from gasoline within automobile cabins. J Expo Anal Environ Epidemiol 2(1):79-96 (1992).

36. Lindstrom AB, Highsmith VR, Buckley TJ, Pate WJ, Michael L. Gasoline-contaminated ground water as a source of residential benzene exposure: a case study. J Expos Anal Environ Epidemiol 4:(2)183-196 (1994).

37. Ashley DL, Bonin MA, Carkinali L, McCraw JM, Wooten JV. Blood concentrations of volatile organic compounds in a nonoccupationally exposed US population and in groups with suspected exposure. Clin Chem 40:1401-1404 (1994).

38. Perbellini I, Faccini GB, Pasini F Cazzoli F, Pistoia S, Rosellini R, Valsecchi M, Brugnone F. Environmental and occupational exposure to benzene by analysis of breath and blood. Br J Ind Med 45:345-352 (1988).

39. Travis CC, Bowers JC. Protein binding of benzene under ambient exposure conditions. Toxicol Ind Health 5(6):1017-1024 (1989).

40. MacLeod AJ, Cave SJ. Volatile flavor compounds of eggs. J Sci Food Agric 26:351-360 (1975).

41. McNeal TP, Nyman PJ, Diachenko GW, Hollifield HC. Survey of benzene in foods by using headspace concentration techniques and capillary gas chromatography. J Assoc Organ Analyt Chem Int 76:(6)1213-1219 (1993).

42. American Petroleum Institute. Analysis of Foods for Benzene. Washington:API, 1992.

43. Government of Canada. Priority Substances List Supporting Document: Benzene. Ottawa:Health Canada, 1993.

44. American Automobile Manufacturers Association. National Fuel Surveys: Gasoline and Diesel Fuel—Winter 1995. Detroit:AAMA, 1995.

45. Sawyer, RF. Trends in auto emissions and gasoline composition. Environ Health Perspect 101(Suppl 6):5-12 (1993).

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