Tumors and DNA Adducts in Mice Exposed to Benzo[a]pyrene and Coal Tars: Implications for Risk Assessment

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Current methods to estimate the quantitative cancer risk of complex mixtures of polycyclic aromatic hydrocarbons (PAH) such as coal tar assume that overall potency can be derived from knowledge of the concentration of a few carcinogenic components such as benzo[a]pyrene (B[a]P). Genotoxic damage, such as DNA adducts, is thought to be an essential aspect of PAH-induced tumorigenesis and could be a biomarker for exposure useful for estimating risk. However, the role of B[a]P and the relationship of adduct formation in tumorigenesis have not been tested rigorously in models appropriate for human health risk assessment. Therefore, we directly compared tumor induction and adduct formation by B[a]P and coal tars in several experimental protocols, including one broadly accepted and used by regulators. We found that B[a]P content did not account for tumor incidences after exposure to coal tars. DNA adducts were found in both tumors and tumor-free tissue and tumor outcomes were not predicted by either quantitation of total DNA adducts or by the DNA adduct formed by B[a]P. These data suggest that risk assessments based on B[a]P content may not predict accurately risk to human health posed by environmental PAH. — Environ Health Perspect 106(Suppl 6):1325–1330 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Supp6/1325-1330goldstein/abstract.html

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Before the advent of the national natural gas distribution network, gas for industrial and residential use was produced locally from destructive distillation of coal and oil. Manufactured gas plants (MGP), as such production facilities were known, were located in over 1500 communities throughout the United States (1). One of the by-products of gas production was coal tar, a chemically diverse mixture characterized by the presence of polycyclic aromatic hydrocarbons (PAH), including known animal carcinogens and probable human carcinogens such as benzo[a]pyrene (B[a]P) (2). Environmental coal tars may represent a potential hazard to exposed individuals. The risk posed to human health by these tars is determined through the process of cancer risk assessment. Risk assessment consists of hazard identification, dose response, and exposure assessment. Hazard identification of coal tars is supported by studies of occupational exposures (3) and laboratory animal studies, mostly in rodents (2). Unfortunately, there is uncertainty in assessing dose and exposure in occupational studies. Further, there are few laboratory animal studies that address dose response to individual PAH using applicable exposure routes such as ingestion, and even fewer studies that delineate outcomes after exposure to complex tar mixtures.

Proposed risk methodologies for complex environmental mixtures of PAH, such as coal tar, emphasize the role of B[a]P in tumorigenesis (4). However, underlying assumptions in such an approach (e.g., independent action, additivity, absence of modulation by other agents) have not been rigorously tested. To overcome these gaps in our understanding and to provide data for possible use in human cancer risk assessment, research was conducted to delineate the role of B[a]P in tumor induction and plausible biologic mechanisms in coal tar-induced tumorigenesis. A series of laboratory studies emphasizing dose response for tumor induction by coal tars and B[a]P was undertaken.

Much consideration was given to the overall experimental design (5,6). A modification of the protocol developed by the U.S. National Toxicology Program (U.S. NTP) (7) to identify potential carcinogens was selected because this protocol has wide regulatory acceptance. The usual U.S. NTP protocol emphasizes hazard identification, and because a major unresolved issue from the perspective of risk assessment was dose response, a modified design that emphasized dose response was adopted. Other experimental approaches used sensitive tumor systems such as liver tumor induction in B6C3F1 mice treated by ip injections at 15 days of age and lung tumor induction in sensitive strain A/J mice. These assays provided information on mechanisms of tumorigenesis to support tumor incidence findings and evaluate candidate short-term assays useful for predicting risk of complex mixtures. One such candidate for a short-term predictive assay and molecular biomarker is quantitation of total and compound-specific DNA adducts; we therefore analyzed DNA adduct formation and related it to tumorigenesis.
Results of these varied experimental approaches indicate that tumor induction by B[α]P and coal tars share some properties but there are many differences. Because of these differences, it appears risk methodologies for evaluating coal tars and other complex mixtures of PAH based on tumorigenesis by B[α]P alone should be reevaluated.

**Materials and Methods**

The studies utilized three exposure protocols: 2-year (chronic) feeding, ip injection, and short-term (14- and 260-day) feeding.

**Two-Year Feeding Studies**

Methodological details used in the 2-year feeding study are discussed by Culp (8). Briefly, groups of 48 five-week-old female B6C3F1 mice were fed one of two composite mixtures of coal tars from different individual environmental sites. One coal tar mixture designated MGP-M7 was formed by combining equal amounts of tars from seven individual environmental sites. It had a final B[α]P content, determined by gas chromatography/mass spectrometry, of 1840 ppm. The coal tar was frozen in liquid nitrogen and blended with powdered NIH-31 feed to make a 10% master mix that was subsequently blended with NIH-31 powdered feed to the desired concentrations. Homogeneity was monitored by measuring the amount of B[α]P by ultraviolet absorbance. Details of the diet preparation are found in Thompson et al. (9). Mice were given feed with a final coal tar concentration of 0.01, 0.03, 0.3, 0.1, 0.6, or 1.0% (i.e., 100–10,000 ppm) that contained 0.22 to 22 ppm B[α]P. Other mice were fed 0.03, 0.1, or 0.3% of a coal tar mixture designated MGP-M3, a composite consisting of two of the tars present in MGP-M7 plus tar from a third tar site not used in MGP-M7. MGP-M3 contained 2760 ppm B[α]P; B[α]P content in the feed was 1 to 11 ppm. Other groups of 48 mice received 5, 25, or 100 ppm B[α]P alone in the diet. The B[α]P was dissolved in acetone, which was subsequently reduced by evaporation prior to use in animal studies.

Two groups of 48 mice served as controls; one group received feed treated with acetone (solvent control) and the other group received untreated food.

The mice were maintained for 2 years. Mice that died before 2 years and all animals in groups where overall survival at 2 years was less than 60% were subjected to full histopathologic examination to determine tumor incidence and nontumor pathology. Tumor incidence and pathology were determined in all control animals. Tumor and control tissues were fixed in 10% buffered formalin and, after processing, were paraffin embedded.

Other groups of four male mice each were fed B[α]P at doses of 2.5 to 50 mg/kg or MGP-M7 at 0.1 to 2% for 3 to 4 weeks. DNA adducts were determined in lung, liver, and forestomach tissue using 32P-postlabeling (10).

**Studies Using Intraperitoneal Injection**

At 15 days of age (birth = day 1), groups of male B6C3F1 mice were administered a) a single ip injection of B[α]P (125, 250, or 375 μg), b) 7980 μg coal tar from a single site (MGP-4) containing 12.5 μg B[α]P, or c) 7980, 3990, or 1995 μg MGP-M7 containing 14.7, 7.8, and 3.9 μg B[α]P, respectively. Twenty-six, 39, and 52 weeks postinjection, groups of 30 mice were sacrificed and tumor incidences in lung, liver, and forestomach were recorded. Tumors were embedded in paraffin using standard histologic techniques after alcohol-based fixation (STAT fix, Stat Path, Riderwood, Massachusetts). Temporaneous groups of six similarly exposed mice were harvested at times postexposure for DNA adduct analyses using 32P-postlabeling and thin layer chromatography (TLC).

In another study, 15-day-old female B6C3F1 mice were administered B[α]P or MGP-4 by single ip injection and assessed for tumor formation at 26, 39, and 52 weeks.

In a third set of studies, individual PAH were combined into a synthetic mixture based on weight percent of 17 marker PAH contained in MGP-4, including B[α]P (whose concentration was varied to deliver a final dose of 26.75 to 126.75 μg/mouse), but omitting indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene, which were not available in sufficient quantities at the start of the study (11,12). The reconstituted mixtures were administered in a single ip injection to groups of thirty 15-day-old males at a dose of 193 mg/kg. In a second approach, the B[α]P content was fixed at 0.09% of the total PAH and the reconstituted mixture given as a single ip injection at 193, 535, and 1041 mg/kg. Tumorigenesis and 32P-postlabeling DNA adduct assays were conducted.

The compositions of MGP-4, MGP-M7, MGP-M3, and the reconstituted mixture are shown in Table 1.

**Studies with 14 to 260 Days of Feeding**

Groups of 21 to 30 female A/J mice 7 weeks of age were fed MGP-M3 (0.1 or 0.25%) or B[α]P (98 or 16 ppm) in a gel diet (13) for 260 days, and the induction of forestomach and lung tumors was determined. Tumor induction was similarly determined in other groups of 30 females given a single ip injection of 1790 μg B[α]P as a positive control. In another study, female A/J mice were fed coal tar and B[α]P diets similar to those in the 2-year feeding

### Table 1. Composition of an environmental coal tar (MGP-4), two mixtures of environmental coal tars (MGP-M7, MGP-M3), and a chemically reconstituted mix.

| Aromatic compound | MGP-4 | MGP-M7 | MGP-M3 | Reconstituted mix |
|-------------------|-------|--------|--------|------------------|
| Indian            | 0.2   | 1.4    | 0.5    | 0.2              |
| Naphthalene       | 29.8  | 27.3   | 31.6   | 23.8             |
| 2-Methylnaphthalene | 21.4  | 13.9   | 10.5   | 23.2             |
| 1-Methylnaphthalene | 12.3  | 8.1    | 5.5    | 13.3             |
| Acenaphthylene    | 6.9   | 3.9    | 5.6    | 7.7              |
| Acenanthrene      | 0.5   | 2.5    | 1.2    | 0.6              |
| Dibenzofuran      | 0.6   | 1.8    | 1.8    | 0.7              |
| Fluorene          | 3.9   | 4.5    | 4.7    | 4.3              |
| Phenanthrene      | 9.7   | 9.4    | 9.9    | 10.5             |
| Anthracene        | 3.1   | 3.1    | 2.8    | 3.4              |
| Fluoranthene      | 2.7   | 6.1    | 6.2    | 2.4              |
| Pyrene            | 3.9   | 6.3    | 7.1    | 4.3              |
| Benzo[a]anthracene | 1.3   | 2.8    | 3.3    | 1.4              |
| Chrysene          | 1.3   | 2.9    | 2.9    | 1.5              |
| Benzo[b]fluoranthene | 0.8   | 2.6    | 2.8    | 0.8              |
| Benzo[k]fluoranthene | 0.3   | 0.9    | 1.0    | 0.9              |
| Benzo[a]pyrene    | 0.9   | 2.3    | 2.7    | 0.9              |
| Indeno[1,2,3-cd]pyrene | 2.8   | 1.6    | 1.9    | 0.0              |
| Dibenz[a,h]anthracene | 1.0   | 0.4    | 0.3    | 0.0              |
| Benzo[g,h,i]perylene | 3.9   | 1.8    | 2.1    | 0.0              |

*Percent quantified total aromatic compounds.*
study. After 2 weeks, DNA adduct formation was evaluated in lung tissue using \(^{32}\)P-postlabeling and separation by high-performance liquid chromatography (HPLC) (14).

Results

Two-Year Feeding Studies

In 2-year feeding studies, MGP-M7 resulted in tumors of the small intestine, forestomach, lung, and liver as well as sarcomas, hemangiosarcomas, and histiocytic sarcomas (Table 2). A similar spectrum of induced tumors (except small intestine tumors, which were found at doses higher than those tested) was found in animals fed MGP-M3 (Table 1). For MGP-M7 and MGP-M3, lung tumors (mostly adenomas) were induced at lower doses than tumors at other organ sites and thus the lung represented the most sensitive site for MGP-induced tumorigenesis. B[a]P behaved as a point of contact carcinogen and induced tumors of the tongue, esophagus, and forestomach (Table 2). Thus, only forestomach tumors were induced by both coal tar and B[a]P. The dose response for B[a]P-induced forestomach tumors was similar to that reported by Neal and Rigdon (15) in CFW mice.

DNA adducts were detected in lung, liver, and forestomach of mice fed 2.5 to 50 mg/kg B[a]P for 3 weeks and 0.1 to 2% MGP-M7 for 4 weeks (Table 3). Adduct maps (not shown) for mice treated with MGP-M7 gave a diffuse pattern (diagonal radioactive zone [DRZ]). The DRZ reflects adducts formed by many PAH in addition to B[a]P in MGP-M7 and perhaps addition of DNA bases other than guanine. Adduct patterns with MGP were typical of patterns observed with complex organic mixtures. Maps of DNA adducts from mice fed B[a]P showed a few discrete spots including one cochromatographic with 10β-deoxyguanosin-\(N^2\)-yl-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (DG-\(N^2\)-BPDE) formed by reacting B[a]P diol epoxide with salmon sperm DNA. Adduct levels in lung, liver, and forestomach in B[a]P-treated mice were similar, suggesting B[a]P reached all three tissues even though only the forestomach exhibited B[a]P-induced tumors. Adduct data are summarized in Table 3.

Studies Using Intrapitoneal Injection

When infant male mice were injected ip with MGP-M7, MGP-4, or B[a]P, liver tumors (mostly adenomas with some hepatocellular carcinomas) but not forestomach

Table 2. Induced tumorigenesis in mice exposed to coal tar or benzo[a]pyrene.

| Experimental model | Exposure group | Dose, mg coal tar | Dose, pg B[a]P | Tumor sites* |
|--------------------|----------------|------------------|---------------|-------------|
| Two-year feeding in B6C3F1 females | MGP-M7 | 25 | 47.8 | Lung, small intestine |
| | | 17 | 31.2 | Lung, forestomach, small intestine, hemangiosarcomas |
| | | 10 | 18.4 | Liver, lung, forestomach, hemangiosarcomas, sarcomas |
| | | 3.5 | 6.4 | None |
| | | 1.0 | 1.8 | None |
| | | 0.36 | 0.6 | None |
| | MGP-M3 | 9.0 | 24.8 | Liver, lung, forestomach, hemangiosarcomas, histiocytic sarcomas |
| | | 3.6 | 9.9 | Lung |
| | | 1.2 | 3.3 | None |
| | | - | 350 | Forestomach, esophagus, tongue |
| | | - | 88 | Forestomach |
| | | - | 17.5 | None |
| Single ip injection, B6C3F1 infant males | MGP-4 | 7.980 | 12.5 | Liver, 43b |
| | | MGP-M7 | 7.980 | 14.7 | Liver, 59b |
| | | | 3.980 | 7.4 | Liver, 26b |
| | | | 1.995 | 3.7 | Liver, 12b |
| | | B[a]P | - | 375 | Liver, 79b |
| | | | - | 259 | Liver, 52b |
| | | | - | 125 | Liver, 45b |
| Single ip injection, B6C3F1 infant females | MGP-4 | 7.980 | 12.5 | Noneb |
| | | B[a]P | - | 375 | Noneb |
| | | | - | 250 | Noneb |
| | | | - | 125 | Noneb |
| Single ip injection, B6C3F1 infant males | Reconstituted mix | 535c | 4.8 | Noneb |
| | | | 1071c | 9.7 | Noneb |
| | | | 193c | 126.74 | Noneb |
| | | | 193c | 101.74 | Noneb |
| | | | 193c | 76.74 | Noneb |
| | | | 193c | 51.74 | Noneb |
| | | | 193c | 26.74 | Noneb |
| | | | 193c | 1.71 | Noneb |
| 260-day-feeding, A/J females | MGP-M3 | 5.9 | 18.3 | Lung: 100, 12.7d |
| | | B[a]P | - | 257 | Lung: 52, 0.59d; forestomach: 100, 4.22d |
| | | | 2.5 | 6.9 | Lung: 70, 1.19d |
| | | | 193c | 40.6 | Lung: 36, 0.48d; forestomach: 100, 4.22d |
| | | B[a]P | - | 1800 | Lung: 100, 15.6d; forestomach: 83, 1.83d |

*p < 0.05 vs control. *Per day per animal. *Percent mice with tumors, 52 weeks postexposure. *Defined PAH, see text. *Percent mice with tumors, tumors per mouse, 260 days postexposure.

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or lung tumors were induced (Table 2). Even though MGP-4 and MGP-M7 contained much less B[a]P (12.5 and 14.7 μg) than the lowest dose of B[a]P administered alone (125 μg), overall tumor incidences were comparable. MGP-M7-induced tumors tended to arise earlier and MGP-4- and MGP-M7-induced tumors were pathologically more advanced than B[a]P-induced tumors (16). Because tumors of lung and forestomach were found in female mice fed B[a]P or MGP-M7 for 2 years but not in infant male mice given single ip injections, the ip injection protocol was repeated using females. No tumors were induced in lungs, forestomach, or, most notably, livers of females treated in this manner (Table 2).

DNA adducts map similar to those after feeding MGP-M7 or B[a]P to adult female mice were found in liver, lung, and forestomach of ip-injected infant mice given B[a]P, MGP-4, or MGP-M7 (Table 3). Thus, DNA adducts were found in liver, lung, and forestomach even though only liver tumors were induced.

To determine the role of B[a]P in tumor induction by MGP residues and whether B[a]P potency was modulated by noncarcinogenic components, male infant mice were administered ip injections of synthetic reconstituted mixtures of PAH formulated to resemble MGP-4 and containing a range of B[a]P concentrations. The reconstituted mixtures, including one with 125 μg B[a]P, a dose of B[a]P that itself caused liver tumors by 52 weeks (16), did not induce liver, lung, or forestomach tumors. In contemporary studies B[a]P administered alone at 250 μg/mouse induced liver tumors but lower doses of B[a]P (31 and 62.5 μg) did not, indicating that the B[a]P was active but that tumor induction was dose dependent. The reconstituted mixture resembling MGP-4 was not tumorigenic even up to a dose of 1071 mg/kg, a much higher dose than with MGP-4 itself. These results are summarized in Table 2.

**Studies with 14 to 260 Days of Feeding**

A high incidence of lung tumors at a high multiplicity was found in male strain A/J mice after ingesting MGP-M3 for 260 days; nonsignificant incidences and multiplicity were found with B[a]P (Table 2). Ingested B[a]P was an effective tumorigen in the forestomach, whereas MGP-M3 was not. B[a]P induced lung tumors when administered as a single ip injection. Thus, in A/J mice, lung tumor induction following MGP-M3 ingestion and forestomach tumor induction following B[a]P ingestion were similar to results found in the modified NTP bioassay (8).

The spot cochromatographing with dG-N'-BPDE in A/J mice fed MGP-M3 for 14 days was investigated further using HPLC (14). Adducted lung DNA gave three peaks (Figure 1), two of which cochromatographed with adducts formed when lung DNA was isolated from mice treated with B[a]P or benzo[a]pyrene and verified for the complex mixtures by the method of Weyand and Wu (17). The identity of the third adduct peak is unknown.

**Table 3. DNA adducts in liver, forestomach and lung of B6C3F1 mice given benzo[a]pyrene or manufactured gas plants residue.**

| Exposure | Total dose | DNA adducts, fmol/mg DNA |
|----------|------------|-------------------------|
|          | B[a]P, μg  | MGP, mg | Liver | Forestomach | Lung |
| B[a]P    | ip         | 375     | 0     | 1023 | 840 | 1851 |
| MGP-4    | ip         | 12.5    | 7.980 | 1290 | 820 | 2664 |
| MGP-M7   | ip         | 14.7    | 7.980 | 417  | 138 | 2319 |
| MGP-M7   | ip         | 7.9     | 3.990 | 297  | 312 | 1290 |
| MGP-M7   | ip         | 3.8     | 1.995 | 210  | 87  | 831  |
| B[a]P    | Oral       | 0       | 0     | 4    | 11  | 4    |
|          |            | 336     | 0     | 33   | 90  | 43   |
|          |            | 819     | 0     | 90   | 137 | 47   |
|          |            | 1638    | 0     | 201  | 199 | 80   |
|          |            | 3255    | 0     | 286  | 360 | 126  |
|          |            | 4050    | 0     | 905  | 446 | 161  |
| MGP-M7   | Oral       | 0       | 0     | 65   | 112 | 22   |
|          |            | 392     | 165.2 | 362  | 234 | 422  |
|          |            | 840     | 344.4 | 714  | 407 | 1259 |
|          |            | 1400    | 582.4 | 1907 | 812 | 3021 |
|          |            | 2156    | 896.0 | 2454 | 1489| 5367 |
|          |            | 3528    | 1470.0| 3121 | 1792| 6776 |

**Discussion**

Even the best available data from animal studies of tumorigenesis by PAH are not particularly appropriate for application to human health risk assessment (4). Because B[a]P has been proposed as a basis for quantitative risk assessment of complex mixtures of PAH (4), these studies directly compared the outcomes from B[a]P alone to those from coal tars that contain B[a]P.

The data indicate significant differences for tumor induction by B[a]P compared to coal tars:

- Both B[a]P and coal tar induced forestomach tumors in the 2-year feeding study. B[a]P, but not coal tar, induced tumors at two sites—tongue and esophagus. Coal tar, but not B[a]P, induced tumors in lung, small intestine, and liver, as well as sarcomas, hemangioendotheliomas, and histiocytic sarcomas in several sites.

- Doses of 125, 250, or 375 μg B[a]P alone or coal tar containing 3.8 to 14.7 μg B[a]P induced liver tumors in infant male mice. Lower doses of B[a]P (31.5 or 62.5 μg) administered alone did not induce liver tumors.

- Neither B[a]P nor MGP-M7 caused tumors in 15-day-old female B6C3F1 mice by single ip injection even though both induced tumors in 15-day-old male mice.

- Tumorigenicity in 15-day-old male mice by coal tar (MGP-4) could not be recapitulated by a reconstituted mixture based on component concentrations. No tumors were found when 125 μg B[a]P was administered as part
of the reconstituted mixture, even though B[a]P administered alone at 125 μg/mouse induces liver tumors.

- Ingested coal tar, but not ingested B[a]P, induced lung tumors at high incidence and high multiplicity in A/J mice.

Data from the 2-year feeding study are likely to be given more weight than the data from the other protocols in human health risk assessments. Because lung tumors were induced at lower doses than tumors at other sites, lung tumor incidence is likely to form the basis of any quantitative risk methodology for coal tar. The choice of lung tumor incidence for risk assessment is consistent with data in humans showing incidence of lung cancer in workers engaged in certain occupations where exposure to PAH was likely to be high (3). Using lung tumorogenesis to assess MGP–PAH risk is supported by studies that attribute a large portion of smoking-related cancers to PAH in cigarette smoke, as well as data indicating lung tissue in rodents is particularly susceptible to genotoxic effects by complex mixtures of PAH (17, 18).

Lung tumorogenesis was induced at high frequencies in A/J mice fed MGP-M3 but not B[a]P. Robinson et al. (19) detected increased lung tumors in strain A mice orally gavaged with high doses of either B[a]P or a coal tar paint preparation. They concluded that B[a]P could not account for the level of tumor formation by the coal tar paint. Increased lung tumor incidence has been reported in studies where B[a]P or other PAH are introduced into the lung by surgical implantation (20), but the relevance of data derived using this exposure route is questionable. Overall, the data suggest that B[a]P is at best a weak lung carcinogen when ingested, and therefore B[a]P may not be a particularly good surrogate for use in human health risk assessments of coal tar.

Formation of DNA adducts and subsequent mutations leading to malignant conversion of cells is a central tenet of carcinogenic mechanisms proposed for B[a]P and other PAH. Our data indicate DNA adduct formation is not sufficient for tumorogenesis. Ingested B[a]P forms adducts but does not induce tumors in lung (Tables 2 and 3). It is possible that adducts in B[a]P-treated mice were in cells other than bronchial epithelium (most MGP-induced tumors were bronchioalveolar adenomas) or occurred primarily in nondividing cells. In ip-injected male mice 15 days of age, DNA adducts were found in liver, lung, and forestomach (Table 3), but only the liver subsequently had tumors (Table 2). The role of DNA adducts in predicting tumorogenesis is uncertain.

One proposed method for establishing quantitative risk estimates for PAH mixtures is based on their relative potencies or toxic equivalency factors (TEF) (21). In a TEF approach for PAH, overall potency of the mixture is expressed relative to B[a]P and contributions of individual carcinogenic PAH are taken to be additive (4). B[a]P is relatively more abundant than other carcinogenic PAH in coal tar (3) and is more potent than other listed carcinogenic PAH except dibenz[a,h]anthracene. B[a]P would account for about two-thirds of the overall toxicity of coal tars when calculating the toxicity equivalency quotient, the sum of TEFs. Using TEFs is questionable, as the data show no evidence for lung tumor induction by B[a]P in any of the assays except by ip injection in sensitive strain A/J mice.

In an alternative approach, coal tar itself rather than carcinogenic PAH is considered the unit of dose in risk assessments for tars and contaminated media. This approach is based on lung tumor incidence and assumes only minor differences in chemical composition of coal tars, coal tar-contaminated soil, and coal tar-contaminated water. However, low-molecular-weight PAH are lost by volatilization or biologically degraded, whereas high-molecular-weight PAH are not volatile and biodegrade only slowly. Aged soil will have relatively more high-molecular-weight PAH and less low-molecular-weight PAH than tars. PAH have a wide range of aqueous solubility; thus, PAH-contaminated water will have relatively more water soluble, low-molecular-weight (3- and 4-ring) PAH and less insoluble, high-molecular-weight (4-, 5-, and 6-ring) PAH than tars. Because of these differences in composition, the risk approach based on chemical composition of coal tars may not hold for media contaminated by tars. In some cases overestimation might result (contaminated water) and in some cases underestimation might result (contaminated soil).

Until an accurate predictor of carcinogenicity is developed, risk assessment approaches based on exposures to known carcinogenic PAH may still be a useful approach. Carcinogenic PAH comprise about 20% of the PAH content of coal tars and up to 70% of the total PAH in aged soils contaminated by coal tars (22). Because we have no evidence that B[a]P deserves a special role or that mixture potency is the sum of potencies normalized to B[a]P, a risk assessment approach based on total carcinogenic PAH rather than weighted TEF likely captures many of the attributes of the actual carcinogenic mixture (i.e., coal tar) without assuming a pivotal role for B[a]P or any other currently identified carcinogen.

In summary, both tumor induction and DNA adduct data from three independent experimental protocols lead us to question the use of TEF in cancer risk assessments for coal tar or other mixtures of PAH. Until mechanisms of carcinogenicity by PAH are better understood, an approach based on naturally occurring carcinogenic PAH may be useful.

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