NO SIGNIFICANT RISK LEVELS (NSRLS) FOR THE PROPOSITION 65 CARCINOGENS BENZ[A]ANTHRACENE (ORAL) AND 7H-DIBENZO[C,G]CARBAZOLE (ORAL)

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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.

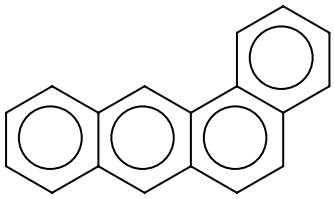
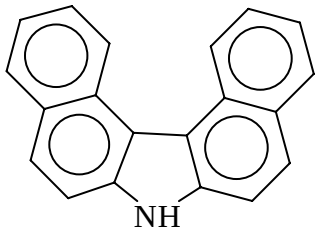
Table 1. Cancer Potencies (Oral) and NSRLs (Oral).

Chemical	Cancer Potency (Oral) $(mg/kg\text{-}day)^{-1}$	NSRL (Oral) (μg/day)
Benz[a]anthracene	21	0.033
7H-Dibenzo[c,g]carbazole	230	0.0030

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.

	Benz[a]anthracene	**7H-Dibenzo[c,g]carbazole**
Formula:	$C_{18}H_{12}$	$C_{20}H_{13}N$
CAS No.:	56-55-3	194-59-2
Mol.Wt.:	228.3	267.3
Structure:		

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]-

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]cabazole 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 $B6AF_1$/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. $B6AF_1$/J mice are an F_1 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 B6AF$_1$/J Mice Treated Orally with Benz[a]anthracene (Klein, 1963).

Experiment Number	Total Dose (mg)	Number of Treatments	Incidence [a]		Median Age at Autopsy
			Hepatomas	Pulmonary Adenomas	
I [b]	0	15	0/38	10/38	444
	22.5	15	18/39 [c]	37/39 [c]	437
IIa	0	15	2/20	7/20	600
	22.5	15	20/20 [c]	19/20 [c]	547
IIb	3	2	16/20 [c]	17/20 [c]	568

[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).

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

* Tumor incidence among mice surviving to 17 weeks.

APPROACH TO DOSE RESPONSE ANALYSIS

The studies by Klein (1963) were conducted with B6AF$_1$/J mice, an F$_1$ 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 B6AF$_1$/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 $B6AF_1/J$ 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 BAF_1 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 contant 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 4. Dose Calculations for Klein (1963) Mouse Studies of Benz[a]anthracene by the Oral Route, Using Doll-Armitage Equivalent Constant Dose.

Interval	Treatment Dose (mg) [a]	Interval Body Weight (kg) [b]	Interval Dose (mg/kg-day)	Adjusted Dose (mg/kg-day) [c]		
				Exp."I"	Exp."IIa"	Exp."IIb"
Week 1-2	1.5	0.0047	136.78 (Exp. IIb: 91.2 [d])	1.353	2.143	1.542
Week 2-3	1.5	0.0054	119.05	1.139	1.817	–
Week 3-4	1.5	0.0077	83.49	0.772	1.240	–
Week 4-5	1.5	0.0104	61.81	0.553	0.894	–
Week 5-6	1.5	0.0167	38.49	0.332	0.542	–
			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 $B6AF_1/J$ 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 q_1 for Experiment IIb was 1.56 $(mg/kg\text{-}day)^{-1}$ for hepatomas. Using this q_1*, the human potency estimate for benz[a]anthracene based on these data is 21 $(mg/kg\text{-}day)^{-1}$.

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

Experiment Number	Tumor	Weighted Dose	Tumor Incidence	Potency Estimate $(mg/kg\text{-}day)^{-1}$ [1]	
				q_1*	q_{human}
I	Hepatomas	0, 4.15	0/38, 18/39	0.216	2.9
IIa	Hepatomas	0, 6.64	2/20, 20/20	0.281[a]	3.7
IIb	Hepatomas	0, 1.54	2/20, 16/20	1.56	**21**

[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).

Strain, Sex	Animal Cancer Potency $[(mg/kg\text{-}day)^{-1}]$ [a]	Human Cancer Potency $[(mg/kg\text{-}day)^{-1}]$ [b]
CBA, male	17.6	**230**
Strong A, female	13.5	190
Strong A, male	7.87	100

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

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).

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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):

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

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 mg/kg-day, the parameters q_1 and q_1^* are given in units $(\text{mg/kg-d})^{-1}$. Details of the estimation procedure are given in Crump (1981) and Crump *et al.* (1977).

To estimate potency in animals (q_{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:

$$q_{animal} = q_1^* \times (T/T_e)^3 \quad (2)$$

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:

$$q_{animal} = q_1^* \times (104/T_e)^3 \quad (3)$$

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 suvival) 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

$$P(Te) = 1\text{-}\exp[-(A + BD)] \tag{4}$$

with

$$D = \frac{1}{T^m \cdot \beta(m - j + 1, j)} \int_0^{T_e} d(t)(T_e - t)^{m-j} t^{j-1} dt \tag{5}$$

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. 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

$$\frac{(T_e - a)^m - (T_e - b)^m}{T^3} \qquad (6)$$

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_{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.

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.*

$$p(d) = 1 - e^{-[q_0 + q_1 d]} \qquad (7)$$

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.*

$$0.05 = p(d)^n \tag{8}$$

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.*

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

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

$$q_{human} = q_{animal} \times (bw_h / bw_a)^{1/3} \tag{10}$$

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 (bw_a) 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 (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

$$I = \frac{R \times bw_h}{q_{human}} \tag{11}$$

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:

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

APPENDIX REFERENCES

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