6395 G-000-307 .26

COMPARATIVE POTENCY APPROACH FOR ESTIMATING THE CANCER RISK ASSOCIATED WITH EXPOSURE TO MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS - (USED AS A REFERENCE IN OU1, OU2 AND OU5 RI REPORTS)

04/01/88

ICF-CLEMENT
75
REPORT

6395

COMPARATIVE POTENCY APPROACH FOR ESTIMATING THE CANCER RISK ASSOCIATED WITH EXPOSURE TO MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS

INTERIM FINAL REPORT

Contract No. 68-02-4403

Prepared by

ICF-Clement Associates
9300 Lee Highway
Fairfax, Virginia 22031

April 1, 1988

COMPARATIVE POTENCY APPROACH FOR ESTIMATING THE CANCER RISK ASSOCIATED WITH EXPOSURE TO MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS

INTERIM FINAL REPORT

Clement Associates, Incorporated

Health and Environmental Science

A Subsidiary of ICF Technology, Inc.

000002

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. INTRODUCTION: A TWO-STAGE MODEL FOR CARCINOGENESIS AND ITS APPLICATION TO POLYCYCLIC AROMATIC HYDROCARBONS

II. ESTIMATION OF CANCER RISK DUE TO INGESTION OR INHALATION EXPOSURE TO BENZO[a]PYRENE USING THE TWO-STAGE MODEL

III. DEVELOPMENT OF AND BASIS FOR DERIVATION OF COMPARATIVE POTENCY ESTIMATES FOR OTHER POLYCYCLIC AROMATIC HYDROCARBONS

IV. ESTIMATION OF COMPARATIVE POTENCIES FOR POLYCYCLIC AROMATIC HYDROCARBONS USING THE LINEARIZED MULTISTAGE MODEL

V. LIMITED VALIDATION OF THE COMPARATIVE POTENCY APPROACH

VI. REFERENCES

APPENDIX A: EFFORTS TO OBTAIN A MORE EXTENSIVE DATA BASE FOR ESTIMATING BENZO[a]PYRENE CANCER RISK

APPENDIX B: PARAMETER ESTIMATES FOR MODEL COMPARISON

APPENDIX C: PAH TUMOR INCIDENCES: OBSERVED VERSUS PREDICTED

EXECUTIVE SUMMARY

This report was prepared in response to a request by the EPA Office of Health and Environmental Assessment to develop a method for assessing the cancer risk of polycyclic aromatic hydrocarbons (PAHs) for which inadequate ingestion or inhalation bioassay data exist, as well as to improve existing risk estimates using methodologies that are consistent with the experimental protocols and observations. A relative potency approach was developed as an alternative to the current practice of assuming that all carcinogenic PAHs are equivalent in potency to benzo[a]pyrene (B[a]P), which has little scientific support. B[a]P has consistently been demonstrated to be one of the most potent carcinogenic PAHs to which people might be expected to be exposed environmentally. As a result, estimates of cancer risk using a B[a]P one-to-one equivalency approach will greatly overestimate the carcinogenic potency of most mixtures of PAHs. Use of a relative potency approach that takes into account the differing potencies of carcinogenic PAHs would yield a more realistic estimate of risk, with a sounder biological basis.

In this report, a new method is developed for estimating the cancer risk associated with exposure to mixtures of PAHs that attempts to rectify the problems inherent in earlier approaches in use by EPA and others. A two-stage mathematical dose-repose model is postulated that is consistent with the biological mechanisms of action of PAHs. The model parameters are estimated using rodent tumor response data following exposure to B[a]P. The two-stage model is a special case of the Moolgavkar and Knudson (1981, 1986) cancer risk model that was adapted by Thorslund et al. (1987) to account for exposure to known levels of carcinogenic agents. The model may also be viewed as a special

000004

case of the classic Armitage and Doll (1954) multistage model in its time-independent form and a restricted case of the Armitage and Doll (1957) two-stage model in both its time-independent or -dependent forms. The advantages of the model are that it is based on a strong theoretical argument derived from biological principles yet is simple enough to obtain estimates for its parameters using very limited data. Using this model, the estimate of cancer potency for B[a]P was changed from 11.53 (EPA 1980) to 3.22 $(mg/kg/day)^{-1}$ for ingestion exposure and from 6.11 (EPA 1980) to 0.453 $(mg/kg/day)^{-1}$ for inhalation exposure.

The second critical element in the development of the method was obtaining estimates of relative potencies for carcinogenic PAHs other than B[a]P using the structural form of the model derived for B[a]P. These estimates were based on bioassay results that were obtained from systems that are not suitable for direct extrapolation to humans because the routes of exposure employed are not comparable to those by which humans are exposed in the environment (with the exception of dermal exposure). Experiments in which carcinogenic PAHs and B[a]P have been tested concurrently can be used to estimate the relative potencies of other PAHs compared with B[a]P. These relative potencies have a specific biological interpretation at low doses under the assumptions of the model: They provide an estimate of the ratio of exposure-induced mutation rates per unit of exposure. These mutations are thought to transform a normal stem cell into a preneoplastic cell and a preneoplastic cell into a malignant cell. Using 11 experimental studies, estimates of potency for carcinogenic PAHs were obtained that ranged from 0.004 to 4.50 as compared with B[a]P. The

estimates were consistent among studies for a particular PAH. Those that were obtained from the most reliable studies are summarized in the table below.

SUMMARY OF RELATIVE POTENCY ESTIMATES
DERIVED FOR PAHs

Anthanthrene	0.320[a]
Benzo[a]pyrene	1.0
Benzo[e]pyrene	0.004[a]
Benz[a]anthracene	0.145[b]
Benzo[b]fluoranthene	0.140[a]
Benzo[j]fluoranthene	0.061[c]
Benzo[k]fluoranthene	0.066[a]
Benzo[ghi]perylene	0.022[a]
Chrysene	0.0044[d]
Cyclopentadieno(cd)pyrene	0.023[c]
Dibenz[ah]anthracene	1.11[d]
Indeno[1,2,3-cd]pyrene	0.232[a]
Pyrene	0.081[e]

[a]Deutsch-Wenzel et al. (1983).
[b]Bingham and Falk (1969).
[c]Habs et al. (1980).
[d]Wynder and Hoffmann (1959).
[e]Wislocki et al. (1986).

The relative potency estimates can be used in conjunction with the B[a]P dose-response model and the dose additivity assumption to obtain estimates of cancer risk associated with any specified exposure to multiple PAHs (for which potency estimates are available). The dose additivity assumption is synonymous with what is referred to by Finney (1964) as simple similar action and advocated for use in the EPA (1986a, 1986b) guidelines for assessing the cancer risk of mixtures when inadequate data on the mixture itself are available. Simple similar action implies that all carcinogenic agents in a mixture induce carcinogenesis by similar mechanisms. Since carcinogenic PAHs appear to be metabolized to similar reactive derivatives, produce comparable adducts with

000006

DNA and histologically similar tumors at the site of introduction and metabolism in experimental animals, the assumption of a common mechanism of action (i.e., dose additivity) is plausible.

Under the assumption of dose additivity, the cancer risk caused by exposure to multiple PAHs can be obtained as follows. The total exposure units equivalent to B[a]P in a mixture to which an individual is exposed is calculated by taking the sum of the products of the relative potencies and the exposure levels for each PAH. These B[a]P-equivalent exposure units are then substituted into the dose-response model for B[a]P to obtain the cancer risk associated with exposure to the PAH mixture. This procedure is evaluated in this document using bioassay results from an experiment conducted by Schmahl et al. (1977) using two different mixtures of PAHs. The resulting predictions were encouragingly close to the tumor rates observed.

For the sake of comparison, the more familiar linearized multistage model has also been used in this report to estimate relative potencies, although it is less defensible biologically. Results reasonably comparable to those from the two-stage model were obtained, suggesting that the results are not highly model dependent.

This study has demonstrated that estimation of the total cancer risk from exposure to a mixture of PAHs should be based on measurements of the concentrations of its carcinogenic components and relative estimates of their potency.

This report is organized as follows. Section I describes the special problems associated with estimating cancer risks of complex mixtures of chemicals in the environment, why adding estimated individual risks together is appropriate for mixtures of PAHs, and why using B[a]P as a surrogate for other

PAHs is not appropriate. The assumptions required for using a two-stage model for PAH carcinogenesis are described and their experimental support presented. A broader context for the development of biologically based cancer risk models is provided and then a specific model for B[a]P is derived. In Section II, the model derived for B[a]P is applied to tumor data from an inhalation bioassay performed using hamsters (Thyssen et al. 1981) and an ingestion bioassay using mice (Neal and Rigdon 1967) and unit risk estimates are obtained. Studies in which the carcinogenicity of other PAHs was evaluated and compared with that of B[a]P are reviewed in Section III and used to establish relative potencies for the other PAHs. The results of Section III are recalculated in Section IV using the standard linearized multistage modeling approach to demonstrate how different biological and mathematical assumptions affect these results. Section V uses the biologically based model and relative potency method to estimate the cancer risk of mixtures of PAHs tested in the laboratory and compares the results with those actually observed.

This report is an interim final report because resources were inadequate to obtain the best cancer potency estimates possible for B[a]P and the best comparative potency estimates possible for the other carcinogenic PAHs. Use of individual animal data and other supplementary data sets, as well as more sophisticated statistical estimation procedures, along with evaluation of the sensitivities of the estimates to the assumptions made in order to derive them, are necessary for the completion of this work. The following list constitutes the specific tasks that remain to be performed in order to finalize this project and provide adequate support for the potency estimates derived.

1. Use individual animal exposure and pathology data from Thyssen et al. (1981) bioassay to recalculate inhalation risk for B[a]P.

000008

a. Estimate individual animal exposure levels from analytical data for B[a]P, exposure durations, and dates of initiation and termination of exposure.

b. Code individual animal exposure and pathology data and place in a computer file to enable computer manipulations.

c. Use individual animal data to derive more precise, target site-specific time-to-tumor dose-response models for inhalation exposure to B[a]P.

2. Estimate ingestion risk for B[a]P using the Thyssen et al. (1981) bioassay data by relating the portion of the inhaled dose that was swallowed following mucociliary clearance and gastrointestinal tract tumor formation.

3. Estimate and improve the precision of the relative potency estimates.

a. Develop methods for the simultaneous estimation of dose-response model parameters within a single bioassay and obtain confidence limits on the relative potencies obtained.

b. Extend the methods developed in (a) to include multiple bioassays.

c. Use the methods developed in (b) to estimate relative potencies for the PAHs used in the mixture bioassay conducted by Schmähl et al. (1977).

d. Examine the time-dependency of the PAH-induced tumor response where time-to-tumor data are available.

4. Improve confidence associated with B[a]P ingestion risk derived from Neal and Rigdon (1967) bioassay.

a. Use the dose-response model derived for the ingestion risk of B[a]P to predict tumor responses for the short-term exposure data and the data from the other Rigdon and Neal bioassays. If the predictions are consistent with what is observed, incorporate these data into the dose-response model parameter estimates.

b. Evaluate the sensitivity of the ingestion risk estimate to the assumption that one-half of the animals in the bioassay were exposed to B[a]P for an earlier exposure duration while one-half were exposed for a later duration.

c. Evaluate the sensitivity of the ingestion risk estimate to the use of surrogate background tumor rates.

d. Derive an ingestion risk estimate for B[a]P from the Triolo et al. (1977) bioassay data for the purposes of comparison to that based on Neal and Rigdon (1967).

5. Extend the model to account for additional PAHs and other biological information:

   a. Develop methods for using historical controls and other assumptions about background tumor rates in the estimation process.

   b. Develop a tri-nomial dose-response model in which the response can be expressed as no tumor, adenoma, or carcinoma (instead of the binomial relationship, with or without tumor).

   c. Develop methods of estimating relative potencies for PAHs for which no cancer bioassay data are available based on, for example, DNA adduct formation or structure-activity relationships.

   d. Refine the dose-response models for B[a]P to include information on potential tumor promotional effects and effects from the saturation of activating enzymes.

   e. Develop a method for the estimation of relative potencies when the transition rate parameters are not proportional between cell stages. Investigate the bias that is introduced when the proportionality assumption is made but is invalid.

6. Evaluate the dose-response relationships for B[a]P and other PAHs in bioassay systems other than those examined in the report for their consistency with the two-stage model. These data may include complex mixture bioassays in which B[a]P was used as a positive control, bioassays using alternative routes of exposure such as intratracheal instillation, and data on experimental PAHs such as 3-methylcholanthrene.

7. Use tumor data for several PAHs administered by different routes in the same bioassay to evaluate the route-independence of relative potency estimates.

8. Using the same techniques that were developed for the estimation of relative potencies for PAHs as compared with B[a]P, estimates of the relative potencies of cigarette smoke condensate, roofing tar emissions, and coke oven emissions, can be obtained using the SENCAR mouse tumor initiation and complete carcinogenesis studies discussed by Albert et al. (1983). Two-stage dose-response models using human epidemiological data would also be obtained for each of these complex mixtures. Estimates of the cancer risk from a single PAH would then be obtained by multiplying the following three factors together:

   a. Relative potency of the PAH as compared with B[a]P;

   b. Relative potency of B[a]P as compared with the complex mixture; and

   c. The unit cancer risk for the complex mixture.

   This approach eliminates the need to extrapolate animal bioassays directly to humans.

000010

I. INTRODUCTION: A TWO-STAGE MODEL FOR CARCINOGENESIS AND ITS APPLICATION TO POLYCYCLIC AROMATIC HYDROCARBONS

The majority of occupational and environmental chemical exposures are to complex mixtures. Emissions from combustion sources and leachates from hazardous waste sites are examples of complex mixtures that contain hundreds of components that have not been completely characterized. Estimating the potential human health hazards associated with such mixtures is difficult because of inadequate chemical and toxicological characterization, as well as the extent of heterogeneity between similar mixtures from different sources.

For complex mixtures of polycyclic aromatic hydrocarbons (PAHs), the standard approach to estimating the human cancer risk associated with exposure has been to assume that the carcinogenic PAH components (excluding heterocyclic compounds such as the nitroaromatics) are equivalent by weight to benzo[a]pyrene (B[a]P) in terms of their carcinogenic potency (EPA 1980, 1984). Based on a limited chemical characterization of the mixture, a number of PAHs are identified and their contributions to cancer risk are estimated based on their relative concentrations in the mixture, their expected contributions to human exposure, and the statistical upper-bound on the potency of B[a]P. This procedure has several serious problems.

The assumption that all the carcinogenic PAHs have potencies equivalent to that of B[a]P is not supported by the scientific literature. In studies that have been performed to evaluate the comparative potencies of PAHs, B[a]P has consistently proved to be one of the most potent carcinogens tested. These studies will be described in detail in Section III. Studies of the carcinogenic potencies of complex mixtures of PAHs as compared with B[a]P have shown that most mixtures are considerably less potent than B[a]P alone. For

example, various kinds of combustion emissions and B[a]P were tested for potency as tumor initiators on the skin of SENCAR mice (Slaga et al. 1980). Table I-1 shows that the mixtures (predominantly composed of PAHs) were much less potent as tumor initiators than B[a]P. The authors calculated relative potency estimates that ranged from 0.007 for coke oven emissions extract to less than 0.002 for diesel engine exhaust extract, using papillomas/mouse-mg as the end point. In another study, the tumorigenicity of an automobile emission condensate (AEC), a diesel emission condensate (DEC), a representative mixture of carcinogenic PAHs, and B[a]P were tested for carcinogenicity by chronic application to mouse skin (Misfeld 1980). The results are shown in Table I-2. Relative potencies were calculated by the authors to be 0.00011, 0.0053, and 0.36 for DEC, AEC, and the PAH mixture, respectively, as compared to B[a]P. Such results make the use of the assumption that all carcinogenic PAHs are as potent as B[a]P to predict the total carcinogenicity of PAH mixtures difficult to support.

1. Dose Additivity for Mixtures

Another problem with the use of B[a]P as a surrogate for the components of PAH mixtures is that it assumes that the total cancer risk of the mixture can be predicted on the basis of response additivity. The EPA guidelines for assessing the cancer risk of chemical mixtures in the absence of combined exposure data, however, advocate the assumption of dose additivity (EPA 1986b). Dose additivity is synonymous with what is referred to by Finney (1964) as simple similar action. When agents induce cancer by the same mechanism, they are characterized by simple similar action and the cancer risk due to combined exposure can be obtained by estimating the risk due to the total additive dose of the agents.

000012

TABLE I-1

RELATIVE TUMOR-INITIATING POTENCY OF VARIOUS EMISSION EXTRACTS AND BENZO[a]PYRENE

Substance[a]	Relative Potency[b] Based on (papillomas/mouse-mg)
Benzo[a]pyrene	1.0
Roofing-tar emission extract	0.004
Coke-oven emission extract	0.007
Caterpillar diesel exhaust extract	[c]0
Oldsmobile diesel exhaust extract	0.002
Nissan diesel exhaust extract	0.007
Mustang gasoline-engine exhaust extract	0.002
Cigarette-smoke condensate	[c]0

[a]Material was applied to Sencar mice once as initiator. Phobol myristate acetate (2 μg), twice a week, was used as promoter. Emission exposures that were used in the relative potency calculations were restricted to the linear portion of the dose-response curve.

[b]As calculated by authors.

[c]These entries refer to potencies that were not significantly different from zero at $p=0.05$.

Sources: Slaga et al. (1980) and NAS (1983).

TABLE I-2

CARCINOGENIC ACTIVITY OF AUTOMOBILE EMISSION CONDENSATE (AEC), DIESEL EMISSION CONDENSATE (DEC), AND PAHs ON MOUSE SKIN

Treatment	%Mice with Tumors	Relative Potency[b]
Solvent control	0	1
Benzo[a]pyrene:		
3.86 μg	32.8	
7.69 μg	60.9	
15.4 μg	89.1	
AEC:[a]		0.0053
290 μg	10.3	
880 μg	44.3	
2,630 μg	83.3	
DEC:[a]		0.00011
4,300 μg	0	
8,600 μg	2.6	
17,150 μg	12.7	
Mixture of PAHs:		0.36
3.5 μg	1.3	
10.5 μg	38.7	

[a]Obtained with leaded fuel.

[b]As calculated by authors.

Sources: Misfeld (1980) and NAS (1983).

000014

To use this method for mixtures of PAHs, estimates of the relative potencies of the components are required. The relative potency of the jth carcinogenic PAH compared to the potency of B[a]P at response level p can be defined as

$$R_j(p) = x(p)/y_j(p)$$

where $x(p)$ and $y_j(p)$ are the number of exposure units of B[a]P and the jth carcinogenic PAH, respectively, required to produce a total carcinogenic response rate of p in the test system.

If the mechanism of action of B[a]P is the same as that of the jth carcinogenic PAH, it follows that $R_j(p) = R_j$ (i.e., the relative potency is independent of the response level).

Under the hypothesis of simple similar action, the joint response to a set of carcinogenic PAHs is dependent upon the total PAH exposure, T, expressed in units equivalent to B[a]P. This exposure may be written as

$$T = \sum_{j=1}^{m} R_j y_j + x \qquad \text{(I-1)}$$

where

- m = the total number of indicator PAHs exclusive of B[a]P;
- y_j = the exposure to the jth indicator PAH;
- x = exposure to B[a]P; and
- R_j = relative potency of the jth indicator PAH compared to B[a]P.

The probability of a cancer response given T is P(T), where P(•) is the dose-response relationship for B[a]P. For this approach to be valid, the

function $P(\cdot)$ must be monotonic in the experimental range with values of x and γ_j expressed in the same units

In the case of mixtures of carcinogenic PAHs, dose additivity has been postulated to be a reasonable assumption because, as a group, PAHs have similar biological mechanisms of action: they appear to be metabolized to similar reactive derivatives that interact with DNA and produce histologically similar tumors at the site of introduction (IARC 1983). Kaden et al. (1979) found a simple additive relationship of the mutagenicity of soot and added B[a]P. Schmahl et al. (1977) tested B[a]P and two mixtures of carcinogenic and weak or noncarcinogenic PAHs when applied to the backs of mice. The tumor response is shown in Section V of this document to be predictable on the basis of additivity of doses of the carcinogenic mixture components. Assuming simple similar action (i.e., dose additivity) for the purpose of assessing the cancer risk of PAH mixtures seems plausible.

2. Biological Activity of PAHs

Choosing a dose-response model to assess the cancer risk of PAHs that is consistent with their biological mechanisms of action requires some important assumptions. The assumptions regarding aspects of PAH metabolism that influence the choice of dose-response model are as follows:

a. Carcinogenic PAHs form adducts with DNA (Sims and Grover 1974).

This step appears to be essential for the production of PAH-induced neoplasia (Gelboin 1980, Weinstein et al. 1978). Pereira et al. (1979) applied single doses of B[a]P topically to ICR/Ha mice and found a linear relationship between the applied dose and the amount of each of the two major B[a]P-DNA

000016

adducts formed in the epidermis over a dose range of 0.01 to 300 mg B[a]P per mouse. The level of adduct formed reached a maximum at 7 hours and remained constant for the next 49 hours, indicating that repair of such lesions is slow.

Adriaenssens et al. (1983) administered single doses of B[a]P orally via intubation to rats and examined the formation of DNA adducts over a dose range of 2 to 1,351 μmol/kg (0.048 to 29.7 μmol/mouse). The rate of formation of the major diol epoxide-DNA adduct was linear with respect to dose in the forestomach, as seen in Figure I-1; and, although the authors stated otherwise, linearity was observed in the lung as well. The extent of the deviation from linearity in the lung was slight and consistent with the level of error associated with this type of measurement.

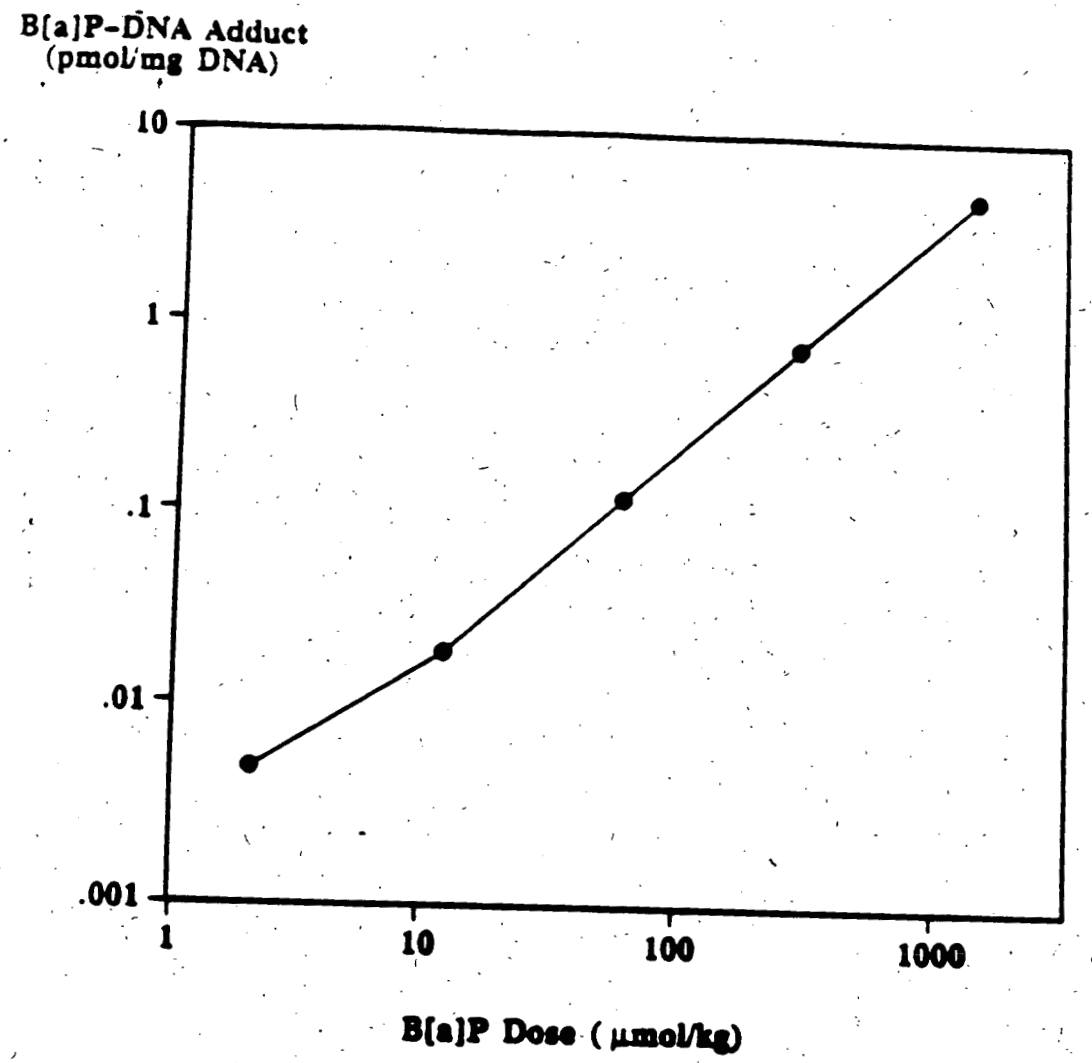
PAH metabolites have been found to bind to DNA in every tissue that has been examined, regardless of species, dose, or route of administration. The physical nature of the adducts formed, the levels at which they occur, and the extent to which they persist unrepaired appear to be similar in various tissues, whether or not those tissues are likely to develop tumors (Stowers and Anderson 1985); the formation of DNA adducts appears to be a necessary, albeit insufficient, condition for PAH-induced carcinogenesis. Since the extent to which PAH adducts are formed appears to be linearly related to administered dose, administered dose can be directly related to the actual dose to the target tissue.

b. PAH-DNA adducts can lead to mutations.

B[a]P diol epoxide is a well-established mutagen in a variety of mammalian (Newbold and Brookes 1976) and bacterial (Eisenstadt et al. 1982) systems. Marshall et al. (1984) have shown that reaction of B[a]P diol epoxide <u>in vitro</u>

FIGURE I-1

DOSE-RESPONSE RELATIONSHIP OBSERVED FOR DNA ADDUCT FORMATION IN THE RAT FORESTOMACH FOLLOWING THE ADMINISTRATION OF BENZO[a]PYRENE BY GAVAGE[a]



[a]The relationship depicted is that of the formation of the deoxyguanosine adduct with (+)-benzo[a]pyrene diol epoxide 1, considered to be the major adduct formed as well as that responsible for benzo[a]pyrene's carcinogenicity.

SOURCE: Adriaenssens et al. 1983.

000018

with DNA can lead to the activation of a transforming ras oncogene, which, when introduced into the DNA of mammalian cells, can lead to transformation. The difference between the oncogene before and after activation is that of a single base-pair substitution.

The extent of PAH-induced mutation has been found not to differ significantly at equivalent levels of DNA adduct formation for several PAHs tested in V79 cells (Wigley et al. 1979, Newbold et al. 1977). Induced mutation frequencies were shown to be linearly related to DNA adduct levels in V79 cells at sublethal doses of B[a]P (Newbold et al. 1979) as well as in Salmonella typhimurium cells (Fahl et al. 1981). The extent to which PAHs form DNA adducts thus appears to correlate well with induced mutation frequency in cells undergoing replication (NAS 1983).

Once a cell has undergone a critical mutation, it may be considered to be initiated. Berenblum and Shubik's (1947) early skin painting studies demonstrated that exposure to an initiating agent produced changes in tissue that persisted for many months following termination of exposure. A second mutational change is thought to be necessary before a partially transformed cell becomes fully malignant (Land et al. 1983). The actual mechanisms involved in these cellular events are unknown, but it is hypothesized that they involve oncogenes (Hennings et al. 1983).

c. Two mutations are required for PAHs to induce cancer.

Experimental evidence appears to support the assumption that at least two mutations are required for PAHs to induce cancer. Lee and O'Neill (1971) applied B[a]P to the backs of albino mice at intervals of 2, 3, or 4 days until the death of the animal or until sacrifice at 70 weeks. The numbers of

carcinomas of the skin were recorded and on the basis of their results the authors concluded,

> "It is certainly not possible for any integral value of θ [i.e., power of dose] but 2 to be consistent with the data so that if the multistage model of Armitage and Doll [1954] were true then benzopyrene affects two stages of the cancer process."

Chu et al. (1987) used a multievent mathematical model to model several assumptions about the nature of skin tumor initiation and promotion. Different assumptions produced different predictions of tumor incidences expected in initiation/promotion and initiation/promotion/initiation bioassays. Analysis of existing tumor data showed them to be in agreement with a two-stage model proposed by Hennings and Yuspa (1985), supporting a "sequential tumor formation hypothesis" and the view that initiated cells become papillomas, some of which are autonomous (as opposed to promoter-dependent), and can become carcinomas (Colburn 1980).

Further support for the two-stage model is provided by the experiments of Nettesheim et al. (1987), who exposed rat tracheal epithelial cells to an alkylating agent in vitro and in vivo and were able to detect a subpopulation of cells that manifested aberrant growth control, lost their responsiveness to negative growth regulators, and ultimately expressed an inappropriate growth factor receptor gene in the neoplastic transformants.

A dose-response model for carcinogenesis that incorporates cell proliferation parameters and that assumes cancer is the result of two discrete, specific, heritable cellular events is consistent with epidemiological observations. When tissue growth kinetics are taken into account, two stages are sufficient to generate the whole spectrum of cancer incidence curves observed in human populations (Moolgavkar and Knudson 1981). In fact, while

000020

initiation-promotion experiments in animals and data on human tumors that occur in both inherited and spontaneous forms suggest that at least two stages are involved in carcinogenesis. It is difficult to give a biological interpretation of more than two events in the process (Moolgavkar and Venzon 1979).

Hereditary cancers such as neuroblastoma and Wilms' tumor segregate in an autosomal dominant fashion. Although every cell in the susceptible tissue carries the cancer gene, only a few cells proceed to malignancy, indicating that at least one other event is necessary for malignant transformation. A hypothesis that is consistent with the dominant inheritance of some cancers is that the first event, probably a mutation, has occurred in a germ cell; a second event gives rise to malignant transformation (Moolgavkar and Knudson 1981). For example, two mutations at a homologous site would confer homozygosity at a gene locus. Hereditary cancers are no different pathogenetically from those that arise sporadically and offer an opportunity to study cancer in general.

The two-stage interpretation of the rate of change of age-specific background cancer rates over time is also a reasonable hypothesis. Rather than interpreting the rate of change as related to the number of stages required for carcinogenesis, as is assumed by the multistage model (with the number of stages dependent upon cell type), it can be viewed as the growth rate of preneoplastic cells. This interpretation does not require that a different mechanism of action for malignant transformation be hypothesized for each cell type, as does the multistage model, and is consistent with the observation of low tumor rates in tissues that have low growth rates, such as nerve cells.

Two molecular stages of carcinogenesis should not be confused with morphological stages of tumor development, although some correspondence may

exist. More than two stages may actually be required for PAH-induced carcinogenesis, although nothing more complicated is needed to explain experimental observations. On the basis of parsimony of parameters, a two-stage model will therefore be used.

The evidence discussed above thus supports the assumption that in the case of carcinogenic PAHs, the papillomas that appear after dermal application using an initiation-promotion protocol may be considered to be nonmalignant and to require further mutation to become malignant.

In the next section, these assumptions are used to support the use of a simple two-stage model of PAH-induced carcinogenesis, in which the rate of transition between stages (mutation) is linearly proportional to the administered dose of the PAH; the ratios of the exposure-dependent mutation rates to the background rate are the same for both stages (which is reasonable if the mutations arise from the same type of adduct).

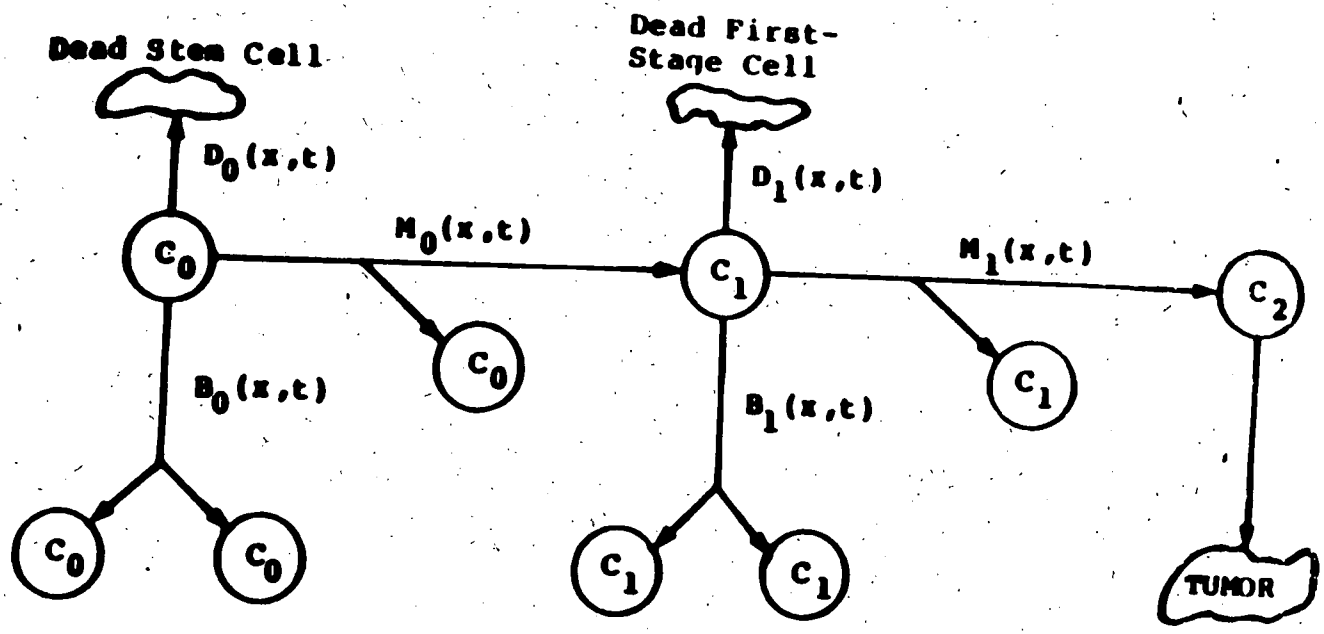
3. Two-Stage Model for Carcinogenesis

A two-stage model of carcinogenesis has been proposed by Moolgavkar, Venzon and Knudson (1979, 1981). This model is depicted in Figure I-2. According to the model, the population of cells at risk is proliferating cells, often referred to as stem cells. Stem cells are those from which most other cells in an organ arise. Once a stem cell has differentiated and left the pool of proliferating cells it is no longer susceptible to heritable alterations of its DNA. A normal, susceptible stem cell may do one of several things. It may divide into two daughter stem cells, terminally differentiate, die, or undergo mutation at a critical site that results in formation of a preneoplastic or intermediate cell. A preneoplastic cell has undergone one of the changes

000022

FIGURE I-2

GENERAL BIOLOGICAL STRUCTURE UNDERLYING EXPOSURE- AND TIME-DEPENDENT M-V-K MODEL



KEY:

C_0 is a normal, susceptible stem cell.
C_1 is a preneoplastic, first-stage cell, which can proliferate into a premalignant clone.
C_2 is a cancerous cell that will eventually develop into a detectable tumor.
$D_0(x,t)$, $B_0(x,t)$, and $M_0(x,t)$ are the exposure- and time-dependent death, birth, and transition or mutation rates for the normal stem cell.
$D_1(x,t)$, $B_1(x,t)$, and $M_1(x,t)$ are the exposure- and time-dependent death, birth, and transition or mutation rates for the first-stage cell.
x is the exposure level, which is assumed to be constant over time.
t is the age of the subject.

000023

6395

necessary to become a cancer cell but is not yet cancerous. The preneoplastic cell may, in turn, divide into two daughter preneoplastic cells, differentiate, die, or undergo further mutation at another critical site to produce a cancerous cell. The cancerous cell will, after a sufficient length of time, divide into enough cells that it becomes a detectable tumor. All of these processes can be described mathematically by postulating specific exposure-dependent rates for the cell changes. Moolgavkar and Knudson (1981) showed that to a close approximation, the age-specific tumor rate at time t for their two-stage model may be expressed as:

$$I(t) = M_0 M_1 \int_0^t C_0(v) \; [\exp (B-D)(t-v)] \; dv \qquad \text{(I-2)}$$

where

- $I(t)$ = age-specific cancer incidence at age t;
- M_0 = transition rate from stem to preneoplastic cell;
- M_1 = transition rate from preneoplastic to cancerous cell;
- $C_0(v)$ = number of susceptible stem cells per individual target organ at age v;
- B = birth rate or rate of cell proliferation of preneoplastic cells; and
- D = death rate of preneoplastic cells.

Essentially, the equation describes the progression from a normal stem cell to a cancerous cell under the assumptions of the two-stage model. This model is a combination of deterministic and stochastic components. The numbers of preneoplastic and fully malignant cells at any time are assumed to be random variables that are dependent upon these event rates, while the number of normal cells at risk of transformation at time v, denoted by $C_0(v)$, is assumed to be deterministic and known.

000024

Using this carcinogenesis model, Moolgavkar and Knudson (1981) successfully described or explained such phenomena as

- genetic predisposition to cancer,
- patterns in childhood cancer rates;
- hormonally influenced changes in breast cancer rates;
- the results of initiation-promotion experiments for multiple agents;
- changes in respiratory cancer rates associated with variable smoking patterns; and
- the fact that for many human carcinomas, the age-specific incidence rates increase roughly with the fourth to the seventh power of age.

The Moolgavkar-Knudson model is thus more general in applicability than the Armitage-Doll model, which was developed specifically for carcinomas in epithelial cells and predicts an age-specific incidence approximately proportional to t^{k-1}, where k is the assumed number of stages.

The biological processes described by equation (I-2) can be affected by the level of exposure to carcinogenic agents, and are more likely to occur as the length of exposure increases; as a result, they are exposure and time dependent. Thorslund et al. (1987) have described an exposure- and time-dependent version of the model. In the context of biological mechanisms of carcinogenesis, the model can be used to predict the risk of agents that exert their effects in a number of different ways. Mutation-inducing initiating agents would affect the transition rates between cell stages (M_0 or M_1), while promoting agents may increase the proliferation rate of preneoplastic cells (increasing B without affecting D). Cocarcinogens may increase the proliferation rate of normal stem cells (C_0), thereby increasing the size of the target for initiating agents. Inhibitors could remove cells

from the populations of susceptible stem or preneoplastic cells through toxicity (e.g., increasing D without affecting B) or by inducing differentiation. Within the context of the model, the following observations can be explained:

- tumor rates at very high experimental doses are often lower than at lower doses, even after adjusting for differential mortality, as a result of high-dose toxicity to stem and preneoplastic cells;
- exposure to multiple carcinogens can give antagonistic, additive, multiplicative, or super-multiplicative tumor rate responses by affecting different model parameters;
- such a response to combined agents appears to be dose-dependent because some responses such as increased stem cell proliferation may both occur and have an impact on tumor rates only at high doses; and
- some preneoplastic, benign cell masses that may be precursors of tumors will regress and disappear upon cessation of exposure as a result of a decline in the birth rate and no change in the death rate of such cells.

The multistage model provides no satisfactory explanation for any of these observations.

4. Restricted Dose-Response Model for B[a]P

Based on the evidence presented earlier, the two-stage model is a useful approach for estimating the cancer risk associated with exposure to B[a]P. Without intermediary experimental information about cell stages and differences in exposure over time, however, it is not possible to estimate the individual background and exposure-induced mutation rates for preneoplastic and transformed cells using tumor rate data from a standard animal carcinogenesis bioassay, nor can the relative transition rates that correspond to each stage be identified. What can be estimated from bioassay data are two exposure-induced relative transition rates and a background transition rate.

000026

In the absence of information to the contrary, the assumption can be made that the transition rates from normal to preneoplastic and preneoplastic to cancerous cells are equally likely, which could occur if the mutations that caused the transformations resulted from the same type of DNA adduct. If these transition rates are linear functions of dose (which is likely at low doses), they may be expressed as $M_0 = \alpha_0 + \beta_0 x$ and $M_1 = \alpha_1 + \beta_1 x$ where α is the background transition rate and β is the PAH-induced transition rate per unit of exposure for each stage. It is reasonable to assume that the factors influencing the background transition rates and the PAH-induced transition rates have the same relative effectiveness for each stage. Under this assumption, $\beta_0/\alpha_0 = \beta_1/\alpha_1 = S$, the relative transition rate, so that $M_0M_1 = \alpha_0\alpha_1(1+Sx)^2 = M(1+Sx)^2$, where $M = \alpha_0\alpha_1$.

Promotional effects result from multiple molecular interactions. As a result, the growth rate of preneoplastic cells G at low doses is virtually independent of exposure level so that $G = B - D$. If this assumption is violated at higher doses, the shape of the curve will have greater curvature than quadratic, and the quadratic model will be rejected. Finally, as a first approximation it is assumed that the number of stem cells post-maturity, $C_0(v)$, is relatively constant and may be taken to be unity (i.e., $C_0(v) = C_0 = 1$).

Substituting these expressions [G, M, S, $C_0(v)$] into equation (I-2) and integrating yields a cumulative hazard function $H(x,t)$, so that the probability that a tumor will develop by time t as a result of exposure to a level of genotoxic agent x can be expressed as:

$$P(x,t) = 1 - \exp[-H(x,t)] = 1 - \exp[-M(1+Sx)^2][\exp(Gt) - 1 - Gt]/G^2, \quad \text{(I-3)}$$

where

M = background tumor rate parameter;

S = fractional increase in the transition rate between cell stages per unit dose, assumed to be the same for each stage;

G = B − D and is the exposure-independent growth rate of preneoplastic cells; and

t = the time (or age) at which risk is evaluated.

The level of x at the target tissue is assumed to be directly related to the administered dose, based on observations of experiments discussed earlier in which the rate of formation of the major B[a]P diol epoxide-DNA adduct was found to be linearly related with respect to dose in the forestomach, lung, and skin (Pereira et al. 1979, Adriaenssens et al. 1983).

The specific dose-response model derived for B[a]P [equation (I-3)] is a restricted form of the model developed by Armitage and Doll (1957), Moolgavkar and Knudson (1981), and Thorslund et al. (1987). It is restricted by assuming that G is independent of x and that the two transition rates are linear functions of x with proportional coefficients. The consequence of these assumptions is that at constant t the dose-response function has only two parameters:

$$P(x) = 1 - \exp\text{-}A(1 + Sx)^2 \qquad \text{(I-4)}$$

where $A = M\,[\exp(Gt)-1-Gt]/G^2$ and t = age at last observation.

This model has a number of advantages. Among the most important are the following:

- At low doses, the model converges to a linear, no-threshold form with slope parameter equal to 2AS.
- The model can be fitted to data from an experiment in which only one exposure group exhibited a positive response.
- The model has only two parameters that have to be estimated, so that X^2 goodness-of-fit tests can be run on the data from the standard bioassay with one control and two exposure groups.
- A stable point estimate of risk can be obtained directly.

000028

- The mathematical form of the model follows directly from what is currently the most widely accepted hypothesis of the mechanisms of cancer induction.
- The model is consistent with the mechanisms of tumorigenesis for B[a]P and other PAHs.

It should be noted that equation (I-4) is also a restricted form of the Armitage-Doll (1954) model, with their two stages affected proportionally by the carcinogen and differing only in their dependance on t. It is not possible to distinguish between the Armitage-Doll (1954) model and the two-stage model discussed here on the basis of the change over time, unless an integer power of t is inconsistent with the data. In such a case, the Armitage-Doll (1954) model would be rejected.

The assumption that the parameters A and S in equation (I-4) are greater than zero avoids many of the disadvantages of the linearized multistage model and as a result, equation (I-3) or (I-4) can be used to derive stable point estimates of low-dose risk. Such estimates are derived for B[a]P in Section II and for other PAHs in Section III.

5. Estimation of the Total Cancer Response to Combined PAH Exposure

In this section, a method for obtaining relative carcinogenic potency estimates for PAHs is derived. This method is dependent upon the structural form of the dose-response model developed for B[a]P. Derivation of relative potency estimates requires that point estimates of risk be obtained because upper bounds on risk cannot be compared. For these reasons, point estimates of risk were derived by fitting equation (I-4) to dose-response data for various PAHs (Section III). The point estimates were compared to the corresponding

estimates for B[a]P to obtain relative potency estimates. These relative potency estimates serve as the parameters Rj in equation (I-1).

As discussed earlier, it is reasonable to assume that the same functional relationship exists for PAHs in all animal test models. The parameter estimates would undoubtedly be species- and tissue-dependent, but the underlying structural relationships would very likely be the same.

Using the low-dose linear two-stage proportional transition rate model developed for B[a]P, the dose-response relationship for a specified test system can be expressed as

$$P(x) = 1-\exp[-A(1+Sx)^2]$$

for B[a]P and

$$P(y_j) = 1-\exp-A(1 + SR_j y_j)^2 \quad j = 1,2, \ldots, m$$

for the jth carcinogenic PAH, where x is the B[a]P exposure level and y_j is the exposure level for the jth PAH (in the same units).

It is possible to obtain 100% efficient estimates of the R_j values by joint maximum likelihood estimation using data from bioassays of individual PAHs conducted simultaneously; however, such estimation procedures are time-consuming to develop and lie beyond the scope of the present project. Fortunately, a simple approximation for the estimates of the R_j values can be obtained. To do so, the value A is estimated from the control data as previously discussed. It can be shown that the final potency estimates are not very sensitive to this estimate. Given this estimated value of A, the term SR_j can be estimated by an approximate goodness-of-fit procedure for each PAH for which data are available. The parameter S is estimated in a comparable manner using B[a]P dose-response data from the same bioassay. The final relative potency estimates R_j, which according to our general theoretical approach are

000030

independent of animal test system used, are obtained from the ratio of the estimates for SR_j and S, determined from the jth PAH and B[a]P, respectively.

For many test systems, the highest dose may be the least relevant for determining potency due to acute toxicity, activation of cell proliferation mechanisms, and/or saturation of metabolism or local penetration. When the highest dose yields a level that is inconsistent with the assumed dose-response model, it will not be used in estimating the parameters. In other situations, only a single dose level for B[a]P may be available. In this case, if B[a]P gives a high response, a more stable estimate may be obtained by simply using the PAH exposure level that gives the closest response rate to that of B[a]P since such a procedure is independent of any assumed parametric dose-response relationship. Even when a control value and a single response for B[a]P and the jth carcinogenic PAH are the only values available, it is possible to obtain an estimate of the relative potency. To demonstrate how such estimates can be obtained, consider the following limited response data:

OBSERVED RATES

Agent	Exposure	Response
Control	0	r_o/n_o
B[a]P	x	r/n
jth PAH	y_j	r_j/n_j

Estimates for the relative potencies can be obtained by equating the observed rate with the function response. This results in the equations

$$r_o/n_o = 1-\exp-A$$

$$r/n = 1-\exp-A(1+Sx)^2$$

$$r_j/n_j = 1-\exp-A(1+SR_jy_j)^2 \quad j = 1, \ldots, m$$

which can be solved algebraically by hierarchical substitution of the parameter estimates for A and S into the equation defining the relative potencies. This approach yields the algebraic solution:

$$Rj = \frac{x}{Y_j} \frac{[\ln(1-rj/nj)/\ln(1-r_o/n^o)]^{1/2} - 1}{[\ln(1-r/n)/\ln(l_o/n_o)]^{1/2} - 1} \qquad j = 1,2\ldots,m.$$

In the next section, cancer risk estimates are derived for ingestion and inhalation exposure to B[a]P. In Section III, the relative potency values (R_j) are estimated for selected PAHs that are likely to be encountered in the environment.

000032

II. ESTIMATION OF CANCER RISK DUE TO INGESTION OR INHALATION EXPOSURE TO BENZO[a]PYRENE USING THE TWO-STAGE MODEL

In this chapter, unit cancer risks for B[a]P are obtained by applying the dose-response model for PAHs derived in the previous chapter to tumor bioassay data following ingestion or inhalation exposure.

1. Dose-Response Relationship for Oral Exposure to Benzo[a]pyrene

Two studies were identified in which B[a]P was administered orally to animals. It may also be possible to estimate ingestion risk on the basis of the dose of B[a]P swallowed as a result of mucociliary clearance following inhalation exposure.

1.1 Triolo et al. (1977)

As part of a study to investigate the tumorigenicity of B[a]P and the inducibility of aryl hydrocarbon hydroxylase in target and nontarget tissues in the mouse, Triolo et al. (1977) administered diets containing 0, 0.2, or 0.3 mg B[a]P per gram food to groups of nine female Ha/ICR mice for 12 weeks. Animals were 9 weeks of age at the start of treatment. After 12 weeks of treatment, animals were sacrificed and forestomachs, livers, and lungs were removed for gross macroscopic observation. Papillomas were observed in the forestomach; other tissues were negative. Table II-1 shows the tumor dose-response relationship observed in the forestomach.

The Triolo et al. (1977) bioassay is considered unreliable as a basis for the derivation of a unit cancer risk for ingestion of B[a]P due to the small number of animals used and its brief duration. Deriving a unit risk would be

TABLE II-1

DOSE-RESPONSE RELATIONSHIP BETWEEN DIETARY B[A]P ADMINISTRATION AND FORESTOMACH TUMOR FORMATION IN MICE

Dose (mg/g food)	Effective No. of Mice	Mice with Tumors (%)	No. of Tumors per Mouse
0	9	0	0
0.2	9	67	1.8
0.3	9	100	4.0

of interest for the purposes of comparison, however, and should be performed when resources permit.

1.2 Neal and Rigdon (1967)

The carcinogenicity of B[a]P via ingestion has also been investigated in a series of experiments conducted by Neal and Ridgon (1967) and Rigdon and Neal (1966, 1969). The most pronounced dose-response relationship obtained under reasonably consistent conditions was observed in the Neal and Rigdon (1967) study. The results of this experiment are reproducted in Table II-2. The end point in this study was either a papilloma or a squamous cell carcinoma in the forestomach (squamous portion of the stomach), although squamous cell carcinomas were far more common.

Derivation of Model

As Table II-2 indicates, Neal and Rigdon (1967) employed an unusual protocol in which the times of initial and final exposures and thus the duration of exposure varied. The cancer dose-response model derived in Section I was modified to account for these variables.

000034

TABLE II-2

FORESTOMACH TUMORS IN MICE FED BENZO[a]PYRENE

Age First Exposed (days)	Milligrams of B[a]P per gram of food	Number of days fed B[a]P	Age killed (in days)	Number with Forestomach Tumors / Number of Mice
--	0.0	--	300	0/289
30	0.001	110	140	0/25
30	0.01	110	140	0/24
116	0.02	110	226	1/23
33-67	0.03	110	143-177	0/37
33-101	0.04	110	143-211	1/40
31-71	0.045	110	143-183	4/40
17-22	0.05	107-197	124-219	24/34
20-24	0.10	98-122	118-146	19/23
18-20	0.25	70-165	88-185	66/73
49	0.25	1	155	0/10
56	0.25	2	162	1/9
49	0.25	4	155	1/10
62	0.25	5	168	4/9
49	0.25	7	155	3/10
91	0.25	30	198	26/26
74	0.10	7	182	0/10
48	0.10	30	156	12/18
98-180	5.0	1	209-294	17/33

SOURCE: Adapted from Neal and Rigdon (1967).

As noted by Thorslund et al. (1987), the age-specific cancer rate (i.e., hazard rate) associated with constant exposure to a carcinogen at level x may be expressed in its general form as

$$h(x,t) = M_1(x,t) \left\{ \int_0^t C_0(x,u)\, M_0(x,u) \right\} \left\{ \exp \int_u^t [B_1(x,y) - D_1(x,y)]\,dy\,du \right\} \qquad \text{(II-1)}.$$

For exposure to variable levels of a genotoxic agent, a time- and exposure-dependent model can be obtained by making the following assumptions:

- In an adult, the number of susceptible stem cells is relatively constant so that $C_0(x,u) = C_0$, which has a constant value that may be taken to be unity [i.e., $C_0 = 1$] without changing the shape of the resulting dose-response relationship.
- The birth and death rates of first stage (preneoplastic) cells are constant (i.e., exposure- and age-independent) so that the net growth rate of a first stage cell clone is $G = B_1(x,y) - D_1(x,y)$.
- The cell transformation rates are linear functions of exposure at an instant in time and the extent of background and exposure-induced transformations are both proportional between stages, i.e.,

$$M_0[x(t),t] = \gamma^{-1} M_1[x(t),t] = \alpha + \beta x(t)$$

where x(t) is the exposure level at time or age t.

Using these approximations and assumptions, equation (II-1) has the exposure- and time-dependent form:

$$h[x(t),t] = \gamma\,[\alpha + \beta x(t)]\; C_0 \int_0^t [\alpha + \beta x(u)] \exp\,[G(t-u)]\,du \qquad \text{(II-2)}.$$

It is possible to evaluate equation (II-2) using a variety of expressions for the time-dependent exposure rate x(t). For the situation found in Neal and Rigdon (1967), x(t) may be viewed as a single constant exposure that is at level x over the interval t_s to t and zero elsewhere. This relationship may be expressed more formally as:

$$x(t) = \begin{cases} 0 & 0 \le t < t_s \\ x & t_s \le t \end{cases} \qquad \text{(II-3)}.$$

000036

Substituting equation (II-3) into equation (II-2) and integrating yields an explicit equation for the hazard rate of the form:

$$h[x(t),t] = \begin{cases} \dfrac{\gamma\alpha^2}{G}[\exp Gt-1] & 0\le t<t_s \\ \dfrac{\gamma\alpha^2}{G}[\exp Gt-1] + \dfrac{\gamma\alpha\beta x}{G}[\exp Gt-1+\exp G(t-t_s)-1] & \\ + \dfrac{\gamma(\beta x)^2}{G}[\exp G(t-t_s)-1] & t_s\le t \end{cases} \qquad \text{(II-4).}$$

The cumulative hazard can be defined as the sum of the hazards from time zero until t or, in mathematical notation,

$$H[x(t),t] = \int_0^t h[x(u),u]du \qquad \text{(II-5).}$$

Substituting equation (II-4) into equation (II-5) and integrating gives an expression for the cumulative hazard of the form:

$$H[x(t),t] = \begin{cases} \dfrac{\gamma\alpha^2}{G^2}[\exp Gt-1-Gt] & t<t_s \\ \dfrac{\gamma\alpha^2}{G^2}[\exp Gt-1-Gt] & \\ + \dfrac{\gamma\alpha\beta x}{G^2}[\exp Gt-\exp Gt_s-G(t-t_s)+\exp G(t-t_s)-1-G(t-t_s)] & \\ + \dfrac{\gamma(\beta x)^2}{G^2}[\exp G(t-t_s)-1-G(t-t_s)] & t_s<t \end{cases} \qquad \text{(II-6).}$$

When exposure is continuous (i.e., $t_s=0$), the model reduces to the less complex form:

$$H[x,t] = \frac{\gamma(\alpha+\beta x)^2}{G^2}\,[\exp Gt-1-Gt] \qquad \text{(II-7)}$$

Since the parameters in equation (II-7) are confounded, it is not possible to estimate all of them individually; however, the model can be reparameterized by defining the following estimable terms: A and S as $\gamma\alpha^2$ and β/α, respectively. The terms in equations (II-6) and (II-7) can then be replaced by the relationships $\gamma\alpha\beta = AS$, $\gamma\beta^2 = AS^2$, and $\gamma(\alpha+\beta x)^2 = A(1+Sx)^2$, which permits the rewriting of equations (II-6) and (II-7) as

$$H'[x(t),t] = \begin{cases} \dfrac{A}{G^2}\,[\exp Gt-1-Gt] & t<t_s \\[2ex] \dfrac{A}{G^2}\,[\exp Gt-1-Gt] \\ \quad + \dfrac{ASx}{G^2}\,[\exp Gt-\exp Gt_s-G(t-t_s)+\exp G(t-t_s)-1-G(t-t_s)] \\ \quad + \dfrac{AS^2x^2}{G^2}\,[\exp G(t-t_s)-1-G(t-t_s)] & t_s<t \end{cases} \qquad \text{(II-8)}$$

and $H(x,t) = \dfrac{A(1+Sx)^2}{G^2}\,[\exp Gt-1-Gt]$, respectively (II-9).

When adjustments for differences in survival times are not required, an additional simplification can be made, yielding the two-parameter form:

$$H(x) = A(1+Sx)^2 \qquad \text{(II-10)}.$$

000038

The cancer risk in the absence of competing mortality by time t is, by definition,

$$P[x(t),t] = 1-\exp-H[x(t),t] \qquad \text{(II-11)}.$$

Application of Model to Data

The Neal and Rigdon (1967) ingestion bioassay of B[a]P had a number of other peculiarities associated with it that make it difficult to fit any dose-response model to the tumor incidence data. Among the more important were:

- A higher response rate was obtained at an exposure level of 0.02 ppm than at 0.03 or 0.04 ppm.
- A very sharp increase in the response rate occurred at levels of 0.045 to 0.05 ppm.
- The higher exposure groups have a response rate that appears to reach a plateau.
- The higher exposure groups were composed of animals that received different durations of exposure, which are unknown except for the extremes.

Previous attempts to use an extensive part of these data to define a dose-response relationship for B[a]P have been unsuccessful. For example, in the EPA water quality documents for B[a]P, all exposure levels greater than 0.045 ppm were discarded since the data did not fit the standard Global multistage modeling procedures.

Due to Neal and Rigdon's (1967) unusual exposure protocol, in order to describe the dose-response relationship for the ingestion of B[a]P and tumor incidence for each exposure group, estimates for each of the exposure variables must be made. Since the age at initial exposure, duration of exposure, and age at sacrifice varied within several of the exposure groups, the cancer risks due to B[a]P exposure for each animal varied within the groups. As a first

approximation for these exposure variables, each mouse was assumed to be exposed to one of two exposure patterns:

(1) those that were youngest at first exposure and were sacrificed at the earliest age (e.g., for the group exposed to 0.04 mg B[a]P in Table II-2, were first exposed at age 33 days and last exposed at age 143 days), and

(2) those that were oldest at first exposure and were sacrificed at the oldest age (e g., for the 0.04 mg/group, were first exposed at 67 days and last exposed at 177 days).

From the data shown in Table II-3, it can be logically inferred that at least one mouse was actually exposed to each of the two hypothesized exposure patterns. In the absence of information to the contrary, one-half of the mice in each exposure group are assumed to have been exposed to each exposure pattern. Using this assumption, the cancer risk for any randomly selected mouse in an exposure group is equal to one-half the sum of the risks for animals receiving each of the exposure patterns. This relationship may be expressed as:

$$P(x) = 0.5\ [1-\exp-H(x,t_{s1},t_1)] + 0.5\ [1-\exp-H(x,t_{s2},t_2)] \qquad \text{(II-12)}$$

where the numerical subscripts indicate one of two exposure scenarios and the hazard function H(x) was defined in equation (II-6).

Using the maximum likelihood method, the data in Table II-2, and the assumption that the tumors are incidental, the parameters in the hypothesized risk equation can be estimated as outlined in Gart et al. (1986, pg. 125, section iii).

The resulting best-fitting model has the parameters estimates $\gamma\alpha^2 \to 0$, $G = 0.194$, and $\gamma\beta^2 = 0.2142$, which results in a cumulative hazard function of the form:

000040

TABLE II-3

VARIABLE EXPOSURE DATA USED TO ESTIMATE PARAMETERS IN THE INGESTION DOSE-RESPONSE MODEL FOR B[a]P

Dose (mg/g food) x	Age first exposed (days) t_s	Age sacrificed (last exposed) (days) t	Number of animals exposed n	Number of fore-stomach tumors	
				Observed	Expected[a]
0.000	--	300	289	0	0.000
0.001	30	140	25	0	0.002
0.01	30	140	24	0	0.232
0.02	116	226	23	1	0.875
0.03	33 67	143 177	37	0	3.091
0.04	33 101	143 211	40	1	5.746
0.045	31 71	141 181	40	4	7.560
0.05	17 22	124 219	34	24	19.692
0.10	20 24	118 146	23	19	14.220
0.25	18 20	88 185	73	66	64.259

[a]It was assumed that one-half the animals in each group were exposed during each of the intervals reported, t_s to t.

$$H(x,t_s;t) = 5.695\ x^2\ \{\exp[0.194(t-t_s)] - 1 - 0.194(t-t_s)\} \qquad \text{(II-13)}$$

Despite the unusual characteristics of the data and experimental protocol, this mathematical model gives a statistically adequate fit (i.e., the resulting χ^2 has a p value greater than 0.01, which is the criterion used for the multistage model by EPA). This fit is shown in Table II-3 and has a χ^2=17.1155 with eight degrees of freedom and a p=0.029.

The resulting model is quadratic and will be assumed to be valid over the longest exposure duration employed for a group of animals, that is, $t-t_s$=(219-22)/7=28.143 weeks. However, the parameter value for G, 0.194, is incompatible with increases in age-specific cancer death rates that have been observed since it implies that the rates are increasing at about the 15th to 16th powers of age (since h(t)=exp Gt-1 ~ $\alpha\ t^{15.5}$). These rates are greater than the highest known, which is that for human prostate cancer (k=13). Values for k are usually between 3 and 5. Thus to extrapolate the partial lifetime exposure durations to a full lifetime (from 28.143 weeks to an assumed lifespan of 104 weeks), the standard EPA time correction factor of t^3 will be employed. This time correction factor is controversial, although since it is consistent with EPA's standard procedures, it will be used here. This correction is made by obtaining a coefficient for the quadratic term in the hazard function and extrapolating it to 104 weeks, assuming exposure was constant from t_s=0 to t=28.143 weeks. The hazard at 28.1 weeks was

$$H(x,0,28.143) = 1,307.44\ x^2.$$

Extrapolating to 104 weeks can be achieved by multiplying the hazard at 28.1 weeks by the factor $(104/28.143)^3$=50.46, thereby obtaining a quadratic term of (1,307.44)(50.46)=65,979.74 x^2 for a full lifetime exposure to B[a]P.

000042

Although this is the best-fitting dose-response model mathematically, taking $\alpha=0$ implies that the background tumor rate is zero and that a linear term does not exist in the model. In reality, a background tumor rate must exist and as a result, so must a linear term. The model can be modified by obtaining the largest possible α that is not statistically different from the best-fitting model at the 95% level. This modification results in the following parameter estimates:

$\gamma\alpha^2 = 3.683\times10^{-8}$,

$\gamma\beta^2 = 0.2739$, and

$G = 0.1589$.

Substituting these values into the cumulative hazard function in which exposure is considered to be continuous from 0 to 28.143 weeks results in the numerical expression:

$$H(x,0,28.143) = 1.19638\times10^{-4} + 0.652489x + 889.64x^2.$$

To extrapolate to a full lifetime, the same correction factor used earlier of $(104/28.14)^3=50.46$ can be multiplied by the above expression to obtain the relationship:

$$H(x,0,104) = 6.037\times10^{-3} + 32.9246x + 44,891.40x^2.$$

Conversion of exposure units to mg/kg/day can be achieved by multiplying each term in the polynomial by the factor 0.00769^k (i.e., multiply by 10^{-3} to convert from g to kg food and divide by 0.13, the fraction of a mouse's body weight that it consumes in food per day), where k is the degree of the term in the polynomial. Making this conversion results in a cumulative hazard function for mice in terms of mg/kg/day exposure of the numerical form:

$$H(x,0,104) = 6.037\times10^{-3} + 0.2533x + 2.6563x^2.$$

A final adjustment for differences in surface area between species is required to obtain an expression for human exposure in terms of mg/kg/day and in order to be consistent with current risk assessment methodology. The low-dose linear term for humans is thus:

$$H(x,0,70) = 0.2533\ \sqrt[3]{70/0.034}\ x = 3.22x\ [\text{mg/kg/day}]^{-1}.$$

This term is a function of exposure level x and includes the unit risk estimate for B[a]P ingestion generated from this data set.

An independent check of the model could be obtained when resources permit by using the data from the short-term exposure groups (see Table II-2). If the responses predicted using the model were consistent with those observed for these groups, they could be incorporated into the estimates of model parameters.

1.3 Thyssen et al. (1981)

It may be possible to obtain an ingestion risk for B[a]P based on the inhalation bioassay conducted by Thyssen et al. (1981) that is described in detail as the basis for the inhalation unit risk for B[a]P in Section II.2. Doing so would require estimating the dose of B[a]P received by the digestive tract of the exposed hamsters. Several of the hamsters developed exposure-related neoplasms in the larynx, esophagus, and forestomach. These tumors presumably developed as a result of exposure to B[a]P that was swallowed following mucociliary clearance. By making a number of assumptions, several observations in the literature can be combined to obtain estimates of the doses

000044

of B[a]P delivered to the gastrointestinal tract. This section describes the steps that could be taken to obtain these estimates if resources were available to complete the calculations.

1.3.1 Estimate Administered Dose

In the Thyssen et al. (1981) study, the authors estimated the total administered doses of B[a]P received by each exposure group based on the average concentration of B[a]P in air, the average length of survival of each group, and a hamster minute volume of 110 ml air/minute. The resulting exposure estimates were as follows:

Group	Average Exposure Level (mg/m^3)	Total Average Dose (mg/animal)
1	0	0
2	2.2	29
3	9.5	127
4	46.5	383

The original exposure and pathology data have been obtained from the authors and could be reanalyzed on an individual animal basis. These total average administered doses should be adjusted upon completion of such an analysis.

1.3.2 Estimate Retained Dose

The actual doses received by each hamster can be estimated by determining the total respiratory tract deposition of the B[a]P aerosol. Total respiratory tract deposition of particles depends upon the size of the particles. B[a]P was administered on a sodium chloride aerosol with particles size 0.2-0.5 μ (80% of which were between 0.2-0.3 μ). Total respiratory tract deposition of inhaled particles as a function of particle size has been determined for the

hamster by Raab et al. (1977) and Yeh et al. (1980), and is summarized in Schlesinger (1985). The relationship is depicted in Figure II-1a. By extrapolating the information shown in the graph, it can be estimated that approximately 15-20% of the inhaled dose of B[a]P was actually deposited in the hamster respiratory tract. It is possible that deposition increases for lower particle sizes; however, by analogy to observations made in humans and guinea pigs (see Figure II-1b and II-1c), the increase does not occur until particles are much smaller than 0.2 μ. As a result, the total respiratory tract deposition of B[a]P in hamsters will be conservatively assumed to be the lower bound on that which was estimated to occur, 15%. The actual doses of B[a]P that each hamster received can thus be estimated by assuming that they were equal to 15% of the administered doses specified in the previous section.

1.3.3 Estimate Dose to Gastrointestinal Tract

To estimate the proportions of the actual B[a]P dose that reach the hamster pharynx, esophagus, and forestomach, the assumptions will be made that the sodium chloride aerosol dissolves when it is deposited on respiratory tract tissue and mucus. This is a reasonable assumption in view of the weak nature of the chemical association between B[a]P and the sodium chloride particles as well as the high aqueous solubility of the latter. The transport and fate of inhaled B[a]P can then be modelled on the basis of information on B[a]P itself, not on particles associated B[a]P.

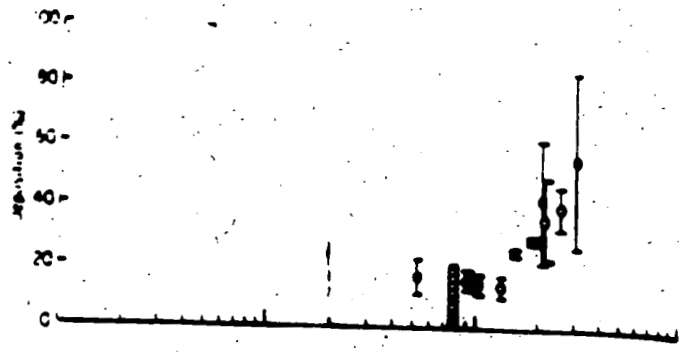
Sun et al. (1982) studied the biological fate of pure inhaled B[a]P in the rat. Since a similar study has not been conducted in the hamster, the hamster will be assumed to resemble the rat for the purposes of dose estimation. These authors exposed rats for 30 minutes to a pure B[a]P aerosol labelled with

000046

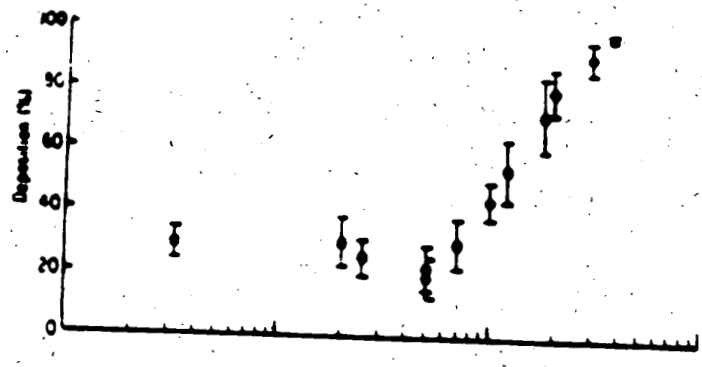
FIGURE II-1

RELATIONSHIPS BETWEEN TOTAL RESPIRATORY TRACT DEPOSITION AND PARTICLE SIZE

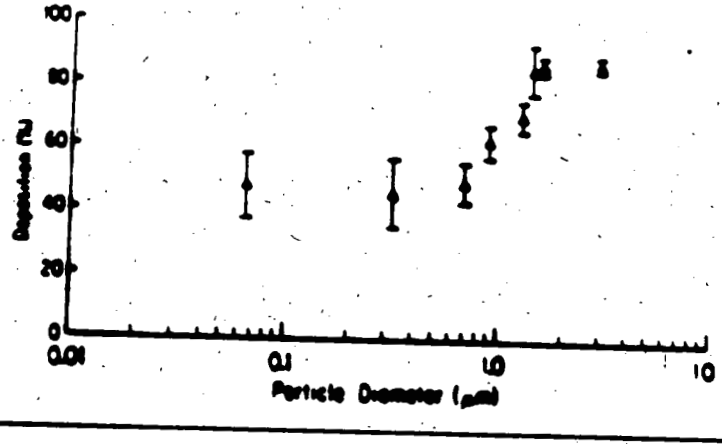
a. Hamster



b. Human (nasal)



c. Guinea pig



SOURCE: Schlesinger 1985

tritium and then followed the retention of the label in various organs, including the esophagus and stomach. The maximum levels of radioactivity in these organs were reached one-half and two hours post-exposure, as shown by the following data; profiles of the absorption and elimination of B[a]P are shown in Figure II-2.

Time Post-Exposure	^{3}H-B[a]P (ng equivalents ± SD) (y_t)	
	Esophagus[a]	Stomach[a]
0.5 hours	0.60±0.43	0.25±0.16
2 hours	1.01±1.38	2.97±3.40
6 hours	0.44±0.20	2.21±1.30
12 hours	0.84±0.71	2.12±1.37
24 hours	0.79±0.51	1.45±1.30
48 hours	0.58±0.70	0.60±0.41
5 days	0.14±0.02	0.09±0.11
8 days	0.11±0.04	0.19±0.14
16 days	0.08±0.03	0.12±0.10

[a]N = 4.

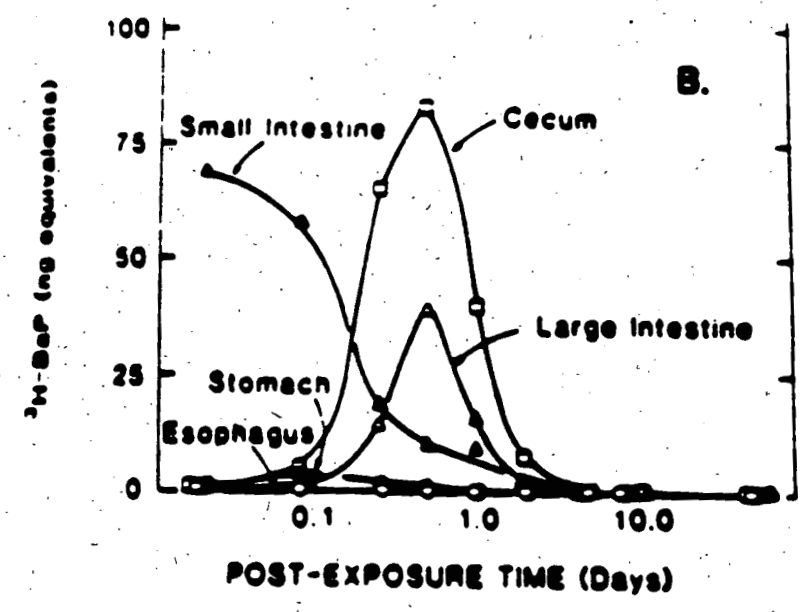
The retention of B[a]P in the hamster gastrointestinal tract can be estimated using the profiles for the stomach and esophagus, making adjustments for the differences in administered dose and length of exposure, and by assuming that the forestomach is approximately 40% of the entire stomach. The pharynx was considered to be approximately one-half the size of the esophagus on the basis of surface area, so the dose of B[a]P to the pharynx can be estimated to be 50% of that to the esophagus.

To estimate the total dose (D) of B[a]P to the esophagus and stomach, its absorption and elimination from each organ can be assumed to follow that of first-order kinetics in a one-compartment system, as is suggested by

000048

FIGURE II-2

ABSORPTION AND ELIMINATION OF BENZO[a]PYRENE
FROM THE RAT GASTROINTESTINAL TRACT
FOLLOWING INHALATION EXPOSURE



SOURCE: Sun et al. 1982

Figure II-2. The behavior of a substance in a one-compartment biological system in which its absorption and elimination are first-order (exponential) can be described by the following equation (Filov et al. 1979):

$$y_t = \frac{Dk_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t})$$

where D is the total dose to the organ and k_1 and k_2 are the rate constants for the absorption and elimination processes, respectively.

The data in the table can be used to estimate the unknown parameters D, k_1, and k_2 for the esophagus and stomach by weighted least square estimation using the computer program General Model fit (GMF) (Thorslund et al. 1987), in which the weights are the reciprocal of the square of the standard deviation.

The total dose to the digestive tract can then be obtained by adding together that for the esophagus, 50% of that for the esophagus to account for the pharynx, and 40% of that to the stomach to account for the forestomach only.

The actual (deposited) inhaled dose of B[a]P received by the rat can be compared to the digestive tract dose to obtain a ratio, which can be assumed to be constant in the hamster. Sun et al. (1982) calculated that a total inhaled mass of 4.9 μg B[a]P was received by each rat and of that, 10% was retained, giving an actual dose of 0.5 μg. The ratio of digestive tract dose to actual dose can then be applied to the individual hamster exposure data to obtain their digestive tract doses.

1.3.4 Estimate Biologically Active Proportion of Dose

In order to extrapolate the B[a]P-induced digestive tract cancer dose-response relationship from the inhalation to the oral route of exposure, the

000050

proportion of B[a]P that is still biologically active (i.e., capable of being metabolized to a carcinogenic derivative) when it reaches the gastrointestinal tract can be estimated. To make such an estimate, the experiments of Dahl et al. (1983) will be used. These investigators instilled B[a]P onto the nasal mucosa of hamsters and collected and analyzed any material that reached the esophagus. Approximately 50% of collected material was reported to have been B[a]P metabolites such as tetrols, diols, quinones, oxides, and phenols; these metabolites are not likely to be activated to the carcinogenic 7,8-diol 9,10-epoxide of B[a]P (with the exception of the 7,8-diols, which represented less than 2% of the metabolites detected). Approximately 50% of the digestive tract doses may thus be assumed to be available for metabolic activation. The assumption must also be made that the extent to which B[a]P is metabolized and swallowed following nasal instillation is not substantially different from that following inhalation.

1.2.5 Estimate Actual Dose

The actual doses of B[a]P to the gastrointestinal tract of the hamsters following inhalation exposure in the Thyssen et al. (1981) study can be estimated by applying the factor obtained in the previous sections to the average exposure levels (shown in Section 1.3.1) or to the individual animal exposure levels, once calculated. A dose-response relationship could then be described for ingested B[a]P level and gastrointestinal tract tumors in the hamster and used to generate an alternative and potentially more reliable unit risk for ingestion exposure to B[a]P than that based on Neal and Rigdon (1967).

2. Dose-Response Relationship for Inhalation Exposure to Benzo[a]pyrene

The preferred route of exposure for chronic studies conducted to assess the risk of cancer posed by airborne B[a]P is inhalation. However, such studies have difficult methodological problems associated with them. As a result, much of the work on this chemical has been done using intratracheal instillation of B[a]P alone or in combination with other agents as a surrogate route of exposure for inhalation. Although much valuable qualitative information has been obtained by this approach, its use in quantitative risk assessments has not met with much success.

B[a]P has elicited lung tumors in several bioassay systems following inhalation exposure or intratracheal instillation. Its carcinogenic potency is enhanced by coadministration of particulate matter or some gases (IARC 1983). For example, a positive response was obtained in rats using a combination of B[a]P and the atmospheric irritant sulfur dioxide; sulfur dioxide by itself was not carcinogenic (Laskin et al. 1970). The tumors induced in this experiment were squamous cell carcinomas; a proportion of the bronchogenic carcinomas found in humans are of this type.

A well-conducted study by Thyssen et al. (1981) provides the most clear-cut evidence of the dose-response relationship between inhaled B[a]P and tumorigenesis. In this experiment, Syrian golden hamsters were exposed throughout their lives to B[a]P in a sodium chloride aerosol for 4.5 hours per day, 7 days per week for 10 weeks and then 3 hours per day thereafter. Respiratory tract tumors were induced in the nasal cavity, larynx, and trachea. The dose-response relationship obtained for these tumors is shown in Table II-4.

000052

TABLE II-4

DATA USED TO ESTIMATE DOSE-RESPONSE RELATIONSHIP BETWEEN INHALED B[a]P AND RESPIRATORY TRACT TUMORS

Exposure Rate (x) (mg B[a]P/m^3)	Average Survival (t) (in weeks)	Effective Number of Animals	Number of Animals with Respiratory Tract Tumors	
			Observed	Predicted
0	96.4	27	0	0.73
2.2	95.2	27	0	1.88
9.5	96.4	26	0	9.06
46.5	59.5	25	13	12.59

NOTE: Based on low-dose linear two-stage, dose- and time-dependent, identical transition rate model:

$$P(x,t) = 1-\exp\left[-0.000115(1+0.312x)^2\right]\left[\exp(0.057t)-1-0.057t\right]$$

SOURCE: Thyssen et al. 1981

Because survival times varied widely among the groups, the data in Table II-4 are fitted to the time-dependent form of the two-stage model [equation (I-3)], yielding estimates of the three parameters M, S, and G. For this purpose, the average survival time in each group is used as an estimate of the variable t in equation (I-3); this approximation would be subject to error if survival times varied substantially within groups, which was not reported. The fitted model slightly underpredicts the curvature of the observed dose-response curve, but the differences are not statistically significant ($\chi^2 = 2.81$, df = 1, $p > 0.1$), indicating a reasonable fit.

The risk at the end of an average lifetime is obtained from the equation shown in Table II-3 by evaluating P(x,t) at t=96.4 weeks, the average survival time in the control population. This gives the following time-independent lifetime risk relationship:

$$P(x) = 1-\exp[-0.0272(1+0.312x)^2].$$

For low levels of exposure, the linear term of the model times the exposure level is a close approximation of the estimated risk. The linear term in this case may be expressed as

$$q_1 = (2)(0.0272)(0.312) = 0.0170\ (mg/m^3)^{-1}.$$

To extrapolate this value to human exposure for 24 hours per day, the average length of exposure per day over the approximate two-year experimental period is calculated. The weighted mean of the two exposure periods is

000054

$[(10)(4.5)+(92)(3)]/102 = 3.147$ hr/day. Thus, exposure 24 hours per day has a potency value associated with it of

$$q_1 = (0.0170)(24/3.147) = 0.1295\ (mg/m^3)^{-1}.$$

This calculation is based on the assumption that inhalation exposures are equivalent across species (i.e., mg/metabolic rate = mg/surface area). To convert this exposure level to $(mg/kg/day)^{-1}$ to be able to compare it to exposure via ingestion, the absorption rates for inhalation and ingestion are assumed to be the same and the average person is assumed to weigh 70 kg and inhale 20 m^3 air per day. Under these assumptions, exposure to 1 mg/m^3 for 24 hours results in an exposure level of $[(1)(20)]/70 = 0.2857$ mg/kg/day. Thus, on a mg/kg/day basis the estimated slope is

$$q_1 = 0.1295/0.2857 = 0.4533\ (mg/kg/day)^{-1}.$$

A considerably improved cancer risk estimate could be obtained by using the individual animal data assembled in Appendix A. Because each animal in the experiment had a unique exposure level, exposure duration, and survival time, specific exposure information can be generated and used with the model to provide a much more accurate unit risk. When resources permit, this recalculation should be an important priority.

III. THE BASIS FOR AND DERIVATION OF COMPARATIVE POTENCY ESTIMATES

This chapter describes eleven experiments in which B[a]P and other PAHs were tested concomitantly for carcinogenesis using several animal species and different methods of administration, as well as two experiments in which the levels of these chemicals that interact with cellular DNA were determined. Unlike the experiments using B[a]P described in Section II, most of these studies used methods of administration that cannot be quantitatively compared to those by which humans would be expected to be exposed. As a result, dose-response relationships for these PAHs cannot be described mathematically in a manner that is useful for the prediction of human risk unless they are expressed relative to B[a]P. Cancer potencies relative to B[a]P are derived for each PAH from each experiment in the following section and are summarized at the end.

A. CARCINOGENESIS

It is difficult to compare directly the results of carcinogenesis bioassays because of inter-laboratory differences, varying susceptibilities and metabolic capabilities among species used, and differences in techniques and exposure routes. As a result, the only studies that are used in this document to make relative potency estimates meet the criteria that B[a]P was tested in the same bioassay system as the other PAHs, in the same laboratory, and at the same time. In this section, individual studies that meet these criteria are summarized and evaluated regarding quality, and relative potency estimates are derived for each of the PAHs for which adequate data are available. For each study discussed, a table is included that summarizes all

080056

of the results obtained; another table presents the data actually used to derive the relative potency estimates, for ease of verification. The order in which the studies are discussed corresponds to the reverse of the chronological order in which they were performed, with the most recent study presented first. Relative potency estimates were derived by the method described in Section II. To do so, the operational assumption was made that within the context of the model, papillomas and other benign tumors are clones of first stage cells that are large enough to be observable. Since only the most advanced stage of a tumor is usually recorded at necropsy and since carcinomas can develop from papillomas, rendering the initial papilloma unobservable, the observation of either a papilloma or a carcinoma can be viewed as evidence that at least one transformation has taken place. Thus, in cases where benign and malignant tumors were combined in reporting, the risk equation used to calculate comparative potencies is of the simple form

$$P(x) = 1\text{-}\exp\text{-}A(1+Sx).$$

The model described earlier for which two transformations are required, of the form

$$P(x) = 1\text{-}\exp\text{-}A(1+Sx)^2,$$

will be restricted in its use to cases where only carcinomas were reported separately. Using carcinomas as the end point for dose-response modeling is preferable to using papillomas, since not all papillomas may be capable of developing into carcinomas. Strong evidence for this interpretation of the two-stage model in the case of skin tumors has recently been provided by Chu et al. (1987) and by Hennings and Yuspa (1985). For each of the following

studies, one of these two models was used to estimate the relative potencies of the PAHs tested. When data were reported in a form for which either model could be used, only the two-stage model was employed and applied to the incidence of carcinomas.

1. LaVoie et al. 1987

The tumorigenicities of the benzofluoranthenes and indeno[1,2,3-cd]pyrene were evaluated in the newborn mouse assay and compared with that of B[a]P. Solutions of each PAH in DMSO were administered intraperitoneally on days 1, 8, and 15 of age to groups of 30-40 CD-1 mice (males and females). Mice were weaned on day 21, separated by sex, and received no further treatment until sacrifice at 1 year. Histological examination was performed on all livers and any gross lesions. Table III-1 shows the results and indicates that treatment-related effects on the incidences of lung adenomas were seen for mice treated with B[a]P and benzo[j]fluoranthene and of hepatic adenomas for male mice treated with these compounds as well as with benzo[b]fluoranthene.

Comparative potency estimates were based upon the incidences of hepatic tumors in males (females were not affected) and of lung tumors in both sexes combined (since the sexes were not statistically different). Organizing the data in this manner yielded the most pronounced dose-effect data and the most stable estimates. The resulting rearranged data are displayed in Tables III-2 to III-4, which show only the results from those groups for which positive responses were obtained. In the absence of data on the dose-response relationship for B[a]P in this type of bioassay, little confidence can be placed in the relative potency estimates obtained for the other PAHs.

000058

TABLE III-1

TUMOR INCIDENCE FOLLOWING INTRAPERITONEAL ADMINISTRATION OF PAHs IN NEWBORN MICE[a]

Treatment Group		Total Dose (μmol)	Hepatic Adenoma	Hepatocellular Carcinoma	Lung Adenoma
Control (DMSO)	M	-	0/17	1/17(6)	0/17
	F	-	0/18	0/18	0/18
Benzo[a]-pyrene	M	1.1	9/17(53)[b]	4/17(24)	14/17(82)[b]
	F	1.1	0/14	0/14	9/14(64)[b]
Benzo[b]-fluoranthene	M	0.5	6/15(40)[b]	2/15(13)	2/15(13)
	F	0.5	0/17	0/17	3/17(18)
Benzo[j]-fluoranthene	M	1.1	8/21(38)[b]	3/21(14)	11/21(52)[b]
	F	1.1	0/18	0/18	4/18(22)[b]
Benzo[k]-fluoranthene	M	2.1	2/16(13)	1/16(6)	1/16(6)
	F	2.1	0/18	0/18	3/18(17)
Indeno-[1,2,3-cd]-pyrene	M	2.1	0/11	0/11	1/11(9)
	F	2.1	0/9	0/9	0/9

[a]Newborn CD-1 mice received i.p. injections of PAHs in DMSO on days 1, 8, and 15 of age. Surviving animals were sacrificed at 1 year.

[b]Significantly different from controls using the Fisher exact test with $P < 0.05$.

Source: LaVoie et al. 1987

TABLE III-2

POSITIVE TUMOR DATA IN NEWBORN MOUSE LUNGS AND RELATIVE POTENCY ESTIMATES[a]

Treatment Group	Total Dose (μmol)	Lung Adenomas[b] (Combined Sexes)	Estimated Transition Rate (SRj)	Estimated Relative Potency (Rj)
Control (DMSO)	0	0/35	--	--
Benzo[a]-pyrene	1.1	23/31	93.140	1.0
Benzo[b]-fluoranthene	0.5	5/32	21.616	0.232
Benzo[j]-fluoranthene	1.1	15/39	29.766	0.320
Benzo[k]-fluoranthene	2.1	4/34	3.666	0.040
Indeno-[1,2,3-cd]-pyrene	2.1	1/20	1.221	0.013

[a]The one-hit (single transition) model was used: $P(x) = 1-\exp-A(1+SR_j x)$
$A = -\ln(1-0.5/36) = 0.014$

[b]No carcinomas were observed.

Source: LaVoie et al. 1987

000060

TABLE III-3

POSITIVE TUMOR DATA IN NEWBORN MOUSE LIVERS AND RELATIVE POTENCY ESTIMATES[a]

Treatment Group	Total Dose (μmol)	Liver Tumors (Male)	Estimated Transition Rate (SRj)	Estimated Relative Potency (Rj)
Control (DMSO)	0	1/17	--	--
Benzo[a]-pyrene	1.1	13/17	21.697	1.0
Benzo[b]-fluoranthene	0.5	8/15	23.143	1.067
Benzo[j]-fluoranthene	1.1	11/21	10.217	0.471
Benzo[k]-fluoranthene	2.1	3/16	2.107	0.097

[a]The one-hit (single transition) model was used: $P(x)=1-\exp-A(1+SR_j x)$

Source: LaVoie et al. 1987

TABLE III-4

POSITIVE TUMOR DATA IN NEWBORN MOUSE LIVERS AND RELATIVE POTENCY ESTIMATES[a]

Treatment Group	Total Dose (μmol)	Hepatocellular Carcinoma (Male)	Estimated Transition Rate (SRj)	Estimated Relative Potency (Rj)
Control (DMSO)	0	1/17	--	--
Benzo[a]-pyrene	1.1	4/17	3.114	1.0
Benzo[b]-fluoranthene	0.5	2/15	2.721	0.874
Benzo[j]-fluoranthene	1.1	3/21	1.402	0.450
Benzo[k]-fluoranthene	2.1	1/16	0.136	0.044

[a]The two-stage model for carcinomas was used: $P(x) = 1\text{-}\exp\text{-}A(1+SR_j x)^2$

Source: LaVoie et al. 1987

000062

2. Wislocki et al. 1986

Several PAHs were tested for tumorigenicity along with their nitrated derivatives in the newborn mouse assay. Solutions of each compound in DMSO were injected intraperitoneally into groups of 90-100 male and female newborn CD-1 mice on days 1, 8, and 15 of age. Mice were weaned at age 25-27 days, separated by sex, and received no further treatment. Surviving mice were sacrificed at one year. All mice that survived weaning were subjected to complete necropsies and are included in the results. Treatment-related liver tumors (adenomas or carcinomas) were observed in some groups of mice treated with pyrene, benzo[a]anthracene, chrysene, or B[a]P (see Table III-5) while an elevated incidence of lung adenomas was seen only in mice treated with B[a]P. The reduced survival observed in some of the treated groups was attributed to animal husbandry problems and maternal cannibalism and was not treatment-related (P. Wislocki, personal communication).

The data were treated in the same manner as those from the LaVoie et al. (1987) study described in the previous section. The comparative potency estimates obtained are shown in Tables III-6 to III-8. These data are unusual in that with the exception of chrysene-induced hepatocellular carcinoma, no dose-related positive trends in tumor yields were observed. As a result of the absence of positive trends and since no dose-response data for B[a]P exist for this type of bioassay, the comparative potency estimates obtained may result from experimental artifact and not accurately reflect biological relationships. Little confidence should be placed in the estimates.

000063

TABLE III-5

TUMOR INCIDENCE FOLLOWING INTRAPERITONEAL ADMINISTRATION OF PAHs IN NEWBORN MICE[a]

Treatment Group		Total Dose (nmol)	Number of mice with liver tumors (%)		Number of mice with lung tumors (%)	
			Adenomas only	Carcinomas	Adenomas only	Carcinomas
Control (DMSO)	M	-	7/73(10)	0/73	3/73(4)	2/73(3)
	F	-	0/65	0/65	1/65(2)	1/65(2)
Pyrene	M	200	0/29	0/29	1/29(3)	0/29
	F	200	0/31	0/31	1/31(3)	0/31
	M	700	0/25	3/25(12)[b]	2/25(8)	0/25
	F	700	0/49	0/49	5/49(10)	0/49
	M	2,800	2/14	1/14(7)	1/14(7)	0/14
	F	2,800	0/18	0/18	1/18(6)	0/18
Benz[a]-anthracene	M	2,800	6/39(15)	25/39(64)[b]	5/39(13)	1/39(3)
	F	2,800	0/32	0/32	6/32(19)	0/32
Chrysene	M	700	8/35(23)	2/35(6)	5/35(14)	1/35(3)
	F	700	0/33	0/33	2/33(6)	0/33
	M	2,800	1/34(3)	13/34(38)[b]	7/34(21)	0/34
	F	2,800	0/24	0/24	1/24(4)	0/24
Benzo[a]-pyrene	M	560	11/37(30)	7/37(19)[b]	13/37(35)[b]	0/37
	F	560	0/27	0/27	13/27(48)[b]	0/27

[a]Newborn CD-1 mice received i.p. injections of PAHs in DMSO on days 1, 8, and 15 of age. Surviving animals were sacrificed at 1 year.
[b]Significantly different from controls using the Fisher exact test with $P < 0.05$.

Source: Wislocki et al. 1986

000064

TABLE III-6

POSITIVE TUMOR DATA IN NEWBORN MOUSE LUNGS AND RELATIVE POTENCY ESTIMATES[a]

Treatment Group	Total Dose (μmol)	Lung Adenomas (Combined Sexes)	Estimated Transition Rate (SRj)	Estimated Relative Potency (Rj)
Control (DMSO)	0	7/138	--	--
Benzo[a]-pyrene	0.56	26/64	16.097	1.0
Pyrene	0.20 0.70 2.80[b]	2/60 7/74 2/32	1.298	0.081
Benz[a]-anthracene	2.80	12/71	0.913	0.057
Chrysene	0.70 2.80[b]	8/68 8/58	2.006	0.125

[a]The one-hit (single transition) model was used: $P(x) = 1-\exp-A(1+SR_j x)$.

[b]Does not fit model; observation not used in estimate.

Source: Wislocki et al. 1986

TABLE III-7

POSITIVE TUMOR DATA IN NEWBORN MOUSE LIVERS AND RELATIVE POTENCY ESTIMATES[a]

Treatment Group	Total Dose (μmol)	Liver Tumors (Male)	Estimated Transition Rate (SRj)	Estimated Relative Potency (Rj)
Control (DMSO)	0	7/73	--	--
Benzo[a]-pyrene	0.56	18/37	10.021	1.0
Pyrene	2.80	3/14	0.497	0.050
Benz[a]-anthracene	2.80	31/39	5.255	0.524
Chrysene	0.70 2.80[b]	10/3[illegible] 14/34	3.340	0.333

[a]The one-hit (single transition) model was used: $P(x) = 1-\exp-A(1+SR_j x)$.

[b]Does not fit model; observation not used in estimate.

Source: Wislocki et al. 1986

000066

TABLE III-8

POSITIVE TUMOR DATA IN NEWBORN MOUSE LIVERS AND RELATIVE POTENCY ESTIMATES[a]

Treatment Group	Total Dose (μmol)	Hepatocellular carcinoma (Male)	Estimated Transition Rate (SRj)	Estimated Relative Potency (Rj)
Control (DMSO)	0	0/73	--	--
Benzo[a]-pyrene	0.56	7/37	8.078	1.0
Pyrene	0.70 2.80[b]	3/25 1/14	4.732	0.586
Benz[a]-anthracene	2.80	25/39	4.003	0.496
Chrysene	0.70 2.80	2/35 13/34	2.692	0.333

[a]The two-stage model for carcinomas was used: $P(x) = 1-\exp-A(1+SR_j x)^2$.

[b]Does not fit model; observation not used in estimate.

Source: Wislocki et al. 1986

3. Deutsch-Wenzel et al. (1983)

In this study, eight environmentally ubiquitous PAHs were tested for carcinogenicity by direct implantation into the lungs of female Osborne-Mendel rats. Test compounds were prepared in solution with trioctanoin and molten beeswax. Upon injection into the left lobe of the lung, the mixture congealed into a pellet, from which the test compound diffused over time into the surrounding lung tissue. Epidermoid carcinomas and pleomorphic sarcomas were observed and dose-response relationships were obtained. The treatment method involved some trauma to the animals. Relative potency estimates would not be affected, however, since this factor was constant for all treatment groups. Tumor induction times were difficult to observe, so survival time and the number of tumor-bearing animals were the responses evaluated. Table III-9 shows the results for the PAHs tested.

The transition rate parameter R_jS was estimated for each of the carcinogenic PAHs from the data in Table III-9. The measure of response used for modeling was the number of animals bearing epidermoid carcinomas of the lung at the completion of the study. (Pleiomorphic sarcomas were excluded in the interest of consistency and because their etiology probably differs from that of carcinomas.) The numerical form of the best-fitting model and the goodness of fit are shown in Table III-10. No tumors were observed in the control rats, so the parameter A was obtained by Bayesian estimation, as discussed in Section II (i.e., $A = -\ln(1-0.5/36) = 0.014$). The highest exposure groups were omitted for B[a]P and IND, since the response was significantly less than that predicted by the model. This discrepancy is most likely due to toxicity and reduced survival in those high exposure groups. A more complex analysis could use median survival times as an additional

000068

TABLE III-9

CARCINOGENICITY AND DOSE-RESPONSE RELATIONSHIPS OF EIGHT FREQUENTLY OCCURRING ENVIRONMENTAL PAHs IN RAT LUNGS

Compound[a]	Dose (mg)	Number of Animals	Median Survival Time, Weeks (95% Confidence Interval)	Number of Animals Bearing Epidermoid Carcinomas/ Number of Animals Bearing Pleomorphic Sarcomas	Tumor Incidence[b] (%)
B[b]F	0.1	35	110(95-118)	0/1	0
B[b]F	0.3	35	113(100-121)	1/2	2.9
B[b]F	1.0	35	112(98-126)	9/4	25.7
B[e]P	0.2	35	117(105-123)	0/0	0
B[e]P	1.0	30	111(91-126)	0/1	0
B[e]P	5.0	35	104(92-113)	1/0	2.9
B[j]F	0.2	35	110(91-118)	1/0	2.9
B[j]F	1.0	35	117(103-125)	3/0	8.6
B[j]F	5.0	35	89(75-105)	18/0	51.4
B[k]F	0.16	35	114(101-122)	0/0	0
B[k]F	0.83	31	95(81-104)	3/0	9.7
B[k]F	4.15	27	98(87-108)	12/0	44.4
IND	0.16	35	116(95-123)	3/1	8.6
IND	0.83	35	109(98-116)	8/0	22.9
IND	4.15	35	92(82-108)	21/0	60.0
ANT	0.16	35	102(91-116)	1/0	2.9
ANT	0.83	35	88(72-103)	19/0	54.3
B[ghi]P	0.16	35	109(101-121)	0/0	0
B[ghi]P	0.83	35	114(103-123)	1/0	2.9
B[ghi]P	4.15	34	106(93-117)	4/0	11.8
B[a]P	0.1	35	111(95-120)	4/6	11.8
B[a]P	0.3	35	77(68-99)	21/2	60.0
B[a]P	1.0	35	54(46-64)	33/0	94.3
BW-TC	--	35	104(91-121)	0/0	0
Untreated control	--	35	118(104-133)	0/0	0

[a]Abbreviations: B[b]F, benzo[b]fluoranthene; B[e]P, benzo[e]pyrene; B[j]F, benzo[j]fluoranthene; B[k]F, benzo[k]fluoranthene; IND, indeno(1,2,3-cd)pyrene; ANT, anthanthrene; B[ghi]P, benzo[ghi]perylene; B[a]P, benzo[a]pyrene; BW-TC, beeswax and trioctanoin (vehicle).

[b]Carcinomas only.

SOURCE: Deutsch-Wenzel et al. (1983).

TABLE III-10

DATA USED AND ESTIMATES OF RELATIVE POTENCY OBTAINED FROM RAT LUNGS FOLLOWING INTRAPULMONARY INJECTION

Compound[a] Exposure Levels in mg (x)	No. Rats Exposed	Epidermoid Carcinomas		Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
		Observed	Predicted		
B[a]P:				22.28	1.0
0.1	35	4	4.79		
0.3	35	21	19.82		
1.0	35	([b])	([b])		
B[b]F:				3.12	0.140
0.1	35	0	0.83		
0.3	35	1	1.73		
1.0	35	9	7.40		
B[e]P:				0.088	0.004
5.0	35	1	1.00		
B[k]F:				1.48	0.066
0.16	35	0	0.75		
0.83	31	3	2.09		
4.15	27	12	13.79		
IND:				5.17	0.232
0.16	35	3	1.66		
0.83	35	8	11.41		
4.15	35	([b])	([b])		
ANT:				7.12	0.320
0.16	35	1	2.17		
0.83	35	19	17.06		
B[ghi]P:				0.486	0.022
0.16	35	0	0.57		
0.83	35	1	0.95		
4.15	34	4	4.07		
Control				--	--
0	35	0	--		

[a]Abbreviations: B[a]P, benzo[a]pyrene; B[b]F, benzo[b]fluoranthene; B[e]P, benzo[e]pyrene; B[k]F, benzo[k]fluoranthene; IND, indeno(1,2,3-cd)pyrene; ANT, anthanthrene; B[ghi]P, benzo[ghi]perylene.

[b]Omitted because of lack of fit, excess mortality.

Model: $P(x) = 1-\exp-A(1+SR_j x)^2$, $A = -\ln(1-0.5/36) = 0.014$.

Source: Deutsch-Wenzel et al. (1983).

000070

variable; this would most likely reduce the relative potency estimates somewhat and increase precision.

4 LaVoie et al. (1982)

The tumor-initiating activities of the benzofluoranthenes were tested on Swiss albino CD-1 female mouse skin and compared with that of B[a]P. Compounds were applied in 10 doses (1 every other day) to the backs of mice and followed 10 days later with applications of the tumor promoter phorbol myristate acetate (TPA) 3 times a week for 20 weeks, where upon animals were sacrificed. Table III-11 shows the total initiating dose of each of the compounds tested and numbers of tumors and tumor-bearing animals obtained. Tumors were principally squamous cell papillomas, with some keratoacanthomas observed as well. Benzo[b]fluoranthene was approximately ten times as potent as a tumor initiator than benzo[j]fluoranthene, which was only somewhat more potent than benzo[k]fluoranthene. B[a]P was more potent than all three.

Two factors make the data obtained in this experiment difficult to interpret. The application of the promotor TPA adds an additional element of complexity to the evaluation. The possibility of a qualitatively different interaction between TPA and the various PAHs exists. Furthermore, this experimental protocol may allow only the first and not the second mutation that is hypothesized to occur during PAH-induced carcinogenesis and would therefore not fit a two-stage model. The one-hit (single transition) model was consequently used. These potential problems are compounded by having only a single level of B[a]P available upon which to base the potency estimate. To minimize the latter problem, the data used in the evaluation will be restricted to the single exposure level of each of the PAHs that gives a tumor response

TABLE III-11

TUMOR INITIATING ACTIVITY OF BENZOFLUORANTHENES

Compound[a]	Total Initiating Dose (μg)	Number of Skin Tumor-Bearing Animals/Number of Animals	Skin Tumors[b] per Animal
B[b]F	10	9/20	0.9
B[b]F	30	12/20	2.3
B[b]F	100	16/20	7.1
B[j]F	30	6/20	0.6
B[j]F	100	11/20	1.9
B[j]F	1,000	19/20	7.2
B[k]F	30	1/20	0.1
B[k]F	100	5/20	0.4
B[k]F	1,000	15/20	2.8
B[a]P	30	17/20	4.9
Acetone (Vehicle)	--	0/20	0

[a]Abbreviations: B[b]F, benzo[b]fluoranthene; B[j]F, benzo[j]fluoranthene; B[k]F, benzo[k]fluoranthene; B[a]P, benzo[a]pyrene.

[b]Skin tumors were principally papillomas.

Source: LaVoie et al. (1982).

000072

closest to the 85% B[a]P tumor response rate. This procedure yields results that are less model-dependent. The relative potencies calculated on the basis of these restricted data are displayed in Table III-12.

5. Habs et al. (1980)

Six environmentally relevant PAHs were tested in order to determine their dose-response relationships as carcinogens when topically applied to the backs of female NMRI mice throughout their lifetimes. Table III-13 lists the compounds tested, doses used, frequency of application, and results obtained. Although it was not clearly stated by the authors, it will be assumed that the tumors observed were all carcinomas on the basis of their statement, "Animals at an advanced state of macroscopically clearly infiltrative growth were killed." Clear-cut dose-response relationships were seen for B[a]P and benzo[b]fluoranthene. Benzo[j]fluoranthene proved to be weakly carcinogenic, as did cyclopentadieno(c,d)pyrene and coronene. Benzo[k]fluoranthene and indeno(1,2,3-cd)pyrene were inactive as carcinogens in this test system.

The relative transition rate was calculated using the low-dose linear two-stage model for each of the six test agents, as shown in Table III-14 (coronene was not included due to the different protocol and solvent employed). The estimates obtained for B[j]F, CP, B[k]F, and IND are based upon very weak or perhaps nonexistent dose-response relationships. Even so, the response data observed are consistent with the underlying response model. Estimates obtained using these data would, if erroneous, tend to overestimate the relative transition rates and, as a result, the relative potencies because the actual tumor response may be much lower or non-existent.

TABLE III-12

DATA USED AND ESTIMATES OF RELATIVE POTENCY OBTAINED FROM MOUSE SKIN INITIATION-PROMOTION EXPERIMENT

Compound[a]	Total Dose (μg)	Number of Skin Tumor-Bearing Animals/ Number Exposed	Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
B[a]P	30	17/20	2.602	1.0
B[b]F	100	16/20	0.671	0.258
B[j]F	1,000	19/20	0.124	0.048
B[k]F	1,000	15/20	0.057	0.022

[a]Abbreviations: B[a]P, benzo[a]pyrene; B[b]F, benzo[b]fluoranthene; B[j]F, benzo[j]fluoranthene; B[k]F, benzo[k]fluoranthene.

Model: $P(x) = 1\text{-}\exp\text{-}A(1 + SR_j x)$, $A = -\ln[1-0.5/21] = 0.024$.

Source: LaVoie et al. (1982).

000074

TABLE III-13

PAH-INDUCED SKIN TUMOR RATES IN MICE[b]

Compound[a]	Solvent	Frequency of Application	Dose (mg/animal)	Number of Tumor-Bearing Animals/ Total Number of Animals	Tumor Incidence (%)
B[a]P	Acetone	2x/week	1.7	8/34	23.5
			2.8	24/35	68.6
B[b]F	Acetone	2x/week	4.6	22/36	61.1
			3.4	2/38	5.3
B[j]F	Acetone	2x/week	5.6	5/34	14.7
			9.2	20/37	54.1
B[k]F	Acetone	2x/week	3.4	1/38	2.6
			5.6	1/35	2.9
IND	Acetone	2x/week	9.2	2/38	5.3
			3.4	1/39	2.6
CP	Acetone	2x/week	5.6	0/38	0
			9.2	0/38	0
COR	DMSO	4x/week	1.7	1/36	2.8
			6.8	0/37	0
--	Acetone	2x/week	27.2	0/37	0
			5.0	0/34	0
--	DMSO	4x/week	15.0	0/35	0
			--	3/38	7.9
			--	1/39	2.6
				2/40	5.0
				0/35	0
				0/36	0

[a]Abbreviations: B[a]P, benzo[a]pyrene; B[b]F, benzo[b]fluoranthene; B[j]F, benzo[j]fluoranthene; B[k]F, benzo[k]fluoranthene; IND, indeno[1,2,3-cd]pyrene; CP, cyclopentadieno(cd)pyrene; COR, coronene; DMSO, dimethylsulfoxide.

[b]Assumed to be carcinomas (see text).

Source: Adapted from Habs et al. (1980).

TABLE III-14

DATA USED AND ESTIMATES OF RELATIVE POTENCY OBTAINED FROM MICE SKIN PAINTING STUDY

Compound[a] Dose mg/animal (x)	No. Mice Exposed	No. of Tumor-Bearing Animals		Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
		Observed	Predicted		
B[a]P:				3.92	1.0
1.7	34	8	10.36		
2.8	35	24	20.60		
4.6	--	([b])	([b])		
B[b]F:				0.656	0.167
3.4	38	2	2.38		
5.6	34	5	4.30		
9.2	--	([b])	([b])		
B[j]F:				0.241	0.061
3.4	38	1	0.77		
5.6	35	1	1.18		
9.2	38	2	2.36		
CP:				0.091	0.023
1.7	34	0	0.28		
6.8	35	0	0.56		
27.2	38	3	2.74		
B[k]F:				0.078	0.020
3.4	39	1	0.43		
5.6	38	0	0.59		
9.2	38	0	0.86		
IND:				0.081	0.021
3.4	36	1	0.36		
5.6	37	0	0.48		
9.2	37	0	0.69		

[a]Abbreviations: B[a]P, benzo[a]pyrene; B[b]F, benzo[b]fluoranthene; B[j]F, benzo[j]fluoranthene; CP, cyclopentadieno(cd)pyrene; B[k]F, benzo[k]fluoranthene; IND, indeno[1,2,3-cd]pyrene.

[b]Omitted because of lack of fit.

Model: $P(x) = 1-\exp-A(1+SR_j x)^2$, $A = -\ln(1-0.5/81) = 0.006192$.
Source: Habs et al. (1980).

000076

6. Pfeiffer (1977)

As part of an experiment designed to evaluate the interactions between carcinogenic and noncarcinogenic PAHS, single doses of B[a]P or dibenz[ah]anthracene dissolved in tricaprylin were injected subcutaneously between the shoulder blades of female NMRI mice. Animals were palpated weekly for tumor development and allowed to survive up to 114 weeks. Table III-15 shows the incidence of sarcomas obtained and the doses of PAHs used. The control tumor incidence was not reported, although a group of "non-carcinogenic" PAHs was tested and will be included here as a pseudo-control group. Furthermore, the numbers of effective or surviving animals were not reported, so the tumor incidences may not reflect potential toxicity at the higher doses.

The dose-response curves for B[a]P and D[ah]A increase at rates that are less than linear, presumably due either to a reduced rate of absorption or saturation of metabolic activating systems at high doses. This effect is more pronounced for D[ah]A than B[a]P, so that relative potencies for D[ah]A estimated at high doses are lower than those estimated at lower doses. The lack of a true control population and the presence of a tumor response from the population receiving a group of hypothesized noncarcinogenic PAHs also make fitting the data to the low-dose linear two-stage model difficult to interpret. As a result, the relative potency was determined by relating results at comparable observed response levels and using the noncarcinogenic PAHs as the pseudo-vehicle control. The data used for these comparisons are shown in Table III-16. Two estimates of the relative potency were obtained; however, the estimate corresponding to the lower exposure levels (i.e., 2.81) is probably more reasonable to use for low-dose extrapolations.

TABLE III-15

TUMOR INCIDENCE FOLLOWING SUBCUTANEOUS INJECTION OF BENZO[A]PYRENE OR DIBENZ[AH]ANTHRACENE

Compound[a]	Dose (μg)	Number of Tumor-Bearing Animals / Number of Animals	Tumor Incidence (%)
B[a]P	3.12	9/100	9
	6.25	35/100	35
	12.5	51/100	51
	25.0	57/100	57
	50.0	77/100	77
	100.0	83/100	83
D[ah]A	2.35	37/100	37
	4.7	39/100	39
	9.3	44/100	44
	18.7	56/100	56
	37.5	65/100	65
	75.0	69/100	69
Pseudo-control[b]	273.975	6/100	6
	549	8/100	8
	1,098.37	6/100	6
	2,196.75	4/100	4
	4,394	13/100	13
	8,787	5/100	5

[a]B[a]P, benzo[a]pyrene; D[ah]A, dibenz[ah]anthracene.

[b]10 "noncarcinogenic" PAHs. The "noncarcinogenic" PAH group included benz[a]anthracene, benzo[ghi]perylene, and chrysene, which are generally considered to be weakly carcinogenic; at the dose levels used, however, these compounds cannot account for the tumor response observed. The other PAHs in this group were benzo[e]pyrene, phenanthrene, anthracene, pyrene, fluoranthene, perylene, and coronene.

NOTE: Control animals were included in the experiment; however, whether they were vehicle or untreated controls was not specified, and their tumor incidence was not reported.

Source: Pfeiffer (1977).

TABLE III-16

DATA USED AND ESTIMATES OF RELATIVE POTENCY OBTAINED FROM SUBCUTANEOUS INJECTION STUDY WITH FEMALE MICE

Compound[a]	Dose (μg) (x)	Number Exposed	Number Mice with Tumors	Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
Low dose:					
B[a]P	6.25	100	35	0.2297	1.0
D[ah]A	2.35	100	37	0.6480	2.82
High dose:					
B[a]P	25.00	100	57	0.0964	1.0
D[ah]A	18.70	100	56	0.1264	1.31
Pseudo-control	0	600	42	--	--

[a]Abbreviations: B[a]P, benzo[a]pyrene; D[ah]A, dibenz[ah]anthracene.

Model: $P(x) = 1-\exp-A(1+SR_j x)^2$, $A = -\ln[1-42/600] = 0.0726$.

Source: Pfeiffer et al. (1977).

7. Bingham and Falk (1969)

Graded concentrations of B[a]P or benz[a]anthracene (B[a]A) were applied topically to the backs of C3H/He mice 3 times a week for 50 weeks at which time animals were sacrificed and local tumors were quantitated. B[a]P was dissolved in decalin, benz[a]anthracene was dissolved in toluene and 50 mg by weight of each solution was administered at each dosing; however, the method of application was not specified. Table III-17 shows the results of this experiment. No solvent controls were included. The identity of the tumors obtained was not specified by the authors and will be assumed to be carcinomas (E. Bingham, personal communication). From these results, benz[a]anthracene appears to be less potent than B[a]P, although the use of different solvents for each agent introduces a confounding factor that cannot be directly evaluated.

An extremely shallow dose-response relationship for B[a]A was obtained over a two order-of-magnitude dose range. The most likely explanation for this response is that the administered dose and the biologically active dose at the target site (reactive form of the agent at the site of action) are not linearly related because of saturation of metabolic activating systems or permeability. In this case, the responses obtained at the lowest exposure levels would be the most directly comparable between agents. The data used to obtain the relative potency estimate for B[a]A are the single levels of agent giving the lowest response for both B[a]P and B[a]A. The data used and the relative potency estimate derived are shown in Table III-18. Incorporation of the other exposure levels of B[a]A into the relative potency estimate would decrease the estimate for that agent.

000080

TABLE III-17

TUMOR INCIDENCE FOLLOWING DERMAL EXPOSURE OF MICE TO BENZO[A]PYRENE AND BENZ[A]ANTHRACENE

Compound[a]	Dose (% Concentration)	Number of Animals Bearing Malignant Tumors/Number of Animals	Tumor Incidence (%)
B[a]P	0.02	5/12	42
	0.002	0/20	0
	0.0002	0/21	0
	0.00002	0/18	0
B[a]A	1.0	5/29	17
	0.2	3/32	9
	0.02	1/18	6
	0.002	0/32	0

[a]Abbreviations: B[a]P, benzo[a]pyrene; B[a]A, benz[a]anthracene.

Source: Bingham and Falk (1969).

TABLE III-18

DATA USED AND ESTIMATE OF RELATIVE POTENCY OBTAINED FOR BENZ(A)ANTHRACENE FROM MOUSE SKIN PAINTING STUDY

Compound[a] Dose (% concentration (x)	No. Mice Exposed (Survived 50 weeks)	No. Mice with Malignant Tumor	Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
B[a]P:				
0.02	12	5	187.0	1.0
B[a]A:				
0.02	18	1	27.16	0.145[b]
0.20	32	3	5.13	0.027
1.0	29	5	1.81	0.0097
Control[c]:				
0	20	0	--	--

[a]Abbreviations: B[a]P, benzo[a]pyrene; B[a]A, benz[a]anthracene.

[b]Value used for relative potency estimate.

[c]Assumed value.

Model: $P(x) = 1\text{-}\exp\text{-}A(1 + SR_j x)^2$, $A = -\ln(1-0.5/21) = 0.024$.

Source: Bingham and Falk (1969).

000082

8. Van Duuren et al. (1966)

As part of an experiment to evaluate the tumorigenic potential of tobacco leaf and smoke constituents, several PAHs were tested for tumor initiating activity. The compounds were applied as single doses in 0.1 ml of solvent to the backs of female ICR/Ha Swiss mice. This treatment was followed by applications of 25 mg croton resin in acetone as a promoter three times a week for 63 weeks (or until death). The compounds tested and the experimental results obtained are presented in Table III-19. B[a]P is clearly more potent than any of the other PAHs tested.

The data used to obtain potency estimates for CH and B[b]F are shown in Table III-20. The response employed for the estimates was number of malignant tumor-bearing animals by the 63rd week of the test. It was not possible to combine the incidences of papillomas and carcinomas in this experiment since individual animal data were unavailable.

The necessity of using a promoter to obtain a carcinogenic response in the test system is a factor that would tend to reduce the credibility of the potency estimates if no other information was available; however, since carcinomas were observed, it was assumed that use of the promoter allowed the expression of cells that had undergone two PAH-induced transitions and therefore that use of the two-stage model was appropriate. The potency estimate for CH in this system is uncertain, since the response level is not clearly different from the croton resin control.

TABLE III-19

PAH-INDUCED SKIN TUMOR INITIATION IN MICE USING CROTON RESIN AS A PROMOTER

Compound[a]	Dose	Solvent	Papillomas: Number of Tumor-Bearing Animals/ Number of Animals	Papillomas: Tumor Incidence (%)	Carcinomas: Number of Tumor-Bearing Animals/ Number of Animals	Carcinomas: Tumor Incidence (%)
B[a]P	150 mg	0.1 ml acetone	20/20	100	6/20	30
CH	1 mg	0.4 ml acetone	16/20	80	2/20	10
B[b]F	1 mg	0.1 ml acetone	18/20	90	5/20	25
B[mm]F	1 mg	0.1 ml acetone	4/20	20	0/20	0
Control (promotion with acetone only)	--	--	5/20	25	1/20	5
Control (untreated)	--	--	0/100	0	0/20	0

[a]Abbreviations: B[a]P, benzo[a]pyrene; CH, chrysene; B[b]F, benzo[b]fluoranthene; B[mm]F, benzo[mm]fluoranthene.

Source: Van Duuren et al. (1966).

000080084

TABLE III-20

DATA USED AND ESTIMATES OF RELATIVE POTENCY OBTAINED FROM MOUSE SKIN INITIATION PROMOTION EXPERIMENT

Compound[a]	Dose[b] (mg)	No. Exposed	No. with Cancer by 63rd Week	Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
B[a]P	0.15	20	6	10.91	1.0
CH	1.0	20	2	0.433	0.040
B[b]F	1.0	20	5	1.368	0.125
Promotor control	0	20	1	--	--

[a]Abbreviations: B[a]P, benzo[a]pyrene; CH, chrysene; B[b]F, benzo[b]fluoranthene.

[b]PAH administration was followed by 25 mg croton resin applied three times weekly for 63 weeks.

Model: $P(x) = 1-\exp-A(1+SR_j x)^2$, $A = -\ln(1-1/20) = 0.0513$.

Source: Van Duuren et al. (1966).

9. Hoffmann and Wynder (1966)

Two different types of experiment were performed:

(1) "Complete" carcinogenesis experiments, in which PAHs were topically administered for 1 year and

(2) Initiation-promotion experiments, in which PAHs were administered for only 3 weeks, followed by application of a tumor-promoting agent for the duration of the experiment.

For the "complete" carcinogenicity evaluations, female Ha/ICR/mil Swiss albino mice received three weekly topical applications of B[a]P, benzo[ghi]perylene, or indeno(1,2,3-cd)pyrene for one year. The number of papillomas observed at each dose for each group is shown in Table III-21. B[a]P and benzo[ghi]perylene were dissolved in dioxane, and indeno(1,2,3-cd)pyrene was dissolved in acetone. No tumors were observed in the dioxane vehicle controls; no acetone control was included. The results show that B[a]P is much more potent as a carcinogen than either of the other PAHs tested in this model.

For the initiation-promotion experiments, ten doses of the same PAHs tested above were applied in dioxane 2 days apart to the backs of mice for a total dose of 0.25 mg per mouse and followed by 2.5% croton oil in acetone. The frequency and duration of croton oil administration were unclear. Papillomas were observed and the results of these experiments are found in Table III-22. Again, B[a]P was shown to be much more potent than either of the other PAHs tested.

For each of the compounds used in the first experiment, the lowest dose that gave a positive response was employed in the relative potency estimation. Since B[a]P gave a much higher response than the other PAHs and the slope appears to be very low, the potency estimate, if in error, is most likely

000086

TABLE III-21

CARCINOGENIC ACTIVITY OF SEVERAL PAHs ON MOUSE SKIN[b]

Compound[a]	Dose (% Concentration)	Number of Tumor-Bearing Animals/ Number of Animals	Tumor Incidence (%)
B[a]P	0.05	17/20	85
	0.1	19/20	95
IND	0.01	0/20	0
	0.05	0/20	0
	0.1	6/20	30
	0.5	7/20	35
B[ghi]P	0.05	1/20	5
	0.1	0/20	0

[a]Abbreviations: B[a]P, benzo[a]pyrene; IND, indeno(1,2,3- cd)pyrene; B[ghi]P, benzo[ghi]perylene.

[b]Papillomas were observed.

Source: Hoffmann and Wynder (1966).

TABLE III-22

INITIATION-PROMOTION ACTIVITY OF SEVERAL PAHs ON MOUSE SKIN

Compound[a]	Total Dose[b] (mg)	Number of Tumor-Bearing Animals/ Number of Animals	Tumor Incidence (%)
B[a]P	0.25	24/30	80
IND	0.25	5/30	17
B[ghi]P	0.25	2/27	7
Control[c]	0	2/30	7

[a]Abbreviations: B[a]P, benzo[a]pyrene; IND, indeno(1,2,3- cd)pyrene; B[ghi]P, benzo[ghi]perylene.

[b]Administration of PAHs was followed by 2.5% croton oil as a promoter.

[c]Croton oil was administered in the absence of PAH treatment.

Source: Hoffmann and Wynder (1966).

overestimated. These estimates were obtained using the one-hit model and are shown in Table III-23.

The initiation-promotion experiment yielded results that would tend to overestimate risk for two reasons. First, the responses for IND and B[ghi]P may be spurious, since they are not statistically elevated over the control response; second, the response for B[a]P is in a high range in which a lower percent of the applied dose may be converted to a biologically active target dose, as compared with lower applied dose levels. The relative potency estimates obtained from this experiment are displayed in Table III-24, and were obtained using the one-hit model as well.

10. Wynder and Hoffmann (1959)

As part of a study of the carcinogenicity of tobacco and its constituents, several PAHs were tested as complete carcinogens on the skin of mice. Female Swiss mice received various concentrations of the test substances dissolved in acetone three times a week applied to their backs with a brush throughout their lifetimes. Table III-25 shows the compounds tested, concentrations used, and the results of these experiments. No solvent control group was reported; however, since no papillomas or carcinomas were obtained for several of the PAHs tested, a solvent control group would most likely have been negative as well. The results indicate that B[a]P and dibenz[ah]anthracene are somewhat similar in potency in this model and chrysene is much less potent.

In this experiment, the survival rate was much less for B[a]P-treated animals than for those receiving the other PAHs. To adjust for the difference in survival rates, the relative potency estimates will be based on comparisons of tumor rates observed at 14 months using number of animals bearing carcinomas

TABLE III-23

DATA USED AND ESTIMATES OF RELATIVE POTENCY OBTAINED FROM MOUSE SKIN COMPLETE CARCINOGENESIS EXPERIMENT

Compound[a]	Dose (% Concentration) (x)	No. of Animals Exposed	No. of Tumor-Bearing Animals	Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
B[a]P	0.05	20	17	1554.4	1.0
IND	0.10	20	6	138.0	0.089
B[ghi]P[b]	0.05	20	1	22.6	0.015
Control	0	20	0	--	--

[a]Abbreviations: B[a]P, benzo[a]pyrene; IND, indeno(1,2,3- cd)pyrene; B[ghi]P, benzo[ghi]perylene.

[b]Response was not dose related.

Model: $P(x) = 1-\exp-A(1+SR_j x)$, $A = -\ln(1-0.5/21) = 0.0241$.

Source: Hoffmann and Wynder (1966).

000090

TABLE III-24

DATA USED AND ESTIMATES OF RELATIVE POTENCY OBTAINED FROM MOUSE SKIN INITIATION-PROMOTION EXPERIMENT

Compound[a]	Dose[b] (ng)	No. of Animals Exposed	No. of Tumor-Bearing Animals	Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
B[a]P	0.25	30	24	89.30	1.0
IND[c]	0.25	30	5	6.57	0.074
B[ghi]P[c]	0.25	27	2	0.46	0.005
Control	0	30	2	--	--

[a]Abbreviations: B[a]P, benzo[a]pyrene; IND, indeno(1,2,3- cd)pyrene; B[ghi]P, benzo[ghi]perylene.

[b]The PAH dose was followed by 2.5% croton oil as a promoter.

[c]Response was not statistically elevated over control.

Model: $P(x) = 1-\exp-A(1+SR_j x)$, $A = -\ln(1-2/30) = 0.0690$.

Source: Hoffmann and Wynder (1966).

TABLE III-25

CARCINOGENIC ACTIVITY OF PAHs ON MOUSE SKIN

Compound[a]	Dose (% Concentration)	Incidence of Papillomas (%)	Incidence of Carcinomas (%)
B[a]P	0.001	43	3
	0.005	73	63
	0.01	95	95
D[ah]A	0.001	30	30
	0.01	95	90
	0.1	90	75
CH	1.0	45	40
AN	10.0	0	0
PY	10.0	0	0
FL	0.1	0	0

[a]Abbreviations: B[a]P, benzo[a]pyrene; D[ah]A, dibenz[ah]anthracene; CH, chrysene; AN, anthracene; PY, pyrene; FL, fluoranthene.

Source: Wynder and Hoffmann (1959).

as the response. The data used and the relative potency estimates generated for D[ah]A and CH are shown in Table III-26. Estimates obtained from this experiment are considered to be of high quality because of two factors: First, the response spans the full range from zero to almost 100% for both B[a]P and D[ah]A; second, the model is consistent with the data for the full range of B[a]P exposures.

11. Bryan and Shimkin (1943)

A study was conducted to establish dose-response curves for several PAHs following subcutaneous injection into the right axilla of male C3H mice. The solvent used was tricaprylin, although no solvent control group was reported. Animals were palpated frequently following a single injection of the test compound in order to detect tumors; 99% of those detected proved to be spindle-cell sarcomas (the remaining 1% were carcinomas or mixed). Animals were observed throughout their lifetimes. Table III-27 shows the results obtained for B[a]P and dibenz[ah]anthracene. Dibenz[ah]anthracene appears to be somewhat more potent than B[a]P in this system.

Although this classic experiment with 12 exposure levels per compound gave a full range of response for each compound, several factors make it difficult to interpret. An exposure level of 0.0039 mg appears to have been included for both B[a]P and D[ah]A; however, no results were reported for this dose, and no explanation for the omission was included. Vehicle control response levels were also not reported. This problem is exacerbated by the abnormality of a positive response at the lowest exposure level for B[a]P (0.00195 mg) in the absence of a response at exposure levels 4, 8, or 16 times higher. For the analysis conducted here, the data will be restricted to the monotonically

TABLE III-26

DATA USED AND RELATIVE POTENCIES OBTAINED FROM MOUSE SKIN CARCINOGENESIS EXPERIMENT

Compound[a] Dose (% concentration)(x)	No. Animals Exposed	No. Animals with Carcinomas by 14 Months		Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
		Observed	Predicted		
B[a]P:				822.0	1.0
0.001	29	0	2.23		
0.005	30	19	14.01		
0.01	20	19	17.42		
D[ah]A:				912.0	1.11
0.001	20	2	1.69		
0.01	20	18	18.31		
0.1	([b])	([b])	([b])		
CH:					
1.0	20	8	8.00	3.60	0.0044
Control:					
0	20	0	0.48	--	--

[a]Abbreviations: B[a]P, benzo[a]pyrene; D[ah]A, dibenz[ah]anthracene; CH, chrysene.

[b]Omitted because of lack of fit.

Model: $P(x) = 1-\exp-A(1+SR_j x)^2$, $A = -\ln(1-0.5/21) = 0.0241$.

Source: Wynder and Hoffmann (1959).

TABLE III-27

TUMOR INCIDENCE OBSERVED IN MICE FOLLOWING SUBCUTANEOUS INJECTION OF BENZO[A]PYRENE OR DIBENZ[AH]ANTHRACENE

Compound[a]	Dose (mg)	Number of Tumor-Bearing Animals/ Number of Animals	Tumor Incidence (%)
D[ah]A	0.00195	2/79	3
	0.0078	6/40	15
	0.0156	6/19	32
	0.031	16/21	76
	0.062	20/20	100
	0.125	21/23	91
	0.25	19/21	90
	0.5	20/21	95
	1.0	22/22	100
	2.0	19/19	100
	4.0	17/20	85
	8.0[b]	16/21	76
B[a]P	0.00195	2/81	2
	0.0078	0/40	0
	0.0156	0/19	0
	0.031	0/16	0
	0.062	4/20	20
	0.125	15/19	79
	0.25	14/21	67
	0.5	19/19	100
	1.0	18/20	90
	2.0	19/19	100
	4.0	16/19	84
	8.0	20/21	95

[a]D[ah]A, dibenz[ah]anthracene; B[a]P, benzo[a]pyrene.

[b]Volume of solvent was 0.5 ml for the 8.0 mg dose of D[ah]A and 0.25 ml for all other doses.

Source: Bryan and Shimkin (1943).

increasing portion of the curve, which is similar to the approach taken by the authors in their fitting of the probit model. The only difference is that the authors of the study included the 0.125 mg level for D[ah]A in their analysis, which would have the effect of causing a slight decrease in the relative potency estimates and yielding a worse fit. In addition, for the purpose of the present analysis, the assumption will be made that the lowest dose of B[a]P (0.00195 mg) actually represents a control level. This assumption is consistent with the observed response data. The dose-response data used in the potency determination are shown in Table III-28. Under the conditions of this experiment, the potency of D[ah]A is estimated to be 4.5 times that of B[a]P.

B. SURROGATE APPROACHES: DNA ADDUCT FORMATION

Interaction between reactive PAH metabolites and DNA has been established as a necessary (albeit not sufficient) event for PAH-induced tumorigenesis (Gelboin 1980, Weinstein et al. 1978). The formation of B[a]P diol-epoxide DNA adducts has been shown to be linearly related to administered dose for several organs in rats and mice (Pereira et al. 1979, Adriensens et al. 1983), and related to a quadratic increase in tumor formation (Hennings and Yuspa 1985, Lee and O'Neill 1971). It is reasonable to suggest that in the absence of quantitative tumor dose-response information for carcinogenic PAHs that can be readily compared to B[a]P, the formation of DNA adducts could be used as a surrogate basis for establishing relative potency estimates.

It is recognized that the DNA adduct level in a tissue is not directly related to carcinogenic response. Other factors such as the DNA repair and cell turnover rates in the target tissue can have a greater effect than the actual number of adducts on the ultimate probability of tumor formation.

000096

TABLE III-28

DATA USED TO OBTAIN A RELATIVE POTENCY ESTIMATE FOR DIBENZ[AH]ANTHRACENE FROM A SUBCUTANEOUS INJECTION EXPERIMENT

Compound[a] Dose (mg)	No. of Mice Exposed	Tumors		Estimated Transition Rate Parameter (SRj)	Estimated Relative Potency (Rj)
		Observed	Expected		
B[a]P:				42.8	1.0
0.031	16	0	2.05		
0.062	20	4	5.73		
0.125	19	15	12.15		
D[ah]A:				192.4	4.50
0.0078	40	6	5.85		
0.0156	19	6	6.33		
0.031	21	16	14.84		
0.062	20	20	19.71		
Pseudo-control[b]:					
0.00195	160	4	4.0	--	--

[a]Abbreviations: B[a]P, benzo[a]pyrene; D[ah]A, dibenz[ah]anthracene.

[b]Combined response at a dose level of 0.00195 mg of B[a]P or D[ah]A.

Model: $P(x) = 1-\exp-A(1+SR_j x)^2$, $A = -\ln(1-4/160) = 0.0253$.

Source: Bryan and Shimkin (1943).

However, under the assumptions of the current model of PAH tumor induction, the transition rate is linearly related to the DNA adduct formation rate. As a result, the relative potency measured as the ratio of DNA adducts per unit administered dose for two PAHs is assumed to be independent of tissue type, route of exposure, and test system as long as the ratios of the levels ofdistribution, metabolism, and elimination for B[a]P and the PAH in question remain unchanged. Furthermore, DNA adduct formation may reflect cancer risk from PAHs at low, environmental levels of exposure more accurately than dose-response curves based on tumor rates produced at high levels of exposure in the laboratory, when additional biological mechanisms may play a role in enhancing or suppressing tumor rates.

This section describes the studies in which the levels of DNA adduct formation were determined for several PAHs, including B[a]P. The studies are described in reverse chronological order, and relative potencies are determined for the PAHs tested.

1. Reddy et al. (1984)

A total of 28 compounds were tested for ability to bind to DNA using a very sensitive technique (detection limit of 1 aromatic DNA adduct in 10^8 normal adducts) for the detection of DNA adducts employing ^{32}P-postlabelling following administration to rats and mice. The PAHs tested were applied to the dorsal skin of female BALB/c mice as four doses of 1.2 umole each in acetone at 0, 6, 30, and 54 hours. Animals were sacrificed 24 hours after the last treatment. DNA was isolated from treated skin, digested, enzymatically labelled with ^{32}P (using T4 polynucleotide kinase and [γ-^{32}P]ATP), resolved using thin-layer chromatography and detected using autoradiography and

000098

scintillation counting. Relative adduct labelling was determined by comparing the radioactivity of the adducts to that of normal nucleotides. Absolute levels of adduct formation were not given, and results were expressed as the ranges depicted in Table III-29; as a result, point estimates of relative potency are not obtainable. The only quantitative conclusion that can be drawn from this study is that B[a]P is the same potency or up to three orders of magnitude more potent than the other PAHs tested. The authors noted, however, that there was a good correlation between adduct level and carcinogenic potency on mouse skin (although D[ah]A has been found to be more potent than B[a]P in some cases) and that the noncarcinogens failed to form detectable adducts.

2. Phillips et al. (1979)

The levels of binding of seven radioactively-labelled PAHs to mouse skin DNA following topical application were determined. One μmole of each PAH in acetone (see Table III-30 for compounds tested) was applied to the backs of male C57BL mice and the animals were sacrificed at the time of maximal DNA binding (from 19 to 72 hours following administration, depending on the compound, which for the purpose of the model is assumed to be proportional to steady-state binding levels under conditions of continuous exposure). DNA was isolated, hydrolyzed enzymatically and chromatographed; levels of PAH binding were determined from the amount of radioactivity eluting with the fractions containing DNA-bound PAHs. The extent of DNA binding for the PAHs tested is shown in Table III-31.

7,12-Dimethylbenz[a]anthracene, considered to be the most carcinogenic PAH, was found to bind to DNA to the greatest extent; the weakest carcinogens, benz[a]anthracene and dibenz[a,c]anthracene, were bound the least. The extent

TABLE III-29

RELATIVE DNA BINDING OF PAHs TO MOUSE SKIN

Compound[a]	Relative Level of Binding[b]
B[a]P	+++
B[a]A	++
D[ah]A	++
B[ghi]P	++
CH	++
AN	ND
PY	ND

[a]Abbreviations: B[a]P, benzo[a]pyrene; B[a]A, benz[a]anthracene; D[ah]A, dibenz[ah]anthracene; B[ghi]P, benzo[ghi]perylene; CH, chrysene; AN, anthracene; PY, pyrene.

[b]Relative binding levels: +++, 1 adduct in 10^4 - $5x10^5$ nucleotides; ++, 1 adduct in $5x10^5$ - 10^7 nucleotides.

ND = not detected; detection level = 1 adduct in 10^8 nucleotides.

Source: Reddy et al. (1984).

000100

TABLE III-30

EXTENT OF PAH BINDING TO DNA

Compound[a]	Extent of Reaction with DNA (pmole/mg DNA)	Estimated Relative Potency $\frac{\text{pmoles DNA-PAH/mg DNA}}{\text{pmoles DNA-B[a]P/mg DNA}}$ (R_j)
B[a]A	2	0.07
D[ac]A	10	0.37
D[ah]A	15	0.56
7-MBA	25	0.93
3-MC	25	0.93
B[a]P	27	1.0
7,12-DMBA	43	1.6

[a]Abbreviations: B[a]A, benz[a]anthracene; D[ac]A, dibenz[ac]anthracene; D[ah]A, dibenz[ah]anthracene; 7-MBA, 7-methylbenz[a]anthracene; 3-MC, 3-methylcholanthrene; B[a]P, benzo[a]pyrene; 7,12-DMBA, 7,12-dimethylbenz[a]anthracene.

Source: Phillips et al. (1979).

TABLE III-31

BINDING OF PAHs TO DNA IN VITRO

Compound[a]	Extent of Reaction with DNA (μmoles/mole DNA)	Estimated Relative Potency $\frac{\text{μmoles DNA-PAH/mole DNA}}{\text{μmoles DNA-B[a]P/mole DNA}}$ (R_j)
B[a]P	1.41	1.0
D[ah]A	0.44	0.31
B[a]A	0.70	0.50
PY	0.31	0.22
PH	0.05	0.04

[a]Abbreviations: B[a]P, benzo[a]pyrene; D[ah]A, dibenz[ah]anthracene; B[a]A, benz[a]anthracene; PY, pyrene; PH, phenanthrene.

Source: Grover and Sims (1968).

000102

of binding of the intermediate compounds also appeared to correlate well with what are thought to be their carcinogenic potencies. B[a]P is approximately two orders of magnitude and twice as potent, respectively, as the other PAHs tested, benz[a]anthracene and dibenz[ah]anthracene.

3. Grover and Sims (1968)

The extent to which PAHs react with DNA was determined using an *in vitro* system comprised of a microsomal enzyme activation system, salmon sperm DNA, and 0.5 mg of radioactively labeled PAHs. Following incubation for one hour, the amount of PAH bound to DNA was determined using scintillation counting. The PAHs tested and results obtained are shown in Table III-30. B[a]P was again shown to be the most active PAH; it was at least twice as potent as the other indicator PAHs.

Establishing a credible biological basis for use of an *in vitro* system on which to base relative potency estimates is difficult, since many of the factors affecting adduct formation *in vivo*, such as DNA repair, are absent; however, the information derived from this system may be used in qualitative support of other data.

C. SELECTION OF RELATIVE POTENCY ESTIMATES

In the previous sections, relative potency estimates were derived for carcinogenic PAHs. These estimates were based on the results obtained in fourteen separate studies using six different experimental systems. The potency estimates and the systems from which they were derived are summarized in Table III-32. Note that up to seven independent estimates of relative potency were obtained for the PAHs.

TABLE III-32

SUMMARY OF RELATIVE POTENCY ESTIMATES
FOR INDICATOR PAHs

Compound	Test System					
	Mouse Skin Carcinogenesis	Subcutaneous Injection into Mice	Intrapulmonary Administration to Rats[a]	Initiation-Promotion on Mouse Skin	Intraperitoneal Injection in Newborn Mice[a]	DNA Adduct Formation[l]
Anthanthrene			0.320			
Benzo[a]pyrene	1.0	1.0	1.0	1.0	1.0	1.0
Benzo[e]pyrene			0.004			
Benz[a]anthracene	0.145[c]				0.037,0.524,0.496[k]	0.07
Benzo[b]fluoranthene	0.167[b]		0.140	0.256[h],0.125[i]	0.232,1.067,0.874[j]	
Benzo[j]fluoranthene	0.061[b]			0.048[h]	0.320,0.471,0.450[j]	
Benzo[k]fluoranthene	0.020[b]		0.066	0.022[h]	0.040,0.097,0.044[j]	
Benzo[ghi]perylene	0.015[e]		0.022	0.005[e]		
Chrysene	0.0044[d]			0.040[i]	0.125,0.33[k]	
Cyclopentadieno[cd]pyrene	0.023[b]					
Dibenz[ah]anthracene	1.11[d]	2.82[e],4.50[f]				0.56
Indeno[1,2,3-cd]pyrene	0.021[b],0.189[c]		0.232	0.074[e]	0.013[j]	
Pyrene					0.081,0.050,0.586[k]	

[a] Bingham and Falk (1969).
[b] Hahn et al. (1980).
[c] Hoffmann and Wynder (1966).
[d] Wynder and Hoffmann (1959).
[e] Pfeiffer (1977).
[f] Bryan and Shimkin (1943).
[g] Deutsch-Wenzel et al. (1983).
[h] LaVoie et al. (1982).
[i] Van Duuren et al. (1966).
[j] LaVoie et al. (1987).
[k] Wislocki et al. (1986).
[l] Phillips et al. (1979).
[m] Where more than one potency estimate is shown, they were derived from the same study using different tumor types as end points.

A composite estimate of the relative potency is required for each PAH. Major qualitative differences exist between the studies from which the estimates are derived concerning their precision and applicability to continuous human ingestion or inhalation exposure. One approach to obtaining composite estimates would be to obtain a numerical estimate based upon a weighted average of the studies. Alternatively, the most relevant study could be selected based upon certain objective criteria and any particular unique condition that is noted. A provisional selection is presented here; a more appropriate method of selection might entail a consensus of opinion from a panel of recognized experts employing a formal system of weighing such as that suggested by DuMouchel and Harris (1983).

Some of the criteria considered for making the selection are:

(1) The relevance of the route of exposure employed in the bioassay to anticipated human exposure.

(2) The duration of exposure (longer exposures are preferred).

(3) The sample sizes used.

(4) The inclusion of a vehicle control group.

(5) A dose-response relationship obtained that is consistent with the underlying theoretical model, which is linear-quadratic in form.

(6) The extent to which the observed responses cover the entire response range.

(7) The absence of additional complicating variables such as promoters.

The extent to which each of the studies fulfills these criteria, in a relative sense, is indicated in Table III-33. The scale used is as follows: +++, above average; ++, average; +, below average.

Based on this information and some factors specific to individual studies, the relative potency estimates that are considered to have the most reliable basis were selected and are shown in Table III-34.

TABLE III-33

EXTENT TO WHICH STUDIES USED TO DERIVE RELATIVE POTENCY ESTIMATES FULFILL APPLICABILITY CRITERIA[a]

Reference	Test System	Route of Exposure	Continuous Exposure	Sample Size	Quality of Controls Used	Absence of Complicating Factors	Full Range of Responses	Fits Dose-Response Model for 2 or More Doses
LaVoie et al. 1987	Intraperitoneal injection in newborn mice	•	•	++	++	+	+	+
Wislocki et al. 1986	Intraperitoneal injection in newborn mice	•	+	+++	++	+	+	•
Deutsch-Wenzel et al. 1983	Intrapulmonary administration	+++	+++	++	+++	++	+++	+++
LaVoie et al. 1980	Initiation-promotion on skin	++	•	+	++	•	+	+
Habs et al. 1980	Skin carcinogenesis	++	+++	++	+++	++	++	+++
Pfeiffer 1977	Subcutaneous injection	+	+	+++	•	++	+++	•
Bingham and Falk 1969	Skin carcinogenesis	++	+++	+	+	++	+	•
Van Duuren et al. 1966	Initiation-promotion on skin	+	•	+	+++	+	+	•
Hoffmann and Wynder 1966	Skin carcinogenesis or	++	+++	•	•	++	+	•
	Initiation-promotion on skin	++	•	++	++	•	+	+
Wynder and Hoffmann 1959	Skin carcinogenesis	++	+++	+	•	++	+++	+++
Bryan and Shimkin 1943	Subcutaneous Injection	•	+	++	•	++	+++	+++

[a]Criteria are discussed more fully in the text.

TABLE III-34

SUMMARY OF RELATIVE POTENCY ESTIMATES
DERIVED FOR PAHs

Anthanthrene	0.320[a]
Benzo[a]pyrene	1.0
Benzo[e]pyrene	0.004[a]
Benz[a]anthracene	0.145[b]
Benzo[b]fluoranthene	0.140[a]
Benzo[j]fluoranthene	0.061[c]
Benzo[k]fluoranthene	0.066[a]
Benzo[ghi]perylene	0.022[a]
Chrysene	0.0044[d]
Cyclopentadieno(cd)pyrene	0.023[c]
Dibenz[ah]anthracene	1.11[d]
Indeno[1,2,3-cd]pyrene	0.232[a]
Pyrene	0.081[e]

[a]Deutsch-Wenzel et al. (1983).

[b]Bingham and Falk (1969).

[c]Habs et al. (1980).

[d]Wynder and Hoffmann (1959).

[e]Wislocki et al. (1986).

The basis for each of the selections in Table III-34 is as follows:

Anthanthrene. Only one study was performed from which a relative potency could be derived.

Benz[a]anthracene. The newborn mouse bioassay (Wislocki et al. 1986) provided unreliable data; the mouse skin bioassay results (Bingham and Falk 1969) are more consistent.

Benzo[b]fluoranthene and Benzo[k]fluoranthene. Both the mouse skin carcinogenesis (Habs et al. 1980) and intrapulmonary administration (Deutsch-Wenzel et al. 1983) studies were found to be of comparable quality; the estimates based on the latter study were chosen because of the relevance of this route of exposure to that of the B[a]P study on which the dose-response model is based. Both studies are more reliable than the newborn mouse bioassay (LaVoie et al. 1987). This choice results in an approximately 15% lower estimate for B[b]F, but in a 3-fold greater estimate for B[k]F.

Benzo[j]fluoranthene. This compound was tested in the newborn mouse bioassay (LaVoie et al. 1987) and for its skin carcinogenicity (Habs et al. 1980); the potency based on the latter study will be used since it is of greater quality.

Benzo[ghi]perylene. IARC (1983) considers this compound to be a non-carcinogen. However, evidence from several studies indicates that it has weak carcinogenic potential. Therefore, for the purpose of conservatism, B[ghi]P will be considered to be carcinogenic and a relative potency estimate derived. The intrapulmonary administration study (Deutsch-Wenzel et al. 1983) proved to fulfill many more of the adequacy criteria than did the skin carcinogenesis study (Hoffmann and Wynder 1966), therefore, the former was used as the basis for estimation.

000108

Chrysene. The skin carcinogenesis study (Wynder and Hoffmann 1959) was considered to be more appropriate than the initiation-promotion study (Van Duuren et al. 1966) or the newborn mouse bioassay (Wislocki et al. 1986) because of the additional variables introduced in the latter two types of study. Furthermore, the tumor response observed in the Van Duuren et al. (1966) study was not statistically significant.

Cyclopentadieno(cd)pyrene. This compound was tested only for mouse skin carcinogenesis (Habs et al. 1980).

Dibenz[ah]anthracene. On the basis of the extent to which each of the studies of this compound fulfill the criteria for study adequacy, the estimate derived from the skin carcinogenesis study (Wynder and Hoffmann 1959) will be used. Estimates of two to four times higher were obtained from studies that employed subcutaneous injection as a method of administration; however this system is less relevant to human exposure.

Indeno[1,2,3-cd]pyrene. As was true for the benzofluoranthenes, both the Habs et al. (1980) skin carcinogenesis study and the Deutsch-Wenzel et al. (1983) intrapulmonary administration study fulfill more of the adequacy criteria than the newborn mouse bioassay (LaVoie et al. 1987). The estimate based on the Deutsch-Wenzel et al. (1983) study was chosen because of its relevance to the route of exposure used in the study of B[a]P from which the dose-response model was derived. Five independent potency estimates were made and the largest chosen. Consistency between estimates was apparent; the three largest estimates varied by no more than a factor of one-third.

Pyrene. This compound was tested only in the newborn mouse bioassay (Wislocki et al. 1986). The estimates are inconsistent in the liver, varying by an order of magnitude. The estimate based on lung tumors was chosen as an alternative.

IV. ESTIMATION OF COMPARATIVE POTENCIES FOR POLYCYCLIC AROMATIC HYDROCARBONS USING THE LINEARIZED MULTISTAGE MODEL

The linearized multistage model can be used in several ways to obtain estimates of relative cancer potency; as a result of serious practical and theoretical problems, however, the utility of such approaches is limited. One measure of potency of a particular PAH is the reciprocal of its exposure level that results in a specified tumor response rate. For example, the reciprocal of the exposure level that induced a 10% tumor response based on point estimates from the multistage model is used by EPA as a measure of potency for reportable quantities of potential carcinogens (Cogliano 1986). The ratio of two potencies so calculated might be used to obtain a relative potency estimate. Relative potencies calculated in this manner may vary at different response levels and as a result, are unreliable because they are highly dependent on the tumor response level chosen. For response levels that are much smaller than those in the observable range (i.e., increases in tumor rates expected at environmental levels of exposure), a very minor shift in the tumor response data in an experiment can be shown to lead to orders of magnitude changes in the relative potency estimates at environmental levels using this approach. As a result, this approach has little to recommend it for assessing low-dose potencies over a wide range of potential environmental exposure levels.

Using the 95% upper bound linear term obtained from the multistage model to derive relative potencies instead of point estimates has a superficial level of appeal. This is the measure of potency most often used by EPA in various regulatory settings (EPA 1986c). The ratios of upper bound estimates are not good measures of relative potency, however, since they are sometimes more

000110

dependent upon sample size considerations than on tumor response levels. Since unit risks (i.e., 95% upper bound linear terms) are often used to represent the potencies of carcinogens at environmental levels of exposure, using them to estimate relative potencies is an informative exercise nonetheless.

Using the tumor data from Section III, the parameters in the linearized multistage model were estimated and a Chi-square goodness-of-fit statistic obtained. The parameter estimates and χ^2 values are shown in Appendix B. In cases where the models did not fit the data, as measured by the approximate χ^2 goodness-of-fit test, the tumor rate obtained at the highest dose was omitted and the models were fitted to the reduced data set.

Comparison of the results in Appendices B and C with the corresponding results for the one- or two-stage models presented in Section III reveals a number of similarities and differences. The multistage model provided adequate fits to the data in all but four cases. In all four of these cases, the two-stage model also fitted poorly to the full data sets and calculations of relative potency were based on selected subsets of the data (Tables III-12, III-16, III-22, and III-28); in at least of two of these cases, the multistage model gave adequate fits to similar subsets. In a number of cases, the multistage model yielded a linear-quadratic dose-response relationship similar to that assumed in the two-stage model. In other cases, however, the multistage model included only a linear term or the last term in the nth degree polynomial. In several cases, the multistage model yielded slightly better fits to the data than the two-stage model (e.g., Tables C-1, C-3, C-7, and C-9). However, these "improvements" in fit resulted from the larger numbers of parameters in the multistage model with resulting variability in the number of terms (i.e., in the shape of the dose-response curve). With only two

exceptions, the two-stage model yielded reasonable fits to the data after elimination of one or more high-dose terms. The exceptions are D[ah]A in Table III-15 and both D[ah]A and B[a]P, shown in Table III-27; in these cases a multistage model could not fit the data either. Thus, the available dose-response data are not sufficient to determine which of these two models provides the better description of the data. The two-stage model is preferred for parameter estimation, for two reasons: (a) it has the same form in all cases, whereas the multistage model invokes different numbers of stages to fit different data sets, making relative potency estimates dose-dependent; and (b) it is based on a specific model of the two affected transitions, which has strong empirical and theoretical support in the case of PAHs, whereas the multistage model leaves both the nature and the number of the affected transitions unspecified.

The 95% upper bound linear term was used to obtain relative potency estimates, as shown in Appendix B. The ratio of these parameters to that derived for B[a]P yields the relative potency estimates.

Table IV-1 summarizes the relative potency estimates derived for a large sample of the PAHs from the bioassay data in Section III using the multistage model 95% linear term upper bounds (q_1^*). The estimates obtained using the two-stage model are shown in parentheses for comparison. To evaluate these estimates and their consistency as compared to those obtained using the two-stage model, the following analyses were conducted.

Obtaining average relative potencies is an approach that appeals to some investigators instead of attempting to select the best study on which to base the relative potency estimates. To illustrate the results of this approach, the geometric means of the potencies obtained for each PAH using both models

000112

TABLE IV-1

ESTIMATES OF RELATIVE POTENCY DERIVED USING THE MULTISTAGE AND TWO-STAGE MODELS[a]

Experimental Study	B[a]P	B[b]F	B[j]F	CP	B[k]F	IND	B[ghi]P	D[ah]A	B[a]A	CH
Deutsch-Wenzel et al. (1983)	[b]1(1)	0.105 (0.140)	--	--	0.085 (0.066)	0.246 (0.232)	[b]0.025 (0.022)	--	--	--
LaVoie et al. (1982)	1(1)	[b]0.562 (0.258)	[b]0.134 (0.023)	--	0.0236 (0.022)	--	--	--	--	--
Habs et al. (1980)	[b]1(1)	[b]0.201 (0.167)	0.0648 (0.061)	0.0268 (0.023)	0.0279 (0.020)	0.0302 (0.021)	--	--	--	--
Pfeiffer (1977)	[b]1(1)	--	--	--	--	--	--	[b]1.17 (2.82)	--	--
Bingham and Falk (1969)	1(1)	--	--	--	--	--	--	--	0.0137 (0.145)	--
Van Duuren et al. (1966)	1(1)	<0.001 (0.125)	--	--	--	--	--	--	--	<0.001 (0.040)
Hoffmann and Wynder (1966)	1(1)	--	--	--	--	[b]0.0292 (0.189)	0.0250 (0.015)	--	--	--
Hoffmann and Wynder (1966)	1(1)	--	--	--	--	0.131 (0.074)	0.0665 (0.085)	--	--	--
Wynder and Hoffmann (1959)	1(1)	--	--	--	--	--	--	[b]4.05 (1.11)	--	0.0132 (0.0044)
Bryan and Shimkin (1943)	1(1)	--	--	--	--	--	--	[c]0.488 (4.50)	--	--

[a]Estimates obtained using the two-stage model are shown in parentheses.

[b]Without high dose group.

[c]Did not substantially change after dropping high dose group.

Abbreviations: B[a]P, benzo[a]pyrene; B[b]F, benzo[b]fluoranthene; B[j]F, benzo[j]fluoranthene; CP, cyclopentadieno[c,d]pyrene; B[k]F, benzo[k]fluoranthene; IND, indeno[c,d]pyrene; B[ghi]P, benzo[ghi]perylene; D[ah]A, dibenz[ah]anthracene; B[a]A, benz[a]anthracene; CH, chrysene.

000113

6395

are shown in Table IV-2 for all PAHs that have three or more independent estimates. Also displayed are the geometric means of each relative potency for each method of estimation and for methods of estimation among PAHs. The geometric means for the same estimation methods indicate that on average, both approaches yield comparable results.

An additional comparison of relative potencies from the "best" studies (see Section III) using both estimation methods is shown in Table IV-3. For these estimates, the two-stage approach yielded relative potencies close to those obtained using the multistage method. This comparison amplifies the assertion that the general two-stage approach has not introduced a selective bias that would uniformly reduce risk. In view of the superior mathematical and biological bases for the two-stage model as compared to the linearized multistage upper bound procedure, the comparative potency estimates obtained using the two-stage model are recommended.

000114

TABLE IV-2

GEOMETRIC MEANS OF RELATIVE POTENCIES BY AGENT AND ESTIMATION METHOD

PAH	Multistage	Two-Stage
B[b]F	0.228	0.182
B[k]F	0.0384	0.0307
B[ghi]P	0.0346	0.0118
IND	0.0729	0.0909
D[ah]A	1.323	2.415
Geometric Mean	0.1239	0.1077

Abbreviations: B[b]f, benzo[b]fluoranthene; B[k]F, benzo[k]fluoranthene; IND, indeno[c,d]pyrene; B[ghi]P, benzo[ghi]perylene; D[ah]A, dibenz[ah]anthracene.

TABLE IV-3

COMPARISON OF RELATIVE POTENCY ESTIMATES DERIVED FROM THE "BEST" STUDIES USING THE TWO-STAGE AND MULTISTAGE LINEAR 95% UPPER BOUND METHODS

PAH	Two-Stage	95% Upper Bound
B[a]P	1.0	1.0
B[a]A	0.145	0.014
B[b]F	0.140	0.105
B[j]F	0.061	0.0648
B[k]F	0.066	0.085
B[ghi]P	0.022	0.025
CH	0.0044	0
D[ah]A	1.11	4.05
IND	0.232	0.246

Abbreviations: B[a]P, benzo[a]pyrene; B[b]f, benzo[b]fluoranthene; B[j]F, benzo[j]fluoranthene; B[k]F, benzo[k]fluoranthene; IND, indeno[c,d]pyrene; B[ghi]P, benzo[ghi]perylene; D[ah]A, dibenz[ah]anthracene; B[a]A, benz[a]anthracene; CH, chrysene.

000116

V. LIMITED VALIDATION OF THE COMPARATIVE POTENCY APPROACH

This chapter describes how the appropriateness of the two-stage model and the accuracy of the comparative potency estimates for PAHs can be partially validated using experimental data from the literature. Tumor rates predicted by the two-stage model and comparative potencies developed in this report can be compared to the actual tumor rates observed in experiments using combinations of PAHs to induce cancer in laboratory animals. The criteria for a useful study for such a comparison are discussed below, and a study in which PAHs were tested for carcinogenesis using mice is evaluated. The model predicts tumor rates reasonably well in this experiment at lower doses, where deviations due to biological interactions are less likely.

An unbiased, sensitive, independent validation of the predictions of the low-dose additive two-stage or any other low-dose extrapolation model is not possible using currently available experimental techniques. The tumor rates predicted by the model in the low-dose range are far below those that can be practically measured using animal bioassays. As a result, direct experimental evidence for the model must be obtained at exposure levels far in excess of those expected in the environment. Use of high experimental levels of exposure, however, can lead to biological interactions that would not be expected to occur at environmental levels and that result in deviations from what is predicted.

Among the more important interaction mechanisms anticipated at high doses are exposure saturation and enzyme saturation. In the first case, PAHs are applied to mouse skin at such high levels that they accumulate on the surface and are absorbed at relative rates that are lower than those at lower doses.

As a result, higher doses administered to the animal are proportionally greater than that which is actually absorbed and predicted to reach target sites, leading to overpredictions of tumor risk at high doses. In the second case, if the levels of metabolizing enzymes are insufficient to convert all of the carcinogenic PAHs in a mixture to their reactive derivatives, the number of reactive derivatives will be fewer when PAHs are administered together as a mixture than would be predicted for the sum of the PAHs had they been administered separately; tumor rates would consequently be less than those predicted by the hypothesis of dose-additivity.

An additional factor that makes direct verification of the predictions of the model difficult is that the validation is dependent upon the combined exposure experiments that are available in the literature. Such experiments do not include all the PAHs for which relative potency estimates have been obtained. In spite of these limitations, using the low-dose additive two-stage model and relative potency estimates to predict a carcinogenic response to multiple PAHs can provide partial verification of the approach.

1. Criteria for Useful Combined Exposure Studies

For a study to provide a useful evaluation of the comparative potency method, two essential elements are required. First, B[a]P must have been included in the assay and have demonstrated a dose-response relationship. Second, two or more PAHs must have been tested simultaneously. In addition, an experiment in which high-dose interaction did not affect the tumor response is desirable. Results where a quadratic dose-response relationship exists up to the highest response levels demonstrate the absence of high-dose interaction under the assumed model. Evaluating an experiment in which high-dose

000118

interaction occurred would require additional information and assumptions concerning the nature of the mechanisms of interaction. Sufficiently detailed biological information with which to postulate such mechanisms of interaction is not likely to be available.

One study has been identified that contains sufficient information to allow an attempt to verify the basic model. This experiment is discussed and evaluated in the next section.

2. Carcinogenicity of Combined PAHs

Schmahl et al. (1977) investigated the carcinogenicity of combinations of PAHs as part of a study to evaluate automobile exhaust. Eleven PAHs were selected (not all carcinogens), combined in proportions thought to reflect those in automobile exhaust, and applied twice weekly to the backs of NMRI mice. Animals were killed if a carcinoma appeared at the site of application, or were observed until their natural deaths. Table V-1 shows the PAHs tested and their relative proportions by weight in the mixtures. Table V-2 shows the results of the experiments. The tumor rates observed in the experiments can be compared to those predicted using the comparative potency method. In order to do so, a dose-response relationship for B[a]P is derived in the next section and used to predict the response to a mixture given its exposure in units equivalent to B[a]P.

3. Derivation of the B[a]P Dose-Response Model

In Section II of this report the following quadratic form of the dose-response model for B[a]P was derived:

TABLE V-1

TREATMENT GROUPS FOR CARCINOGENESIS EXPERIMENT USING COMBINED PAHs

		Dose (μg)[a]			
Control: Acetone		as solvent			
Benzo[a]pyrene		1.0	1.7		3.0
Mixture 1:					
Benzo[a]pyrene		1.0	1.7		3.0
Dibenz[a,h]anthracene		0.7	1.2		2.1
Benz[a]anthracene		1.4	2.4		4.2
Benzo[b]fluoranthene		0.9	1.5		2.7
	Total	4.0	6.8		12.0
Mixture 2:					
Phenanthrene		27.0	81.0	243.0	729.0
Anthracene		8.5	25.5	76.5	229.5
Fluoranthene		10.8	32.4	97.2	291.6
Pyrene		13.8	41.4	124.2	372.6
Chrysene		1.2	3.6	10.8	32.4
Benzo[e]pyrene		0.6	1.8	5.4	16.2
Benzo[ghi]perylene		3.1	9.3	27.9	83.7
	Total	65.0	195.0	585.0	1755.0

[a]Animals received the total mixture doses shown, composed of the amounts of PAHs indicated, twice weekly throughout their lifetimes.

SOURCE: Schmähl et al. (1977).

000120

TABLE V-2

RESULTS OF CARCINOGENESIS EXPERIMENT
USING COMBINED PAHs

Application	Dose (μg)[a]	Number of Animals With Carcinoma/ Effective Number of Animals	
		Observed	Predicted
Control	--	0/81	1/81
Benzo[a]pyrene	1.0	10/77	9/77[b]
	1.7	25/88	23/88
	3.0	43/81	45/81
Mixture 1	4.0	25/81	29/81
	6.8	53/88	60/88
	12.0	63/90	87/90[c]
Mixture 2 (Case 1)	65.0	1/85	1/85
	195.0	0/84	2/84
	585.0	1/88	6/88
	1755.0	15/86	29/86

[a]This amount was applied twice weekly to the backs of NMRI mice (see Table V-1).

[b]$P(x)=1-\exp-[0.006116(1+3.52x)^2]$

[c]Significant deviation from expected at $p = 0.01$ using χ^2 goodness-of-fit test.

Source: Schmähl et al. (1977)

$$P(x) = 1-\exp\,[-A(1+Sx)^2],$$

where A is the background transition rate parameter and S is the exposure-related transition rate parameter. The control data from the Schmahl et al. (1977) study were used to estimate A, employing the Bayesian procedure discussed previously. Given a tumor rate of 0/80 in the control group, the Bayesian estimate of A was obtained from the following relationship:

$$(0.5)/(80+1) = 1-\exp-A \text{ or } A = 0.006116.$$

The parameter S was estimated using an approximate likelihood method that yielded a relative transition rate of S = 3.52. The dose-response model thus has the numerical form:

$$P(x) = 1-\exp-[0.006116(1+3.52x)^2]. \qquad \text{(V-1)}$$

The predicted number of carcinoma-bearing animals was obtained by multiplying the tumor rate obtained from equation (V-1) times the effective number of animals in each dose group. The results of these calculations are shown in the last column of Table V-2. A standard χ^2 goodness-of-fit test yielded the result $\chi^2 = 0.677$ with 4-2 = 2 degrees of freedom, which has a "p" value of 0.73 associated with it and therefore indicates an adequate fit.

Since the quadratic model fits the B[a]P data well in the observed dose range of 0 to 3 mg, high-dose interactive effects are not expected to be important factors in that range. The predictions that are the most consistent

000122

with low-dose additivity would therefore be for mixtures composed of PAHs with a combined dose equivalent to 3 ng of B[a]P or less.

4. Predictions of Tumor Rates Due to Exposure to PAH Mixtures

In order to predict the tumor rates expected as a result of exposure to combined PAHs, the potencies of the PAHs in each mixture in the Schmahl et al. (1977) study were calculated in terms of B[a]P equivalents; these are shown in Tables V-3 and V-4. The lowest experimental dose was used for the calculations, as well as the relative potencies obtained in Section III. Since the relative proportions of PAHs in each mixture were constant for all exposure levels, the B[a]P equivalent units at higher exposure levels were obtained by simple proportional multiplication. These B[a]P exposure units were substituted into equation (V-1) and multiplied by the effective number of animals in each dose group to obtain the predicted number of animals with carcinomas. The predicted numbers were then compared to the observed numbers, which are shown graphically in Figure V-1. It should be emphasized that the predicted values were obtained without using the observed values in any manner.

The following observations can be made from the information given in Table V-2. The two lower Mixture 1 exposure levels yielded predictions that were slightly larger but statistically consistent with the observed numbers of tumor-bearing animals. The highest dose led to an overprediction of the observed number by a significant amount. However, exposure at this level was more than twice that of the dose range used for B[a]P when administered alone and as a result is likely to have led to a high-dose interaction phenomenon. The relevance of this value to low-dose extrapolation is therefore questionable.

TABLE V-3

CALCULATIONS OF B[a]P-EQUIVALENT EXPOSURE UNITS
FOR MIXTURE 1

Chemical	Relative Potency[a]	Dose (μg)[b]	B[a]P Equivalent
Mixture 1			
Benzo[a]pyrene	1.000	1.0	1.000
Dibenz[a,h]anthracene	1.110	0.7	0.777
Benzo[a]anthracene	0.145	1.4	0.203
Benzo[b]fluoranthene	0.140	0.9	0.126
Total		4.0	2.106

NOTE: Doses equivalent to B[a]P for other exposure levels were obtained by using simple ratios:

Mixture Dose (μg)	Equivalent B[a]P Dose (μg)
4.0	2.106
6.8	3.580
12.0	6.318

[a]From Table III-34

[b]From Table V-1

000124

TABLE V-4

CALCULATIONS OF B[a]P-EQUIVALENT EXPOSURE UNITS FOR MIXTURE 2

Chemical	Relative Potency[a]	Dose (μg)[b]	B[a]P Equivalent
Phenanthrene[c]	0.0	27.0	0.0
Anthracene[c]	0.0	8.5	0.0
Fluoranthene[c]	0.0	10.8	0.0
Pyrene[c]	0.081	13.8	1.1178
Chrysene	0.0044	1.2	0.00528
Benzo[e]pyrene[c]	0.004	0.6	0.0024
Benzo[ghi]perylene	0.022	3.1	0.0682
Total		65.0	1.19368

NOTE: Doses equivalent to B[a]P for other exposure levels were obtained by using simple ratios:

	Mixture Dose (μg)	Equivalent B[a]P Dose (μg)
Case 1	65.0	1.19368
	195.0	3.58104
	585.0	10.74312
	1755.0	32.22936
Case 2[d]	65.0	0.07588
	195.0	0.22764
	585.0	0.68292
	1755.0	2.04876

[a]From Table III-34

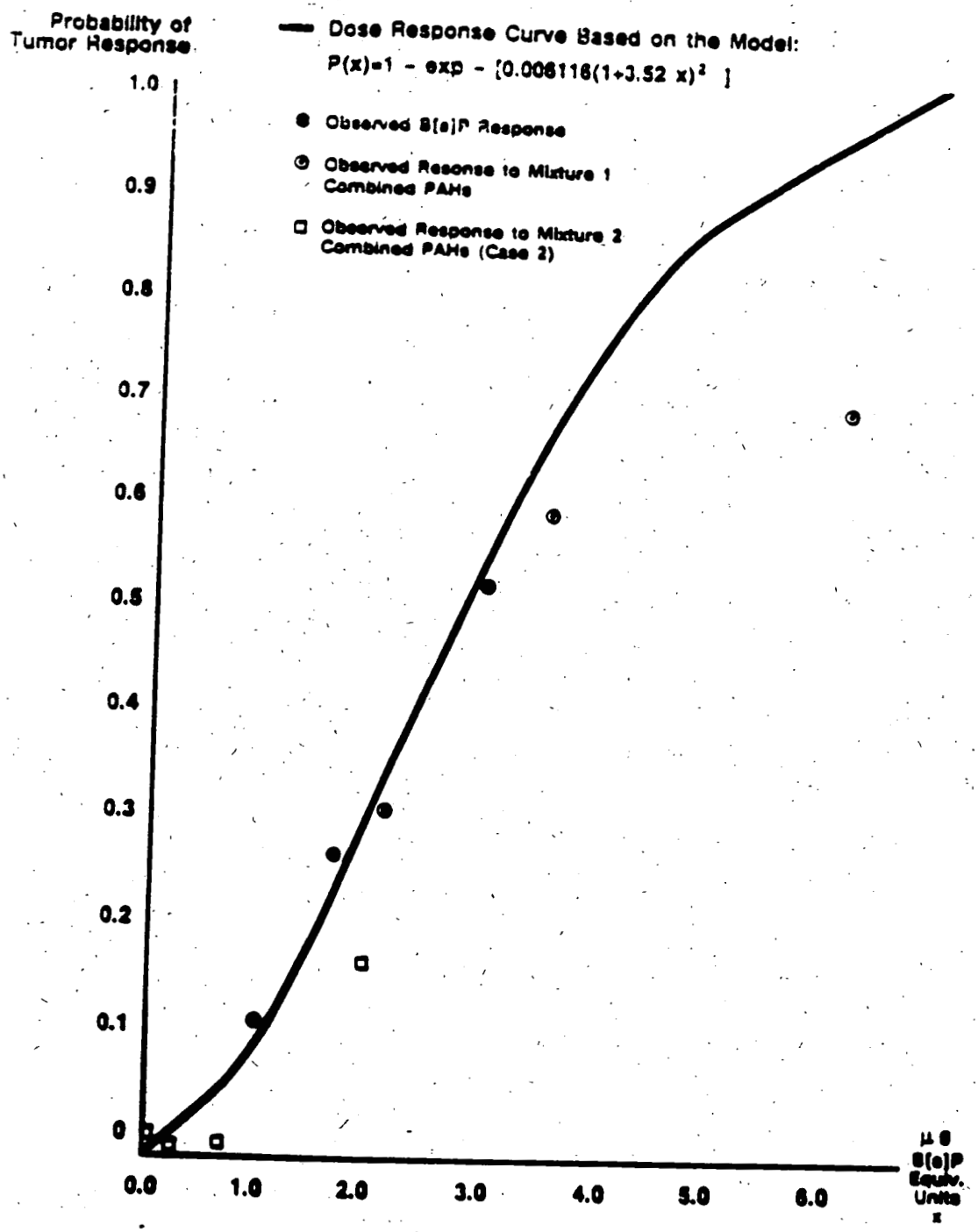
[b]From Table V-1

[c]Considered noncarcinogenic by IARC (1983) although relative potencies were available for some.

[d]Assumes a zero relative potency for pyrene.

FIGURE V-1

COMPARISONS OF OBSERVED[a] AND PREDICTED TUMOR RATES FOR MICE EXPOSED TO PAH MIXTURES



[a]Observed tumor rates are based on Schmähl et al. (1977).

000126

Mixture 2 was analyzed in two different ways. Case 1 includes all components of the mixture and results in a B[a]P-equivalent dose almost sixteen times greater than that obtained for Case 2, from which pyrene has been omitted. Pyrene is considered noncarcinogenic by IARC (1983), although since some tumors were observed following intraperitoneal injection into newborn mice (Wislocki et al. 1986), a relative potency was calculated. The number of tumors observed was statistically insignificant as compared to controls, however, so the relative potency obtained is very unreliable and is likely to greatly overestimate actual risk. The numbers of tumors predicted in each case as compared to those observed are shown in Table V-5. Clearly, including pyrene greatly overestimates risk.

Overestimation of tumor rates for Mixture 2 may also have resulted from high-dose interactions or overestimation of the potency of benzo[ghi]perylene. Pyrene and benzo[ghi]perylene are the main contributors to the potency of Mixture 2. The data from the Schmähl et al. (1977) bioassay can be used in conjunction with the data from the bioassays on which the potencies were based (described in Section III) to obtain more realistic relative potencies for pyrene and benzo[ghi]perylene. A good fit could be obtained by reducing the potency estimates for these compounds to be consistent with the observed tumor data and still be consistent with the data in the studies from which the potencies were derived.

5. Summary

Schmähl et al. (1977) tested B[a]P and two mixtures of both carcinogenic and noncarcinogenic PAHs for carcinogenicity when applied to the backs of mice. For each dose-group it was possible to predict tumor rates without using the

TABLE V-5

OBSERVED AND PREDICTED TUMOR RATES FOR MIXTURE 2 WITH OR WITHOUT PYRENE

	Equivalent B[a]P Dose (μg)	Number of Animals With Carcinoma/ Effective Number of Animals	
		Observed	Predicted
Case 1	1.19368	1/85	13/85
	3.58104	0/84	57/85
	10.74312	1/88	88/88
	32.22936	15/86	86/86
Case 2[a]	0.07588	1/85	1/85
	0.22764	0/84	2/84
	0.68292	1/88	6/88
	2.04876	15/86	29/86

[a]Assumes a zero relative potency for pyrene.

000128

experimental data from this specific study in any manner. The method yielded good estimates for combinations of the carcinogens benzo[a]pyrene, dibenz[a,h]anthracene, benz[a]anthracene, and benzo[b]fluoranthene in the same dose range observed for B[a]P alone (Mixture 1). Using potency estimates for pyrene, chrysene, benzo[e]pyrene, and benzo[ghi]perylene, the effects of the agents in Mixture 2 (weak carcinogens or noncarcinogens) were systematically overestimated. In general, the method tends to overestimate at high exposure combinations and give good results at more moderate levels. As a result, the approach may be viewed as conservative for the mixtures evaluated, which in turn enhances its overall credibility.

VI. REFERENCES

ADRIAENSSENS, P.I., WHITE, C.M., and ANDERSON, M.W. 1983. Dose-response relationships for the binding of benzo[a]pyrene metabolites to DNA and protein in lung, liver, and forestomach of control and butylated hydroxyanisole-treated mice. Cancer Res. 43:3712-3719

ARMITAGE, P., and DOLL, R. 1954. The age distribution of cancer and a multi-stage theory of carcinogenesis. Brit. J. Cancer 8:1-12

ARMITAGE, P., and DOLL, R. 1957. A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. Brit. J. Cancer 11:161-169

BERENBLUM, I., and SHUBIK, P. 1947. A new, quantitative approach to the study of the stages of chemical carcinogenesis in the mouse's skin. Br. J. Cancer 1:383-391

BINGHAM, E., and FALK, H.L. 1969. The modifying effect of carcinogens on the threshold response. Arch. Environ. Health 19:779-783

BRYAN, W.R., and SHIMKIN, M.B. 1943. Quantitative analysis of dose-response data obtained with three carcinogenic hydrocarbons in strain C3H male mice. J. Natl. Cancer Inst. 9:503-531

CHU, K.C., BROWN, C.C., TARONE, R.E., and TAN, W.Y. 1987. Differentiating among proposed mechanisms for tumor promotion in mouse skin with the use of the multi-event model for cancer. J. Natl. Cancer Inst. 79:789-796

COGLIANO, V.J. 1986. The U.S. EPA's methodology for adjusting the reportable quantities of potential carcinogens. Proceedings of Superfund '86 Conference: Management of Uncontrolled Hazardous Waste Sites, Washington, D.C.

COLBURN, N.H. 1980. Tumor promotion and preneoplastic progression. In Slaga, T.J., ed. Carcinogenesis. Vol. 5. Modifiers of chemical Carcinogenesis. New York, Raven Press

DAHL, A.R., COSLETT, D.S., BOND, J.A., and HESSELTINE, G.R. 1985. Metabolism of benzo[a]pyrene on the nasal mucosa of Syrian hamsters: comparison to metabolism by other extrahepatic tissues and possible role of nasally produced metabolites in carcinogenesis. J. Natl. Cancer Inst. 75:135-139

DEUTSCH-WENZEL, R.P., BRUNE, H., GRIMMER, O., DETTBARN, G., and MISFELD, J. 1983. Experimental studies in rat lungs on the carcinogenicity and dose-response relationships of eight frequently occurring environmental polycyclic aromatic hydrocarbons. J. Natl. Cancer Inst. 71:539-544

DUMOUCHEL, W.H., and HARRIS, J.E. 1983. Bayes methods for combining the results of cancer studies in humans and other species. J. Amer. Statist. Assoc. 78:293-315

000130

EISENSTADT, E., WARREN, A.J., PORTER, J., ATKINS, D., and MILLER, J.H. 1982. Carcinogenic epoxides of benzo[a]pyrene and cyclopenta[cd]pyrene induce base substitutions via specific transversions. Proc. Natl. Acad. Sci. 79:1945-1949

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1980. Ambient Water Quality Criteria for Polynuclear Aromatic Hydrocarbons. Prepared by the Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Water Regulations and Standards, Washington, D.C. EPA 440/5-80-069. NTIS PB 81-117806

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1984. Health Effects Assessment for Polycyclic Aromatic Hydrocarbons (PAH). Environmental Criteria and Assessment Office, Cincinnati, Ohio. EPA 540/1-86-013

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1986a. Guidelines for Carcinogen Risk Assessment. Federal Register 51(185): 33991-34003. September 24, 1986

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1986b. Guidelines for the Health Risk Assessment of Chemical Mixtures. Federal Register 51(185):34013-34025. September 24, 1986

ENVIRONMENTAL PROTECTION AGENCY (EPA). 1986c. Health Effects Assessment Summary Table. Revised Summary Tables for 58 Health Effects Assessment Documents (HEAs). May 6, 1986

FAHL, W.E., SCARPELLI, D.G., and GILL, K. 1981. Relationship between benzo[a]pyrene-induced DNA base modification and frequency of reverse mutations in mutant strains of Salmonella typhimurium. Cancer Res. 41:3400-3406

FILOV, V.A., GOLUBEV, A.A., LIUBLINA, E.I., and TOLOKONTSEV, N.A., eds. 1979. Quantitative Toxicology. John Wiley & Sons, New York

FINNEY, D.G. 1964. Statistical Methods in Biological Assay. Second Edition. Hafner Publishing Co. New York

GART, J.J., KREWSKI, D., LEE, P.N., TARONE, R.E., and WAHRENDORF, J. 1986. Statistical Methods in Cancer Research. Volume III. The Design and Analysis of Long-Term Animal Experiments. IARC Scientific Publication No. 79. Lyon, France

GELBOIN, H.W. 1980. Benzo[a]pyrene metabolism, activation, and carcinogenesis: Role and regulation of mixed-function oxidases and related enzymes. Physiol. Rev. 60:1107-1166

GROVER, P.L., and SIMS, P. 1968. Enzyme-catalysed reactions of polycyclic hydrocarbons with deoxyribonucleic acid and protein *in vitro*. Biochem. J. 110:159-160

HABS, M., SCHMAHL, D., and MISFELD, J. 1980. Local carcinogenicity of some environmentally relevant polycyclic aromatic hydrocarbons after lifelong topical application to mouse skin. Arch. Geschwulstforsch. 50:266-274

HENNINGS, H., SHORES, R., WENK, M.L., SPANGLER, E.F., TARONE, R., and YUSPA, S.H. 1983. Malignant conversion of mouse skin tumors is increased by tumor initiators and unaffected by tumor promoters. Nature 304:67-69

HENNINGS, H., and YUSPA, S.H. 1985. Two-stage tumor promotion in mouse skin: An alternative interpretation. J. Natl. Cancer Inst. 74:735-740

HOFFMANN, D., and WYNDER, E.L. 1966. Beitrag zur carcinogenen Wirkung von Dibenzopyrenen. Z. Krebsforsch. 68:137-149

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). 1983. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Volume 32. Polynuclear Aromatic Compounds. Part 1. Chemical, Environmental and Experimental Data. World Health Organization, Lyon, France

KADEN, D.A., HITES, R.A., and THILLY, W.G. 1979. Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to Salmonella typhimurium. Cancer Res. 39:4152-4159

LAND, H., PARADA, L.F., and WEINBERG, R.A. 1983. Cellular oncogenes and multistep carcinogenesis. Science 222:771-777

LASKIN, S., KUSCHNER, M., and DREW, R.T. 1970. Studies in pulmonary carcinogenesis. In Hanna, M.G., Nettesheim, P., and Gilbert, J.R., eds. Inhalation Carcinogenesis: Proceedings of the U.S. Atomic Energy Commission Symposium Series. Oak Ridge, Tennessee

LaVOIE, E.J., AMIN, S., HECHT, S.S., FURUYA, K., and HOFFMAN, D. 1982. Tumour initiating activity of dihydrodiols of benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene. Carcinogenesis 3:49-52

LaVOIE, E.J., BRALEY, J., RICE, J.E., and RIVENSON, A. 1987. Tumorigenic activity of non-alternant polynuclear aromatic hydrocarbons in newborn mice. Cancer Lett. 34:15-20

LEE, P.N., and O'NEILL, J.A. 1971. The effect both of time and dose applied on tumor incidence rate in benzopyrene skin painting experiments. Brit. J. Cancer 25:759-770

MARSHALL, C.J., VOUSDEN, K.H., and PHILLIPS, D.H. 1984. Activation of c-Ha-ras-1 proto-oncogene by in vitro modification with a chemical carcinogen, benzo(a)pyrene diol-epoxide. Nature 310:586-589

MISFELD, J. 1980. The tumor-producing effects of automobile exhaust condensate and of diesel exhaust condensate. In Pepelko, W.E., Danner, R.M., and Clarke, N.A., eds. Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium. Vol. 2. Environmental Protection Agency, Cincinnati, Ohio. EPA 600/9-80-057b. Pp. 1012-1025

MOOLGAVKAR, S.H. 1986. Carcinogenesis modeling: From molecular biology to epidemiology. Ann. Rev. Public Health 7:151-169

MOOLGAVKAR, S.H., and KNUDSON, A.G. 1981. Mutation and cancer: A model for human carcinogenesis. J. Natl. Cancer Inst. 66:1037-1052

MOOLGAVKAR, S.H., and VENZON, D.J. 1979. Two-event models for carcinogenesis: Incidence curves for childhood and adult tumors. Math. Biosci. 47:55-77

NATIONAL ACADEMY OF SCIENCES (NAS). 1983. Polycyclic Aromatic Hydrocarbons: Evaluation of Sources and Effects. National Academy Press, Washington, D.C.

NEAL, J., and RIGDON, R.H. 1967. Gastric tumors in mice fed benzo[a]pyrene: A quantitative study. Tex. Rep. Biol. Med. 25:553-557

NETTESHEIM, P., FITZGERALD, D.J., KITAMURA, H., WALKER, C.L., GILMER, T.M., BARRETT, J.C., and GRAY, T.E. 1987. In vitro analysis of multistage carcinogenesis. Environ. Health Perspec. 75:71-79

NEWBOLD, R.F., and BROOKES, P. 1976. Exceptional mutagenicity of a benzo(a)pyrene diol epoxide in cultured mammalian cells. Nature 261:52-54

NEWBOLD, R.F., WIGLEY, C.B., THOMPSON, M.H., and BROOKES, P. 1977. Cell-mediated mutagenesis in cultured Chinese hamster cells by carcinogenic polycyclic hydrocarbons: Nature and extent of the associated hydrocarbon-DNA reaction. Mutat. Res. 43:101-116

NEWBOLD, R.F., BROOKES, P., and HARVEY, R.G. 1979. A quantitative comparison of the mutagenicity of carcinogenic polycyclic hydrocarbon derivatives in cultured mammalian cells. Int. J. Cancer 24:203-209

PEREIRA, M.A., BURNS, F.J., and ALBRTY, R.E. 1979. Dose response for benzo[a]pyrene adducts in mouse epidermal DNA. Cancer Res. 39:2556-2559

PFEIFFER, E.H. 1977. Oncogenic interaction of carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons. In Mohr,V., Schmahl, D., and Tomatis, L., eds. Air Pollution and Cancer in Man. IARC Scientific Publication No. 16. Lyon, France. Pages 69-77

PHILLIPS, D.H., GROVER, P.L., and SIMS, P. 1979. A quantitative determination of the covalent binding of a series of polycyclic hydrocarbons to DNA in mouse skin. Int. J. Cancer 23:201-208

RAAB, O.G., YEH, H.C., NEWTON, G.J., PHALEN, R.F., and VELASQUES, D.J. 1977. Deposition of inhaled monodisperse aerosols in small rodents. In Walton, W.H., ed. Inhaled Particles IV, part 1. Oxford, Pergamon

REDDY, M.V., GUPTA, R.C., RANDERATH, E., and RANDERATH, K. 1984. ^{32}P-Postlabelling test for covalent DNA binding of chemicals *in vivo*: Application to a variety of aromatic carcinogens and methylating agents. Carcinogenesis 5:231-243

RIGDON, R.H., and NEAL, J. 1969. Relationship of leukemia to lung and stomach tumors in mice fed benzo[a]pyrene. Proc. Soc. Exptl. Biol. N.Y. 130:146-152

RIGDON, R.H., and NEAL, J. 1966. Gastric carcinomas and pulmonary adenomas in mice fed benzo[a]pyrene. J. Natl. Cancer Inst. 34:297-305

SCHLESINGER, R.B. 1985. Comparative deposition of inhaled aerosols in experimental animals and humans: a review. J. Toxicol. Environ. Health 15:197-214

SCHMAHL, D., SCHMIDT, K.G., and HABS, M. 1977. Syncarcinogenic action of polycyclic aromatic hydrocarbons in automobile exhaust gas condensates. In Mohr, U., Schmahl, D., and Tomatis, L., eds. Air Pollution and Cancer in Man. IARC Publication 16. World Health Organization, Lyon, France. Pages 53-59

SIMS, P., and GROVER, P.L. 1974. Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. Adv. Cancer Res. 20:165-274

SLAGA, T.J., TRIPLETT, L.L., and NESNOW, S. 1980. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: Two-stage carcinogenesis in skin tumor sensitive mice (SENCAR). In Pepelko, W.E., Danner, R.M., and Clarke, N.A., eds. Health Effects of Diesel Engine Emissions. Proceedings of an International Symposium. Vol. 2. Environmental Protection Agency, Cincinnati, Ohio. EPA 600/9-80-057b. Pp. 874-897

STOWERS, S.J., and ANDERSON, M.W. 1985. Formation and persistence of benzo[a]pyrene metabolite-DNA adducts. Environ. Health Perspect. 62:31-39

SUN, J.D., WOLFF, R.K., and KANAPILLY, G.M. 1982. Deposition, retention, and biological fate of inhaled benzo[a]pyrene adsorbed onto ultrafine particles and as a pure aerosol. Toxicol. Appl. Pharmacol. 65:231-244

THORSLUND, T.W., BROWN, C.C., and CHARNLEY, G. 1987. Biologically motivated cancer risk models. Risk Analysis 7:109-119

THYSSEN, J., ALTHOFF, J., KIMMERLE, G., and MOHR, U. 1981. Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. J. Natl. Cancer Inst. 66:575-577

TRIOLO, A.J., APONTE, G.E., and HERR, D.L. 1977. Induction of aryl hydrocarbon hydroxylase and forestomach tumors by benzo(a)pyrene. Cancer Res. 37:3018-3021

VAN DUUREN, B.L., SIVAK, A., SEGAL, A., ORRIS, L., and LANGSETH, L. 1966.

The tumor-promoting agents of tobacco leaf and tobacco smoke condensate. J. Natl. Cancer Inst. 37:519-526

WEINSTEIN, I.B., JEFFREY, A.M., LEFFLER, S., PULKRABEK, P., YAMASAKI, H., and GRUNBERGER, D. 1978. Interactions between polycyclic aromatic hydrocarbons and cellular macromolecules. In Ts'O, P.O.P., and Gelboin, H.V., eds. Polycyclic Hydrocarbons and Cancer. Vol. 2: Molecular and Cell Biology. Academic Press, New York. Pp. 3-36

WIGLEY, C.B., NEWBOLD, R.F., AMOS, J., and BROOKES, P. 1979. Cell mediated mutagenesis in cultured Chinese hamster cells by polycyclic hydrocarbons: Mutagenicity and DNA reaction related to carcinogenicity in a series of compounds. Int. J. Cancer 23:691-696

WISLOCKI, P.G., BAGAN, E.S., LU, A.Y.H., DOOLEY, K.L., FU, P.P., HAN-HSU, H., BELAND, F.A., and KADLUBAR, F.F. 1986. Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene and benzo[a]pyrene in the newborn mouse assay. Carcinog. 7:1317-1322

WYNDER, E.L., and HOFFMANN, D. 1959. A study of tobacco carcinogenesis. VII. The role of higher polycyclic hydrocarbons. Cancer 12:1079-1086.

YEH, H.C., BARR, E.B., and ESPARZA, D.C. 1980. Deposition of inhaled dual aerodynamically similar aerosols in Syrian hamsters. In Yeh, H.C., ed. Respiratory Tract Deposition Models. Final report to NIEHS. Springfield, VA, Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute