NO SIGNIFICANT RISK LEVELS (NSRLS) FOR THE PROPOSITION 65 CARCINOGENS BENZO[B]FLUORANTHENE, BENZO[J]FLUORANTHENE, CHRYSENE, DIBENZO[A,H]PYRENE, DIBENZO[A,I]PYRENE, AND 5-METHYLCHRYSENE BY THE ORAL ROUTE

May 2003

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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 i.p. studies than in 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.

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 (q_1) of the multistage model fit to dose response data for a given site represents the cancer potency for that site. 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 the various PAHs was derived through Monte Carlo analysis. The upper 95 percent confidence bounds indicated by the combined distribution for these treatment-related tumor sites, adjusted downward by a factor of 75, were taken as the cancer potencies.

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).

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

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.

Studies of nitro-arene compounds have shown that the i.p. injection route results in a tumorigenic response similar to that seen after oral treatments if the species, dose regimen, and age at treatment are comparable (King, 1988; Imaida *et al.*, 1991a; Imaida *et al.*, 1991b). This is assumed true for the PAHs that are the subject of this report. For nitro-arenes it was also shown that the neonatal mouse model produced a greater tumor response relative to oral studies in adults (King, 1988; Wislocki *et al.*, 1986). The source of this difference is uncertain, but may be related to the life stage at which the experimental animals were exposed. 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

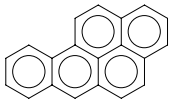
Crouch, 1980; U.S. EPA, 1993). The use of comparative potency calculations for PAHs has been examined previously (Clement Associates, 1988). Estimates obtained in this way are assumed applicable for oral route exposures.

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.

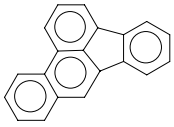
Table 2. Physicochemical Properties.

Compound	Molecular Weight	CAS No.	Formula	Melting Point	Boiling Point
Benzo[b]fluoranthene	252.3	205-99-2	$C_{20}H_{12}$	168.3°C	-
Benzo[j]fluoranthene	252.3	205-82-3	$C_{20}H_{12}$	165.4°C	-
Chrysene	228.3	218-01-9	$C_{18}H_{12}$	255 – 256°C	448°C
Dibenzo[a,h]pyrene	302.4	189-64-0	$C_{24}H_{14}$	317°C	-
Dibenzo[a,i]pyrene	302.4	189-55-9	$C_{24}H_{14}$	281.5 - 282.5°C	275°C (@ 0.05 mm Hg)
5-Methylchrysene	242.3	3697-24-3	$C_{19}H_{14}$	117 - 118°C	-
Benzo[a]pyrene	252.3	50-32-8	$C_{20}H_{12}$	176.5°C	495°C

Figure 1. Molecular Structures.



Benzo[a]pyrene



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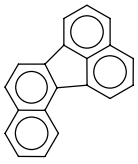

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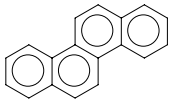
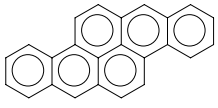
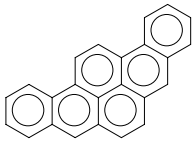


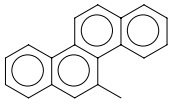
Chrysene



Dibenzo[a,h]pyrene



Dibenzo[a,i]pyrene



5-Methylchrysene

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. Subcutaneous injection or implantation routes are also less suitable for potency estimation because of the difficulty of extrapolating to other routes of exposure.

For some related carcinogenic compounds, the potencies generated from i.p. route carcinogenicity data do not differ greatly from those observed by the oral route, provided that other variables such as the species and age at the time of exposure are similar (King, 1988; Imaida *et al.*, 1991a; Imaida *et al.*, 1991b). Intraperitoneal studies have been used here for potency estimation for benzo[b]fluoranthene, benzo[j]fluoranthene, 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]fluoranthene, benzo[j]fluoranthene, 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]fluoranthene, or indeno[1,2,3-cd]pyrene dissolved in DMSO. Total doses given were 0.5 μmoles for benzo[b]fluoranthene, or 1.1 μmoles for benzo[a]pyrene or benzo[j]-

fluoranthene, 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]fluoranthene, and benzo[j]fluoranthene (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]fluoranthene.

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

Group	Compound	Dose (µmol)	Liver Tumors [a]		Lung Adenomas [a]	
			Male	Female	Male	Female
1	**Control**	0	1/17 (1)	0/18	0/17	0/18
2	**Benzo[a]pyrene**	1.1	13/17 [b] (4)	0/14	14/17 [b]	9/14 [b]
3	**Benzo[b]fluoranthene**	0.5	8/15 [b] (2)	0/17	2/15	3/17
4	**Benzo[j]fluoranthene**	1.1	11/21 [b] (3)	0/18	11/21 [b]	4/18 [c]
5	**Benzo[k]fluoranthene**	2.1	3/16 (1)	0/18	1/16	3/18
6	**Indeno(1,2,3-cd)pyrene**	2.1	0/11	0/9	1/11	0/9

[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] $p = 0.052$.

Wislocki et al. (1986)

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).

Compound	Dose (nmol)	Sex	Liver Tumors [a]			Lung Tumors [a]		
			Adenoma	Carcinoma	Combined	Adenoma	Carcinoma	Combined
Control	0	M	2/28	0/28	2/28	1/28	0/28	1/28
		F	0/31	0/31	0/31	0/31	0/31	0/31
Benzo[a]pyrene	560	M	11/37 [c]	7/37 [c]	18/37 [b]	13/37 [b]	0/37	13/37 [b]
		F	0/27	0/27	0/27	13/27 [b]	0/27	13/27 [b]
Chrysene	700	M	8/35	2/35	10/35 [c]	5/35	1/35	6/35
		F	0/33	0/33	0/33	2/33	0/33	2/33
	2800	M	1/34	13/34 [b]	14/34 [b]	7/34 [c]	0/34	7/34 [c]
		F	0/24	0/24	0/24	1/24	0/24	1/24

[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 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 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).

Compound	Total Dose (nmol)	Hepatic Tumors		Lung Tumors	
		Male	Female	Male	Female
Control	0	0/32	0/39	7/32	11/39
Dibenzo[a,h]pyrene	87.5	11/25 *	1/14	25/25 *	13/14 *
Dibenzo[a,i]pyrene	87.5	21/39 *	0/21	37/39 *	21/21 *

* 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-methyl-chrysene 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).

Treatment Group (Total Dose in nmol)	Hepatic Tumors		Lung Tumors	
	Male	Female	Male	Female
0	1/48	1/41	2/48	3/41
56	8/35 [a]	6/48 [b]	7/35 [c]	10/48 [b]

Significance relative to control (Fisher's exact test):
[a] $p < 0.005$
[b] $0.1 < p < 0.05$
[c] $p < 0.05$

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. Lung tumor incidences were increased among both males and females

in the two highest dose groups. The average numbers of tumors per mouse also increased with dose (data not presented).

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).

Treatment Group (Total Dose in μmol)	Total Tumors		Alveolar / Bronchiolar Carcinomas	
	Male	Female	Male	Female
0	8/33	7/33	6/33	7/33
0.110 [a]	18/37 [b]	8/29	8/37	6/29
0.275	20/34 [c]	14/32 [b]	17/34 [c]	14/32 [b]
1.10	25/25 [d]	35/38 [d]	25/25 [d]	35/38 [d]

[a] Low-dose animals were only examined by gross necropsy.
Significance relative to controls by Fisher's exact test:
[b] $p < 0.05$
[c] $p < 0.01$
[d] $p << 0.001$

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.

APPROACH 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 99.9^{th} 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 $(mg/kg\text{-}day)^{-1}$ 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 $(mg/kg\text{-}day)^{-1}$, somewhat higher than the estimate based on lung tumor incidence in female mice [110 $(mg/kg\text{-}day)^{-1}$]. 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 $(mg/kg\text{-}day)^{-1}$], 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 $(mg/kg\text{-}day)^{-1}$.

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 $(mg/kg\text{-}day)^{-1}$ 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 $(mg/kg\text{-}day)^{-1}$.

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 $(mg/kg\text{-}day)^{-1}$ (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 $(mg/kg\text{-}day)^{-1}$]. 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 $(mg/kg\text{-}day)^{-1}$ 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 $B6C3F_1$ 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.

There are several additional issues to consider. For nitropyrenes, the mouse was observed to have greater sensitivity than the rat (Wislocki *et al.,* 1986; King, 1988). In addition, 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 these reasons, 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 $(\text{mg/kg-day})^{-1}$] to that of benzo[a]pyrene used for regulatory purposes [11.7 $(\text{mg/kg-day})^{-1}$ (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_{human}) of PAHs in (mg/kg-day)$^{-1}$ Based on Newborn Mouse i.p. Injection Studies.

Compound	Study	Sex	Dose (mg/kg-day)	Tumor Site & Incidence	q_{animal}	Combined q_{animal}	q_{human} [a]
Benzo[a]pyrene	Wislocki *et al.*, 1986	M	0, 0.0341	Liver 2/28, 18/37	26.46	40.46	540
				Lung 1/28, 13/37	18.57		
		F	0, 0.0350	Lung 0/31, 13/27	28.95		410
	LaVoie *et al.*, 1987	M	0, 0.0670	Liver 1/17, 13/17	33.80	67.31	890
				Lung 0/17, 14/17	41.77		
		F	0, 0.0688	Lung 0/18, 9/14	25.48		360
Benzo[b]fluoranthene	LaVoie *et al.*, 1987	M	0, 0.0304	Liver 1/17, 8/15	41.56		550
Benzo[j]fluoranthene	LaVoie *et al.*, 1987	M	0, 0.0670	Liver 1/17, 11/21	16.96	30.70	410
				Lung 0/17, 11/21	17.75		
		F	0, 0.0688	Lung 0/18, 4/18	7.552		110
	LaVoie *et al.*, 1994	M	0, 0.00670, 0.0167, 0.0670	Lung 6/33, 8/37, 17/34, 25/25	34.48		460
		F	0, 0.00688, 0.0172, 0.0688	Lung 7/33, 6/29, 14/32, 35/38	32.49		460
Chrysene	Chang *et al.*, 1983	M	0, 0.0426	Liver 0/52, 6/27	10.82		140
	Buenig *et al.*, 1979	M	0, 0.0449	Liver 0/21, 6/24	11.76		160
	Wislocki *et al.*, 1986	M	0, 0.0386, 0.1542	Liver 2/28, 10/35, 14/34	5.225	6.94	92
				Lung 1/28, 6/35, 7/34	2.703		
Dibenzo[a,h]pyrene	Chang *et al.*, 1982	M	0, 0.00626	Liver 0/32, 11/25	147.7		2000
				Lung 7/32, 25/25	>313.5 [b]		>4200
		F	0, 0.00643	Lung 11/39, 13/14	703.9		9900
Dibenzo[a,i]pyrene	Chang *et al.*, 1982	M	0, 0.00626	Liver 0/32, 21/39	175.1	792.5	10500
				Lung 7/32, 37/39	662.8		
		F	0, 0.00643	Lung 11/39, 21/21	>269.9 [b]		>3800
5-Methylchrysene	Hecht *et al.*, 1985	M	0, 0.00144	Liver 1/48, 8/35	293.9	466.0	6200
				Lung 2/48, 7/35	246.6		

[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.).

NO SIGNIFICANT RISK LEVELS FOR BENZO[B]FLUORANTHENE, BENZO[J]-FLUORANTHENE, CHRYSENE, 5-METHYLCHRYSENE, DIBENZO[A,H]PYRENE AND DIBENZO[A,I]PYRENE

The recommended potency estimates for the oral route, in units $(mg/kg\text{-}day)^{-1}$, derived from data on tumor incidence after i.p. injection of the PAHs into mice are shown in Table 9. Daily oral intake levels (in μg) associated with lifetime cancer risks of 10^{-5} are also shown.

Table 9. Adjusted Cancer Potencies and Risk Specific Intake Levels for PAHs.

Compound	"Neonatal" i.p. Potency $[(mg/kg\text{-}day)^{-1}]$	Adjusted Potency (Oral) [a] $[(mg/kg\text{-}day)^{-1}]$	Risk Specific Intake Level (Oral) [b] (μg/day)
Benzo[b]fluoranthene	550	7.3	0.096
Benzo[j]fluoranthene	460	6.1	0.11
Chrysene	150	2.0	0.35
Dibenzo[a,h]pyrene	9900	130	0.0054
Dibenzo[a,i]pyrene	10500	140	0.005
5-Methylchrysene	6200	83	0.0084

[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.

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APPENDIX: METHODOLOGY USED TO DERIVE RISK-SPECIFIC INTAKE LEVELS FOR BENZO[B]FLUORANTHENE, BENZO[J]FLUORANTHENE, CHRYSENE, DIBENZO[A,H]PYRENE, DIBENZO[A,I]PYRENE, AND 5-METHYLCHRYSENE 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 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):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_i d^i)] \quad (1)$$

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 $(\text{mg/kg-day})^{-1}$. 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

$$P(T_e) = 1 - \exp[-(A + BD)] \tag{2}$$

with

$$D = \frac{1}{T^m \cdot \beta(m-j+1, j)} \int_0^{T_e} d(t)(T_e - t)^{m-j} t^{j-1} dt \tag{3}$$

where T_e is the time to observation, and β 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

$$D = d \cdot \left[\frac{(T_e - a)^m - (T_e - b)^m}{T^m} \right]. \tag{4}$$

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_{animal}, is given by the upper 95% confidence bound on β. 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.

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

$$p(d) = 1 - \exp[-(q_0 + q_1 d)] \tag{5}$$

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

$$0.05 \le p(d)^n \quad or \quad p(d) \ge (0.05)^{1/n} \tag{6}$$

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

$$q_1 \ge -\frac{\ln(1 - p(d)) + q_0}{d} \tag{7}$$

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

$$q_1 \ge -\frac{\ln\left(1 - (0.05)^{1/n}\right) + k}{d} \tag{8}$$

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). 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.

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_{human}) can be achieved by multiplying the animal potency (q_{animal}) by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-third power when animal potency is expressed in units $(mg/kg\text{-}day)^{-1}$:

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3} \tag{9}$$

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 (bw_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

$$I = \frac{R \times bw_h}{q_{human}} \tag{10}$$

where bw_h is the body weight, and q_{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

$$NSRL = \frac{10^{-5} \times 70\,kg}{q_{human}} \tag{11}$$

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