NO SIGNIFICANT RISK LEVELS (NSRLS) FOR THE PROPOSITION 65 CARCINOGENS

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Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment (OEHHA)
California Environmental Protection Agency

SUMMARY OF FINDINGS
The oral cancer potencies of six polycyclic aromatic hydrocarbons (benzo[b]fluoranthene, benzo[j]fluoranthene, chrysene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, and 5-methylchrysene) were estimated from cancer dose-response data obtained from animal studies. Potency estimates obtained from newborn mouse intraperitoneal (i.p.) injection studies were converted to oral equivalent potencies. The conversions were based on data for benzo[a]pyrene for which both oral adult mouse data and i.p. newborn mouse data are available. Benzo[a]pyrene is 75 times more potent in newborn mouse i.p. studies than in adult oral studies. Therefore, to obtain oral potency estimates, the potencies estimated from the newborn mouse i.p. studies for these six polycyclic aromatic hydrocarbons were adjusted downward by a factor of 75. Differences in potencies between the oral and newborn i.p. studies is likely to be due to pharmacokinetic differences from different routes of administration and greater sensitivity of the neonatal mice relative to the adult mice. Risks from perinatal PAH exposure may be underestimated by the potencies presented here because exposures early in life can result in greater cancer risks than exposures occurring in adulthood and studies of adult oral benzo[a]pyrene were used in developing the adjustment factor employed here.

The cancer potency estimates correspond to the upper 95 percent confidence bound on the linear term of the multistage model fit to cancer dose-response data from i.p. studies in animals, which were then adjusted downward, as described above, by a factor of 75. Dose calculations were adjusted by Doll-Armitage analysis for variable dosing over time. In cases where multiple tumor sites contribute to the cancer potency, a probability distribution of cancer potency estimates was derived using likelihood theory. The linear term (q1) of the multistage model fit to dose response data for a given site represents the cancer potency for that site. When the chemical resulted in cancer at multiple sites, the cancer potencies for the affected sites were summed probabilistically, according to their distributions, to obtain a combined distribution. This combined distribution representing cancer potency for sites affected by a given PAH was derived through Monte Carlo analysis. The upper 95 percent confidence bound indicated by the
combined distribution for these treatment-related tumor sites, adjusted downward by a factor of 75, was taken as the cancer potency for the PAH.

The method used to develop cancer potencies for the PAHs presented here is equivalent to a potency equivalency factor (PEF) approach, based on potencies relative to benzo[a]pyrene. The oral PEFs are as follows: benzo[b]fluoranthene, 0.62; benzo[j]fluoranthene, 0.52; chrysene, 0.17; dibenzo[a,h]pyrene, 11; dibenzo[a,i]pyrene, 12; 5-methylchrysene, 7.0.

The potency derivation takes into account body size differences between humans and experimental animals. The Proposition 65 “no significant risk level” (NSRL) is defined in regulation as the daily intake level posing a $10^{-5}$ lifetime risk of cancer. The potency estimates, and the corresponding NSRLs, are given in Table 1.

**Table 1. Cancer Potencies (Oral) and NSRLs (Oral).**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Cancer Potency (Oral) (mg/kg-day)$^{-1}$</th>
<th>NSRL (Oral) (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>7.3</td>
<td>0.096</td>
</tr>
<tr>
<td>Benzo[j]fluoranthene</td>
<td>6.1</td>
<td>0.11</td>
</tr>
<tr>
<td>Chrysene</td>
<td>2.0</td>
<td>0.35</td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>130</td>
<td>0.0054</td>
</tr>
<tr>
<td>Dibenzo[a,i]pyrene</td>
<td>140</td>
<td>0.0050</td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>83</td>
<td>0.0084</td>
</tr>
</tbody>
</table>

**INTRODUCTION**

The six polycyclic aromatic hydrocarbons (PAHs) discussed here have been listed as chemicals known to the State to cause cancer under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code Section 25249.5 et seq.). Listing occurred as follows: benzo[b]fluoranthene and benzo[j]fluoranthene on July 1, 1987; dibenzo[a,h]pyrene and dibenzo[a,i]pyrene on January 1, 1988; 5-methylchrysene on April 1, 1988; and chrysene on January 1, 1990. This document describes the derivation of cancer potency values by the oral route and the corresponding NSRLs for these six PAHs.

To derive cancer potencies, we used studies of tumor incidences after i.p. injection into newborn mice. Injection studies would not usually be used for potency estimation, but no oral or inhalation data are available for these compounds. Unlike the skin application and subcutaneous injection studies which have been reported for these compounds, i.p. injection produces tumors remote from the site of application and does not involve the use of additional chemical treatments, *i.e.*, promoters. Intraperitoneal injection of PAHs is expected to result in widespread distribution of the compounds, including absorption into the hepatic portal bloodstream. This route may therefore be more like oral exposures than routes where the compound is initially placed in a compartment from which distribution is slow and possibly incomplete, such as the skin.
The potency estimates for PAHs based on i.p. data were therefore compared to estimates for benzo[a]pyrene based on i.p. data from the same series of experiments, and additional estimates for benzo[a]pyrene based upon oral data. Potency estimates for benzo[a]pyrene have been extensively reviewed (Zeise and Crouch, 1980; U.S. EPA, 1993). The use of comparative potency calculations for PAHs has been examined previously (Clement Associates, 1988). Estimates obtained in this way are assumed applicable for oral route exposures.

The PAHs under consideration here are soluble in various organic solvents, such as benzene, and sparingly soluble in alcohol, but are insoluble in water (IARC, 1983). The International Agency for Research on Cancer (IARC) describes the compounds as having needle-shaped or plate-like crystals, often with a characteristic colored fluorescence. The molecular weights, molecular formulae, boiling and melting points, and chemical structures for the compounds under consideration here are presented in Table 2 and Figure 1 below.

Table 2. Physicochemical Properties.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Weight</th>
<th>CAS No.</th>
<th>Formula</th>
<th>Melting Point</th>
<th>Boiling Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>252.3</td>
<td>205-99-2</td>
<td>C_{20}H_{12}</td>
<td>168.3°C</td>
<td>-</td>
</tr>
<tr>
<td>Benzo[j]fluoranthene</td>
<td>252.3</td>
<td>205-82-3</td>
<td>C_{20}H_{12}</td>
<td>165.4°C</td>
<td>-</td>
</tr>
<tr>
<td>Chrysene</td>
<td>228.3</td>
<td>218-01-9</td>
<td>C_{18}H_{12}</td>
<td>255 – 256°C</td>
<td>448°C</td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>302.4</td>
<td>189-64-0</td>
<td>C_{24}H_{14}</td>
<td>317°C</td>
<td>-</td>
</tr>
<tr>
<td>Dibenzo[a,i]pyrene</td>
<td>302.4</td>
<td>189-55-9</td>
<td>C_{24}H_{14}</td>
<td>281.5 - 282.5°C</td>
<td>275°C @ 0.05 mm Hg</td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>242.3</td>
<td>3697-24-3</td>
<td>C_{19}H_{14}</td>
<td>117 - 118°C</td>
<td>-</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>252.3</td>
<td>50-32-8</td>
<td>C_{20}H_{12}</td>
<td>176.5°C</td>
<td>495°C</td>
</tr>
</tbody>
</table>
PAHs are generated by combustion or pyrolysis of organic materials, and occur widely as environmental pollutants, food contaminants (especially of smoked or grilled food) and components of soots, tars and other wastes and by-products of industrial processes (IARC, 1973; IARC, 1983). They are found in the particulate fractions of engine exhausts and in materials such as crude oil, coal, carbon blacks, coal tar, and in some mineral oils. All six carcinogens which are discussed in this report have been specifically identified as components of cigarette smoke (IARC, 1983).

This document discusses the studies available for cancer dose-response assessment and summarizes the derivation of the cancer potency estimates and NSRLs. A description of the methodology is provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

The experimental evidence for carcinogenicity of benzo[b]fluoranthene, benzo[j]fluoranthene, chrysene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, and 5-methylchrysene in animals was reviewed originally by IARC (1973). A number of positive studies by various routes in mice, and for some compounds in rats and hamsters, were described. IARC concluded that there was sufficient evidence of carcinogenicity of these substances in animals. Searches of more recent literature identified some additional studies of these compounds that are consistent with these conclusions. The animal cancer bioassay data are relied upon to derive quantitative estimates of cancer potency.

In selecting studies as bases for potency estimation, those using routes of exposure corresponding to likely human exposures are normally preferred. However, for the compounds considered here, no studies by the oral or inhalation routes were found. Although skin application studies are of interest in establishing the relative potency of different topically applied carcinogens, use of these studies to obtain direct potency estimates is difficult, due to problems in determining actual dose received and in extrapolating from dermal to other routes of exposure.
exposure. Subcutaneous injection or implantation routes are also less suitable for potency estimation because of the difficulty of extrapolating to other routes of exposure.

Intraperitoneal studies have been used here for potency estimation for benzo[b]fluorantheme, benzo[j]fluorantheme, chrysene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, and 5-methylchrysene. To provide a basis for comparison of the cancer potency estimates for PAHs based on i.p. experiments with those relevant to the oral route, i.p. potencies as well as oral potencies were derived for benzo[a]pyrene, a well-known and extensively studied carcinogenic PAH.

The following sections describe all the i.p. carcinogenicity studies for benzo[b]fluorantheme, benzo[j]fluorantheme, chrysene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, and 5-methylchrysene which were found in the literature.

_LaVoie et al. (1987)_

Groups of 30-40 newborn CD-1 mice received i.p. injections of benzo[a]pyrene, benzo[b]-, benzo[j]- or benzo[k]fluorantheme, or indeno[1,2,3-cd]pyrene dissolved in DMSO. Total doses given were 0.5 µmoles for benzo[b]fluorantheme, or 1.1 µmoles for benzo[a]pyrene or benzo[j]-fluorantheme, in each case divided so that the dose volumes were 5 µl on day one of life, 10 µl on day eight and 20 µl on day 15. The effective group size was defined as animals surviving to at least 35 weeks old. All animals were killed at 52 weeks of age. Liver sections from all animals were examined histologically, as were all gross lesions. Hepatic tumors were diagnosed as either “hepatomas” (hepatocellular carcinomas) or adenomas. Significant increases in liver tumor incidence were observed in male mice treated with benzo[a]pyrene, benzo[b]fluorantheme, and benzo[j]fluorantheme (see Table 3). Lung adenomas were also observed in many treated animals. Significant increases in lung tumor incidence were observed in male and female mice treated with benzo[a]pyrene and benzo[j]fluorantheme.

Table 3. Liver and Lung Tumor Incidences in CD-1 Mice Receiving Intraperitoneal Injections of Benzo-fluoranthenes, Indeno[1,2,3-cd]pyrene or Benzo[a]pyrene as Newborns and Sacrificed at One Year (LaVoie et al., 1987).

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>Dose (µmol)</th>
<th>Liver Tumors a</th>
<th>Lung Adenomas a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>0</td>
<td>1/17</td>
<td>(1)</td>
</tr>
<tr>
<td>2</td>
<td>Benzo[a]pyrene</td>
<td>1.1</td>
<td>13/17</td>
<td>b</td>
</tr>
<tr>
<td>3</td>
<td>Benzo[b]fluorantheme</td>
<td>0.5</td>
<td>8/15</td>
<td>b</td>
</tr>
<tr>
<td>4</td>
<td>Benzo[j]fluorantheme</td>
<td>1.1</td>
<td>11/21</td>
<td>b</td>
</tr>
<tr>
<td>5</td>
<td>Benzo[k]fluorantheme</td>
<td>2.1</td>
<td>3/16</td>
<td>b</td>
</tr>
<tr>
<td>6</td>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>2.1</td>
<td>0/11</td>
<td>0/9</td>
</tr>
</tbody>
</table>

a Effective group size is animals surviving to 35 weeks. Reported incidences for liver tumors are combined “hepatomas” and adenomas. Numbers in parentheses indicate number of “hepatomas” alone.

Pairwise comparison with controls, Fisher’s exact test:

b p < 0.005.
c p = 0.052.
Newborn CD-1 mice received i.p. injections of various PAHs (including chrysene and benzo[a]pyrene) and nitro-derivatives. The materials were dissolved in DMSO; a control group received only DMSO. A second control group was started 10 weeks after the first control and test groups, although in this report all findings are related to the concurrent controls only. Total doses of 700 or 2800 nmol of chrysene, or 560 nmol of benzo[a]pyrene were given, divided so that one-, two-, and four-sevenths of the total dose were given within 24 hours of birth and on day eight and 15 of life, respectively. Initial group sizes of 90-100 animals (males and females) were reduced somewhat by early mortality: group sizes were reported as those surviving past weaning. Animals were observed for one year after dosing. The combined incidences of liver adenomas and carcinomas were significantly increased among male mice treated with benzo[a]pyrene or chrysene (see Table 4). The incidences of lung adenomas were increased among both male and female mice treated with benzo[a]pyrene and male mice treated with chrysene.

Table 4. Liver and Lung Tumor Incidences in CD-1 Mice Receiving Intraperitoneal Injections of Chrysene or Benzo[a]pyrene as Newborns and Sacrificed at One Year (Wislocki et al., 1986).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (nmol)</th>
<th>Sex</th>
<th>Liver Tumors a</th>
<th>Lung Tumors a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>M</td>
<td>2/28 0/28 2/28</td>
<td>1/28 0/28 1/28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0/31 0/31 0/31</td>
<td>0/31 0/31 0/31</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>560</td>
<td>M</td>
<td>11/37 7/37 18/37</td>
<td>13/37 0/37 13/37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0/27 0/27 0/27</td>
<td>13/27 0/27 13/27</td>
</tr>
<tr>
<td>Chrysene</td>
<td>700</td>
<td>M</td>
<td>8/35 2/35 10/35</td>
<td>5/35 1/35 6/35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0/33 0/33 0/33</td>
<td>2/33 0/33 2/33</td>
</tr>
<tr>
<td></td>
<td>2800</td>
<td>M</td>
<td>1/34 13/34 14/34</td>
<td>7/34 0/34 7/34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0/24 0/24 0/24</td>
<td>1/24 0/24 1/24</td>
</tr>
</tbody>
</table>

a Effective group size is the number of animals surviving past weaning.

b Significantly different from control by Fisher's exact test, p < 0.01.

c Significantly different from control by Fisher's exact test, p < 0.05.

Chang et al. (1983)

Newborn Swiss-Webster BLU:Ha (ICR) mice were given three i.p. injections of 0.2, 0.4, and 0.8 µmol chrysene in DMSO on day one, eight, and 15 of life, respectively (1.4 µmol total dose). Control animals received injections of DMSO only. Effective group sizes were defined as the number of animals alive at weaning (25 days). All animals were killed at 37-41 weeks of age. A representative number of pulmonary tumors, all hepatic tumors, and any other tissues showing suspected pathology were examined histologically. A significant increase in incidence of hepatic tumors was observed in male mice relative to controls (6/27, treated vs. 0/52, control; p = 0.001). A slight, but not statistically significant, increase in the incidence of pulmonary tumors was also observed in males (4/27, treated; 4/52, control; p = 0.26). No increases in tumor incidence were observed.
observed in females. Additional treatment groups received 1,2-dihydrodiol and 1,2-dihydrodiol-3,4-epoxide derivatives of chrysene, which also caused increased incidences of pulmonary and/or hepatic tumors. These compounds are considered possible metabolites of chrysene and may include the active intermediate responsible for the carcinogenic action of chrysene.

*Buenig et al. (1979)*

Newborn Swiss-Webster BLU:Ha (ICR) mice were given i.p. injections of 0.2, 0.4, and 0.8 µmol chrysene in DMSO on day one, eight, and 15 of life, respectively (1.4 µmol total dose). Control animals received injections of DMSO only. All animals were killed at 38-42 weeks of age. Effective group sizes were defined as the number of animals alive at the termination of the experiment, since it was not reported that animals dying during the observation period were autopsied. A representative number of pulmonary tumors, all hepatic tumors, and any other tissues showing suspected pathology were examined histologically. A significant increase in the incidence of hepatic tumors was observed in male mice (6/24, treated vs. 0/21, control; p = 0.017). Slight increases in the incidences of pulmonary tumors (5/24, treated vs. 2/21, control; p = 0.27) and localized splenic lymphosarcomas (4/24, treated vs. 0/21, control; p = 0.07) were also observed in exposed male mice but these effects were not statistically significant. No increases in tumor incidence were observed in females.

*Chang et al. (1985)*

Newborn Swiss-Webster BLU:Ha (ICR) mice were given three i.p. injections of 7.1, 14.3, and 28.6 nmol dibenzo[a,h]pyrene in DMSO on day one, eight, and 15 of life, respectively (50 nmol total dose). Control animals also received injections of DMSO only. All animals also received an injection of DMSO 10 minutes before the injection of dibenzo[a,h]pyrene in DMSO. Other groups not described here received phenolic compounds which were being tested for modifying effects on dibenzo[a,h]pyrene or benzo[a]pyrene carcinogenesis. Effective group sizes were not given exactly, but the percentage of the starting number of mice alive at the termination of the experiment was reported. All animals were killed at 45-49 weeks of age. Dibenzo[a,h]pyrene treated animals showed an increased incidence of lung tumors [reported as the “percent of mice with tumors” (95% in treated mice vs. 30% in control mice)].

*Chang et al. (1982)*

Groups of 80 newborn Swiss-Webster BLU:Ha (ICR) mice (males plus females) were given i.p. injections of 12.5, 25, and 50 nmol dibenzo[a,h]pyrene or dibenzo[a,i]pyrene in DMSO on day one, eight, and 15 of life, respectively (87.5 nmol total dose). Control animals received injections of DMSO only. All animals were killed at 49-54 weeks of age. A representative number of pulmonary tumors, all hepatic tumors and other tissues with suspected pathology was examined histologically. The effective group size was the number of mice in each sex and treatment group alive at termination. Increased incidences of pulmonary and hepatic tumors (“type A or neoplastic nodules”) were reported in male mice treated with either dibenzo[a,h]-pyrene or dibenzo[a,i]pyrene (see Table 5). In females treated with dibenzo[a,h]pyrene or dibenzo[a,i]pyrene, only an increased incidence of lung tumors was observed. Incidences were reported as “percentage of mice with tumors” by the authors (along with the average number of tumors per mouse), permitting calculation of the absolute numbers of tumor-bearing animals from the reported group sizes. In addition to the lung and liver tumors, two female mice treated with dibenzo[a,h]pyrene had sarcomas of the skin, and one had an adenocarcinoma in the small intestine. Two female mice treated with dibenzo[a,i]pyrene had hemangiomas of the
uterus, and one male treated with dibenzo[a,i]pyrene had a thymic lymphoma. No such tumors were observed in control animals.

Table 5. Liver and Lung Tumor Incidences in ICR mice Receiving Intraperitoneal Injections of Dibenzo[a,h]- or Dibenzo[a,i]pyrene as Newborns and Surviving to the Time of Sacrifice at 49-54 Weeks (Chang et al., 1982).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Total Dose (nmol)</th>
<th>Hepatic Tumors</th>
<th>Lung Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0/32</td>
<td>0/39</td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>87.5</td>
<td>11/25 *</td>
<td>1/14</td>
</tr>
<tr>
<td>Dibenzo[a,i]pyrene</td>
<td>87.5</td>
<td>21/39 *</td>
<td>0/21</td>
</tr>
</tbody>
</table>

* Significantly different from control by Fisher's exact test, p << 0.001.

Peacock and Peacock (1966)

Small groups of BALB/c mice were injected once intraperitoneally at birth with approximately 50 µg of one of several PAHs including: benzo[a]pyrene, dibenzo[a,l]pyrene, dibenzo[a,e]-pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, benz[a]anthracene, dibenz[a,h]anthracene and dibenz[a,j]anthracene. The lungs of treated animals were examined after 12 weeks. Alveolar hyperplasia was observed in some treated groups, as follows; dibenzo[a,e]pyrene, 1/3; dibenzo[a,i]pyrene, 1/8; dibenz[a,h]anthracene, 4/6. Other treated groups did not show this lesion; benzo[a]pyrene, 0/7, dibenzo[a,l]pyrene, 0/9, dibenzo[a,h]pyrene, 0/9, benz[a]anthracene, 0/4. One of eight mice injected with dibenz[a,j]anthracene developed both alveolar hyperplasia and a papillary adenoma of the lung. Neither type of pulmonary lesion was observed in 26 untreated control mice.

Hecht et al. (1985)

One hundred newborn ICR/Ha mice were given i.p. injections of 8, 16, and 32 nmol 5-methylchrysene in DMSO on day one, eight, and 15 of life, respectively (56 nmol total dose). Control animals (n=100) received injections of DMSO only. All animals were killed at 35 weeks of age. The number of mice in each sex and treatment group alive at termination was the effective group size reported by the authors. Liver and lung tumors were counted, and representative lesions were examined histologically. The authors only reported the percentage of mice with tumors, so these values were used as the basis for calculating the tumor incidence among the mice. Increased incidences of liver adenomas and lung alveogenic adenomas were observed in exposed male mice (see Table 6). Marginal increases in lung and liver tumor incidences were also observed in female mice, but these were not statistically significant.
Table 6. Liver and Lung Tumor Incidences in ICR mice Receiving Intraperitoneal Injections of 5-Methylchrysene as Newborns and Surviving to the Time of Sacrifice at 35 Weeks of Age (Hecht et al., 1985).

<table>
<thead>
<tr>
<th>Treatment Group (Total Dose in nmol)</th>
<th>Hepatic Tumors</th>
<th>Lung Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>0</td>
<td>1/48</td>
<td>1/41</td>
</tr>
<tr>
<td>56</td>
<td>8/35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6/48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significance relative to control (Fisher's exact test):

- <sup>a</sup> p < 0.005
- <sup>b</sup> 0.1 < p < 0.05
- <sup>c</sup> p < 0.05

LaVoie et al. (1994)

Groups of 80 newborn CD-1 mice of each sex received i.p. injections of benzo[j]fluoranthene and six synthesized diol-epoxides of benzo[j]fluoranthene in DMSO. Only the portion of the experiment involving the parent compound, benzo[j]fluoranthene is described below. Injections were given on day one, eight, and 15 of life, with total doses delivered for each dose group, respectively, of 0 (vehicle), 0.110, 0.275 and 1.10 µmole benzo[j]fluoranthene. The effective number of animals used in the bioassay count was mice surviving to 52 weeks at which time the study was terminated. The incidences of total tumors and lung tumors among the surviving mice are presented in Table 7.

Table 7. Tumor Incidences Among CD-1 Mice Receiving Intraperitoneal Injections of Benzo[j]fluoranthene as Newborns and Surviving to the Time of Sacrifice at 52 Weeks (LaVoie et al., 1994).

<table>
<thead>
<tr>
<th>Treatment Group (Total Dose in µmol)</th>
<th>Total Tumors</th>
<th>Alveolar / Bronchiolar Carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>0</td>
<td>8/33</td>
<td>7/33</td>
</tr>
<tr>
<td>0.110&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18/37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8/29</td>
</tr>
<tr>
<td>0.275</td>
<td>20/34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14/32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.10</td>
<td>25/25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>35/38&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Low-dose animals were only examined by gross necropsy.

Significance relative to controls by Fisher's exact test:

- <sup>b</sup> p < 0.05
- <sup>c</sup> p < 0.01
- <sup>d</sup> p << 0.001
You et al. (1994) and Nesnow et al. (1995)

Groups of 20 male A/J mice, six to eight weeks old, were treated with a single i.p. injection of 5-methylchrysene in tricaprylin (vehicle) and observed for eight months (You et al., 1994). Doses administered were 0 (vehicle), 10, 50, 100 and 200 mg/kg. The incidences of “surface lung tumors” (as described by the authors) in the mice were 55%, 65%, 100%, 100% and 100% for the dose groups, respectively. The average number of lung tumors per mouse increased dramatically with increasing dose: 0.6, 1.8, 39.0, 93.1 and >100, for vehicle control and increasing doses, respectively. Using equivalent methodology, Nesnow et al. (1995) reported studies with several other PAHs, including benzo[a]pyrene, benzo[b]fluoranthene, dibenzo[a,h]-anthracene, 5-methylchrysene, and cyclopenta[c,d]pyrene. Tumor incidences (e.g., proportion of mice with tumor) were not reported, presumably because the incidence was 100% in nearly all cases. Instead, the authors reported the numbers of lung adenomas per mouse, which increased exponentially with dose for all compounds.

APPRAOCH TO DOSE RESPONSE ANALYSIS

PAHs are generally recognized as genotoxic agents (IARC, 1973). There is insufficient information on the precise mechanism of carcinogenicity to permit the development of a biologically based model for cancer potency estimation. There are also insufficient data to support dose adjustments based on pharmacokinetic models. Therefore, the default approach (i.e., a linearized multistage model incorporating the Doll-Armitage correction for variable dosing, adjustments for less-than-lifetime study duration, and interspecies scaling) has been applied. The approach used is described in detail in the Appendix.

DOSE-RESPONSE ASSESSMENT

Potencies were calculated for each compound, sex, and tumor site where tumor incidence was significantly elevated relative to controls for the following studies: Wislocki et al., 1986; LaVoie et al., 1987; LaVoie et al., 1994; Chang et al., 1983; Chang et al., 1982; Buenig et al., 1979; Hecht et al., 1985; the data from the remaining studies are considered less suitable for potency estimation for the following reasons. In the study by Chang et al. (1985), tumor incidences were only quoted in percentages, and the precise numbers of tumor-bearing animals or total animals in each group were not given. The data from the study by Peacock and Peacock (1966) were also considered less suitable in view of the very small size of the exposed groups, the short period of observation, and the limited reporting of this study.

Several studies also showed significant increases in tumor incidence at multiple sites within a given sex, species and study (Wislocki et al., 1986; LaVoie et al., 1987; Chang et al., 1982). For these data sets, a methodological approach using Monte Carlo analysis was used to combine potency estimates across sites (see Appendix). For each tumor site, a distribution of estimates corresponding to the 0.1 through 99.9th percentiles of the linear term ($q_1$) of the multistage model (Appendix, Equation 1) was generated with the MSTAGE computer program (Crouch, 1998), which had been modified to tabulate percentile values. A combined distribution was created by adding $q_1$ for each tumor site, according to its distribution, through at least 100,000 Monte Carlo simulations (Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The upper 95 percent confidence bound of the combined distribution was taken as the basis of the cancer potency estimate for the combined tumor sites.
In two data sets from the studies by Chang et al. (1982) of dibenzopyrenes all the treated animals developed tumors – male mice treated with dibenzo[a,h]pyrene and female mice treated with dibenzo[a,i]pyrene – precluding the determination of upper bound estimates of $q_1$ (see Appendix). In these cases, lower bound estimates were obtained and compared with estimates of $q_1$ from other data sets for these chemicals. In each of these cases the lower bound estimates were lower than, and therefore consistent with, those produced by the more traditional analyses of the tumors occurring in the mice of the opposite sex in the same study.

In addition to potency values for the compounds which are the subject of this report, potency estimates for benzo[a]pyrene were derived from two i.p. studies in newborn mice (LaVoie et al., 1987 and Wislocki et al., 1986) for comparison with previously reported potency estimates for this compound by non-injection routes. Potency estimates for benzo[a]pyrene have previously been established using studies showing the development of stomach tumors in mice resulting from exposure to the compound in feed.

Cancer potencies derived directly from all the i.p. studies analyzed are shown in Table 8. The neonatal i.p. potency estimate for benzo[b]fluoranthene is 550 (mg/kg-day)$^{-1}$ based on liver tumor incidence in male mice (LaVoie et al., 1987).

For benzo[j]fluoranthene, the combined neonatal i.p. potency based on the incidences of liver and lung tumors of LaVoie et al. (1987) in male mice is 410 (mg/kg-day)$^{-1}$, somewhat higher than the estimate based on lung tumor incidence in female mice [110 (mg/kg-day)$^{-1}$]. The response per dose observed in LaVoie et al. (1994) was greater than that of LaVoie et al. (1987) and may reflect the longer study duration. These higher neonatal i.p. potency estimates for male and female mice, which were the same after rounding [460 (mg/kg-day)$^{-1}$], serve as the neonatal i.p. potency estimate for benzo[j]fluoranthene.

Two neonatal i.p. potency estimates for chrysene based on liver tumor incidence in male mice from Chang et al. (1983) and Buenig et al. (1979) are nearly identical and are slightly higher than the combined potency for liver and lung tumors derived from the Wislocki et al. (1986) study. Since the individual potencies are for the same species, sex, and tumor site and the studies are of similar quality, a reasonable estimate of the neonatal i.p. cancer potency of chrysene is the geometric mean of the two potencies, 150 (mg/kg-day)$^{-1}$.

The lung was the most sensitive site of tumor development for both dibenzo[a,h]pyrene and dibenzo[a,i]pyrene (Chang et al., 1982). In these studies, 100% incidence of lung tumors was observed for male mice treated with dibenzo[a,h]pyrene and for female mice treated with dibenzo[a,i]pyrene. Since these results cannot be used to calculate upper-bound potency estimates, lower 5% confidence bounds on the probability that all animals in the dosed groups were tumor-bearing were calculated (see Appendix). For the reasons stated previously, however, ultimately none of the estimates calculated by this method was adopted. For dibenzo[a,h]pyrene, the female mouse appeared more sensitive, with a potency of 9900 (mg/kg-day)$^{-1}$ for lung tumors. For dibenzo[a,i]pyrene, liver and lung tumors were significantly increased in male mice, thus the combined potency estimate for these two sites forms the basis of the neonatal i.p. potency estimate of 10500 (mg/kg-day)$^{-1}$.

Liver and lung tumor incidences in male mice provided the data to produce a combined neonatal i.p. cancer potency estimate for 5-methylchrysene of 6200 (mg/kg-day)$^{-1}$ (Hecht et al., 1985).
The individual potency estimates for benzo[a]pyrene based on liver tumors in male mice or lung tumors from i.p. injection of neonatal mice of either sex are similar, almost falling within a two-fold range. Combining the potency estimates from the two studies that produced both liver and lung tumors (Wislocki et al., 1986, and LaVoie et al., 1987), however, produced somewhat higher potency estimates, with that derived from the male mice in the LaVoie et al. study being the highest [890 (mg/kg-day)^-1]. This value is near the high end of the range of reported values based on induction of forestomach tumors by oral benzo[a]pyrene (Zeise and Crouch, 1980), and is at the high end of the range for long-term exposure studies, and is high compared to the estimated range of 4.5 to 11.7 (mg/kg-day)^-1 adopted by U.S. EPA (1993). The bases for the U.S. EPA range of potency estimates were analyses of tumors of the forestomach which developed in mice treated with benzo[a]pyrene in their diet (Neal and Rigdon, 1967) (low end of range) and tumors of the forestomach, esophagus, and larynx which developed in rats treated with benzo[a]pyrene by gavage (Brune et al., 1981) (high end of range).

Since the adoption of these cancer potency values, additional data from a more recent long-term bioassay have supported the carcinogenic potential of benzo[a]pyrene. Culp et al. (1998) reported that female B6C3F1 mice fed diet containing 0, 5, 25, or 100 ppm benzo[a]pyrene for two years developed tumors of the forestomach, tongue, esophagus, and larynx. Initial estimates of the cancer potency from this study suggest that the potency would fall within the range previously adopted by U.S. EPA.

Zeise and Crouch (1980) noted a roughly 10-fold increase in the potency of benzo[a]pyrene at higher dose rates (> 7 mg/kg-day). The neonatal mice used in these studies may be as sensitive at low or moderate doses as adult animals dosed at high levels since many detoxification enzymes do not reach adult levels until after the first 20 days of life. For this reason, the potencies derived from the neonate studies are expected to be higher than if they had been derived from chronic oral studies.

For purposes of the present assessment of PAHs studied only by the i.p. route in the neonatal mouse model, the potencies which were calculated directly from the data were adjusted based on the following relationship:

\[
\text{adult oral potency} = \text{neonatal i.p. potency} \times \left( \frac{\text{adult oral B[a]P potency}}{\text{neonatal i.p. B[a]P potency}} \right)
\]

Thus, potencies are adjusted downward by dividing by a factor of 75, which is roughly the ratio of the potency of benzo[a]pyrene derived from the neonate i.p. studies [890 (mg/kg-day)^-1] to that of benzo[a]pyrene used for regulatory purposes [11.7 (mg/kg-day)^-1 (U.S. EPA, 1993)]. Cancer potencies for those compounds studied only in the neonatal mouse model could be recalculated by this relationship should a revised estimate of the adult potency for benzo[a]pyrene be issued.
Table 8. Human Cancer Potency Estimates ($q_{\text{human}}$) of PAHs in (mg/kg-day)$^{-1}$ Based on Newborn Mouse i.p. Injection Studies.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Study</th>
<th>Sex</th>
<th>Dose (mg/kg-day)</th>
<th>Tumor Site &amp; Incidence</th>
<th>$q_{\text{animal}}$</th>
<th>Combined $q_{\text{animal}}$</th>
<th>$q_{\text{human}}^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]pyrene</td>
<td>Wislocki et al., 1986</td>
<td>M</td>
<td>0, 0.0341</td>
<td>Liver 2/28, 18/37</td>
<td>26.46</td>
<td>40.46</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung 1/28, 13/37</td>
<td>18.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0, 0.0350</td>
<td>Lung 0/31, 13/27</td>
<td>28.95</td>
<td></td>
<td>410</td>
</tr>
<tr>
<td></td>
<td>LaVoie et al., 1987</td>
<td>M</td>
<td>0, 0.0670</td>
<td>Liver 1/17, 13/17</td>
<td>33.80</td>
<td>67.31</td>
<td>890</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung 0/17, 14/17</td>
<td>41.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0, 0.0688</td>
<td>Lung 0/18, 9/14</td>
<td>25.48</td>
<td></td>
<td>360</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>LaVoie et al., 1987</td>
<td>M</td>
<td>0, 0.0304</td>
<td>Liver 1/17, 8/15</td>
<td>41.56</td>
<td></td>
<td>550</td>
</tr>
<tr>
<td>Benzo[j]fluoranthene</td>
<td>LaVoie et al., 1987</td>
<td>M</td>
<td>0, 0.0670</td>
<td>Liver 1/17, 11/21</td>
<td>16.96</td>
<td>30.70</td>
<td>410</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung 0/17, 11/21</td>
<td>17.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0, 0.0688</td>
<td>Lung 0/18, 4/18</td>
<td>7.552</td>
<td></td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>LaVoie et al., 1994</td>
<td>M</td>
<td>0, 0.00670, 0.0167, 0.0670</td>
<td>Lung 6/33, 8/37, 17/34, 25/25</td>
<td>34.48</td>
<td>460</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung 7/33, 6/29, 14/32, 35/38</td>
<td>32.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0, 0.00688, 0.0172, 0.0688</td>
<td>Lung 7/33, 6/29, 14/32, 35/38</td>
<td>32.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysene</td>
<td>Chang et al., 1983</td>
<td>M</td>
<td>0, 0.0426</td>
<td>Liver 0/52, 6/27</td>
<td>10.82</td>
<td></td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Buenig et al., 1979</td>
<td>M</td>
<td>0, 0.0449</td>
<td>Liver 0/21, 6/24</td>
<td>11.76</td>
<td></td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Wislocki et al., 1986</td>
<td>M</td>
<td>0, 0.0386, 0.1542</td>
<td>Liver 2/28, 10/35, 14/34</td>
<td>5.225</td>
<td>6.94</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung 1/28, 6/35, 7/34</td>
<td>147.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>Chang et al., 1982</td>
<td>M</td>
<td>0, 0.00626</td>
<td>Liver 0/32, 11/25</td>
<td>147.7</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung 7/32, 25/25</td>
<td>&gt;313.5$^b$</td>
<td>&gt;4200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0, 0.00643</td>
<td>Lung 11/39, 13/14</td>
<td>703.9</td>
<td></td>
<td>9900</td>
</tr>
<tr>
<td>Dibenzo[a,i]pyrene</td>
<td>Chang et al., 1982</td>
<td>M</td>
<td>0, 0.00626</td>
<td>Liver 0/32, 21/39</td>
<td>175.1</td>
<td>792.5</td>
<td>10500</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung 7/32, 37/39</td>
<td>662.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>0, 0.00643</td>
<td>Lung 11/39, 21/21</td>
<td>&gt;269.9$^b$</td>
<td>&gt;3800</td>
<td></td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>Hecht et al., 1985</td>
<td>M</td>
<td>0, 0.00144</td>
<td>Liver 1/48, 8/35</td>
<td>293.9</td>
<td>466.0</td>
<td>6200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lung 2/48, 7/35</td>
<td>246.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Estimates on which the recommended potencies are based are indicated by shading.
$^b$ Lower 5% confidence bound on $q_1$. See Appendix for method for estimation of a lower bound on cancer potency for data sets with 100% tumor incidence (Combined site analyses cannot be performed with these sets.).

B[b]F, B[j]F, chrysene,
DB[a,h]P, DB[a,i]P, and 5-MC NSRLs

May 2004
OEHHA
This method is equivalent to a potency equivalency factor (PEF) approach using the i.p. studies for these PAHs and benzo[a]pyrene as a basis for determining potencies relative to benzo[a]pyrene. PEFs for the inhalation route are based on dermal studies. Intraperitoneal studies are a more reliable basis for deriving relative potencies for the oral route the oral route results in systemic exposure, and the i.p. route results in more systemic exposure than the dermal route.

Oral PEFs for the PAHs are as follows:

**Table 9. Oral Potency Equivalency Factors (PEFs) for PAHs.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>PEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>0.62</td>
</tr>
<tr>
<td>Benzo[j]fluoranthene</td>
<td>0.52</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.17</td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>11</td>
</tr>
<tr>
<td>Dibenzo[a,i]pyrene</td>
<td>12</td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Multiplying the PEFs by the oral cancer potency for benzo[a]pyrene \([11.7 \text{ (mg/kg-day)}^{-1}]\) results in the same cancer potencies as given in Table 10 below (rounded to two significant figures).


The recommended potency estimates for the oral route, in units \((\text{mg/kg-day})^{-1}\), derived from data on tumor incidence after i.p. injection of the PAHs into mice are shown in **Table 10**. Daily oral intake levels (in \(\mu\text{g}\)) associated with lifetime cancer risks of \(10^{-5}\) are also shown.
Table 10. Adjusted Cancer Potencies and Risk Specific Intake Levels for PAHs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>“Neonatal” i.p. Potency (mg/kg-day)^−1</th>
<th>Adjusted Potency (Oral) a (mg/kg-day)^−1</th>
<th>Risk Specific Intake Level (Oral) b (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>550</td>
<td>7.3</td>
<td>0.096</td>
</tr>
<tr>
<td>Benzo[j]fluoranthene</td>
<td>460</td>
<td>6.1</td>
<td>0.11</td>
</tr>
<tr>
<td>Chrysene</td>
<td>150</td>
<td>2.0</td>
<td>0.35</td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>9900</td>
<td>130</td>
<td>0.0054</td>
</tr>
<tr>
<td>Dibenzo[a,i]pyrene</td>
<td>10500</td>
<td>140</td>
<td>0.005</td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>6200</td>
<td>83</td>
<td>0.0084</td>
</tr>
</tbody>
</table>

a The “neonatal” i.p. potency estimate was adjusted downward by a factor of 75 based on the difference in tumor response in mice treated with benzo[a]pyrene by the i.p. route compared to the response in mice treated with benzo[a]pyrene by the oral route (see text).

b Based on adjusted potency.

Studies using the neonatal mouse model of benzo[a]pyrene carcinogenicity have demonstrated a greater potency from early-in-life exposures by the i.p. route relative to that by the oral route. The source of this difference is unknown, but likely is tied to greater sensitivity of neonatal animals to the carcinogenic properties of this compound. Possible factors include the immaturity of detoxification pathways or increased growth and cell proliferation at this stage of development.

For routes of exposure of concern for human health (e.g., oral, inhalation), cancer potencies for a subset of PAHs cannot be determined directly based on the data available. A reasonable approach to the calculation of adult oral cancer potencies for these compounds is through a calibration method using the relative potencies of benzo[a]pyrene derived from studies of both neonatal and adult animals.

REFERENCES


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B[b,F, B[j,F, chrysene, DB[a,h]P, DB[a,i]P, and 5-MC NSRLs

May 2004

OEHHA


Procedures for the development of Proposition 65 NSRLs are described in regulation (California Code of Regulations, Title 22, Sections 12701 and 12703). Consistent with these procedures, the specific methods used to derive the NSRLs for benzo[b]fluoranthene, benzo[j]fluoranthene, chrysene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, and 5-methylchrysene by the oral route are outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

“Multistage” Polynomial

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (CDHS, 1985; U.S. EPA, 1996; Anderson et al., 1983):

\[ p(d) = 1 - \exp[-(q_0 + q_1d + q_2d^2 + \ldots + q_id^i)] \] (1)

with constraints,

\[ q_i \geq 0 \text{ for all } i \]

The \( q_i \) are parameters of the model which are taken to be constants and are estimated from the data. The parameter \( q_0 \) represents the background lifetime incidence of the tumor. The parameter \( q_1 \), or some upper bound, is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as \( q_1^* \), the upper 95% confidence bound on \( q_1 \) (CDHS, 1985), estimated by maximum likelihood techniques. When dose is expressed in units of mg/kg-d, the parameters \( q_1 \) and \( q_1^* \) are given in units (mg/kg-day)^{-1}. Details of the estimation procedure are given in Crump (1981) and Crump et al. (1977).

Calculation of the Lifetime Average Dose

In order to convert the dose levels reported to units of amount given per animal body weight, mouse body weights at various ages shortly after birth were required. These data were not provided by the authors of the studies, but for some mouse strains, strain and sex specific mean chronic body weights are available from the work of Poiley (1972), as cited by U.S. EPA (1988). Growth curves for numerous strains of mouse are available from this source, including a hybrid CD strain for which the body weights were 1.43 g (males) and 1.38 g (females) at one day, 4.93 g (males) and 4.82 g (females) at seven days, and 6.35 g (males) and 6.22 g (females) at 14 days. These weights, which are typical for newborn mice, were assumed to be applicable to the CD-1 mice used by LaVoie et al. (1987) and Wislocki et al. (1986), and the Swiss-Webster (ICR) mice used by Chang et al. (1982, 1983), Buenig et al. (1979) and Hecht et al. (1985). Each of the three administered doses in each study was converted to a per body weight basis, then averaged over the course of the week, providing three weekly dose rates in mg/kg-day. Each of these weekly dose rates was then subjected to adjustment using the Doll-Armitage correction for variable dosing described below.
Variable Dosing Doll-Armitage Analysis

The Armitage and Doll (1954) mathematical description of carcinogenesis as expressed by Crouch (1983) and Crump and Howe (1984) allows for the analysis of data sets with variable dosing over time. The model assumes that cancer derives from a single cell after it has undergone a series of transformations. The model has been used to describe cancer dose response data in animal bioassays as well as in the general population. This methodology was used to allow for the fact that the studies described in this report involved periods of dosing much shorter than the nominal lifetime of the test animals, or the overall observation period of the experiment.

Assumptions are required for the application of the Doll-Armitage model regarding: 1) the mathematical relationship between applied dose and the probability that a “stage transition” has occurred, 2) the stage affected by the carcinogen and 3) the number of “stages.” For the particular forms used to fit the tumor data in this report, a linear relationship is assumed between dose and cell transformation, and the PAH carcinogens are assumed to affect an early stage of the cancer process.

As discussed by Crouch (1983), if the probability per unit time of the stage transformation depends linearly on dose rate \(d(t)\), and the carcinogen only affects a single “stage,” the probability of tumor by time \(T_e\) under Armitage and Doll (1954) becomes

\[
P(T_e) = 1 - \exp[-(A + BD)]
\]

with

\[
D = \frac{1}{T^m \cdot \beta(m-j+1,j)} \int_0^{T_e} d(t)(T_e - t)^{m-j} t^{j-1} dt
\]

where \(T_e\) is the time to observation, and \(\beta\) is Euler's beta function. Following Anderson et al. (1983), the natural lifetime of the test animal, \(T\), is assumed to be two years for rats and mice. The integer \(m\) (the number of “stages”) specifies the rate of increase in incidence with time and \(j\) is the “stage” affected by the carcinogen. The compounds considered in this assessment are assumed to act only as initiators \((j = 1)\). For \(j = 1\), the solution to Equation 3 describing the constant daily dose \((D)\) equivalent to a daily dose \(d\) given over a time interval from \(a\) to \(b\) becomes

\[
D = d \cdot \left[ \frac{(T_e - a)^m - (T_e - b)^m}{T^m} \right]
\]

In the calculations described here, the intervals used to calculate the adjustment factor for each of the three administered doses are zero to one, one to two, and two to three weeks.

To adjust for less than lifetime experiments in estimating cancer potency, the hazard function is assumed to increase with the third power of age. This corresponds to a value for \(m\) of 3.0. This assumption was made for the purposes of this report since no contrary information was available. The potency in animals, \(q_{\text{animal}}\), is given by the upper 95% confidence bound on \(\beta\). This method of calculation allows for both abbreviated and variable dosing schedules, and for observation periods less than the nominal lifetime of the test animals.

DB[a,h]P, DB[a,i]P, and 5-MC NSRLs OEHHA
For purposes of the Doll-Armitage variable dosing calculation for the various PAHs, three dosing intervals of one week were assumed to occur in each of the experiments in which the compound was administered on the first, eighth, and fifteenth day of life. The doses were averaged over the week (1/7) and divided by an estimate of the body weight for that week (see above) to produce an unadjusted interval dose in milligrams per kilogram per day. The Doll-Armitage adjustment factors (see Equation 4 above) for each interval were calculated as follows: for the first week, interval, $a$ and $b$ were zero and one, respectively, for the second week, one and two, respectively, and so on. The experimental length or time to observation ($T_e$) was indicated in each experiment and the natural lifespan of the animals ($T$) was assumed to be 104 weeks. The adjustment factor for each interval was then multiplied by the corresponding (unadjusted) interval dose to produce an adjusted dose for that interval. The three adjusted interval doses were then summed to produce the weighted dose total for the experiment.

**Potency Estimates from Data Sets with 100% Site-Specific Tumor Incidence**

If an animal carcinogenicity experiment consists of two groups whereby at study termination the incidence in the control group (the fraction $k$) is less than 100% tumor bearing animals and the dosed group consists entirely of tumor-bearing animals at the site of interest, then conventional methods for determining potency estimates fail. In the case of one control and one group of treated animals, only two parameters can be estimated and the multistage polynomial (Equation 1) to be fit reduces to

$$p(d) = 1 - \exp[-(q_0 + q_1d)] \quad (5)$$

When site-specific tumor incidence in the treated animals is 100%, the maximum likelihood estimate of $q_1$ is not finite. A lower bound estimate on $q_1$ can be obtained as follows. The number of tumor-bearing animals in a dose group consisting of $n$ animals is assumed to be a binomial random variable. The lower 5% confidence bound on $p(d)$ is given by

$$0.05 \leq p(d)^n \quad \text{or} \quad p(d) \geq (0.05)^{1/n} \quad (6)$$

Once $p(d)$ is determined, then a lower bound on $q_1$ is obtained from Equation 5:

$$q_1 \geq -\frac{\ln(1 - p(d)) + q_o}{d} \quad (7)$$

$q_1$ can then be used as a lower bound estimate of potency in this instance. For simplicity, $p(0)$ is assumed to be estimated by $k$, and a lower bound on $q_1$ is therefore given by

$$q_1 \geq -\frac{\ln(1 - (0.05)^{1/n}) + k}{d} \quad (8)$$

**Combining Potencies across Sites Using Monte Carlo Analysis**

For chemicals which significantly increase tumor incidence at multiple sites within a given sex, species and study, a methodological approach using Monte Carlo analysis has been used to combine potency estimates across sites. For each tumor site, a distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term ($q_1$) of the multistage model was generated with the MSTAGE 2.01 computer program (created by Edmund Crouch), which had been modified to tabulate percentile values. A combined distribution was created by adding $q_1$ for each tumor site, according to its distribution, through one hundred thousand Monte Carlo trial simulations (Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado).
upper 95 percent confidence bound of the combined distribution was taken as the basis of the
cancer potency estimate for the combined tumor sites.

A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human
potency is estimated. As described in the California risk assessment guidelines (CDHS, 1985), a
dose in units of milligram per unit surface area is assumed to produce the same degree of effect
in different species in the absence of information indicating otherwise. Under this assumption,
scaling to the estimated human potency ($q_{\text{human}}$) can be achieved by multiplying the animal
potency ($q_{\text{animal}}$) by the ratio of human to animal body weights ($b_{\text{h}}/b_{\text{a}}$) raised to the one-third
power when animal potency is expressed in units (mg/kg-day)$^{-1}$:

$$q_{\text{human}} = q_{\text{animal}} \times \left(\frac{b_{\text{h}}}{b_{\text{a}}}\right)^{1/3} \quad (9)$$

In interspecies scaling calculations, the mean chronic body weight of 30 g for male mice and 25 g for
female mice was used. Human body weight ($b_{\text{h}}$) is assumed to be 70 kg.

A.3 Risk-Specific Intake Level Calculation

The intake level ($I$, in mg/day) associated with a cancer risk $R$, from exposure to a carcinogen is

$$I = \frac{R \times b_{\text{h}}}{q_{\text{human}}} \quad (10)$$

where $b_{\text{h}}$ is the body weight, and $q_{\text{human}}$ the theoretical cancer potency estimate for humans.

Daily intake levels associated with lifetime cancer risks above $10^{-5}$ exceed the no significant risk
level for cancer under Proposition 65 (Title 22 California Code of Regulations, Section 12703).
Thus for a 70 kg person, the NSRL is given by

$$\text{NSRL} = \frac{10^{-5} \times 70 \text{ kg}}{q_{\text{human}}} \quad (11)$$

REFERENCES


Anderson EL (1983). Quantitative approaches in use to assess cancer risk [developed with U.S.

Armitage P, Doll R (1954). The age distribution of cancer and a multistage theory of

Buenig MK, Levin W, Karle JM, Yagi J, Jerina DM, Conney AH (1979). Tumorigenicity of bay-
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Risk Assessment and their Scientific Rationale*. State of California; Health and Welfare Agency,
Sacramento, CA (Note: Refer inquiries to the Reproductive and Cancer Hazard Assessment Section, Cal/EPA Office of Environmental Health Hazard Assessment).


NO SIGNIFICANT RISK LEVELS (NSRLS) FOR THE PROPOSITION 65 CARCINOGENS BENZ[a]ANTHRACENE (ORAL) AND 7H-Dibenzo[c,g]CARBAZOLE (ORAL)

May 2004

Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment (OEHHA)
California Environmental Protection Agency

SUMMARY OF FINDINGS
Carcinogenic potencies were estimated from oral carcinogenicity studies of benz[a]anthracene, which induced liver tumors in treated mice (Klein, 1963), and 7H-dibenzo[c,g]carbazole, which induced forestomach tumors in treated mice (Armstrong and Bonser, 1950). In the case of benz[a]anthracene, dose calculations were adjusted by Doll-Armitage analysis for variable dosing over time.

The potency derivation takes into account body size differences between humans and experimental animals. The Proposition 65 “no significant risk level” (NSRL) is defined in regulation as the daily intake level posing a $10^{-5}$ lifetime risk of cancer. Cancer potency estimates and the corresponding NSRLs are given in Table 1.

Table 1. Cancer Potencies (Oral) and NSRLs (Oral).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Cancer Potency (Oral) (mg/kg-day)$^{-1}$</th>
<th>NSRL (Oral) (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benz[a]anthracene</td>
<td>21</td>
<td>0.033</td>
</tr>
<tr>
<td>7H-Dibenzo[c,g]carbazole</td>
<td>230</td>
<td>0.0030</td>
</tr>
</tbody>
</table>

INTRODUCTION
The two polycyclic aromatic hydrocarbons (PAHs) discussed here have been listed as chemicals known to the State to cause cancer under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code Section 25249.5 et seq.). Listing occurred as follows: benz[a]anthracene on July 1, 1987, and 7H-dibenzo[c,g]carbazole on January 1, 1988. This document describes the derivation of cancer potency values by the oral route and the corresponding NSRLs for these two PAHs. For the discussion which follows, it is noted that 7H-dibenzo[c,g]carbazole is a heterocyclic polyaromatic compound, rather than a
polycyclic aromatic hydrocarbon (PAH), but is included among the PAHs by convention and because of properties similar to non-heterocyclic PAHs.

<table>
<thead>
<tr>
<th></th>
<th>Benz[a]anthracene</th>
<th>7H-Dibenzo[c,g]carbazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula:</strong></td>
<td>C_{18}H_{12}</td>
<td>C_{20}H_{13}N</td>
</tr>
<tr>
<td><strong>CAS No.:</strong></td>
<td>56-55-3</td>
<td>194-59-2</td>
</tr>
<tr>
<td><strong>Mol.Wt.:</strong></td>
<td>228.3</td>
<td>267.3</td>
</tr>
<tr>
<td><strong>Structure:</strong></td>
<td><img src="image" alt="Structure of Benz[a]anthracene" /></td>
<td><img src="image" alt="Structure of 7H-Dibenzo[c,g]carbazole" /></td>
</tr>
</tbody>
</table>

These PAHs are soluble in various organic solvents, such as benzene, ketones, and ethers (IARC, 1983a; IARC, 1983b). Benz[a]anthracene is described by IARC as having colorless plate-like crystals with a greenish-yellow fluorescence; 7H-dibenzo[c,g]carbazole has needle-shaped crystals.

PAHs are generated by combustion or pyrolysis of organic materials, and occur widely as environmental pollutants, food contaminants (especially of smoked or grilled food) and components of soots, tars and other wastes and by-products of industrial processes (IARC, 1973; IARC, 1983a; IARC, 1983b). They are found in the particulate fractions of engine exhausts and other emissions from mobile or stationary combustion sources. PAHs also occur in materials such as crude oil, coal, carbon blacks, coal tar, and in some mineral oils. Both benz[a]anthracene and 7H-dibenzo[c,g]carbazole have been specifically identified as components of cigarette smoke.

**STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT**

Data on the effects of human exposure to benz[a]anthracene and 7H-dibenzo[c,g]carbazole as pure substances for use in dose-response evaluations are not available. Benz[a]anthracene is a major component of various mixtures of polycyclic hydrocarbons for which positive carcinogenicity data have been obtained after occupational exposure (see, for example, IARC, 1983c), and both compounds are components of tobacco smoke, a known human carcinogen. However, because tobacco smoke is a mixture of PAHs and numerous other carcinogenic substances, the data available on PAH concentrations in tobacco smoke and human cancer incidence in smokers do not permit independent dose-response evaluations for the compounds discussed here.

Numerous carcinogenesis bioassays by various routes of exposure and in different species are available for these compounds (CancerChem, 2000). The large number of positive studies supports the identification of these agents as carcinogens, but the use of most of these studies in assessing the dose response is difficult, as outlined below.

In selecting animal studies as bases for potency estimation, those using routes of exposure corresponding to likely human exposures are considered the most suitable (*e.g.*, oral, inhalation, dermal). Inhalation studies are not available for benz[a]anthracene and 7H-dibenzo[c,g]-

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**Benz[a]anthracene and 7H-Dibenzo[c,g]carbazole NSRLs**

May 2004

OEHHA
carbazole. Oral studies are available and are considered to provide better estimates of potencies for inhalation exposures than those by the other routes for which bioassay information is available. One study in A/J mice in which 7H-dibenzo[c,g]carbazole was administered via intraperitoneal (i.p.) injection was located, but not evaluated. Although skin application studies are of interest in establishing the relative potency of different topically applied carcinogens, the dose received by the target tissue likely would be difficult to determine in such a study, or relate to other routes of exposure. Thus, the various studies using skin painting, injection or implantation routes were not selected for potency estimation, although they add to the overall weight of evidence for hazard identification. The available studies of the carcinogenicity of benz[a]anthracene and 7H-dibenzo[c,g]carbazole, as tested by the oral route, are discussed below.

**Benz[a]anthracene**

Multiple studies have examined the carcinogenicity of benz[a]anthracene by the oral route in experimental animals, although several suffer from limitations for the purpose of estimating cancer potency. These include small size and lack of control incidence data (White and Eschenbrenner, 1945), lack of statistically significant increases in tumor incidence and limited examination of tissues or inadequate dosing period and follow-up (Bock and King, 1959; Huggins and Yang, 1962).

One set of studies reported data on the carcinogenicity of benz[a]anthracene which are suitable for cancer potency estimation (Klein, 1963). In these studies groups of seven- to eight-day old B6AF1/J male mice received by oral gavage 0.05 ml doses of 3% benz[a]anthracene in 0.1% methocel-Aerosol OT, three times weekly for five weeks. B6AF1/J mice are an F1 hybrid strain produced from female C57BL/6J female mice and A/J male mice. Two experiments (“I” and “II”) were run concurrently but had different sacrifice units, numbers of animals and control groups. Those in Experiment I were killed at median ages of 340-444 days, and in Experiment II, 547-600 days. All animals received 15 doses except for one group of animals in Experiment II (“IIb”), which received only two doses. Control groups received the vehicle, methocel-Aerosol OT, only. Substantial increases in the incidences of hepatomas and pulmonary adenomas were observed in all treated groups. Two treated animals in Experiment I were also found to have papillomas of the forestomach. Detailed incidence data for hepatomas and lung adenomas are given below in Table 2.
Table 2: Incidence of Liver and Lung Tumors in Male B6AF1/J Mice Treated Orally with Benz[a]anthracene (Klein, 1963).

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Total Dose (mg)</th>
<th>Number of Treatments</th>
<th>Incidence a</th>
<th>Median Age at Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hepatomas</td>
<td>Pulmonary Adenomas</td>
</tr>
<tr>
<td>I b</td>
<td>0</td>
<td>15</td>
<td>0/38</td>
<td>10/38</td>
</tr>
<tr>
<td></td>
<td>22.5</td>
<td>15</td>
<td>18/39 c</td>
<td>37/39 c</td>
</tr>
<tr>
<td>IIa</td>
<td>0</td>
<td>15</td>
<td>2/20</td>
<td>7/20</td>
</tr>
<tr>
<td></td>
<td>22.5</td>
<td>15</td>
<td>20/20 c</td>
<td>19/20 c</td>
</tr>
<tr>
<td>IIb</td>
<td>3</td>
<td>2</td>
<td>16/20 c</td>
<td>17/20 c</td>
</tr>
</tbody>
</table>

a Incidence given as number of animals with tumor over total number of animals autopsied.

b Two animals in the treated group were also found with forestomach papillomas.

c Statistical significance for Fisher’s exact test comparing results in treated animals with those of the appropriate control group (p < 0.05).

7H-Dibenzo[c,g]carbazole

Few studies have examined the carcinogenicity of 7H-dibenzo[c,g]carbazole in experimental animals. One was limited in its usefulness for the estimation of carcinogenic potency, as it examined lung tumor endpoints following intraperitoneal injection in the Strain A/J mouse, a strain bred to be sensitive to this carcinogenic effect by chemical carcinogens and which is generally used for screening purposes (Warshawsky et al., 1996).

A single publication reported data suitable for the estimation of carcinogenic potency of 7H-dibenzo[c,g]carbazole (Armstrong and Bonser, 1950). In these studies, male and female mice of two strains (CBA and Strong A) received doses of 7H-dibenzo[c,g]carbazole in arachis oil by gavage twice weekly, starting at 12 weeks of age. Treatment was continued until death (CBA mice) or until signs of liver toxicity became severe (Strong A mice). All mice showed signs of liver toxicity, and many died early in the experiment from liver necrosis, particularly the CBA females. The length of the dosing period, and in some cases the dose level, was adjusted to promote survival, so the total dose received varied somewhat between individual animals. For each animal, the authors reported the total weeks of treatment, the weeks of survival (assumed to be since onset of treatment at 12 weeks of age) and the total dose of 7H-dibenzo[c,g]carbazole received. Male and female Strong A mice received average total doses of 19.0 and 17.2 mg of 7H-dibenzo[c,g]carbazole, respectively. Male CBA mice received an average of 13.1 mg 7H-dibenzo[c,g]carbazole. Average daily dose rates were calculated using the total dose received (in mg) averaged over the total experimental length (weeks before first dose plus average survival); animal body weights were taken to be 0.03 kg for male mice and 0.025 for female mice (see Table 3). Tumor data were reported for all animals surviving for 17 weeks or longer (the time when the first forestomach tumors appeared), the maximum survival period being 59 weeks. Only a single female CBA mouse survived this long.
High incidences of forestomach papillomas, including some carcinomas, were reported in treated Strong A and CBA mice (see Table 3 below). The authors reported high incidences of liver tumors in treated animals in both strains. Benign and malignant hepatomas were observed; the criterion for malignancy appears to have been the observation of metastasis (a very stringent test compared to the morphological criteria used currently). Incidence of bile-duct cystadenomas was 100% in both strains in treated animals. All exposed Strong A mice were found to have lung adenomas, whereas no such tumors were seen in CBA mice. Strong A mice are essentially the same as Strain A and A/J; these mice have been bred to be sensitive to the development of lung tumors from exposure to numerous chemical carcinogens. No control groups were described, so the control rates for liver, bile-duct, and lung tumors cannot be estimated. Because the background incidence of liver and lung tumors is highly variable among mouse strains, estimation of these endpoints for a potency calculation was not considered appropriate. However, with the forestomach tumors, the authors, citing historical data, imply that they did not expect to see the forestomach tumors in the absence of treatment. Forestomach tumors are rare among control populations of other mouse strains (NTP Historical Control Information available at http://ehp.niehs.nih.gov/ntp/docs/ntp_hcrs.html). An assumption that no forestomach tumors were expected to develop in putative control groups of either mouse strain permits the use of the data on the development of these tumors in 7H-dibenzo[c,g]carbazole treated mice for the estimation of cancer potency.

Table 3: Incidence of Forestomach Tumors in Strong A and CBA Mice Administered 7H-Dibenzo[c,g]carbazole by the Oral Route (Armstrong and Bonser, 1950).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Total Dose (mg)</th>
<th>Age of 1st Dose (weeks)</th>
<th>Average Treatment (weeks)</th>
<th>Average Survival (weeks)</th>
<th>Average Daily Dose (mg/kg-day)</th>
<th>Forestomach Tumors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA</td>
<td>M</td>
<td>13.1</td>
<td>12</td>
<td>30.1</td>
<td>30.1</td>
<td>1.48</td>
<td>21/30</td>
</tr>
<tr>
<td>Strong A</td>
<td>F</td>
<td>17.2</td>
<td>12</td>
<td>37.4</td>
<td>51.5</td>
<td>1.55</td>
<td>12/13</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>19.0</td>
<td>12</td>
<td>42.7</td>
<td>48.5</td>
<td>1.50</td>
<td>8/11</td>
</tr>
</tbody>
</table>

* Tumor incidence among mice surviving to 17 weeks.

**APPROACH TO DOSE RESPONSE ANALYSIS**

The studies by Klein (1963) were conducted with B6AF1/J mice, an F1 hybrid strain produced as the offspring of female C57BL/6J female mice and A/J male mice. Strain A/J mice are highly sensitive to the development of lung adenomas by numerous chemicals. Because of the possible extreme sensitivity of the hybrid B6AF1/J strain used in these experiments to lung tumors, the lung tumor data (and resulting potency estimates) were excluded from the dose response analysis. Both benz[a]anthracene and 7H-dibenzo[c,g]carbazole are genotoxic (IARC, 1983a; IARC, 1983b). There are insufficient data available for either of these chemicals to support dose adjustments based on pharmacokinetic models. Therefore, default approaches (i.e., a linearized multistage model, use of adjustments for less-than-lifetime exposure and interspecies scaling) have been applied. The approaches used are described in detail in the Appendix.
DOSE-RESPONSE ASSESSMENT

Benz[a]anthracene

Cancer potency estimates for liver tumors in male B6AF1/J mice treated orally with benz[a]anthracene were derived from the studies of Klein (1963), described above. Dose calculations are summarized in Table 4, and cancer potency estimates are summarized in Table 5.

In experiment IIa, benz[a]anthracene induced a 100% incidence of hepatomas, a case in which a probability distribution and potency estimate could not be obtained by fitting to the “multistage” polynomial. Here, the lower 5% confidence bound for the probability that all animals in the dosed group are tumor-bearing was used to calculate a potency (see Appendix).

Since the dosing periods in this study were short and concentrated in the earliest part of the animals’ lifetime, the time-dependent version of the Armitage-Doll model was used, as described in the Appendix. The period of observation was defined as the median age at autopsy reported by the study authors, since no other information relevant to this parameter was provided. The authors did not provide any data on the body weights of the mice at the time of dosing, so body weight estimates during weekly dosing intervals for each of weeks one through six for male BAF1 mice were derived from Poiley (1972). Treatments were assumed to have begun on postnatal day seven of life. Average doses (in mg/kg-day) for weekly intervals were calculated based upon these assumptions (see Table 4). Because of the variable dosing over time, the Doll-Armitage correction factors were applied to these doses to produce equivalent constant doses for each interval, producing what is termed here the “adjusted dose” (see Table 4 and Appendix). The weekly equivalent doses were summed to produce the “weighted dose” for the entire study.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Treatment Dose (mg)</th>
<th>Interval Body Weight (kg)</th>
<th>Interval Dose (mg/kg-day)</th>
<th>Adjusted Dose (mg/kg-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp.”I”</td>
<td>Exp.”IIa”</td>
<td>Exp.”IIb”</td>
<td></td>
</tr>
<tr>
<td>Week 1-2</td>
<td>1.5</td>
<td>0.0047</td>
<td>136.78</td>
<td>1.353</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Exp. IIb: 91.2</td>
<td>2.143</td>
<td>1.542</td>
</tr>
<tr>
<td>Week 2-3</td>
<td>1.5</td>
<td>0.0054</td>
<td>119.05</td>
<td>1.139</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.817</td>
</tr>
<tr>
<td>Week 3-4</td>
<td>1.5</td>
<td>0.0077</td>
<td>83.49</td>
<td>0.772</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.240</td>
</tr>
<tr>
<td>Week 4-5</td>
<td>1.5</td>
<td>0.0104</td>
<td>61.81</td>
<td>0.553</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.894</td>
</tr>
<tr>
<td>Week 5-6</td>
<td>1.5</td>
<td>0.0167</td>
<td>38.49</td>
<td>0.332</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.542</td>
</tr>
</tbody>
</table>

Weighted Dose = 4.15, 6.64, 1.54

a Mice were treated orally three times per week, except animals in Experiment IIb, which received only two doses.
b Mouse body weights for first weeks of life were adopted from Poiley (1972).
c Interval dose adjusted using Doll-Armitage weighting for early-in-life and variable exposure. These values represent the lifetime (104 wks) equivalent dose received during the defined interval; the sum, or weighted dose, represents the total lifetime exposure, using the mean sacrifice time of the treated group as the time to observation and two years as the natural lifespan of the animals (see Appendix for details of the adjustment).
d Experiment IIb involved only two treatments during the first week, thus the calculated “interval dose” for an assumed one week of exposure is 91.2 mg/kg-day (= 1.5 mg × (2 days/7 days) ÷ 0.0047 kg).

For interspecies scaling, a lifetime mean body weight for male B6AF1/J mice of 0.0302 kg was used, as calculated by U.S. EPA (1988) from the Poiley (1972) data series. Potency estimates, given in Table 5, were calculated separately for Experiments I, IIa, and IIb. The incidence of hepatomas in Experiment IIa (treated animals received 15 doses of benz[a]anthracene) was 100%, so a probability distribution and potency estimate could not be obtained by fitting to the “multistage” polynomial. Instead, the lower 5% confidence bound on the probability that all animals in this group would develop tumors was calculated. The 95% upper confidence limits on q1 for Experiment IIb was 1.56 (mg/kg-day)-1 for hepatomas. Using this q1*, the human potency estimate for benz[a]anthracene based on these data is 21 (mg/kg-day)-1.

Table 5: Oral Potency Estimates for Benz[a]anthracene Based on Klein (1963).

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Tumor</th>
<th>Weighted Dose</th>
<th>Tumor Incidence</th>
<th>Potency Estimate (mg/kg-day)-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Hepatomas</td>
<td>0, 4.15</td>
<td>0/38, 18/39</td>
<td>0.216</td>
</tr>
<tr>
<td>IIa</td>
<td>Hepatomas</td>
<td>0, 6.64</td>
<td>2/20, 20/20</td>
<td>0.281a</td>
</tr>
<tr>
<td>IIb</td>
<td>Hepatomas</td>
<td>0, 1.54</td>
<td>2/20, 16/20</td>
<td>1.56</td>
</tr>
</tbody>
</table>

a Lower five percent confidence bound for the probability that all animals in the dosed group are tumor-bearing.

**Bolding** indicates value selected as the basis of the NSRL.
7H-Dibenzo[c,g]carbazole

The studies of oral 7H-dibenzo[c,g]carbazole carcinogenicity in mice by Armstrong and Bonser (1950) are the only source of such data which was identified; however, these studies have a number of serious deficiencies as a basis for a potency estimate. Since no control groups were reported and the background rates for liver and lung tumors in mice of various strains are substantial and variable, it is not possible to use the liver or lung tumor incidences in these experiments for potency analysis. The authors implied that historical data on forestomach tumors indicate a background incidence of zero for this site in each of the mouse strains employed in these studies. If this assumption is made, then the incidence data for forestomach tumors in male and female Strong A and male CBA mice can be used as bases for potency estimates (assuming that the control groups had identical numbers of animals as the dosed groups) (see Table 6). These estimates are less reliable than would be obtained from a study of more recent design and with more complete reporting. They may also underestimate the true carcinogenic potency of 7H-dibenzo[c,g]carbazole, since 100% incidence of bile-duct cystadenomas (possibly a neoplastic lesion) was noted.

Table 6. Derivation of Cancer Potencies by the Oral Route for 7H-Dibenzo[c,g]carbazole Based upon Forestomach Tumors Observed in the Studies of Armstrong and Bonser (1950).

<table>
<thead>
<tr>
<th>Strain, Sex</th>
<th>Animal Cancer Potency [(mg/kg-day)^-1]</th>
<th>Human Cancer Potency [(mg/kg-day)^-1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA, male</td>
<td>17.6</td>
<td>230</td>
</tr>
<tr>
<td>Strong A, female</td>
<td>13.5</td>
<td>190</td>
</tr>
<tr>
<td>Strong A, male</td>
<td>7.87</td>
<td>100</td>
</tr>
</tbody>
</table>

a Animal potencies for lifetime exposure were calculated using experimental length based on average animal survival times (30, 52, and 48 weeks for male CBA, female Strong A and male Strong A mice, respectively). Putative control groups were assigned incidences of 0/30, 0/13, and 0/11 for the male CBA, female Strong A and male Strong A mice, respectively.

b Extrapolation to human potency was based on assumed body weights of 0.030, 0.025, and 70 kg for male mice, female mice, and humans, respectively (see Appendix).

Bolding indicates value selected as the basis of the NSRL.

The cancer potency values estimated from the data of Armstrong and Bonser (1950) on forestomach tumors in Strong A male, Strong A female and CBA male mice are shown in Table 6. The male CBA mouse was the most sensitive strain and sex tested by Armstrong and Bonser (1950) in their studies of 7H-dibenzo[c,g]carbazole. As noted by CDHS (1985), it is appropriate to use the most sensitive strain and sex as the basis of estimates of human cancer potency. This applies especially in this case where other data which cannot be analyzed indicate that the actual potency may be higher than estimated here. The estimated value for the human cancer potency of 7H-dibenzo[c,g]carbazole by the oral route is 230 (mg/kg-day)^-1.
NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of $10^{-5}$. The cancer potency estimates derived above for oral exposures were used to calculate NSRLs for benz[a]anthracene by the oral route (0.033 µg/day) and 7H-dibenzo[c,g]carbazole by the oral route (0.0030 µg/day).
REFERENCES


APPENDIX: METHODOLOGY USED TO DERIVE RISK-SPECIFIC INTAKE LEVELS FOR BENZ[A]ANTHRACENE AND 7H-DIBENZO[C,G]CARBAZOLE BY THE ORAL ROUTE

Procedures for the development of Proposition 65 NSRLs are described in regulation (California Code of Regulations, Title 22, Sections 12701 and 12703). Consistent with these procedures, the specific methods used to derive the NSRLs for benz[a]anthracene and 7H-dibenzo[c,g]carbazole are outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

“Multistage” Polynomial

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (CDHS, 1985; U.S. EPA, 1987; Anderson, 1983):

\[ p(d) = 1 - \exp[-(q_0 + q_1d + q_2d^2 + \ldots + q_id^i)] \]  

with constraints

\[ q_i \geq 0 \text{ for all } i. \]

The \( q_i \) are parameters of the model which are taken to be constants and are estimated from the data. The parameter \( q_0 \) represents the background lifetime incidence of the tumor. The parameter \( q_1 \) or some upper bound, is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as \( q_1^* \), the upper 95% confidence bound on \( q_1 \) (CDHS, 1985), estimated by maximum likelihood techniques. When dose is expressed in units mg/kg-day, the parameters \( q_1 \) and \( q_1^* \) are given in units (mg/kg-d)^{-1}. Details of the estimation procedure are given in Crump (1981) and Crump et al. (1977).

To estimate potency in animals (\( q_{\text{animal}} \)) from experiments of duration \( T_e \), rather than the natural lifespan of the animals (\( T \)), it is assumed that lifetime incidence of cancer increases with the third power of age:

\[ q_{\text{animal}} = q_1^* \times (T/T_e)^3 \]  

Following Gold and Zeiger (1997) and the U.S. Environmental Protection Agency (U.S. EPA, 1988), the natural lifespans of mice and rats are assumed to be two years, so that for experiments lasting \( T_e \) weeks in these rodents:

\[ q_{\text{animal}} = q_1^* \times (104/T_e)^3 \]  

To estimate risk at low doses, potency is multiplied by average daily dose. The risk estimate obtained is referred to by the U.S. EPA (Anderson et al., 1983) as “extra risk,” and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.
**Calculation of the Lifetime Average Dose for 7H-Dibenzo[c,g]carbazole**

The lifetime average daily doses for the studies of 7H-dibenzo[c,g]carbazole were calculated based upon the information provided by Armstrong and Bonser (1950) and assumed mouse body weights. Average total doses administered to the mice and provided by the authors (13.1 mg for male CBA mice, 17.2 mg for female Strong A and 19.0 mg for male Strong A) were divided by the total experimental length (age at dosing plus average survival) and divided by mouse body weight estimate assumptions (0.030 and 0.025 kg, for male and female mice, respectively) to produce estimates of the lifetime average daily dose.

**Variable Dosing Doll-Armitage Analysis for Benz[a]anthracene**

The Armitage and Doll (1954) mathematical description of carcinogenesis as expressed by Crouch (1983) and Crump and Howe (1984) allows for the analysis of data sets with variable dosing over time. The model assumes that cancer derives from a single cell after it has undergone a series of transformations. The model has been used to describe cancer dose response data in animal bioassays as well as in the general population. This methodology was used in the analysis of the study by Klein (1963) of benz[a]anthracene carcinogenicity, to allow for the fact that the experiment involved periods of dosing much shorter than the nominal lifetime of the test animals, or the overall observation period of the experiment.

Assumptions are required for the application of the Doll-Armitage model regarding: 1) the mathematical relationship between applied dose and the probability that a “stage transition” has occurred, 2) the stage affected by the carcinogen and 3) the number of “stages.” For the particular forms used to fit the tumor data in the Klein (1963) study, a linear relationship is assumed between dose and cell transformation, and benz[a]anthracene is assumed to affect an early stage of the cancer process.

As discussed by Crouch (1983), if the probability per unit time of the stage transformation depends linearly on dose rate \(d(t)\), and the carcinogen only affects a single “stage,” the probability of tumor by time \(T_e\) under Armitage and Doll (1954) becomes

\[
P(T_e) = 1 - \exp[-(A + BD)]
\]

with

\[
D = \frac{1}{T^m \cdot \beta(m - j + 1, j)} \int_0^{T_e} d(t)(T_e - t)^{m-j} t^{j-1} dt
\]

where \(T_e\) is the time to observation, and \(\beta\) is Euler's beta function. Following Anderson et al. (1983), the natural lifetime of the test animal, \(T\), is assumed to be two years for rats and mice. The integer \(m\) (the number of “stages”) specifies the rate of increase in incidence with time and \(j\) is the “stage” affected by the carcinogen. Benz[a]anthracene is assumed to act only as an initiator \((j = 1)\). For \(j = 1\), the solution to the equation describing the equivalent constant dose correction factor becomes
For a given time interval from \(a\) to \(b\).

The value of \(m\) is normally assumed to be 3.0; this assumption was made for the purposes of this report since no contrary information was available. The potency in animals, \(q_{\text{animal}}\), is given by the upper 95% confidence bound on \(\beta\). This method of calculation allows for both abbreviated and variable dosing schedules, and for observation periods less than the nominal lifetime of the test animals.

Estimating the total lifetime weighted dose of benz[a]anthracene from the Klein (1963) studies involved three sets of calculations for the three experiments (I, IIa, and IIb). For purposes of the Doll-Armitage variable dosing calculation, five dosing intervals of one week were assumed to occur in Experiments I and IIa, with three doses per week averaged over the week \((3/7)\) then divided by the interval body weight (adopted from Poiley, 1972, see Table 4 above) to produce an unadjusted interval dose in mg/kg-day. The Doll-Armitage adjustment factors (see Equation 6 above) for each interval were calculated as follows: for the first week interval, \(a\) and \(b\) were 1 and 2, respectively, for the second week interval, 2 and 3, respectively, and so on. The experimental length or time to observation \((T_e)\) was the median age at autopsy in weeks for the experiment as reported by Klein (1963) (see Table 2) and the natural lifespan of the animals \((T)\) was assumed to be 104 weeks. The adjustment factor for each interval was then multiplied by the corresponding (unadjusted) interval dose to produce an adjusted dose for that interval. The five adjusted interval doses were then summed to produce the weighted dose total for the experiment. In Experiment IIb only two doses were administered, so only a single interval of one week was assumed \((a = 1, \ b = 2)\), and the dose administered was adjusted accordingly \((2/7)\).

**Potency Estimates from Data Sets with 100% Tumor Incidence**

If an animal carcinogenicity experiment consists of two groups whereby at study termination the control group consists of some percentage (say \(k\%\), but less than 100%), of tumor bearing animals and the dosed group consists entirely of tumor-bearing animals, then conventional methods for determining potency estimates fail. The number of tumor-bearing animals in a dose group consisting of \(n\) animals is assumed to be a binomial random variable with the probability of tumor at administered dose \(d\) denoted by \(p(d)\). The probability of tumor is assumed to be multistage Weibull, \(i.e.\)

\[
p(d) = 1 - e^{-[q_0 + q_1d]} \tag{7}
\]

For the above described experimental situation, the observed data indicate that \(p(0)\) equals \(k\%\) and \(p(d)\) equals 1. Parameter estimates for \(q_0\) and \(q_1\) that would result in a perfect fit to the observed data are \(-\ln[1 - p(0)]\) and infinity, respectively. Since the estimate of \(q_1\) is not finite, conventional methods of using the upper 95% confidence bound of \(q_1\) cannot be employed. One method to circumvent the estimation problems associated with complete tumor response in the dosed group is to use the lower 5% confidence bound for the probability that all animals in the dosed group (of \(n\) animals) are tumor-bearing, \(i.e.\)
0.05 = \( p(d)^n \) \hspace{1cm} (8)

Once \( p(d) \) is determined, then by employing the multistage Weibull form for the probability of tumor at dose \( d \) and solving for \( q_1 \), a finite estimate for \( q_1 \) is obtained, \( i.e. \)

\[
q_1 = -\frac{\ln \left[ \frac{1 - p(d)}{1 - p(0)} \right]}{d}
\] \hspace{1cm} (9)

\( q_1 \) can then be used as an estimate of potency in this instance.

This methodology was applied to the analysis of benz[a]anthracene induced liver tumor dose-response data from Experiment IIa of Klein (1963).

A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. As described in the California risk assessment guidelines (CDHS, 1985), a dose in units of milligram per unit surface area is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Under this assumption, scaling to the estimated human potency (\( q_{\text{human}} \)) can be achieved by multiplying the animal potency (\( q_{\text{animal}} \)) by the ratio of human to animal body weights (\( \frac{b_{wh}}{b_{wa}} \)) raised to the one-third power when animal potency is expressed in units (mg/kg-day)\(^{-1} \):

\[
q_{\text{human}} = q_{\text{animal}} \times \left( \frac{b_{wh}}{b_{wa}} \right)^{1/3}
\] \hspace{1cm} (10)

In order to interpret the study of benz[a]anthracene by Klein (1963), more detailed body weight information was required, including the chronic study mean value (and also the weights at various ages shortly after birth discussed earlier for the Doll-Armitage adjustment). These data were not provided by the author, so the value observed by Poiley (1972) (and cited by U.S. EPA, 1988) of 0.0302 kg for male BAF\(_1\) hybrid mice was used as the chronic study mean value. For the interspecies scaling of the 7H-dibenzo[c,g]carbazole potencies derived from the Armstrong and Bonser (1950) studies, body weights (\( b_{wa} \)) of 0.030 and 0.025 kg for male or female mice, respectively (U.S. EPA, 1988), were used in the absence of more detailed information. Human body weight (\( b_{wh} \)) is assumed to be 70 kg.

A.3 Risk-Specific Intake Level Calculation

The intake level (\( I \), in mg/day) associated with a cancer risk \( R \), from exposure to a carcinogen is

\[
I = \frac{R \times b_{wh}}{q_{\text{human}}}
\] \hspace{1cm} (11)

where \( b_{wh} \) is the body weight, and \( q_{\text{human}} \) the theoretical cancer potency estimate for humans.
Daily intake levels associated with lifetime cancer risks above $10^{-5}$ exceed the no significant risk level for cancer under Proposition 65 (Title 22 California Code of Regulations, Section 12703). Thus for a 70 kg person, the NSRL is given by:

$$\text{NSRL} = \frac{10^{-5} \times 70 \text{ kg}}{q_{\text{human}}}$$  \hspace{1cm} (12)

**APPENDIX REFERENCES**


