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ALLEVIATION OF BENZENE LEUKOPENIA IN RATS SIMULTANEOUSLY EXPOSED TO TOLUENE

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ABSTRACT

Four parallel sets of dynamic flow type chambers were used to study the effect of exposure to a mixture of benzene and toluene vapours. Preliminary experiments revealed that the amounts of phenol excreted in the urine of exposed rats were linearly related to benzene vapour concentrations. When the animals were exposed to the vapour of benzene and/or toluene, the conversion of benzene to phenol in animals exposed to the mixture of vapours was lower than that in the animals exposed to benzene alone. The metabolic suppression was dose dependent; it depended not only on the toluene/benzene ratio (greater suppression with a higher ratio), but also on the actual concentrations of the two compounds (greater suppression with higher vapour concentrations). When rats were exposed to benzene (160–170 ppm), toluene (800–1 000 ppm) or to the mixture (i.e., benzene 160–170 ppm + toluene 800–1 000 ppm) 6 hours a day, 5 days a week for 3 weeks, the decrease of peripheral leukocyte counts in animals exposed to the mixture was significantly lower than that observed in the benzene exposed animals, indicating that simultaneous exposure to toluene has a protective effect against leukopenic action of benzene *in vivo*.

It is well known that factory workers in organic solvent workshops (e.g. those in the printing or painting plant) are exposed to a mixture of vapours of various organic solvents rather than to a single solvent vapour. The toxicity, as well as the metabolism, of one compound may be modified by the presence of another. Previous studies on *in vitro* hydroxylation with rat liver preparation revealed that aromatic hydroxylation of aniline is suppressed by the addition of p-nitrotoluene and that aniline reversely inhibits the side-chain hydroxylation of p-nitrotoluene[6]. This mutual metabolic suppression between two aromatic compounds as observed *in vitro* could be confirmed *in vivo* as well; the conversion of benzene (given i.p. to rats) to phenol was suppressed by i.p. co-administration of toluene, while the biotransformation of toluene to hippuric acid was inhibited by benzene[7]. Such studies were further expanded to reach a conclusion that the mutual metabolic suppression will be observed when both of the two compounds given together undergo microsomal oxidation, while no interaction may take place in case at least one of the two is rather unsusceptible to oxidation[4]. It remains to argue that the results observed after i.p. injection may

not always be applicable to occupational exposure where the main route of exposure is inhalation, that the metabolic suppression may be dependent on the doses of the two compounds given together, and that the modification of metabolism may not necessarily mean changes in toxicity. In the present study the experiments were conducted with specified vapour exposure to benzene and toluene at the concentrations observed in the workshops.

The main metabolic pathways of benzene and toluene are summarized as follows[10]:

benzene → phenol → conjugated phenols
toluene → benzyl alcohol → benzoic acid → hippuric acid

MATERIALS AND METHODS

Male Wistar rats (200–250 g) were maintained on commercial laboratory chow and water *ad libitum*. When indicated, they were given 75 mg/kg/day of sodium phenobarbital i.p. for 4 days before the experiment[5] and also during the exposure period. The animals were exposed to organic solvent vapour 6 hours a day for the period as indicated in four parallel vapour exposure chambers (60 cm × 60 cm × 60 cm, each) of dynamic flow type. When necessary, three chambers were used for the exposure to benzene, toluene, and the mixture of the two, respectively, while the fourth chamber was saved for the sham exposure to fresh air. The vapour of required concentration was prepared by introducing semi-saturated vapour (produced by bubbling of air through liquid solvent at room temperature in a modified impinger) into the chamber where the vapour was diluted with massive fresh air. After the exposure, the vapour in air was removed by activated charcoal before discharge into outdoor atmosphere. The vapour concentration was monitored every hour during the exposure by injecting 1 ml air sample into a Hitachi Model 163 gas-liquid chromatograph equipped with flame ionization detectors (supply of H_2 and air, 0.8 and 1.6 kg/cm^2, respectively) and stainless steel columns (2 mm in inner diameter and 2 m in length, packed with 20% tetracyanoethylated pentaerythritol on Chromosorb WAW, 60–80 mesh). The temperatures of the oven and the injection port were 100 °C and 120 °C, respectively, and flow rate of the carrier N_2 was 50 ml/min.

Urine from individual rats was collected, separately from the faeces, during six hours of exposure and also during the 18-hour period thereafter. The sample was analyzed for phenol after hydrolysis and ether extraction[3] followed by system I gas-liquid chromatography[8].

Blood samples were taken from the tip of the tail at a given time between 9 and 10 a.m. on Saturday morning to avoid possible diurnal variation in cell counts[2]. White blood cells were immediately counted with a diluter-equipped Model D Coulter counter.

RESULTS AND DISCUSSION

Linear relationship between benzene vapour concentration and amounts of phenol excreted in urine

The rats (four animals per group) were exposed for 6 hours to various concentrations of benzene vapour and urine samples collected 24 hours from the beginning of the exposure were analyzed for phenol. At benzene concentrations of 0, 38.2 ± 3.0, 78.3 ± 7.3, and 143.0 ± 11.8 ppm ($\bar{X}$ ± S.D.; six determinations each at one hour-intervals), the respective amounts of urinary phenol were 1.5 ± 0.7, 9.5 ± 1.2, 18.5 ± 5.4, and 32.7 ± 6.9 mg/kg body weight of rats ($\bar{X}$ ± S.D.; four determinations each). The results clearly indicate that under the conditions studied, the amounts of phenol excreted in urine are linearly related to the concentrations of benzene.

When the rats were pre-treated with phenobarbital for 4 days before the exposure, the amount of phenol excreted during the 6 hour exposure was elevated by about 50%, the increase being statistically significant ($p < 0.05$).

Suppression of benzene metabolism by co-exposure to toluene

When exposures were measured at fixed benzene concentration of 160 – 170 ppm coupled with various toluene concentrations (Table 1), the amounts of urinary phenol varied depending on the concentration of toluene (Experiment I).

TABLE 1
Benzene metabolism suppressed by co-exposure to increasing concentrations of toluene.

	Experiment I[d]				Experiment II[e]	
	1	2	3	4	1	2
Benzene concentrations (ppm)[a]	165 ± 5	162 ± 21	163 ± 6	160 ± 11	169 ± 33	162 ± 32
Toluene concentrations (ppm)[a]	0	156 ± 6	423 ± 24	665 ± 50	0	952 ± 123
Relative toluene concentrations[b]	0	0.9	2.6	4.2	0	5.9
Relative urinary phenol excretion[c]	100 ± 12	66 ± 8	61 ± 9	53 ± 4	100 ± 37	13 ± 6

[a] $\bar{X}$ ± S.D. (n = 6 in Experiment I, n = 15 in Experiment II).
[b] Toluene concentration divided by benzene concentration.
[c] Amount of phenol excreted during 24 hours after initiation of a single 6-hour exposure. The amount after exposure to benzene only (Experiment I–1 of II–1) was taken as 100.
[d] Single 6-hour exposure (n = 4).
[e] 6 hour/day, 5 days (Monday to Friday)/week, for 3 weeks. Results from 3 Monday exposures of 5 rats each were pooled (n = 15).

At the highest toluene concentration of 952 ppm where the ratio of toluene concentration over benzene concentration was as high as six, phenol in urine decreased down to 13% of that after the exposure to benzene alone (Experiment II).

Exposures to various concentrations of mixed benzene-toluene vapours in which the toluene/benzene ratio was set constant at six (Experiments III and IV

in Table 2 and Experiment II in Table 1) revealed that the metabolic suppression was more prominent when the concentrations of both benzene and toluene were high (Experiment II), while less inhibition was observed at lower concentrations (Experiment III). At the exposure to 50 ppm of benzene and 450 ppm of toluene (the toluene/benzene ratio was nine), the reduction in benzene metabolism (down to 38%) was more marked than that observed with lower toluene concentrations (48% remaining with 50 ppm of benzene and 340 ppm of toluene; the toluene/benzene ratio was six), but less extensive than that with 160 ppm of benzene and 950 ppm of toluene (the toluene/benzene ratio was six; Experiment II). All these findings indicate that the extent of metabolic suppression depends not only on the ratio of the two vapour concentrations, but also on the actual concentrations. It is worth noting that the suppression exists even at concentrations of benzene and toluene as low as 13 ppm and 75 ppm, respectively, the concentrations which could actually be observed in the factories.

TABLE 2
Benzene metabolism suppressed by co-exposure to toluene with the constant benzene/toluene ratio.

	Experiment III[d]		Experiment IV[d]		
	1	2	1	2	3
Benzene concentrations (ppm)[a]	13 ± 2	13 ± 3	50 ± 4	53 ± 3	49 ± 5
Toluene concentrations (ppm)[a]	—	75 ± 9	—	336 ± 16	451 ± 18
Relative toluene concentrations[b]	—	5.8	—	6.3	9.2
Relative urinary phenol excretion[c]	100 ± 7	83 ± 18	100 ± 16	48 ± 5	38 ± 2

[a] $\bar{X} \pm$ S.D. (n = 6).
[b] Toluene concentration divided by benzene concentration.
[c] Amount of phenol excreted during 24 hours after initiation of a single 6-hour exposure. The amount after exposure to benzene only (Experiment I–1 or II–1) was taken as 100.
[d] Single 6-hour exposure (n = 4).

Protective effect of co-exposure to toluene against benzene-induced leukopenia

Rats were exposed to benzene (160–170 ppm), toluene (800–1 000 ppm), or to the combination 6 hours a day, 5 days (Monday to Friday) a week, for three weeks, and the leukocytes in peripheral blood were counted to detect the effect of co-exposure to toluene on benzene leukopenia. The entire experiments were repeated twice with different batches of animals (Experiments I and II in Table 3). The exposure to benzene alone induced a significant decrease in leukocyte counts, while the exposure to toluene resulted in no change. With the exposure to the mixture of vapours, the decrease in leukocyte counts was much less if any, than that observed with benzene exposure. The observation indicates that simultaneous exposure to toluene has a protective effect against leukopenic action of benzene *in vivo*. Bearing in mind that toluene suppresses the over-all metabolism of benzene *in vivo* (Tables 1 and 2) that toluene reduces the level of benzene metabolites in the bone marrow without affecting the level of benzene there[1], and that benzene is thought to be metabolized by benzene oxide[8], the

TABLE 3
Leukocyte counts after repeated exposure to benzene and/or toluene.

Solvent exposed	$\bar{X} \pm$ S.D.[f] (N = 15)	Experiment I[e]			$\bar{X} \pm$ S.D.[f] (N = 15)	Experiment II[e]		
		1st w.	2nd w.	3rd w.		1st w.	2nd w.	3rd w.
Benzene	176 ± 37	66 ± 13[b]	71 ± 15[b]	74 ± 10[b]	169 ± 33	81 ± 7	74 ± 11[a]	83 ± 11[b]
Toluene	824 ± 83	91 ± 12	94 ± 8	101 ± 17	882 ± 87	89 ± 17	89 ± 14	99 ± 16
Benzene + toluene	164 ± 30 939 ± 126	87 ± 10[c]	79 ± 18	87 ± 14	162 ± 32 912 ± 123	91 ± 8[c]	108 ± 19[d]	110 ± 8[d]
Control	—	99 ± 12	98 ± 14	105 ± 8		91 ± 14	97 ± 19	113 ± 15

[a] Decrease from the control values significant ($p < 0.05$), tested by U-test.
[b] Decrease from the control values significant ($p < 0.01$), tested by U-test.
[c] Increase over the values for the "benzene" group significant ($p < 0.05$), tested by U-test.
[d] Increase over the values for the "benzene" group significant ($p < 0.01$), tested by U-test.
[e] Values (other than those for concentrations) in the table are relative leukocyte counts, taking pre-exposure values as 100. Six rats per group were used in Experiment I, and five in Experiment II.
[f] Vapour concentrations (ppm)

probable active metabolite, one of the possible mechanisms of anti-leukopenic effect of toluene is a suppression of benzene oxide formation. Experiments are in progress in our laboratory to evaluate this hypothesis.

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