Environmental Health Perspectives
Vol. 82, pp. 259-266, 1989

Benzene Adducts with Rat Nucleic Acids and Proteins: Dose-Response Relationship After Treatment *In Vivo*

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The dose-response relationship of the benzene covalent interaction with biological macromolecules from rat organs was studied. The administered dose range was 3.6×10^7 starting from the highest dosage employed, 486 mg/kg, which is oncogenic for rodents, and included low and very low dosages. The present study was initially performed with tritium-labeled benzene, administered by IP injection. In order to exclude the possibility that part of the detected radioactivity was due to tritium incorporated into DNA from metabolic processes, ^{14}C-benzene was then also used following a similar experimental design. By HPLC analysis, a single adduct from benzene-treated DNA was detected; adduct identification will be attempted in the near future. Linear dose-response relationship was observed within most of the range of explored doses. Linearity was particularly evident within low and very low dosages. Saturation of benzene metabolism did occur at the highest dosages for most of the assayed macromolecules and organs, especially in rat liver. This finding could be considered as indicative of the dose-response relationship of tumor induction and could be used in risk assessment.

Introduction

Many data are available on benzene toxicity and carcinogenicity. The primary reason for the interest in the behavior of this chemical is the widespread occupational and environmental human exposure because of its large production and use (*1*). Toxic effects of benzene on bone marrow and blood cells have been observed both in rodents and humans; anemia, lymphocytopenia, and bone marrow aplasia are the most frequent diseases. Carcinogenic action, however, seems to have different targets in humans (with the induction of acute myelogenous leukemia) and rodents (induction of Zymbal gland, oral cavity, and stomach carcinomas, hepatocarcinomas, and lymphoid tumors mainly). Nevertheless, evaluation of the all available experimental and epidemiological carcinogenicity data, from a recent work by Grilli et al. (*2*), points out a similar saturable metabolism of benzene both for rodents and man. Saturation occurs following inhalation exposure to dosages ranging from 10 to 100 ppm. The flattening of the dose-response curve seems to suggest carcinogenic activity at low doses 2- to 3-fold higher than that reckoned by linear extrapolation from data in the 100 ppm range. Thus, evaluation of the dose-response relationship at low doses is very useful; it is a hard task, although the assessment of the associated risk to human occupational and, mostly, nonoccupational exposure is a matter of great interest (*3*).

Benzene is a weak genotoxic compound that forms *in vivo* adducts to rat and mouse liver DNA. The covalent binding index (CBI) of benzene calculated in rat liver according to Lutz (*4*) is in the order of units (*5*). The CBI allows an evaluation of pre-initiating activity of compound in a dose range of about 10^6-fold and is sensitive enough to explore low doses. Because of its high sensitivity and of the appreciable correlation with carcinogenicity data (*6*), this test has been previously employed for the evaluation of aromatic amines, nitrosamines, polycyclic aromatic hydrocarbons, and aflatoxins (*7*). All these chemicals require metabolic activation and affect the early stages of carcinogenesis (initiation).

In this paper, we aimed at quantifying the number of

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in vivo adducts to rat DNA of five organs showing different susceptibility to carcinogenesis by this compound. Furthermore, the amount of adducts as a function of the employed dosage was evaluated in order to obtain a surrogate for the cancer dose-response curve in the range of low and very low doses that cannot be explored by long-term assays.

Materials and Methods

Groups of six male Wistar rats (220–250 g) were injected IP with ^{3}H-labeled benzene (0.5 to 108 μCi/animal, radiochemical purity 98%) purchased from The Radiochemical Centre (Amersham, UK); six animals served as a control group. Specific activity of ^{3}H-benzene was 11.6 Ci/mmole; when necessary the activity was lowered by adding cold benzene according to the experimental design. Dosages employed are shown in Tables 1 to 5. Fasted animals were kept in an air-conditioned room with a dark-light cycle of 12 hr and killed 22 hr after injection.

Liver, spleen, kidney, lung, and stomach were collected from each animal and processed in order to obtain DNA, RNA, and proteins. Livers were pooled and processed two by two in order to obtain triplicate values. For the other organs, pools from animals of each dose group were processed. Aliquots (about 100 mg) from each tissue were dissolved in 1 mL lumasolve (Landgroof, the Netherlands) at 40°C in order to measure benzene distribution to various organs as a function of the administered dose. Ten milliliters of Ready-Solve NA (Beckman Analytical, Milan, Italy) and 0.1 mL acetic acid were added to each scintillation vial in order to avoid chemoluminescence effects. Samples were stored at 0 to 5°C for 2 days and counted in a Beckman LS-1801 liquid scintillation spectrometer.

DNA, RNA, and proteins were isolated by phenol extraction. Glycogen was carefully removed from liver DNA by treatment with methoxyethanol and hexadecyltrimethyl ammonium bromide, according to the method by Kinoshita and Gelboin (*8*). Macromolecules were exhaustively washed with organic solvents until all noncovalently bound radioactivity was removed. DNA and RNA recovery and purity were determined by specific colorimetric reactions and ultraviolet absorption measurement (*5*).

Aliquots of 10 to 20 mg DNA and 3 to 4 mg proteins were dissolved in 1 mL lumasolve at 40°C and stored at 0 to 5°C for 2 days. Acetic acid 0.5 mL, and 10 mL Ready-Solv NA were added to samples dissolved in lumasolve, which were counted as described above. RNA, 2 to 5 mg aliquots, dissolved in 1 to 1.5 mL buffer (pH 7.0), were counted in scintillation vials containing 10 mL Ready-Solv MP (Beckman Analytical). All vials were counted 3 to 6 times for a minimum of 20 min. At least 2000 counts were measured for each sample with a background of about 15 cpm and an efficiency of about 33%. Appropriate controls, performed with macromolecules extracted from untreated rats, were systematically counted. The control macromolecules, like all samples from the lowest assayed dose, gave rise to counts similar from a statistical point of view to the instrument background. Binding values to DNA could not be detected at the two lowest doses since the method is not sensitive enough to detect any effect at these doses. On the contrary, covalent binding of benzene to RNA and proteins could be measured even after administration of 1.04×10^{-6} mmole/kg of compound.

More recently, a study with ^{14}C-benzene (purchased from The Radiochemical Centre, specific activity 120.5 mCi/mmole, radiochemical purity 99%) was performed, using an analogous schedule as that previously described for ^{3}H-benzene. The dosages of 6.23×10^{-6}, 6.23×10^{-5}, 1.97×10^{-4}, 6.23×10^{-4}, 1.97×10^{-3}, 6.23×10^{-3}, 6.23×10^{-2}, 6.23×10^{-1}, and 6.23 mmole/kg have been administered to groups of animals. Another animal group served as control. ^{14}C-benzene, 0.178 to 357 μCi, per animal was applied. Macromolecules were processed as reported previously and their labelings were measured as described in the experimental design carried out with ^{3}H-benzene, with a background of about 40 cpm and an efficiency of about 94%.

HPLC analysis of 5′-mononucleotides from liver DNA of animals injected with 6.23×10^{-6} to 6.23×10^{-1} mmole/kg ^{3}H-benzene and with 6.23×10^{-2} mmole/kg ^{14}C-benzene was performed. Six milligrams of DNA were treated with 0.6 mg DNAse type 1 (Sigma Chemical Co., St. Louis, MO) at 37°C for 3 hr in 3.0 mL of 0.1 M potassium phosphate-5 mM $MgCl_2$ -5 mM $CaCl_2$ buffer, pH 7.0. Then pH was raised to 9.0 and 0.6 mg phosphodiesterase type 1 (Sigma) from Crotalus atrox was added to the mixture. Digestion was carried out for 24 hr at 37°C. 5′-mononucleotides from 6 mg DNA were loaded on a preparative ion exchange Ultrasil-Ax column (Serva, Heidelberg, West Germany, dp 5 μm, diameter: 9 mm, L 250 mm), which was eluted with 0.01 M potassium phosphate buffer · HCl, pH 3.1, by means of a Beckman HPLC system (flow: 1.5 mL/min): pump model 114, absorbance detector model 160, controller model 421. For each case, 80 2-mL fractions were collected. UV absorption at 254 nm and radioactivity of each fraction was measured.

Results

The amount of benzene detected in various organs was always proportional to the administered dose. Whatever dosage was employed, the amount of radioactivity measured in the liver was about 0.2% of the administered dose. About 0.01% of administered benzene was found in kidney and 0.001% in the other assayed organs.

Binding values of ^{3}H-benzene to RNA from various organs were usually one order of magnitude higher than those to DNA. Protein labeling was lower than that of RNA and higher than that of DNA (Table 1). The dose-response relationship was linear in the case of liver DNA within a range of changing by 10^{5}-fold (6.23×10^{-6}–6.23×10^{-1} mmole/kg). At a 10-fold higher dosage (6.23 mmole/kg) saturation of benzene binding activity was observed as shown in Figure 1 (logarithmic scale). This effect would be even more evident in normal scale. Moreover, in the case of both RNA and protein binding, a more

Table 1. Dose-repsonse relationship of *in vivo* binding of ^{3}H-benzene to biological macromolecules from rat liver.

Dose, mmole/kg	Adducts, pmole/mg		
	DNA	RNA	Protein
1.73×10^{-7}	UD[a]	UD	UD
1.04×10^{-6}	UD	$1.29 \times 10^{-4} \pm 0.09 \times 10^{-4}$	$3.74 \times 10^{-5} \pm 1.98 \times 10^{-5}$
6.23×10^{-6}	$3.11 \times 10^{-5} \pm 2.36 \times 10^{-5}$ (1.62)[b]	$1.42 \times 10^{-3} \pm 0.25 \times 10^{-3}$	$2.56 \times 10^{-4} \pm 0.71 \times 10^{-4}$
1.97×10^{-5}	$6.84 \times 10^{-5} \pm 4.18 \times 10^{-5}$ (1.13)	$3.56 \times 10^{-3} \pm 0.11 \times 10^{-3}$	$6.94 \times 10^{-4} \pm 0.18 \times 10^{-4}$
6.23×10^{-5}	$2.74 \times 10^{-4} \pm 0.25 \times 10^{-4}$ (1.43)	$1.50 \times 10^{-2} \pm 0.52 \times 10^{-2}$	$2.34 \times 10^{-3} \pm 0.34 \times 10^{-3}$
6.23×10^{-4}	$2.74 \times 10^{-3} \pm 0.23 \times 10^{-3}$ (1.43)	$9.29 \times 10^{-2} \pm 2.98 \times 10^{-2}$	$2.70 \times 10^{-2} \pm 0.39 \times 10^{-2}$
6.23×10^{-3}	$1.55 \times 10^{-2} \pm 0.37 \times 10^{-2}$ (0.81)	$9.19 \times 10^{-1} \pm 2.43 \times 10^{-1}$	$2.67 \times 10^{-1} \pm 0.19 \times 10^{-1}$
6.23×10^{-2}	$2.47 \times 10^{-1} \pm 0.77 \times 10^{-1}$ (1.29)	9.33 ± 1.20	2.67 ± 0.26
6.23×10^{-1}	2.65 ± 0.33 (1.38) (0.86)[c]	$4.76 \times 10^{1} \pm 1.14 \times 10^{1}$	$1.81 \times 10^{1} \pm 0.27 \times 10^{1}$
6.23	$1.05 \times 10^{1} \pm 0.34 \times 10^{1}$ (0.55)	$1.80 \times 10^{2} \pm 0.65 \times 10^{2}$	$7.02 \times 10^{1} \pm 0.23 \times 10^{1}$

[a]UD, undetectable. The labeling values were not significantly different from those of controls. They were used as blanks and taken away from labeling values of the other dosages.
[b]Numbers in parentheses are the covalent binding index; value calculated according to Lutz (*4*).
[c]μmole/mole DNA-phosphate calculated according to Swenson and Lawley (*19*).

appreciable saturation effect was observed starting from the dose 6.23×10^{-1} mmole/kg. Thus, a linear dose-response relationship was found within a range of 6×10^4-fold. The experiment with ^{14}C-benzene is in progress. Nevertheless, the labelings of liver macromolecules were on the same levels as those detected when using ^{3}H-benzene. Also, their variation as a function of the employed dosage is linear up to the two highest dosages. Due to the lower specific activity of the ^{14}C-benzene, binding to macromolecules was detected within a more limited range of dosages (Table 2).

Covalent binding of ^{3}H-benzene to DNA from other all

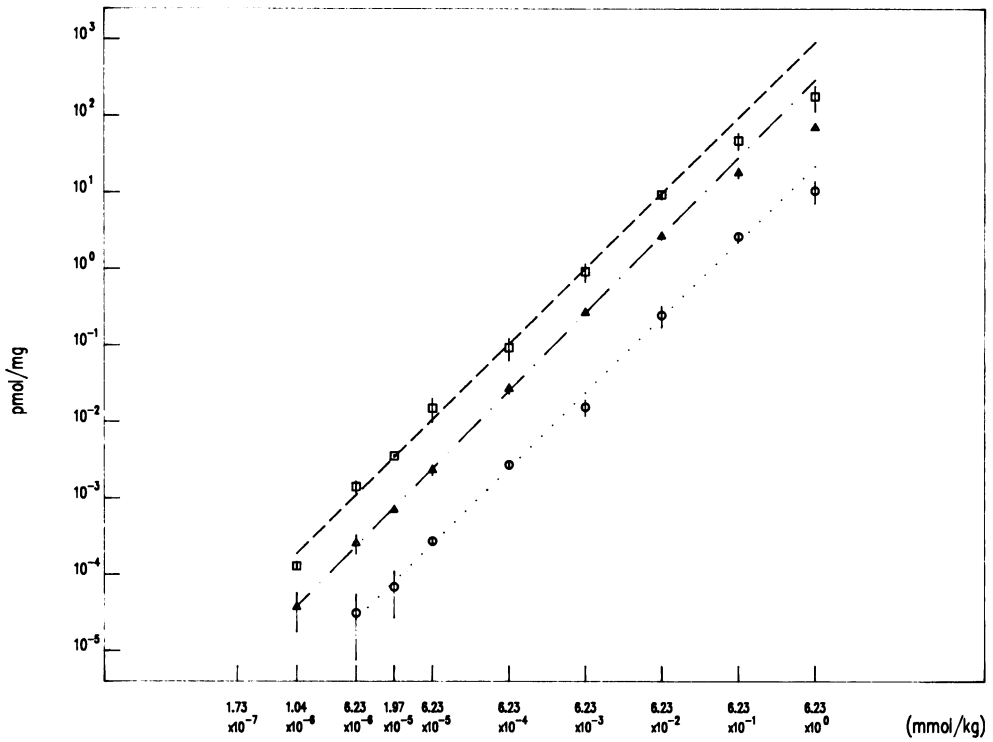

FIGURE 1. Dose-response relationship between ^{3}H-benzene dose and amount of adducts with DNA (○···○), RNA (□--□) or proteins (△···△) of rat liver. Regression lines (log-log scale): $y = 0.5552 + 0.9875x$, $r = 0.9986$, $p < 0.005$ (the value from the highest assayed dose is not considered); $y = 2.1763 + 0.9860x$, $r = 0.9980$, $p < 0.005$ (the values from the two highest assayed doses are not considered); $y = 1.6623 + 1.0165x$, $r = 0.9998$, $p < 0.005$ (the values from the two highest assayed doses are not considered).

Table 2. Dose-response relationship of *in vivo* binding of ^{14}C-benzene to biological marcomolecules from rat liver.

Dose, mmole/kg	Adducts, pmole/mg		
	DNA	RNA	Protein
6.23×10^{-6}	UD[a]	UD	UD
6.23×10^{-5}	UD	UD	UD
1.97×10^{-4}	UD	$7.48 \times 10^{-2} \pm 1.12 \times 10^{-2}$	UD
6.23×10^{-4}	UD	$1.60 \times 10^{-1} \pm 0.10 \times 10^{-1}$	$7.10 \times 10^{-2} \pm 1.49 \times 10^{-2}$
1.97×10^{-3}	$2.35 \times 10^{-2} \pm 0.22 \times 10^{-2}$ (3.90)[b]	$3.40 \times 10^{-1} \pm 0.97 \times 10^{-1}$	$3.17 \times 10^{-1} \pm 0.34 \times 10^{-1}$
6.23×10^{-3}	$3.74 \times 10^{-2} \pm 0.56 \times 10^{-2}$ (1.95)	1.40 ± 0.28	$6.17 \times 10^{-1} \pm 0.11 \times 10^{-1}$
6.23×10^{-2}	$3.03 \times 10^{-1} \pm 0.28 \times 10^{-1}$ (1.60)	$1.17 \times 10^{1} \pm 0.09 \times 10^{1}$	4.95 ± 0.17
6.23×10^{-1}	1.16 ± 0.15 (0.60)	$6.28 \times 10^{1} \pm 1.16 \times 10^{1}$	$2.43 \times 10^{1} \pm 0.12 \times 10^{1}$
6.23	4.62 ± 0.56 (0.24)	$1.31 \times 10^{2} \pm 0.05 \times 10^{2}$	$3.10 \times 10^{1} \pm 0.35 \times 10^{1}$

[a]UD, undetectable. The labeling values were not significantly different from those of controls. They were used as blanks and taken away from labeling values of the other dosages.

[b]Numbers in parentheses are covalent binding index (CBI); value calculated according to Lutz (*4*). A CBI value of 7 was measured in a previous study by Arfellini et al. (*5*) after administration of 6.35×10^{-3} mmole/kg of ^{14}C-benzene.

organs as well as to RNA and proteins of stomach and spleen (Tables 3 and 4) was a linear function of the administered dose within the range of the technique sensitivity (changing up to 10^6-fold in the case of stomach DNA). Tendency to a saturation of metabolism was observed, to a lesser extent than in liver, in the case of RNA and proteins of kidney and lung (Tables 5 and 6). Indeed, saturation was shown in protein labeling when employing the two highest doses.

The extent of ^{3}H-benzene binding to DNA was similar in all assayed organs. CBI values ranged from 1 to 2. Values less than 1 were obtained in lung DNA only. Assuming a random interaction of benzene with DNA molecules, DNA adducts density ranged from 1 per 10^{-11} nucleotides to 0.9 per 10^{-6} nucleotides.

The slope of the regression lines (DNA adducts versus dose, in normal scale) was lower in the liver than in the other target sites (data not shown). This finding is consistent with the higher degree of DNA binding saturation in this organ.

By HPLC analysis, a single adduct was detected in liver DNA (Fig. 2). All radioactivity covalently bound seems to be associated with the last eluting peak, which was not coincident with those from benzene, tritiated water, or normal nucleotides. The same chromatographic behavior was found when analyzing liver DNA from any assayed dose group regardless of the applied dosage.

Discussion

Due to the weak genotoxicity of the chemical, in the first experiment, tritium-labeled benzene was employed for its specific activity higher than that of ^{14}C-benzene. In consequence, we were able to investigate the extent of benzene binding using a wide range of doses.

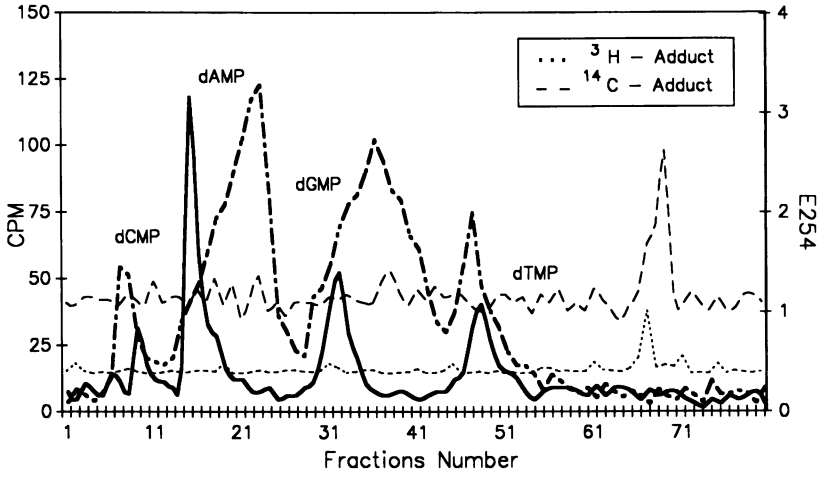

FIGURE 2. HPLC analysis of 5′-mononucleotides from liver DNA. Dashed line represents the UV-absorption of 5′-mononucleotides from 6 mg liver DNA of animal treated with ^{3}H-benzene (6.23×10^{-1} mmole/kg) or ^{14}C-benzene (6.23×10^{-2} mmole/kg). Solid line represents the chromatographic behavior of the standard 5′-mononucleotides, given as reference. In such a chromatographic system tritiated water elutes at the fraction 5 and benzene at the fraction 6.

Table 3. Dose-response relationship of *in vivo* binding of ^{3}H-benzene to biological macromolecules from rat stomach.

Dose, mmole/kg	Adducts, pmole/mg		
	DNA	RNA	Protein
1.73×10^{-7}	UD[a]	UD	UD
1.04×10^{-6}	UD	6.07×10^{-5}	8.09×10^{-5}
6.23×10^{-6}	3.10×10^{-5} (1.62)[b]	4.36×10^{-4}	1.51×10^{-4}
1.97×10^{-5}	ND[c]	1.20×10^{-3}	ND
6.23×10^{-5}	3.74×10^{-4} (1.95)	3.26×10^{-3}	7.96×10^{-4}
6.23×10^{-4}	4.03×10^{-3} (2.10)	4.11×10^{-2}	8.22×10^{-3}
6.23×10^{-3}	5.35×10^{-2} (2.79)	3.30×10^{-1}	6.29×10^{-2}
6.23×10^{-2}	4.62×10^{-1} (2.41)	3.18	8.46×10^{-1}
6.23×10^{-1}	3.32 (1.73)	4.04×10^{1}	7.77
6.23	3.59×10^{1} (1.87)	5.04×10^{2}	5.07×10^{1}

[a]UD, undetectable (see footnote *a* of Table 1).
[b]Numbers in parentheses are covalent binding index; value calculated according to Lutz (*4*).
[c]ND, not determined (lost by accident).

Nevertheless, the possibility that a small amount of labeling could be due to the incorporation of tritium into DNA, from water formed during metabolic processes, has to be considered. According to the report by Lutz and Schlatter (*9*), only a very small percentage of tritiated water is incorporated. The incorporation of ^{3}H-water into normal nucleotides was, however, excluded by HPLC analysis of 5′-mononucleotides from ^{3}H-benzene treated liver. It showed the presence of a single adduct whose identification will be attempted in the near future. Probably, dGMP or dAMP are involved in adduct formulation, as shown *in vitro*, by Snyder et al. (*10*) by using mitoplasts.

The covalent binding of benzene to DNA, RNA, and proteins from all assayed organs was measured at dosages up to 6.23 mmole/kg (486 mg/kg) body weight (about 1/6 of LD_{50}), some of which were higher than those capable of inducing tumors (50 mg/kg when administered lifetime by gavage). Binding values were rather low. Data on ^{3}H-benzene interaction with liver DNA are comparable to those determined in the second experiment (in progress) performed with ^{14}C-labeled benzene. The mean of CBI in rat liver DNA (CBI = 1.2 and 1.7, for ^{3}H- and ^{14}C-benzene, respectively) was slightly less than that measured in a previous report (*5*) with a single dose (6.35 μmole/kg) of ^{14}C-benzene. Benzene binds to various macromolecules in different organs to similar levels. Tendency to saturation is even more evident when employing ^{14}C-benzene whose binding to liver DNA already reaches saturation at a dosage of 6.23×10^{-1} mmole/kg. The extent of binding of lung macromolecules was

Table 4. Dose-response relationship of *in vivo* binding of ^{3}H-benzene to biological macromolecules from rat spleen.

Dose, mmole/kg	Adducts, pmole/mg		
	DNA	RNA	Protein
1.73×10^{-7}	UD[a]	UD	UD
1.04×10^{-6}	UD (0.51)[b]	UD	1.55×10^{-5}
6.23×10^{-6}	9.75×10^{-6} (0.51)[b]	1.20×10^{-3}	9.55×10^{-5}
1.97×10^{-5}	8.88×10^{-5} (1.47)	1.98×10^{-3}	ND[c]
6.23×10^{-5}	2.30×10^{-4} (1.20)	4.83×10^{-3}	1.01×10^{-3}
6.23×10^{-4}	3.18×10^{-3} (1.66)	4.29×10^{-2}	1.08×10^{-2}
6.23×10^{-3}	2.77×10^{-2} (1.45)	4.76×10^{-1}	2.33×10^{-1}
6.23×10^{-2}	3.35×10^{-1} (1.75)	4.17	1.08
6.23×10^{-1}	2.76 (1.45)	3.04×10^{1}	6.15
6.23	2.03×10^{1} (1.07)	ND	6.63×10^{1}

[a]UD, undetectable (see footnote *a* of Table 1).
[b]Numbers in parentheses are covalent binding index; value calculated according to Lutz (*4*).
[c]ND, not determined (lost by accident).

Table 5. Dose-response relationship of *in vivo* binding of ^{3}H-benzene to biological macromolecules from the rat kidney.

Dose, mmole/kg	Adducts, pmole/mg		
	DNA	RNA	Protein
1.73×10^{-7}	UD[a]	UD	UD
1.04×10^{-6}	UD	UD	3.86×10^{-5}
6.23×10^{-6}	UD	1.56×10^{-3}	3.46×10^{-4}
1.97×10^{-5}	UD	1.31×10^{-2}	ND[c]
6.23×10^{-5}	2.34×10^{-4} (1.22)[b]	3.22×10^{-2}	2.99×10^{-3}
6.23×10^{-4}	2.06×10^{-3} (1.07)	4.16×10^{-1}	2.83×10^{-2}
6.23×10^{-3}	ND	ND	ND
6.23×10^{-2}	7.57×10^{-1} (3.95)	1.60×10^{1}	3.34
6.23×10^{-1}	2.57 (1.34)	4.16×10^{2}	1.58×10^{1}
6.23	2.65×10^{1} (1.38)	1.01×10^{3}	5.85×10^{1}

[a]UD, undetectable (see footnote *a* of Table 1).
[b]Numbers in parentheses are covalent binding index; value calculated according to Lutz (*4*).
[c]ND, not determined (lost by accident).

slightly lower than that in macromolecules from other assayed organs. This could be due to either a partial loss of benzene via the lungs and/or to a higher detoxification in that organ. The active metabolite could be formed in the liver and it could be sufficiently stable to reach extrahepatic target sites. Previous work (*5*) showed that only hepatic microsomes are capable of bioactivating benzene. In that same study (*5*), ^{14}C-benzene binding to DNA in the mouse bone marrow reached values similar to those detected in the liver DNA, whereas the labeling of rat bone marrow was even five times higher than that of liver DNA.

The present study further supports the evidence of saturation of benzene metabolism at the upper doses (in the 10–100 ppm range), as previously discussed by Grilli et al. (*2*) and confirmed by Parodi et al. (*11*). The saturation level corresponds to 6.23 mmole/kg (486 mg/kg) body weight [approximately 600 ppm by inhalation according to EPA computations (*12*)], in the case of liver DNA and kidney, and lung RNA, Saturation seems to occur at 0.623 mmole/kg (48.6 mg/kg) (approximately 60 ppm by inhalation), in the case of liver RNA and liver, kidney and lung proteins. All other relationships are linear within the whole range of sensitivity of the technique. However, results reported here are not quite comparable with those obtained from inhalation-administered animals that were kept in a chamber with a constant concentration of benzene. Moreover, we are aware that chronic administration of benzene could affect the number of adducts formed, especially at the highest dosages.

The relationship between adduct formation to DNA, RNA, and protein of the assayed organs and benzene-administered dose stayed linear within a wide range of dosages less than 48.6 mg/kg. The behavior of benzene, a weak genotoxic compound, resembles that of potent genotoxic compounds that belong to the main classes of

Table 6. Dose-response relationship of *in vivo* binding of ^{3}H-benzene to biological macromolecules from rat lung.

Dose, mmole/kg	Adducts, pmole/mg		
	DNA	RNA	Protein
1.73×10^{-7}	UD[a]	UD	UD
1.04×10^{-6}	UD	4.21×10^{-5}	UD
6.23×10^{-6}	UD	4.34×10^{-4}	1.71×10^{-4}
1.97×10^{-5}	3.33×10^{-5} (0.55)[b]	ND[c]	ND
6.23×10^{-5}	4.42×10^{-4} (2.31)	4.02×10^{-3}	9.65×10^{-4}
6.23×10^{-4}	5.00×10^{-4} (0.26)	4.11×10^{-2}	9.51×10^{-3}
6.23×10^{-3}	1.40×10^{-2} (0.73)	5.19×10^{-1}	1.30×10^{-1}
6.23×10^{-2}	1.54×10^{-1} (0.80)	2.11×10^{1}	1.51
6.23×10^{-1}	1.16 (0.61)	4.52×10^{1}	7.76
6.23	2.03×10^{1} (1.06)	1.61×10^{2}	1.95×10^{1}

[a]UD, undetectable (see footnote *a* of Table 1).
[b]Numbers in parentheses are covalent binding index; value calculated according to Lutz (*4*).
[c]ND, not determined (lost by accident).

Table 7. Evidence of linear relationship between administered dose of carcinogen and amount of its DNA adducts.

Compound	Route of administration and species[a]	Range of assayed dose, mole/kg, with linear dose-response relationship	Organ	Reference
t-4-Acetylaminostilbene[b]	PO, rat	5×10^{-10}-5×10^{-7}	Liver	(*7*)
t-4-Dimethylaminostilbene	PO, rat	5×10^{-10}-10^{-4}	Liver, kidney	(*7*)
2-Acetylaminofluorene[c]	PO, rat	10^{-6}-10^{-4}	Liver	(*21*)
Diethynitrosamine	IP, rat	1.2×10^{-4}-2×10^{-3}	Liver	(*22*)
Dimethylnitrosamine	PO, rat	10^{-8}-10^{-6}	Liver	(*23*)
		10^{-8}-10^{-4}	Kidney	
Dimethylnitrosamine[d]	IP, rat	6.8×10^{-6}-3.4×10^{-4}	Liver	(*24*)
Benzo[a]pyrene	PO, mouse	10^{-9}-10^{-4}	Liver	(*25*)
		10^{-9}-10^{-5}	Stomach	
Benzo[a]pyrene	PO, mouse	1.2×10^{-6}-6×10^{-4}	Liver, lung	(*26*)
Benzo[a]pyrene	PC, mouse	1.6×10^{-9}-4.8×10^{-5}	Skin	(*27*)
7,12-Dimethylbenz[a]anthracene	PC, mouse	10^{-6}-4×10^{-5}	Skin	(*27*)
Aflatoxin B_1[e]	IP, rat	3×10^{-11}-10^{-9}	Liver	(*28*)
Aflatoxin B_1	PO, rat	3.2×10^{-12}-3.2×10^{-7}	Liver	(*4*)
Benzene	IP, rat	6.23×10^{-9}-6.23×10^{-4}	Liver	Data from this paper
		1.97×10^{-8}-6.23×10^{-3}	Lung	
		6.23×10^{-8}-6.23×10^{-3}	Kidney	
		6.23×10^{-9}-6.23×10^{-3}	Spleen, stomach	

[a]PO, per os; IP, intraperitoneally; PC, percutaneously.
[b]Binding to liver, kidney, stomach, and lung DNA linearly increases after repeated administration (twelve 5×10^{-6} mole/kg doses within 40 days).
[c]Wund-Bisseret et al. (*20*) found a sublinear shape after IP injection to rat liver, decreasing from 4.7×10^{-6} to 7×10^{-9} mole/kg; binding values were lowered by 29-fold (from 3.178 to 0.109 nmole/mg DNA).
[d]7-Methylguanine production is linear up to 1.4×10^{-7} mole/kg; O^6-methylguanine formation is sublinear within 6.8×10^{-6} and 1.4×10^{-7} mole/kg.
[e]At the dose 3×10^{-9} mole/kg, a flattening of the curves occurs (saturation effect).

chemical carcinogens and require metabolic activation (Table 7). Different shape was observed in the dose-response function of formaldehyde, a directly acting substance: Linearity was found with protein adducts and sublinearity with DNA adducts (*13*). On the other hand, a flattening of the curve of adducts versus dose function has been shown for other carcinogens, such as vinyl chloride (*14*). When the fraction of animals with vinyl chloride-induced angiosarcomas was considered and plotted as a function of DNA adducts, an almost linear curve was obtained (*15*), thus showing a good correlation between metabolite production and carcinogenic action.

Experimental data concerning metabolic activation and mechanisms of action of carcinogens are needed in order to improve the evaluation of human risk (*3,16*). Indeed, the available mathematical models (linear Weibull, logit, probit, multistage, gamma multihit, one-hit) give contrasting information about risk from both occupational and environmental exposure. The degree of uncertainty from the mathematical approaches could be reduced by using data reported here. As previously reported, the curve representing tumor induction as a function of human occupational exposure to benzene shows a flattening at the highest doses (*2*). A recent report on human exposure to benzene at slightly lower occupational doses seems to indicate a sublinear relationship (*17*). The study by Rinsky et al. (*17*) is very important because it accounts for an increased risk of death from leukemia even at 1 ppm (40-year exposure) (that is at an exposure level lower than that previously explored in human studies) and suggests that a reduction in the permissible exposure limits (TLV) needs to be performed.

The shape of the function obtained by logistic regression analysis, which could give a risk estimate lower than that expected from a linear extrapolation at low doses, is not yet sufficiently supported by the scanty number of tumors observed at the lowest exposure levels. Promotionlike effects could account for such a sublinear shape. However, dose-response curves may be linear also at low doses, especially those lower than 1 ppm for a working life, when promotion does not play a prominent role.

The problem of whether the response to a low-dose exposure is linear or sublinear is very important because the number of people exposed at low doses as a consequence of environmental contamination is very high (*18*). However, the estimate of risk associated to environmental exposure, which is much less than occupational exposure, would not be based on direct epidemiological or experimental (long-term assay) data. Therefore, we suggest that a linear extrapolation could be used to quantify the associated risk to the lowest levels of exposure to benzene, starting from the lowest dosages leading to a statistically significant increase of tumor incidence.

This work was supported by grants from Ministero della Pubblica Istruzione and Progetto Finalizzato CNR Oncologia, contract 87.01315.44 and 87.01566.44, Rome, Italy. We thank Carlo Buttazzi for his technical assistance.

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