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Studies on the Applicability of Biomarkers in Estimating the Systemic Bioavailability of Polynuclear Aromatic Hydrocarbons from Manufactured Gas Plant Tar-Contaminated Soils

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The systemic bioavailability of polynuclear aromatic hydrocarbons (PAH) from ingested soils containing manufactured gas plant (MGP) tar was evaluated in mice. Soil and organic extract of each soil were incorporated into a diet and fed to mice for two weeks. 1-Hydroxypyrene levels in urine and chemical:DNA adduct levels in lungs were used as biomarkers of PAH systemic bioavailability. Estimates of PAH relative bioavailability were determined by comparing the bioavailability observed between each soil and corresponding organic extract. In all but one case, bioavailability estimates based on 1-hydroxypyrene levels in urine indicate that the presence of MGP tar on soil results in a considerable decrease in PAH systemic bioavailability (9−75%). Similarly, PAH bioavailability estimates based on chemical:DNA adduct formation ranged from nondetectable to 76%. These results clearly indicate that the bioavailability of PAH is less than 100% when soil contaminated with MGP tar is ingested by mice. In addition, the experimental methods employed in this study appear suitable for evaluating the effects of soil on the gastrointestinal absorption and systemic bioavailability of PAH from soil containing complex organic mixtures.

Introduction

Coal and later oil were used at manufactured gas plants (MGP) as sources of gas for commercial, industrial, and residential uses. The carbon-containing compounds in coal or oil were broken down by heating under reduced oxygen to produce hydrogen and carbon monoxide and, in newer processes, methane. The combustible gases were captured, purified, and stored before being transported by local pipelines to businesses and homes. Coal and oil gasification was a large industry in the United States and Europe that operated from the 1800s to the early 1950s. Coal tars (MGP tars) and coke were also generated as byproducts of this gas manufacturing method, and both products had commercial value. Coke was used in industrial processes such as steel making, while MGP tar was used to pave road surfaces, to roof, and to line and waterproof water pipes. In addition, the chemical diversity of MGP tars made them valuable sources of chemicals used in medicinal agents, dyes, and other commercial products.

MGP tars were collected and stored in holding tanks and/or pits. If there was no commercial market for them they were either disposed of on site or transported off the manufacturing site for disposal. Abundant natural gas resources have eliminated the need of MGP plants for producing methane gas. As a consequence of this, MGP plants and structures containing MGP tar were razed and the land converted to residential, commercial, or industrial uses. There is reason to believe that MGP tars are still found at many of the 2500 former MGP sites known to have operated within the United States (*1*). There is also evidence that gasification plant workers exposed to MGP tars and tar fumes had a higher risk of lung cancer than workers who were not exposed (*2*). In addition, an increased cancer risk was observed in individuals exposed to commercial coal tar products, and some of the chemicals known to be in coal tars are probable human carcinogens (*3*−*7*). Therefore, MGP tars in the environment represent a potential hazard to human health.

Materials similar to MGP tar have been long known to be carcinogenic in laboratory animals (*8*−*13*). More recent studies have clearly demonstrated that neat MGP tar, collected from several MGP sites across the United States, are potent carcinogens in laboratory animals (*14*−*16*). Carcinogenicity is believed to be due to members of a class of organic compounds known as polynuclear aromatic hydrocarbons (PAH) (*17*−*19*). The mechanism by which individual PAH initiate chemical carcinogenesis has been extensively investigated (*20*). PAH are metabolically activated to reactive electrophiles that covalently modify cellular DNA forming chemical:DNA adducts. It is generally accepted that chemical:DNA adduct formation in organs susceptible to PAH carcinogenesis is the first critical step in a multistep process that leads to cancer induction. However, it is also important to note that the formation and presence of chemical:DNA adducts within an organ, susceptible or nonsusceptible to PAH carcinogenesis, does not necessarily mean that cancer will develop within the organ in which the adducts are formed. Thus, the causal relationship between chemical:DNA adduct formation and the susceptibility of an organ to cancer induction is only partially understood.

Human exposure to contaminated soil can occur by inhalation of fine soil particles, ingestion (especially in children), and skin contact during recreational activities such as gardening (*21*). Children playing outdoors are estimated to consume 50−180 mg of soil per day (*22*−*25*). Several studies have assessed the bioavailability of ingested dioxins, halogenated hydrocarbons, and inorganic elements such as lead and arsenic from contaminated soils (*26*−*30*). Goon and co-workers (*31*) reported that the adsorption of benzo[*a*]pyrene (B[a]P) to soil decreased the gastrointestinal availability of B[a]P when administered to animals by gavage. Weathering of soil was also determined to considerably reduce the bioavailability of soil bound B[a]P (*32*). Recent studies performed in our laboratory also indicate that the in vivo bioavailability of PAH within MGP tar can be considerably reduced when adsorbed to soil (*33*).

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TABLE 1. Polynuclear Aromatic Hydrocarbon Content of Soil Samples[a]

	manufactured gas plant site									
	site A				site B			site C		
compound	A1000VL	A1000L	A1000M	A1000H	B1000L	B1000M	B1000H	C1000L	C1000M	C1000H
acenaphthylene	<0.21	<0.15	3.34	46.1	0.18	1.89	40.0	0.90	8.47	70.5
acenaphthene	<0.21	<0.15	0.55	7.29	0.16	0.31	130	0.58	2.59	50.5
fluorene	<0.21	<0.15	0.55	12.3	<0.24	0.25	76.0	1.23	6.07	70.9
phenanthrene	0.12	0.44	7.18	126	0.30	0.77	174	2.30	21.4	895
anthracene	<0.21	<0.15	2.88	37.7	<0.24	0.59	66.5	0.63	4.29	63.1
fluoranthene	0.19	1.00	17.2	192	0.38	2.73	102	2.11	14.6	444
pyrene	0.15	1.09	17.0	193	0.60	5.40	148	3.12	20.5	627
total nPAH	0.46	2.53	48.7	614	1.62	11.9	737	10.9	77.9	2220
benz[*a*]anthracene	<0.21	0.62	10.6	102	0.41	1.55	31.8	1.16	9.17	189
chrysene	0.11	0.63	10.9	115	0.40	3.35	55.4	1.37	7.51	187
benzo[*b*]fluoranthene	<0.21	0.70	15.5	192	0.36	2.68	24.3	1.00	5.71	129
benzo[*k*]fluoranthene	<0.21	0.71	9.62	108	0.23	3.47	10.7	1.21	6.65	123
benzo[*a*]pyrene	<0.21	0.72	11.4	118	0.70	6.51	63.8	1.69	11.0	98.7
indeno[1,2,3-*cd*]pyrene	<0.21	0.67	12.4	155	0.28	2.79	20.9	1.03	6.44	73.1
dibenz[*a,h*]anthracene	<0.21	0.21	2.46	23.6	<0.24	0.27	6.65	0.21	0.52	22.9
benzo[*g,h,i*]perylene	<0.21	0.74	13.3	172	0.41	3.72	24.8	1.20	7.54	72.2
total cPAH	0.11	5.00	86.2	986	2.79	24.3	238	8.87	54.5	895
total PAH	0.57	7.53	135	1600	4.41	36.2	975	19.8	132	3120
concentration unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
quantitation limit	0.21	0.15	0.20	0.62	0.24	0.13	0.16	0.14	0.12	12.5
detection limit	0.08	0.06	0.08	0.25	0.09	0.05	0.06	0.06	0.05	5.02

[a] nPAH: noncarcinogenic polynuclear aromatic hydrocarbons; cPAH: carcinogenic polynuclear aromatic hydrocarbons.

Soil contaminated with MGP tar is of great concern to human health since MGP tar has been demonstrated to be highly carcinogenic to laboratory animals (*14*−*16*) and as much as 1 to 75 acres of soil containing MGP tar can be located at former MGP sites. Since previous studies have demonstrated that the bioavailability of chemicals can be significantly affected by adsorption to soil, it is likely that the effect soil has on the bioavailability of PAH from MGP tar will play a critical role in the overall carcinogenic activity of soil contaminated with MGP tar. Recent guidelines issued by federal and state agencies concerned with the effects of chemicals in the environment encourage assessment of exposure to all significantly impacted media such as air, water, and soil (*34*, *35*). Including these additional evaluations and information concerning the bioavailability of chemicals from various media will likely lead to more reliable human health risk assessments.

In the present investigation, a detailed study was performed to provide quantitative estimates of the systemic bioavailability of PAH from soil containing MGP tar collected from three former MGP sites. Bioavailability was determined by measuring 1-hydroxypyrene levels in urine as well as by analyzing lung tissue for the presence of chemical:DNA adducts. PAH bioavailability observed with soil and organic extract of each soil were compared in order to estimate a relative PAH bioavailability value for each soil sample. These relative bioavailability values provide a better understanding of potential modulating effects of soil on PAH systemic bioavailability. In addition, more reliable estimates of PAH bioavailability from contaminated soil would be particularly useful in risk assessment models to more accurately determine potential human health risks associated with soil containing MGP tar.

Experimental Section

Soil Collection, Preparation, and Analysis. Eight to 12 soil samples were collected from each of three former MGP sites and analyzed for 15 PAH. Sampling locations were selected to contain varying amounts of PAH but have the same or similar geological matrix and relative abundance of PAH. In general, samples collected were close to the surface and subjected to weathering conditions. All of the samples underwent preliminary screening using microscale solvent extraction (MSE), a modification of EPA method 826, followed by analysis using gas chromatography with flame ionization detection (GC/FID) for the simultaneous quantification of monoaromatic hydrocarbons and PAH. The details of this procedure have been previously reported (*36*). The detection and quantitation limits for the 15 PAH evaluated ranged from 0.05 to 12.5 mg/kg (Table 1).

After initial screening, samples from each site were selected for study based on PAH concentration, PAH distribution, and soil type which was based on the visual appearance of each soil. These samples were air-dried and sieved with stainless steel sieves to a grain size of 1000 microns or less. The sieved soils were again analyzed using MSE and GC/FID; results were used to select the final three or four samples to be studied for each site. Organic extracts were prepared for each of the final sieved soils (200 g) using Soxhlet extraction with methylene chloride followed by extract reduction to approximately 10−15 mL using the Kuderna-Danish technique. Due to the high concentration of material in each extract, volumes were further reduced (<5 mL) to a constant weight under a stream of nitrogen. The amount of extractable material present in the methylene chloride extract accounted for 0.057, 0.083, 0.437, 8.000, 0.176, 0.014, 0.776, 0.070, 0.294, and 5.12% of the total original weight (200 g) of each soil sample (A1000VL, A1000L, A1000M, A1000H, B1000L, B1000M, B1000H, C1000L, C1000M, and C1000H, respectively). Organic extracts were analyzed for PAH using waste dilution (EPA Method 3580 Mod.) followed by GC/FID. Since the primary purpose of this study was to evaluate the bioavailability of the high molecular weight PAH, no special effort was made to retain the monoaromatic hydrocarbon fraction originally present in soils. However, the monoaromatic fractions were much lower than the PAH fraction of interest since the soil samples used in this study were collected close to the surface and were subjected to extensive weathering. In addition, the analysis of each soil immediately after site collection and following air-drying and sieving indicates that there was no significant change in the monoaromatic hydrocarbon fraction after soil processing.

TABLE 2. Experimental Protocol

	description	cage no.	no. of mice	diet	description
group 1	powder control	1	4	powder	neg. control
		2	4	powder	neg. control
group 2	extracted soil[a]	3	4	powder	neg control
		4	4	powder	neg. control
group 3	soil no. 1[b]	5	4	powder	experimental
		6	4	powder	experimental
group 4	soil no. 2	7	4	powder	experimental
		8	4	powder	experimental
group 5	soil no. 3	9	4	powder	experimental
		10	4	powder	experimental
group 6	soil no. 4	11	4	powder	experimental
		12	4	powder	experimental
group 7	gel control[c]	13	4	basal gel	neg. control
		14	4	basal gel	neg. control
group 8	org. extract soil no. 1[d]	15	4	basal gel	max. availability
		16	4	basal gel	max. availability
group 9	org. extract soil no. 2	17	4	basal gel	max. availability
		18	4	basal gel	max. availability
group 10	org. extract soil no. 3	19	4	basal gel	max. availability
		20	4	basal gel	max. availability
group 11	org. extract soil no. 4	21	4	basal gel	max. availability
		22	4	basal gel	max. availability

[a] Soil that had the highest methylene chloride extractable material was used to prepare extracted soil diets. This group was used as a negative control. [b] Soil (particle size ≤ 1000 μm) was added to powder rodent diets at a level of 20% (20 g of soil/80 g of food). [c] Clean methylene chloride (5−10 mL) was added to control basal gel diets to control for the methylene chloride used in gel diets (5−10 mL). [d] Soil samples (200 g) from groups 3−6 were extracted with methylene chloride, concentrated under nitrogen, and added to a basal gel diet at a level that would give mice a PAH dose comparable to the dose received by mice ingesting a powder diet containing the corresponding soil sample.

Eight out of the 10 soil samples had monoaromatic hydrocarbon fractions that ranged from nondetectable to 16 ppm, while the two soils (both having the highest level of PAH) had a monoaromatic hydrocarbon fraction of 207 and 307 ppm. Organic extract originating from 200 g of soil along with approximately 500 g of corresponding sieved soil were coded and shipped to Rutgers University. PAH content of soil samples is summarized in Table 1.

Diet Preparation. Coded soil samples and organic extracts from each soil sample were stored at −20 °C. Diets containing soil were prepared by mixing 20 g of soil with 80 g of powder rodent diet (PMI Feeds, Inc., St. Louis, MO) in a glass jar. Soil and food mixtures were gently tumbled until visually homogeneous. Jars were sealed with Parafilm and stored at −20 °C. Organic extracts of each soil sample were incorporated into basal gel diets (Bio-Serv Inc., Frenchtown, NJ) as previously described (*37*). A previous study demonstrated that PAH bioavailability is not altered when MGP tar is administered to animals in either a basal gel or a powder diet (*38*). Thus, the use of both a powder and basal gel diet system is considered appropriate for the current studies. Organic extracts from 200 g of each soil were suspended in 5−10 mL of methylene chloride and added to a basal gel diet containing 3020 mL of water, 100 g of agar, and 1948 g of powder food. Control gel diets were prepared with 5−10 mL of pure methylene chloride. Gel diets and powder diets were stored for 1−2 days at −20 °C prior to initiating experiments.

Animal Treatment. Female B6C3F1 mice 6 weeks old were obtained from Charles River Laboratories (Wilmington, MA). Mice were housed in solid polycarbonate Micro-Isolator cages (Lab Products, Inc., Maywood, NJ) with alpha dry bedding (Shepard Specialty Papers, Kalamazoo, MI) under controlled conditions with a 12 h light-dark cycle. Mice were allowed free access to food and water. Mice scheduled to receive gel diets were acclimated to pure basal gel diets 4 days prior to receiving diets containing organic extracts. At seven weeks of age mice were divided into groups of eight mice (four mice per cage) as outlined in Table 2. Three separate feeding studies were performed using soils collected from three former MGP sites (designated as Site A, B, and C). Mice fed control powder and gel diets along with a powder diet containing soil previously extracted with methylene chloride served as controls and were included in each feeding experiment. Mice were allowed free access to diets for a total of 14 days. Food consumption and animal body weight were monitored throughout each experiment. Mice were moved to plastic Nalgene metabolism cages on day 13, and urine was collected for the last 24 h of diet administration. Urine samples were adjusted to a common volume with 18 MΩ water, labeled according to cage number, and stored at −20 °C for 1-hydroxypyrene analysis. Mice were killed, and lung tissue was removed and pooled according to cage number. DNA was isolated from three lung samples, and remaining lung samples were archived at −20 °C.

1-Hydroxypyrene and Chemical:DNA Adduct Analysis. The level of 1-hydroxypyrene in urine was determined for each cage of mice using a standard method described by Jongeneelen et al. (*39*). In brief, an equal volume of 0.1 M sodium acetate buffer pH 5.0 was added to urine samples (5 mL), and pH was adjusted to 5.0 with concentrated hydrochloric acid. Enzymatic hydrolysis was carried out by adding 25 μL (1250 Fishman units) of β-glucuronidase/arylsulfatase enzyme and incubating at 37 °C for 16 h. Hydrolyzed urine samples were passed through a Sep-Pak C_{18} cartridge and washed with 10 mL of water and metabolites were eluted with 10 mL of methanol. The eluate was dried under vacuum and dissolved in 200 μL of DMSO. Samples (5−10 μL) were analyzed on a C_{18} column (150 × 4.6 mm I.D., Vydac 10 3000A) with gradient elution of methanol and water (40−100% methanol in 15 min). Excitation and emission parameters were set at 347 and 386 nm, respectively.

Levels of chemical:DNA adducts in lung were determined for each cage of mice using the ^{32}P-postlabeling technique (*40*). In brief, 10 μg of each DNA sample was hydrolyzed to nucleotides, ^{32}P-postlabeled, and subjected to five-dimensional poly(ethylenimine)-cellulose (PEI-cellulose) TLC. Following development, radioactivity was located by autoradiography using Kodak Xomat AR film with intensifying screens. The levels of radioactivity on the TLC plates were

TABLE 3. Diet Consumption Values

group	2 week food ingested[a] (g/mouse)	2 week soil/extract[b] ingested per mouse	2 week total PAH dose (mg/mouse)	last 24 h[c] food ingested[d] (g/mouse)	last 24 h soil/extract ingested per mouse
			Site A		
A1000VL	51.9	10.4 g soil	0.006	4.02	0.80 g soil
A1000L	53.0	10.6 g soil	0.080	4.49	0.90 g soil
A1000M	52.0	10.4 g soil	1.40	4.32	0.87 g soil
A1000H	42.8	8.67 g soil	13.7	3.91	0.78 g soil
AR1000VL	120	5.12 mg extract	0.022	7.31	0.33 mg extract
AR1000L	112	7.62 mg extract	0.014	7.12	0.53 mg extract
AR1000M	109	37.5 mg extract	0.812	7.50	1.73 mg extract
AR1000H	100	598 mg extract	20.7	6.02	37.8 mg extract
			Site B		
B1000L	53.7	10.7 g soil	0.047	5.16	1.03 g soil
B1000M	58.6	11.7 g soil	0.424	5.67	1.14 g soil
B1000H	59.1	11.8 g soil	11.5	5.98	1.20 g soil
BR1000L	98.4	0.52 mg extract	0.007	8.60	0.05 mg extract
BR1000M	100	13.1 mg extract	0.290	9.60	1.25 mg extract
BR1000H	96.6	57.4 mg extract	2.72	8.57	5.09 mg extract
			Site C		
C1000L	58.2	11.6 g soil	0.230	3.66	0.73 g soil
C1000M	55.1	11.0 g soil	1.46	3.45	0.69 g soil
C1000H	52.5	10.5 g soil	32.8	3.43	0.69 g soil
CR1000L	110	6.10 mg extract	0.143	11.1	0.62 mg extract
CR1000M	122	27.8 mg extract	1.11	9.97	2.26 mg extract
CR1000H	149	587 mg extract	23.1	9.42	37.0 mg extract

[a] Diet consumption for control mice fed diets for two weeks were as follows: powder control, 41.6−44.9 g/mouse; extracted soil; 50.4−53.4 g/mouse; gel control 88.9−90.5 g/mouse. [b] Extract refers to the material extracted from soil samples using methylene chloride. [c] Values represent the amount of food ingested by mice while in metabolism cages during the last 24 h of the two-week feeding study. [d] Diet consumption for control mice during the last 24 h of the two-week feeding study: powder control, 3.13−3.89 g/mouse; extracted soil; 3.54−4.33 g/mouse; gel control 5.32−10.6 g/mouse.

determined using liquid scintillation counting. Relative adduct levels were determined, and picomoles of adducts were calculated by multiplying relative adduct level values by 0.31×10^7 with the assumption that 1 mg of DNA = 0.31×10^7 pmol of nucleotides. The quantitation limit of the ^{32}P-labeling assay was determined to be 0.003 pmol of adducts/mg of DNA. This limit is based on the amount of background radioactivity observed on TLC maps generated from control groups of mice.

Bioavailability Calculations. Data presented in Tables 2−4 represent the average values from two cages of mice within each group. Since each cage was evaluated separately for food consumption and 1-hydroxypyrene excretion, two independent values representing the average of four mice within each cage were generated for these endpoints. Similarly, two independent values were also generated per group for chemical:DNA adduct levels. However, these two values represent the average of three mice within each cage since a lung from one mouse was archived. These average values were used to estimate the relative bioavailability of PAH from soil.

Fractional urinary excretion (FUE) was calculated as previously outlined by Magee and co-workers (*41*). FUE values estimate the amount of 1-hydroxypyrene excreted in a 24 h urine sample following a dose of pyrene consumed by mice during that same time interval. FUE values for mice ingesting soil (FUE_{soil}) and organic extract of each soil ($FUE_{extract}$) were calculated according to the following equation:

$$FUE = [(\text{amount of 1-hydroxypyrene present in 24 h urine collection})/(\text{amount of pyrene ingested over 24 h})] \times 100$$

FUE values were obtained for each soil and corresponding organic extract. Relative bioavailability of PAH from soil is estimated by comparing FUE_{soil} and $FUE_{extract}$:

$$\text{relative bioavailability} = (FUE_{soil}/FUE_{extract}) \times 100$$

Magee and co-workers (*41*) have previously defined this type of relationship as absorption adjustment factor (AAF). The 1-hydroxypyrene urinary excretion was evaluated using two separate cages of mice for each soil sample and corresponding organic extract (i.e., soil cage 1 and 2 for soil samples and extract cage 1 and 2 for organic extracts). Therefore, relative bioavailability values in Table 4 (Mean ± S.E.) were calculated based on four relative bioavailability values obtained using the following relationship:

$$\text{relative bioavailability} = (FUE_{\text{soil cage 1}}/FUE_{\text{extract cage 1}}) \times 100$$

$$\text{relative bioavailability} = (FUE_{\text{soil cage 1}}/FUE_{\text{extract cage 2}}) \times 100$$

$$\text{relative bioavailability} = (FUE_{\text{soil cage 2}}/FUE_{\text{extract cage 1}}) \times 100$$

$$\text{relative bioavailability} = (FUE_{\text{soil cage 2}}/FUE_{\text{extract cage 2}}) \times 100$$

The formation of chemical:DNA adducts in lung can also be used to estimate PAH bioavailability. Similar to the above approach, a fractional lung adduct (FLA) value can be defined to estimate the amount of carcinogenic PAH (cPAH) absorbed from the gastrointestinal tract, metabolically activated, and bound to DNA in a target organ such as the lung. FLA values for mice ingesting soil (FLA_{soil}) and organic extract of each soil ($FLA_{extract}$) were calculated:

$$FLA = [(\text{chemical:DNA adduct level in lung})/(\text{amount of cPAH ingested over 2 weeks})] \times 100$$

TABLE 4. 1-Hydroxypyrene Levels in Urine and Estimates of Polynuclear Aromatic Hydrocarbon Bioavailability from Soil Contaminated with MGP Tar

	PAH content[a] (ppm)	1-hydroxypyrene excreted[b] (μg/mouse)	pyrene ingested[c] (μg/mouse)	fractional urinary excretion[d] (excreted/ingested) $\times$ 100	relative bioavailability[e]
			Site A		
A1000VL	0.57	0.01 $\pm$ 0.00	0.12 $\pm$ 0.01	8.33	75.0 $\pm$ 5.8
A1000L	7.53	0.09 $\pm$ 0.00	0.98 $\pm$ 0.03	9.18	55.1 $\pm$ 1.7
A1000M	135	0.80 $\pm$ 0.33	14.7 $\pm$ 0.77	5.44	21.13 $\pm$ 4.65
A1000H	1,600	2.64 $\pm$ 0.15	151 $\pm$ 13.5	1.75	29.7 $\pm$ 1.2
AR1000VL		0.02 $\pm$ 0.00	0.18 $\pm$ 0.02	11.1	
AR1000L		0.02 $\pm$ 0.00	0.12 $\pm$ 0.01	16.7	
AR1000M		2.10 $\pm$ 0.00	8.31 $\pm$ 0.78	25.5	
AR1000H		8.97 $\pm$ 0.45	152 $\pm$ 1.51	5.90	
			Site B		
B1000L	4.41	0.03 $\pm$ 0.00	0.62 $\pm$ 0.03	4.84	8.47 $\pm$ 0.95
B1000M	36.2	0.13 $\pm$ 0.01	6.13 $\pm$ 0.07	2.12	10.8 $\pm$ 0.7
B1000H	975	7.08 $\pm$ 3.07	177 $\pm$ 2.45	4.00	30.8 $\pm$ 8.2
BR1000L		0.04 $\pm$ 0.00	0.07 $\pm$ 0.00	57.1	
BR1000M		0.82 $\pm$ 0.07	4.20 $\pm$ 0.04	19.5	
BR1000H		4.53 $\pm$ 0.41	34.8 $\pm$ 1.42	13.0	
			Site C		
C1000L	19.8	0.30 $\pm$ 0.00	2.29 $\pm$ 0.01	13.1	52.3 $\pm$ 4.8
C1000M	132	3.98 $\pm$ 0.49	14.2 $\pm$ 1.01	28.1	111 $\pm$ 17.1
C1000H	3,120	16.6 $\pm$ 0.20	430 $\pm$ 20.9	3.85	25.6 $\pm$ 1.05
CR1000L		0.55 $\pm$ 0.09	2.21 $\pm$ 0.01	25.1	
CR1000M		3.40 $\pm$ 0.76	13.5 $\pm$ 0.36	25.2	
CR1000H		42.2 $\pm$ 1.80	281 $\pm$1.04	15.0	

[a] Individual PAH detected are listed in Table 1. [b] In the case of urine collected from control groups of mice, 1-hydroxypyrene (0.058 μg/mouse) was only detected in urine of mice ingesting a diet containing soil collected from site B and previously extracted with methylene chloride. 1-Hydroxypyrene was not detected in urine collected from any other control group (control powder, control gel, or extracted soil from site A or B). Value represents the mean of two separate determinations $\pm$ range. [c] Value represents the mean of two separate determinations $\pm$ range. [d] Fractional urinary excretion (FUE) = 100 $\times$ [(amount 1-hydroxypyrene in urine)/(amount of pyrene ingested)]. [e] Relative bioavailability = 100 $\times$ ($FUE_{soil}/FUE_{extract}$). Values represent the mean of four values $\pm$ SE as described in the Experimental Section.

The FLA values are then used to estimate relative bioavailability of cPAH from soil by comparing the FLA_{soil} and $FLA_{extract}$ values obtained for each soil sample evaluated. This relationship is defined by

$$\text{relative bioavailability} = (FLA_{soil}/FLA_{extract}) \times 100$$

Similar to above, relative bioavailability values based on chemical:DNA adduct formation were (Table 5, Mean $\pm$ S.E.) calculated using four relative bioavailability values obtained using the following relationship:

$$\text{relative bioavailability} = (FLA_{\text{soil cage 1}}/FLA_{\text{extract cage 1}}) \times 100$$

$$\text{relative bioavailability} = (FLA_{\text{soil cage 1}}/FLA_{\text{extract cage 2}}) \times 100$$

$$\text{relative bioavailability} = (FLA_{\text{soil cage 2}}/FLA_{\text{extract cage 1}}) \times 100$$

$$\text{relative bioavailability} = (FLA_{\text{soil cage 2}}/FLA_{\text{extract cage 2}}) \times 100$$

Results

Mice adjusted well to diets containing soil or organic extract of each soil sample. The amount of diet consumed by mice in each group is summarized in Table 3. Over the two-week time period, mice ingested 9−12 g/mouse of soil and 0.5−598 mg/mouse of organic extract. In the last 24 h of diet administration soil ingested ranged from 0.7 to 1 g/mouse, while the amount of organic extract was 0.3−38 mg/mouse. Body weight gains of mice ingesting diets containing soil or organic extract were similar to control mice (data not shown). Thus, there were no apparent adverse health effects associated with the diets used in this experiment.

Total PAH dose ingested by mice over a two-week time period are also summarized in Table 3. Powder and gel diets containing soil and organic extract of each soil, respectively, were prepared to provide each group of mice with a similar PAH dose. However, in many cases the PAH dose ingested by mice maintained on soil and organic extract of each soil varied. For example, mice ingesting B1000H soil ingested 11.5 mg of PAH, while mice ingesting BR1000H organic extract only ingested a total of 2.72 mg of PAH. In general, mice maintained on diets that contained organic extracts of each soil ingested less PAH than mice maintained on diets containing corresponding soil samples. In these experiments, a methylene chloride extract from 200 g of soil was added to a gel diet at levels that were assumed to be comparable to the amount of PAH present in the 200 g of the same soil added to a powder diet. However, since the methylene chloride extract did not contain the same amount of PAH present in the corresponding soil sample, the PAH dose ingested by mice, when calculated based on the amount of diet consumed, was not comparable. This indicates that the methylene chloride extraction and concentration process used in these experiments did not provide a 100% recovery of PAH during all soil extractions. In addition, the use of coded samples along with not having the PAH concentrations of samples available prior to diet preparation did not allow the PAH levels in the gel diets containing extracts to be adjusted in order to match the levels present in powder diets containing soil samples.

The results obtained do, however, indicate that the amount of 1-hydroxypyrene excretion and chemical:DNA adduct formation in lung was dependent on the amount of PAH ingested by mice. That is, high levels of ingested PAH result in higher levels of chemical:DNA adducts and 1-hy-

TABLE 5. Chemical:DNA Adduct Levels in Lung and Estimates of Polynuclear Aromatic Hydrocarbon Bioavailability from Soil Contaminated with MGP Tar

	PAH content[a] (ppm)	lung DNA adducts[b] (pmol/mg DNA)	cPAH ingested[c] (mg/mouse)	fractional lung adducts[d] (adducts/cPAH ingested) × 100	relative bioavailability[e]
			Site A		
A1000VL	0.57	ND[f]	0.001 ± 0.000	ND[f]	ND[f]
A1000L	7.53	0.007 ± 0.000	0.053 ± 0.001	13.2	16.5 ± 0.350
A1000M	135	0.024 ± 0.000	0.900 ± 0.020	2.67	7.95 ± 0.300
A1000H	1600	0.210 ± 0.032	8.442 ± 0.212	2.49	47.3 ± 3.25
AR1000VL		ND[f]	0.009 ± 0.001	ND[f]	
AR1000L		0.008 ± 0.001	0.010 ± 0.001	80.0	
AR1000M		0.178 ± 0.045	0.530 ± 0.034	33.6	
AR1000H		0.713 ± 0.070	13.53 ± 1.205	5.27	
			Site B		
B1000L	4.41	ND[f]	0.03 ± 0.002	ND[f]	ND[f]
B1000M	36.2	0.011 ± 0.003	0.29 ± 0.012	3.45	14.8 ± 2.35
B1000H	975	0.418 ± 0.034	2.82 ± 0.060	14.9	32.4 ± 3.10
BR1000L		ND[f]	0.005 ± 0.000	ND[f]	
BR1000M		0.049 ± 0.003	0.210 ± 0.001	23.3	
BR1000H		0.349 ± 0.058	0.760 ± 0.011	45.9	
			Site C		
C1000L	19.8	ND[f]	0.103 ± 0.000	ND[f]	ND[f]
C1000M	132	0.019 ± 0.001	0.601 ± 0.012	3.16	75.6 ± 5.38
C1000H	3120	0.070 ± 0.002	9.404 ± 0.353	0.74	19.7 ± 0.80
CR1000L		ND[f]	0.064 ± 0.002	ND[f]	
CR1000M		0.019 ± 0.001	0.455 ± 0.068	4.18	
CR1000H		0.263 ± 0.024	6.980 ± 0.404	3.77	

[a] Individual PAH detected are listed in Table 1. [b] In the case of DNA isolated from lungs of mice maintained in control groups (powder control, gel control or extracted soil), no chemical:DNA adducts were detected using the ^{32}P-postlabeling assay. Value represents the mean of two separate determinations ± range. [c] Value represents the mean of two separate determinations ± range. [d] Fractional lung adducts (FLA) = 100 × [(level of chemical:DNA adducts formed in lung)/(amount of carcinogenic PAH ingested over two weeks)]. [e] Relative bioavailability = 100 × (FLA_{soil}/$FLA_{extract}$). Values represent the mean of four values ± SE as described in the Experimental Section. [f] ND, not detected at the assay detection limits.

droxypyrene excretion. Hence, the differences in PAH doses obtained with soils and corresponding organic extract make it difficult to make a direct comparison between these groups. Although total PAH levels were lower in gel diets containing organic extracts, the relative amount of each hydrocarbon was comparable to the relative amounts present in the corresponding powder diets. Thus, normalization based on the amount of PAH ingested was used in this study to make useful comparisons between groups. However, future studies will utilize chemical analysis prior to diet preparation to minimize variability in PAH dose.

1-Hydroxypyrene was detected in urine of mice ingesting diets containing soil with PAH levels ranging from 0.57 to 3120 ppm (Table 4). 1-Hydroxypyrene was also detected in urine of mice ingesting diets containing extracts of each corresponding soil sample. In general, the amount of 1-hydroxypyrene in urine increased as PAH and pyrene levels of each diet increased. It is difficult to make a direct comparison of the amount of 1-hydroxypyrene excreted by mice ingesting soil and organic extract of each soil since the dose of pyrene ingested by mice was not identical in each group. However, an informative comparison is possible when the amount of 1-hydroxypyrene in urine is normalized based on the amount of pyrene ingested by each group of mice. This relationship is defined as the fractional urinary excretion (FUE). In all but one soil sample (C1000M), the fractional urinary excretion of 1-hydroxypyrene was significantly lower for soil than that observed with the corresponding organic extract. The ingestion of C1000M soil resulted in a FUE value (28.1) comparable to the value obtained with its organic extract (25.2).

FUE values determined with each soil and corresponding organic extract were used to estimate relative bioavailability of PAH from each soil contaminated with MGP tar. Relative bioavailability of PAH from each soil is summarized in Table 4. Excluding sample C1000M, the relative bioavailability of PAH when based on 1-hydroxypyrene urinary excretion ranged from 9 to 75%.

DNA isolated from lungs of mice was evaluated for chemical:DNA adduct formation using the ^{32}P-postlabeling method. Autoradiographs of chemical:DNA adduct maps typically observed in these types of experiments are presented in Figure 1. Maps A, B, and C represent DNA isolated from animals ingesting control powder and gel diets and a diet containing extracted soil, respectively. The lack of a significant amount of radioactivity on these maps indicates that background chemical:DNA adducts, associated with the ingestion of a control diet, are minimal and below the quantitation limit of 0.003 pmol/mg DNA. In addition, the lack of radioactivity on map C indicates that the methylene chloride extraction of soil is effective in removing the chemicals within MGP tar which are responsible for DNA adduct formation. The ingestion of diets containing A1000VL, B1000L, and C1000L soil as well as their corresponding organic extracts (AR1000VL, BR1000L, and CR1000L) for two weeks resulted in no detectable levels of chemical:DNA adducts in lung tissue. Adduct maps 1 and 4 are typical of these observations (Figure 1). Mice in these groups ingested between 0.006 and 0.230 mg/mouse of PAH over 14 days of diet administration. In contrast, the ingestion of the other soil samples and corresponding extracts resulted in a significant level of chemical:DNA adducts. In these samples, a diagonal band of radioactivity with distinct concentrated spots of radioactivity distributed throughout the diagonal band was observed. This adduct pattern is typical of patterns previously observed with MGP tar and other complex mixtures containing PAH (*37*). In many cases, a considerable qualitative difference in the levels of adducts formed was readily apparent in the adduct maps obtained between a soil sample and its corresponding organic extract. For example, considerably more adducts were observed in map 5 (extract) when compared to map 2 (soil). Although the adduct levels

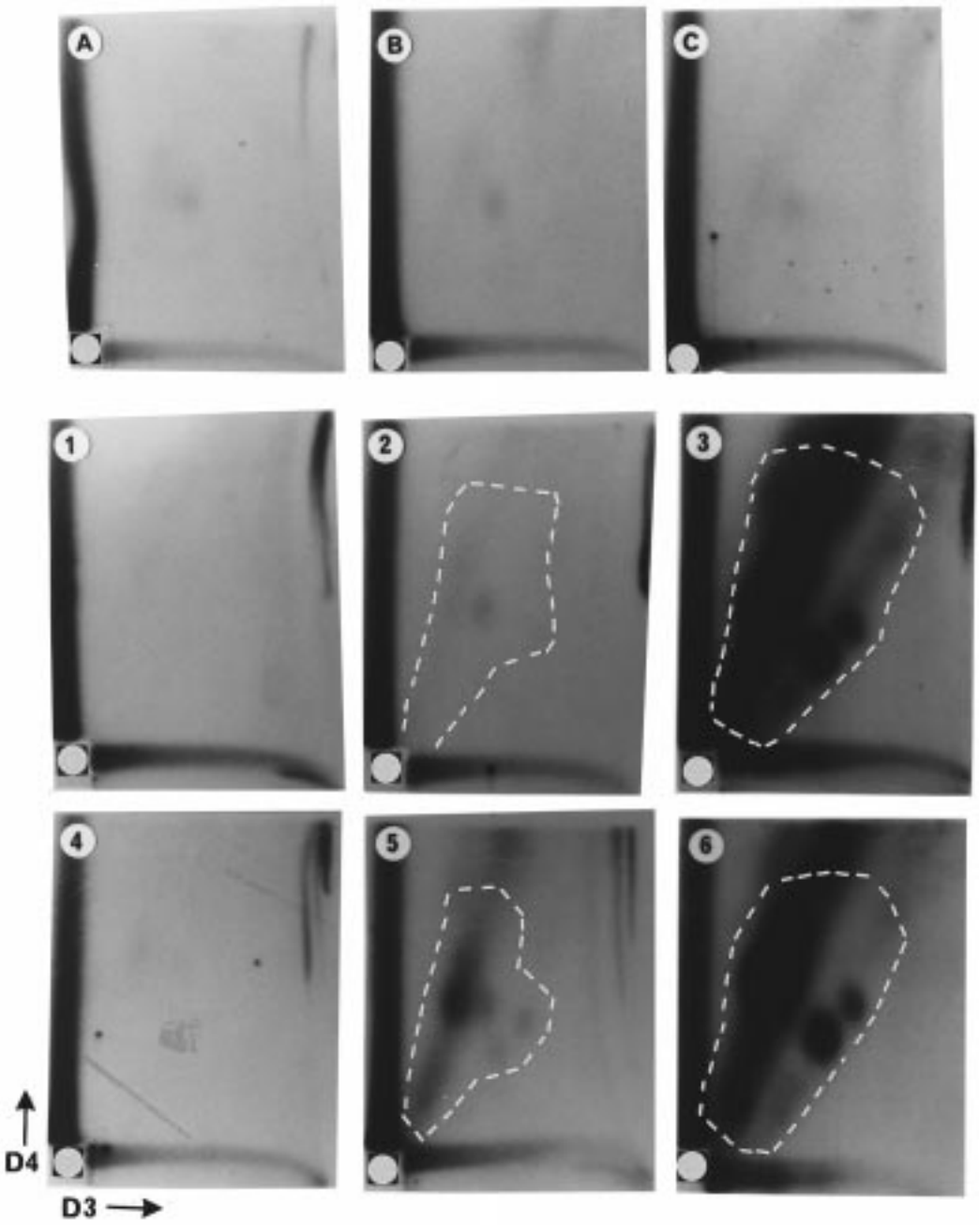

FIGURE 1. Representative autoradiographs of PEI-cellulose TLC maps of DNA isolated from lungs of mice maintained on a control powder (map A), control gel (map B), or a powder diet contaminated with soil (B1000H) previously extracted with methylene chloride (map C). TLC maps 1, 2, and 3, are representative of chemical:DNA adducts formed in lungs of mice ingesting soil contaminated with MGP tar (B1000L, B1000M, B1000H). TLC maps 4, 5, and 6 are representative of chemical:DNA adducts formed in lungs of mice ingesting organic extracts of the same soils (BR1000L, BR1000M, BR1000H). Origins are in the lower left-hand corner, and the dashes define areas of radioactivity that were removed and quantitated.

presented in these maps have not been normalized for PAH dose, they do provide a qualitative assessment of the observed differences between the ingestion of soil and organic extracts of soil. Mice having detectable levels of chemical:DNA adducts ingested between 0.014 and 32.80 mg of PAH over the two-week time period.

Once again it is difficult to directly compare adduct levels observed with mice ingesting soil and organic extract of each soil since the amount of carcinogenic PAH ingested by mice was not similar. However, an informative comparison is possible if observed adduct levels are adjusted for the amount of cPAH consumed. In these calculations, PAH dose is limited to carcinogenic PAH ingested by mice during diet administration because these hydrocarbons (cPAH) are thought to be directly responsible for forming the observed chemical:DNA adducts. This relationship is defined as fractional lung adducts (FLA). The FLA values were lower in mice ingesting soils compared to mice ingesting organic extracts (Table 5). Relative bioavailability values based on chemical:DNA adduct levels in lung are summarized in Table 5. Relative bioavailability of PAH from soil, when based on levels of chemical:DNA adducts detected in lung, ranged from nondetectable to 76% for the 10 soil samples evaluated in this study.

Discussion

The bioavailability of organic chemicals from soil in the gastrointestinal tract has been demonstrated for a number of important environmental contaminants including TCDD,

heavy metals, and PAH (*26*, *27*, *42*, *43*). These studies evaluated the effect of soil on the bioavailability of a single compound by comparing the availability of the compound from soil to that of the pure compound administered in a vehicle. An assumption made in these studies is that the presence of other compounds, if any, in soil does not influence the bioavailability of the compound being studied. This assumption, however, may not hold true in the case of soils that contain complex organic mixtures such as MGP tar. Previous studies have demonstrated that the biological effects of PAH may be modulated by the presence of other compounds within a mixture (*44*). Most of the chemical compounds within MGP tar are unidentified, and, therefore, it is essentially impossible to create a synthetic standard representative of the complex mixture. Lack of a suitable chemical mixture representative of soil contaminants has limited the potential to obtain reasonable estimates of PAH systemic bioavailability from soil contaminated with complex mixtures.

The experimental methods employed in this study were designed to estimate the effect of soil on bioavailability of PAH within a complex mixture adsorbed to soil by comparing the bioavailability of PAH adsorbed to soil to the bioavailability of PAH within an organic extract of the same soil. In this approach, it is assumed that methylene chloride extraction of soil will provide a complex organic mixture representative of the available soil contaminants. Therefore, PAH systemic bioavailability can be estimated based on the availability observed with soil and its corresponding organic extract. This relationship is defined as a relative bioavailability value, which represents a quantitative estimate of the effect of soil on the availability of PAH within MGP tar. The results presented within clearly demonstrate that systemic bioavailability of PAH from the gastrointestinal tract following the ingestion of MGP tar is considerably reduced when adsorbed to a soil matrix.

The present study employed two independent parameters, the detection of 1-hydroxypyrene in urine and chemical:DNA adduct formation in lung, as measures of PAH systemic bioavailability. The urinary excretion of 1-hydroxypyrene has been used extensively as a biomarker to evaluate PAH bioavailability in laboratory mice (*37*) and humans (*39*). However, the use of chemical:DNA adduct formation has not been used previously to estimate PAH bioavailability. Our data indicate that 1-hydroxypyrene excretion and chemical:DNA adduct formation appear to be suitable biomarkers for obtaining quantitative estimates of PAH bioavailability from contaminated soil. Although both biomarkers provide estimates of PAH bioavailability, there is a significant difference in the information obtained. In the case of 1-hydroxypyrene urinary excretion, bioavailability is based on the gastrointestinal absorption, metabolism, and excretion of pyrene. However, pyrene is not carcinogenic and less hydrophobic and smaller in size than carcinogenic hydrocarbons of primary concern such as benzo[*a*]pyrene. Hydrophobicity is thought to play an important role in PAH adsorption to soil. Thus, there is considerable concern over the usefulness and appropriateness of using pyrene as a surrogate for estimating the availability of carcinogenic hydrocarbons within complex organic mixtures. In contrast, detection of chemical:DNA adduct formation in lung tissue provides an assessment of the gastrointestinal absorption of primarily carcinogenic hydrocarbons that are metabolically activated and bound to cellular DNA within lung tissue. Chemical:DNA adduct measurements are particularly meaningful since (1) chemical:DNA adduct formation is considered to be a critical first step in the multistep process of chemical carcinogenesis, (2) chemical:DNA adducts observed primarily arise from high molecular weight carcinogenic hydrocarbons, (3) observed adduct levels result from combined effects of chemicals within the complex mixture that are known to either inhibit and/or promote chemical:DNA adduct formation, and (4) lung tissue is a well established target organ for PAH carcinogenesis. Chemical:DNA adduct analysis has been extensively used to evaluate the metabolic activation of PAH. Therefore, employing chemical:DNA adducts as a biomarker to assess in vivo bioavailability of PAH from contaminated soil is not unreasonable. The use of chemical:DNA adduct formation to estimate PAH bioavailability may provide additional insight into mechanism of action for complex organic mixtures since there is a potential to identify the hydrocarbons responsible for the observed adduct formation.

It is difficult to make a direct comparison of relative bioavailability values estimated based on 1-hydroxypyrene excretion and chemical:DNA adduct formation since they are measurements of very different biological responses. However, there are a number of notable consistencies observed between the two approaches. Relative bioavailability estimates based on 1-hydroxypyrene excretion were higher than estimates based on chemical:DNA adduct formation for 7 out of the 10 soil samples evaluated. This is consistent with the generally accepted concept that pyrene, a four-ring PAH, is more bioavailable from soil than higher molecular weight hydrocarbons which are responsible for chemical:DNA adduct formation and presumably the biological activity of the complex organic mixture containing PAH. In contrast, bioavailability values based on 1-hydroxypyrene values were lower than estimates based on chemical:DNA adduct formation for two of the soil samples. It is not readily apparent why pyrene was much less available in these soils.

In the case of a set of soil samples collected from individual MGP sites relative bioavailability estimates were consistent when based on metabolite urinary excretion or DNA adduct formation. For example, when PAH bioavailability for soils collected from MGP Site B are determined based on 1-hydroxypyrene urinary excretion, relative bioavailability increased with PAH soil concentration (9, 11, and 31%). Likewise, the relative bioavailabilities of PAH from soil also increased when based on chemical:DNA adduct formation (ND, 15, and 32%). In some cases, relative bioavailability estimates based on 1-hydroxypyrene and chemical:DNA adduct formation were also similar for some soil samples. For example, the relative bioavailability of PAH from B1000H soil was 31 and 32%, when based on 1-hydroxypyrene and chemical:DNA adduct levels, respectively.

Relative bioavailability estimates observed with A1000VL, A1000L, B1000L, and C1000L soils, however, were considerably different when based on 1-hydroxypyrene excretion and chemical:DNA adduct formation. Interestingly, these soil samples had the lowest levels of PAH (<20 ppm). The relative bioavailability of PAH from these soil samples was determined to be 75, 55, 9, and 52% when based on 1-hydroxypyrene urinary excretion, respectively. These estimates are considerably higher than expected based on the values observed with soils having higher levels of PAH. In contrast, when the relative bioavailability of PAH from soil is estimated based on chemical:DNA adduct formation in lung tissue, relative bioavailability was ND, 17, ND, and ND percent. These estimates are consistent with soils where PAH relative bioavailability decreased with decreasing PAH levels. These results suggest relative bioavailability estimates based on chemical:DNA adduct formation may be the most suitable method for estimating PAH bioavailability from soils containing low levels of PAH.

The experimental protocol employed in this study is suitable for obtaining quantitative estimates of PAH bioavailability from soil containing MGP tar. The presence of MGP tar on soil resulted in a considerable decrease in the

gastrointestinal absorption and systemic bioavailability of PAH. Quantitative estimates of effect of soil on the bioavailability of PAH varied based on the biomarker used to determine bioavailability. The benefits and disadvantages of using 1-hydroxypyrene urinary excretion and chemical: DNA adduct formation in lung as biomarkers for estimating PAH bioavailability require further study. The wide range of relative bioavailability values obtained with both biomarkers indicate that the level of contamination of soils and the site in which soil samples were collected also affect PAH bioavailability. Factors such as weathering conditions, total organic carbon content, type of soil, particle size, pH, and amount of material consumed need to be evaluated thoroughly in order to understand fully the potential of soil to modulate the in vivo bioavailability of PAH from soils contaminated with complex organic mixtures.

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