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Breath concentration as an index of the health risk from benzene

Studies on the accumulation and clearance of inhaled benzene

by Maths Berlin, MD,[1] John C Gage, PhD,[1] Bo Gullberg, BSc,[2] Stina Holm, BSc,[1] Pernilla Knutsson, Chem Eng,[1] Anders Tunek, BSc [1]

BERLIN M, GAGE JC, GULLBERG B, HOLM S, KNUTSSON P, TUNEK A. Breath concentration as an index of the health risk from benzene: Studies on the accumulation and clearance of inhaled benzene. *Scand j work environ health* 6 (1980) 104—111. Human subjects were exposed to known concentrations of benzene in air for single and repeated daily periods. The breath concentrations measured during repeated exposures approached a maximum after 3 d, and this phenomenon indicated that the tissues were approaching saturation under the experimental conditions. The breath concentrations measured after exposure indicated an initial rapid clearance of benzene with a half-time of 2.6 h, followed by a slower phase with a half-time of 24 h. The decay in breath concentration after prolonged occupational exposure appeared to be slower; the difference between the laboratory and industrial studies was, however, not significant. The hygienic significance of these results was discussed, and it was recommended that control measures be employed when a morning breath concentration exceeds 10 ppb.

Key terms: accumulation, benzene, benzene tissue index, breath concentration, clearance, occupational exposure.

Published evidence indicates that the most serious risks from occupational exposure to benzene are aplastic anemia and leukemia, which appear to be associated with prolonged rather than acute exposure (2). The limited knowledge available on the occupational dose-response relationship with respect to benzene derives from the clinical examination of men working in environments periodically subjected to air analysis. Such measurements are not very satisfactory for assessing the cumulative exposure to a volatile liquid which may produce transient and relatively high concentrations in the work environment.

The application of breath analysis to the assessment of integrated exposure has been proposed for a variety of volatile solvents, including benzene (4, 6, 7). The analytical methods used for benzene in these investigations have not, however, been found to be sufficiently sensitive to be suitable for the breath concentrations associated with the lower hygienic air standards now under consideration by national and international authorities, and a more sensitive method has been developed in this Institute for this purpose (3).

Before the health risks from benzene can be properly assessed, it is necessary to acquire information on the retention of benzene in tissues after single and repeated exposures and knowledge concerning the rate of excretion from tissues after removal from exposure. Some information can be obtained from men occupationally exposed to benzene, but it is also necessary

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to include laboratory studies with human volunteers exposed to known benzene concentrations for measured periods. Such investigations also permit an estimate of the relation between breath concentration and previous exposure to benzene; this information will enable an index of exposure based on breath analysis to be supported by toxicologic data obtained under known environmental conditions and to be related to currently proposed air standards.

Methods

Determination of benzene in air and exhaled breath

The methods used were those described by Gage et al (3). The gas chromatographic peaks were calibrated by means of three benzene standards prepared by injecting amounts of an aqueous solution of benzene into equilibrium tubes, so that the peaks produced bracketed that from the sample under investigation.

Initially, the results from breath samples were rather erratic, and this phenomenon was traced to contamination of the breath sample by volatile compounds present in the plastic lining of the bag, particularly with a new bag. Therefore, before a bag was used, it was placed in an oven at 55°C and cleaned by passing a current of air through it for 2 h. During subsequent use the bag was cleaned out between each sampling by flushing with nitrogen.

The possibility of a significant loss of benzene into the polyethylene lining of the bag was examined. The bag was half-filled with air, a small volume of air containing a known amount of benzene was injected, the bag was then filled with air, and the contents were mixed and passed through a sampling tube. A volume of the dilution of benzene vapor in air, identical with that injected into the bag, was injected into an equilibrium tube. The comparison of the peaks obtained in these two experiments, shown in table 1, enabled an estimate to be made of the loss of benzene into the bag. A similar series of experiments was made with the bag filled with exhaled breath instead of air.

In order to study whether any loss of benzene occurred by absorption into the rubber wall of the face mask, we prepared a known concentration of benzene in a bag in the previously described manner and pumped it through the face mask and then through a silica gel sampling tube.

To confirm that no significant loss occurred in the silica gel sampling tubes during storage, we sampled known benzene atmospheres in pairs of tubes. One tube of a pair was examined at once, and the other was closed with a silicone rubber stopper and examined one week later. The results are shown in table 2.

Analysis by gas chromatography/mass spectrometry

Samples of breath from two smokers and two nonsmokers were analyzed both by the preceding method and by combined gas-chromatography/mass spectrometry (GC/MS). For the latter a Varian 2700 gas chromatograph coupled with a Varian 311A mass spectrometer was used. The

Table 1. Calculated concentration of benzene after the addition of known amounts to air or breath in a sampling bag.

	Benzene (ppm)		
	In bag	Control [a]	Difference (%)
Air	0.185	0.198	+ 6
	0.016	0.021	+ 21
	0.019	0.016	— 19
	0.003	0.003	0
Breath	0.010	0.010	0
	0.008	0.009	+ 11
	0.001	0.001	0

[a] Identical amount of benzene added directly to sampling tube.

Table 2. Loss of benzene (ppm) in sample tube.

Benzene in air sample (ppm)		Change (%)
Immediately after sampling	After 7 d	
0.001	0.001	0
0.003	0.003	0
0.011	0.013	+ 13.6
0.133	0.131	+ 2
0.77	0.73	— 5

25-m capillary column was coated with SE30 at 45°C; the injector and detector temperature was 150°C. A 40-μl sample of headspace air was injected, and the single ion with M/e 78 was measured.

Statistical methods

The statistical methods used were the analysis of covariance and weighted nonlinear regression (9, 10).

Laboratory studies

For the laboratory studies, human volunteers were exposed one or two at a time in a cylindrical chamber 2 m in diameter and 2 m high. The chamber was lined with a polyethylene sheet. An air flow of 40 m^3/h was maintained in the chamber by means of a fan in the air entry duct, and the flow was measured with an anemometer.

The required concentration of benzene vapor in the chamber was obtained with the injection of liquid benzene at a known rate through a fine polyethylene tube which passed into the air entry duct and touched onto a piece of filter paper to aid evaporation. Samples of the atmosphere in the chamber were taken by the insertion of a silica gel sampling tube through an aperture in the wall of the chamber.

With a benzene injection rate of 1 ml/h the expected benzene concentration in the region of 9 ppm was measured in the chamber. With two human subjects in the chamber the concentration fell to about one-third of this value. In the following experiments the injection rate was adjusted to give approximately the desired air concentration.

Experiment 1. Five male and five female subjects between the ages of 20 and 25 a were exposed for a single period. The benzene concentrations and the duration of the experiments are shown in table 3. Breath samples were taken for analysis at the end of the exposure period, and also 15 min and 16 h later.

Experiment 2. Four male nonsmokers were exposed to a benzene concentration for five consecutive daily 6-h periods. Details of the exposure are given in table 4.

Occupational exposure

Duplicate breath samples were taken from five men, ranging in ages between 25 and 60 a and employed in the distillation plant of a gasworks. The samples were taken each morning over a period of 7 d away from work, the first sample being taken soon after the cessation of work.

Results

Loss of benzene to the sampling bag

The analytical results obtained when a known amount of benzene was injected into air or exhaled breath in a sampling bag are shown in table 1. These results in-

Table 3. Benzene concentration in breath after a single exposure.

Sex	Exposure			Benzene concentration in breath [a] (ppm)		
	Benzene concentration (ppm)	Duration (h)	Ppm · h	0 h postexposure	0.25 h postexposure	16 h postexposure
M	3.8	4	15.2	0.07	0.04	0
F	3.8	4	15.2	0.05	0.02	0.001
F	12.5	2	25		0.2	0.004
M	12.5	2	25	0.17	0.18	0.004
F	11.3	3	34	0.20	0.13	0.007
F	11.3	3	34	0.18	0.14	0.012
M	12.5	4	50	0.19	0.10	0.009
M	12.5	4	50	0.23	0.11	0.004
M	11.5	5	57.5	0.35	0.19	0.008
F	11.5	5	57.5	0.32	0.16	0.008

[a] A measured background value has been subtracted.

dicate that there was no systematic loss of benzene into the bag.

Loss of benzene to the face mask

No significant decrease in concentration was observed when air containing 0.1 and 0.001 ppm of benzene was passed through the face mask.

Loss to the storage of sampling tubes

The results in table 2 indicate that any loss in the sampling tube after storage for one week was of little importance.

Normal values

Table 5 shows values for benzene in breath measured from two nonsmokers and two smokers. With three of these determinations there was close agreement between the results obtained by the method (gas-liquid chromatography) of Gage et al (3) and by gas-liquid chromatography combined with mass spectrometry. Breath concentrations of nonexposed nonsmokers are generally in the range 0.001 to 0.002 ppm. When a cigarette was burnt under normal conditions of smoking, 64 to 107 μg of benzene could be measured in the smoke.

Benzene exposure and breath concentration — single exposures

The concentration of benzene in exhaled breath, measured 0, 0.25 and 16 h after a range of daily exposures to benzene, are shown in table 3. Before the exposure a breath analysis was made, and this background value was subtracted from the figures in table 3. In most cases the background did not exceed 0.001 ppm, although in one case it reached 0.005 ppm.

Benzene exposure and breath concentration — repeated exposure

Table 6 shows the progressive build-up of benzene in the breath of two pairs of subjects exposed to benzene for five consecutive daily periods as indicated in table

Table 4. Laboratory exposure to benzene during 5 d.

Day	Subject 1	2	3	4
	(Dose expressed as ppm · h)			
1	15	18	29	25
2	13	41	44	46
3	27	20	36	37
4	26	24	45	45
5	31	28	57	39
Total	132	131	211	192
Mean	26.4	26.2	42.2	38.4

Table 5. Background values for smokers and nonsmokers, determined by gas-liquid chromatography (GLC) and gas-liquid chromatography/mass spectrometry (GLC/MS).

	Breath concentration (ppb) GLC	GLC/MS
Nonsmokers	1.1	1.1
	1.5	1.7
Smokers	5.8	5.7
	3.3	1.5

Table 6. Build-up of morning concentration of benzene in breath during a 5-d exposure period.

Day [a]	Morning breath concentration (ppb) Subject 1	Subject 2	Subject 3	Subject 4
1	4		10.7, 12.5	9.5, 14
2	8	8	9	13, 35
3	14, 17	11, 21	21	17, 34
4	12, 21	21, 27	13, 23	26, 29
5	16.5, 21	17, 17	9, 22	24, 30

[a] Mean daily benzene exposure (ppm · h): subject 1 = 26.4, subject 2 = 26.2, subject 3 = 42.2, and subject 4 = 38.4.

Table 7. Decay in breath concentration of benzene (ppb) after repeated exposure. (see table 6)

Subject [a]												
1			2			3			4			
221,	301	(0)	171		(0)							
16.5,	21	(20)	17,	17	(17)	19,	22	(19)	24,	30	(19)	
9,	14	(27)	6,	7	(24)	14.5,	17.8	(25)	14,	17	(25)	
5,	7	(44)	6,	7	(41)	18.5,	21.6	(41)	34,	34	(39)	
6,	8	(55)	6,	6	(52)	9.3,	10.4	(54)	13,	15	(51)	
5,	6	(69)	3.6,	4.7	(66)	5.5,	7.3	(65)	8.8,	9.1	(63)	
4,	6	(74)	3,	4	(72)	3.6,	4.8	(76)	5.5,	5.8	(70)	
1.4,	1.5	(91)	3.4,	3.4	(89)	2.8,	2.8	(91)	3.9,	4.5	(91)	
1.2,	2	(115)	2,	2	(113)	2.7,	3.2	(113)	1.1,	2.3	(120)	

[a] Figures in parentheses indicate hours since last exposure.

Table 8. Concentration of benzene in breath (ppb) of industrial workers after cessation of occupational exposure.[a]

Subject A			Subject B [b]			Subject C			Subject D [b]			Subject E [b]		
19,	28	(0)	17,	24	(0)	13,	14	(0)	17.4,	26	(0)	10,	11	(4.5)
7.5,	8.8	(10)	16		(9)	3,	14	(7)	11,	12.3	(7)	10,	12	(10.5)
1.3,	4	(70)	10,	11	(26)	5.2,	7.7	(76.5)	8,	9.3	(28)	7.4		(25)
			9,	9.5	(50)	6.8,	7.7	(97.5)	10.7,	13	(55)	6.2,	6.6	(50)
						4,	4.6	(123)	2.5,	3.5	(124.5)	4.5,	5.8	(73.5)
2.7,	2.7	(142)				4.5,	5	(147)				4,	5.4	(97.5)

[a] Figures in parentheses indicate hours after cessation of work.
[b] Smoker.

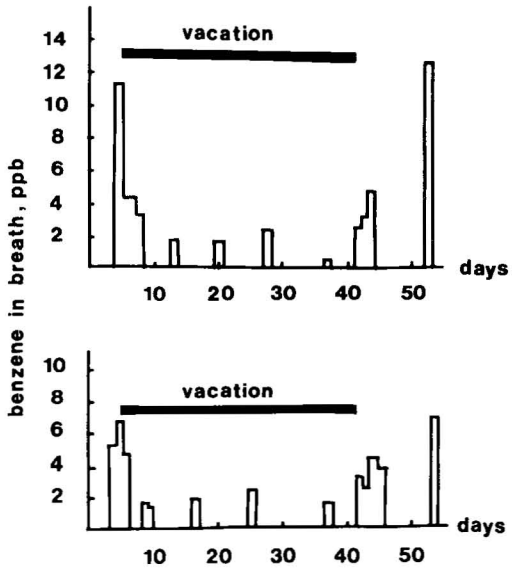

Fig 1. Breath concentrations measured from two workers in a benzene distillery before, during, and after a vacation from work.

4, breath samples being taken 16 h after each daily exposure. Breath samples were also taken at intervals after the end of these exposure periods, and the decay in concentration is shown in table 7.

Occupational exposure

The concentrations of benzene in breath samples from five industrial workers for 7 d after the cessation of exposure to benzene are shown in table 8. The breath concentrations of two other workers from the same distillery, measured before, during and after their vacation, are shown in fig 1.

Discussion

Gage et al (3) claim that their method for the determination of benzene in breath should, under favorable circumstances, provide reliable quantitative results down

to 0.005 ppm and a semiquantitative indication below this level; they recommended that such low concentrations should be confirmed by an independent method. The results in table 5 indicate that the identification of benzene has generally been reliable down to the 0.001 ppm range. In the early stages of the investigation, erratic results were encountered which could be attributed to a contamination of the air sample by volatile compounds in the plastic lining of the sampling bag. When care was taken to remove this source of interference, duplicate determinations showed an acceptable precision in the 0.005 ppm range. Tests have shown no significant losses into the bag and the face mask, nor during storage of the silica gel sampling tube.

Measurements of the concentration of benzene in breath after a single exposure to benzene (table 3), although made when the method was of relatively low precision, indicated a linear relationship between breath concentration and total exposure expressed in ppm · h at a period 16 h after the end of the exposure. The results gave a regression line with a slope of 0.00015 and a correlation coefficient of 0.65. At 0 and 0.25 h after exposure the results, shown in table 3, were not consistent with the hypothesis that the breath concentration is proportional to the total exposure; it seems more likely that the most important factor influencing the breath concentration during these short periods after exposure is the concentration of benzene in air during the exposure.

The concentration of benzene in breath after a single exposure (table 3) confirms observations from earlier investigations (4, 6, 7) that initially there is a rapid fall in the concentration and thereafter the decrease is much slower. This phenomenon is seen more clearly in the breath concentrations measured after five consecutive daily exposures to benzene (table 6), which have been collected together in fig 2 with log-linear coordinates. This graph gives a clear indication of at least two exponential elimination phases; a fast phase with a half-time of about 2.6 h and a slower phase with a half-time of about 24 h. These values are in good agreement with the half-times calculated by Sherwood (6).

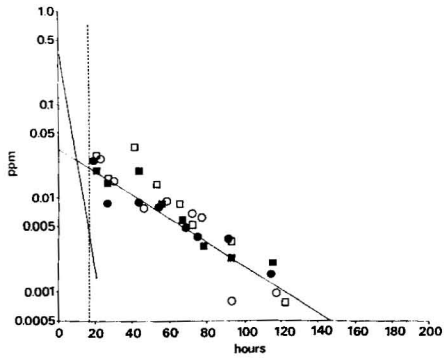

Fig 2. Decay of benzene in breath after a 5-d exposure period. Breath concentrations have been taken from table 7 (corrected for an assumed background of 0.001 ppm); the values from the lower exposure have been multiplied by a factor to make them equivalent to the higher exposure, and the pooled results are presented on log-linear coordinates. The lines show the best fit of a two-stage clearance, with half-times of 2.6 and 24 h ($y = 0.2835e^{-0.2630t} + 0.0330e^{-0.0285t}$).

Subject	Mean daily exposure (ppm · h)
1	26.4
2	26.2
3	42.2
4	38.4

While the four subjects were being exposed to benzene on five successive days, the benzene concentration in breath approached a maximum, as might be expected from a clearance half-time of 24 h (table 6, fig 2). If it is assumed that only one compartment is involved, the accumulation curve is given by an expression of the form

$$A = \alpha(1 - e^{-\beta t}),$$

where A is concentration of benzene in exhaled breath and β is the rate constant for clearance from the compartment; for a half-time of 24 h, $\beta = 0.0286$. The steady state concentration is given by the coefficient α. Curves according to these equations have been fitted to the experimental points (fig 3), and the steady-state concentrations have been calculated to be 0.017 and 0.024 ppm; the ratio of these two concentrations is 1.41, which agrees well with the ratio 1.53 for the exposure doses.

The calculation in the previous paragraph is based on the rapid equilibrium attained by volatile lipid-soluble liquids such as benzene between alveolar spaces and blood so that the concentration in

exhaled breath can be regarded as a measure of the concentration in blood. The build-up of benzene in tissues is a function of the concentration in blood and of the flow of blood through the tissues (1). The rate of filling and of emptying of a compartment is increased by an increase in vascularity or a decrease in the partition coefficient.

In our studies, samples of tidal air were collected, as it would not have been practicable under field conditions to take 4 l of alveolar air. According to Riley & Cournand (5) the physiological dead space in the lungs of healthy supine subjects ranges between 12 and 30 % of the tidal air with a mean of 20 %. In obstructive lung disease the deadspace contribution may be much higher, but no signs of this

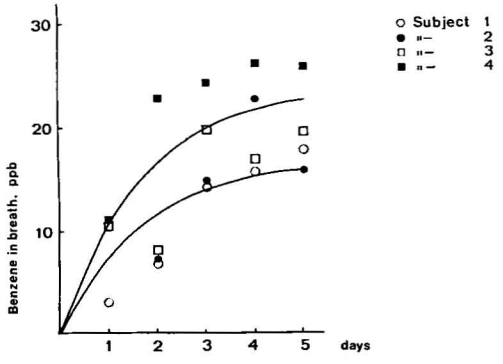

Fig 3. Accumulation curves for benzene over a 5-d period. Breath concentrations have been taken from table 6 (corrected for an assumed background of 0.001 ppm); lines indicate the best fit of theoretical accumulation curves.

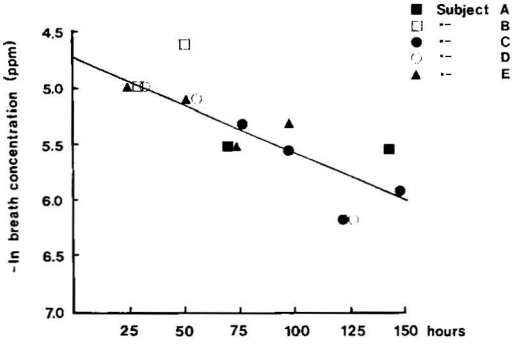

Fig 4. Decay of benzene in breath after occupational exposure. The points correspond to the individuals in table 8; the concentrations from that table have been corrected for an assumed background of 0.001 ppm, pooled to give a single set and presented on log-linear coordinates.

were present in our subjects. If tidal air measurements were used to calculate the blood concentration on the basis of an air/blood partition coefficient, the results would be an average of about 20 % low. But this variation does not invalidate the use of our methods in assessing integrated exposure, as the blood concentration can still be regarded as being approximately proportional to the tidal air concentration. The variation in physiological dead space between individuals, which is not likely to exceed ± 10 %, and the smaller individual variation undoubtedly contribute to the scatter of the results shown in the tables and figures. But this scatter is clearly dominated by other factors.

The results in tables 4 and 7 indicate that the excretion of benzene in breath immediately after exposure may be dominated by the elimination of benzene from a compartment with a short clearance time, and the concentration in breath will then be, to a large extent, influenced by recent exposure. After the clearance of this compartment, the breath concentration may follow the emptying of a compartment with a longer half-time, at a rate that indicates an integrated exposure over a longer period. These results are consistent with studies of breath concentrations after exposure to trichloroethylene (8), and they support the view that breath samples taken on the morning after exposure provide a more useful index of exposure than those taken at the end of a workshift.

Measurements of the breath concentration of industrial workers during a break in their employment in a benzene distillation plant (table 8) suggest that the decay in breath concentration, and therefore in blood concentration, is slower than that observed in laboratory studies. An analysis of covariance showed no significant difference between the slopes recorded for the five industrial workers; therefore the results were pooled to give the single log-linear graph in fig 4.

The slope of the graph in fig 4 corresponds to a clearance half-time of 75 h, or it can be interpreted with equal significance as a two-phase system with half-times of 24 and 175 h. Statistical analysis can demonstrate a significant difference between the results for fig 2 and 4, but, if

the background values likely to be encountered with smokers are subtracted from the measurements of the three subjects known to be smokers in table 8, then the significance of the difference between the two groups disappears. Additional studies are required to establish whether there is a real difference between the two groups; if a slower clearance can be established for the industrial workers, it could reflect differences in metabolism or in blood flow through the tissues. Such a difference in clearance rates would imply physiological differences between a group of industrial workers and a group of students. An alternative hypothesis implies that, if the difference between the two groups is real, it could be attributed to the presence of a slow-emptying compartment not apparent from the experimental results presented in fig 2. However, the results in fig 2 do not exclude the presence of such a phase; it has been calculated that its maximal contribution (95 % confidence interval) could be in the region of 10 %.

The results obtained with both of the experimental groups demonstrate that breath analysis is capable of providing a measure of the accumulated dose of benzene in tissues. The method is, therefore, more suitable for assessing the risk to bone marrow, the tissue most sensitive to the action of benzene, than for environmental air analysis, with its uncertainty on whether the dose absorbed is really being measured, even when a personal sampler is used. Fig 1 demonstrates the sensitivity of the method to changes in work conditions. Not only does the breath concentration fall when the men start their vacation, but it rises again as soon as they return to work.

There is some evidence that chromosome changes may be observed in men who are exposed for long periods to benzene and who excrete about 50 ppb benzene in morning breath (Berlin et al, to be published). According to fig 2, this level corresponds to a daily exposure of about 80 ppm · h, or a time-weighted average over an 8-h day of about 10 ppm. Additional studies are required to confirm these observations, but on the basis of the available evidence it seems undesirable that the morning breath concentration of an individual exposed to benzene for long periods should exceed 10 ppb. In such a case another test should be made on the following morning, and, if the individual is a smoker, no tobacco should have been smoked during the morning prior to the test. If the high breath concentration is maintained, the work environment should be examined for an escape of benzene, and, if necessary, the individual should be transferred to work without a benzene hazard until his breath concentration has returned to the normal range of background values.

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