COMMENTS ON DEVELOPMENT OF A RELATIVE POTENCY FACTOR (RPF) APPROACH FOR POLYCYCLIC AROMATIC HYDROCARBON (PAH) MIXTURES

EXTERNAL REVIEW DRAFT

COMMENTS PREPARED FOR CONSIDERATION BY SCIENCE ADVISORY BOARD PAH MIXTURES REVIEW PANEL

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Abbreviations and Acronyms

3-MC	3-methylchloanthrene
1,2-MBA	11H-benz[b,c]aceanthrylene
3-MC	3-methylchloanthrene
4,5-MC	4H-cyclopenta[d,e,f]chrysene
AA	anthanthrene
ADAF	age-dependent adjustment factor
Ah	Aryl hydrocarbon receptor
AhrR	Aryl hydrocarbon receptor
AIC	Akaike's Information Criterion
AUC	area under the curve
BaA	benz[a]anthracene
BaP	benzo[a]pyrene
BaP-TE	benzo[a]pyrene toxic equivalent
BbcAC	benz[b,c]aceanthrylene, 11H-
BbF	benzo[b]fluoranthene
BeAC	benz[e]aceanthrylene
BeP	benzo[e]pyrene
BghiP	benzo(g,h,i)perylene
BlAC	benz[l]aceanthrylene
BjAC	benz[j]aceanthrylene
BjF	benzo[j]fluoranthene
BkF	benzo[k]fluoranthene
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Modeling Software
BMR	benchmark response
CH	chrysene
CPcdP	cyclopenta[c,d]pyrene
CSF	Cancer Slope Factor
DahA	dibenz[a,h]anthracene
DBA	dibenzanthracene
DBacA	dibenz[a,c]anthracene
DBaeF	dibenzo[a,e]fluoranthene
DBahA	dibenz[a,h]anthracene
DBaeP	dibenzo[a,e]pyrene
DBahP	dibenzo[a,h]pyrene

DBaiP	dibenzo[a,i]pyrene
DBalP	dibenzo[a,l]pyrene
DBelP	dibenzo[e,l]pyrene
DMBA	7,12-dimethyl-benz[a]anthracene
EPA	United States Environmental Protection Agency
EPRI	Electric Power Research Institute
DNA	deoxyribonucleic acid
FA	fluoranthene
GLP	Good Laboratory Practice
HPLC	high-performance liquid chromatography
I123cdP	indeno[1,2,3-cd]pyrene
IARC	International Agency for Research on Cancer
IR	Infrared
IP	intraperitoneal
IRIS	Integrated Risk Information System
mg/kg	milligrams per kilogram
mg/kg/day	milligrams per kilogram per day
mM	millimolar
mol	mole
MTD	maximum tolerated dose
N23eP	naphtho[2,3-e]pyrene
NCTR	National Center for Toxicological Research
nmol	nanomole
NMR	Nuclear Magnetic Resonance
NRC	National Research Council
ODC	ornithine decarboxylase
PAH	polycyclic aromatic hydrocarbon
r^2	Correlation coefficient
RFP	Relative Potency Factor
ug	microgram
umol	micromole
UV	ultraviolet
WHO	World Health Organization

Summary of Key Comments

EPRI's primary concerns are listed below; each of these is discussed in detail in this set of comments.

- EPA did not address the scientific appropriateness of using animal bioassay results from tests that were designed with their sole purpose as hazard identification; the tests were not intended to be used for quantitative dose-response modeling.

- EPA proposed quantitative dose-response criteria on PAHs that neither EPA nor IARC has classified as known, probable, or even possible human carcinogens. EPA has not performed a weight-of-evidence assessment in accordance with EPA (2005) criteria as is necessary before calculating quantitative dose-response criteria for any substance.

- EPA has derived RPFs for many PAHs based on an outdated and scientifically unjustifiable Cancer Slope Factor (CSF) for benzo[a]pyrene (BaP). There was no discussion in the report regarding the need to update the CSF for BaP despite the fact that a guideline-compliant two-year feeding study in mice (performed at the National Center for Toxicological Research) has been available for such purpose for more than ten years.

- EPA did not perform any model validation of the proposed RPFs using high quality studies on PAH-containing mixtures.

- EPA did not adequately assess the data quality of the studies used for RPF derivation, such as whether there is any reasonable degree of certainty about the identity and purity of the test substance studied in the individual scientific reports.

- EPA did not include critical information from studies that compared the tumorigenic potency of multiple PAHs unless BaP was concurrently tested despite the fact that other well-known and well-studied positive control PAHs have been tested concurrently in many of these studies. In addition, it was not determined what useful information would be available in such studies, even for validation purposes.

- EPA has developed a protocol with procedures that are not adequately described or justified.

- EPA has derived dose-response curve slopes for a majority of the PAH data sets with data in which only one dose group was tested, thus generating slope factors with extremely high uncertainties.

I. Charge Questions

General Charge Questions

1. Please comment on whether the report is logical, clear and concise. Please comment on whether EPA has clearly synthesized the scientific evidence for the derivation of relative potency factors for individual PAHs.

   EPRI Comment: EPA (2010) has summarized a large number of studies on PAHs but several major issues, which EPRI outlined in the previous section, were not adequately addressed. Other shortcomings in EPA's report are discussed below.

2. Please comment on whether the report provides adequate context for how the proposed RPF approach could be used in a PAH mixtures risk assessment.

   EPRI Comment: The EPA (2010) report does not discuss the issue of implementation. The report implies that the RPFs will be used to assess each PAH using a CSF for BaP, but the CSF for BaP is not discussed except in an example calculation. In that example calculation, the current CSF, derived years ago based on 1960's vintage data from poorly designed and executed studies is used. Curiously, EPA (2010) discusses the studies upon which the IRIS CSF of 7.3 $(mg/kg/day)^{-1}$ is based: "Earlier, a number of related studies were conducted to evaluate the carcinogenicity of benzo[a]pyrene in feed in Ah-responsive white Swiss mice (Rigdon and Neal, 1969, 1966; Neal and Rigdon 1967). These studies were not conducted using standard, modern toxicological methods and have several limitations, including inconsistent dosing protocols; varying ages of the animals; use of benzene as a solvent; small numbers of animals; and evaluation of only a limited number of tissues. These studies do, however, provide useful dose-response information on benzo[a]pyrene carcinogenicity." The criticisms of the studies do not match the conclusion that the studies "provide useful dose-response information on benzo[a]pyrene carcinogenicity." Clearly, the CSF for BaP needs to be updated with modern, robust animal toxicology studies, as discussed elsewhere in these comments.

Chapter 2. Rationale for Recommending an RPF Approach

2a. Please comment on whether the report provides adequate justification for using an RPF approach as a scientifically defensible method to assess the cancer risk associated with exposure to PAH mixtures.

EPRI Comment: EPA's Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures (EPA 2000), states: "Whenever possible, the preferred approach to the health risk evaluation of chemical mixtures is to perform the assessment using health effects and exposure data on the whole mixture." EPA (2010) acknowledges this fact: "*The Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000) indicates that approaches based on whole mixtures are preferred to component approaches, such as the RPF approach. Risk assessment approaches based on toxicity evaluations of whole mixtures inherently address specific interactions among PAHs and account for the toxicity of unidentified components of PAH mixtures. They also do not require assumptions regarding the toxicity of individual components (e.g., dose additivity or response additivity)."

However, EPA (2010) states that while preferred, this whole mixture approach is not practical for several reasons and proceeds to develop the RPF approach. Some of EPA's reasons for so stating that the whole mixture approach is not practical are reasonable, but some are not, as noted below:

(a) Concern: "There are very few toxicity data available for whole PAH mixtures..." Response: There are toxicity data available on numerous PAH-containing mixtures, such as coal tar, creosote, diesel exhaust, cigarette smoke and others. Epidemiological studies and health surveys have been performed over the years on workers exposed to these and other PAH-containing mixtures. Of particular interest is a well designed and executed two-year feeding study funded by EPRI with two site-generated coal tar mixtures from which CSFs can be and have been derived. These CSFs have great utility for many human health risk assessments, and CSFs could also be derived from epidemiological studies with workers exposed to PAH-containing mixtures. EPA should have, at a minimum, discussed the quantitative dose-response functions from these mixtures and used them to validate their proposed RPF scheme.

(b) Concern: "…chemical analyses of the composition of mixtures are limited." Response: Chemical analyses of the mixtures that have been well studied are far more comprehensive than the analyses of typical environmental media for which the RPF scheme is intended for use. In fact, EPA has proposed RPFs for about 15 PAHs for which there is extremely little quantitative data and for which there are no know

analytical methods for standard environmental characterization studies. EPA should consider limiting any RPF scheme to only those PAHs that are known to be present in the environment and for which there is a reasonable quantity of chemical composition data in typical PAH-containing mixtures.

(c) Concern: "…PAH- containing mixtures tend to be very complex." Response: This is true and is the reason that risk assessment of mixtures should be restricted to the use of quantitative dose-response data on the mixtures themselves.

(d) Concern: "…the composition of these mixtures appears to vary across sources releasing these mixtures to the environment and in various environmental media in which they occur." Response: It is true that PAH content of mixtures varies among sources and environmental conditions. This is why well-designed studies of mixtures of interest and under actual environmental conditions should be performed, such as the EPRI-funded two-year feeding study in mice of composite coal tar samples that were collected from actual sites, defining coal tar in real world situation after weathering for years in the environment. Given that EPA's standardized analytical methods identify and quantify less than half of the PAHs for which EPA has derived RPFs in this document, the fact that mixtures vary in content from site to site is no justification for proposing an RPF scheme.

2b. Please comment on whether the choice of benzo[a]pyrene as the index compound is scientifically justified and appropriately described. Please identify and provide the rationale for any alternative index compound(s) that should be considered.

<u>EPRI Comment:</u> EPA (2010) states that BaP is the only logical choice to be the index PAH for a PAH RPF scheme. However, many studies have used 3-methylchloanthrene (3-MC) or 7,12-dimethyl-benz[a]anthracene (DMBA) as positive controls instead of or in addition to BaP. In the limited time available to evaluate the RPF document, EPRI was unable to search the literature to determine if there are adequate studies to support RPF derivation on the basis of 3-MC or DMBA, but it is likely that RPFs could be derived in this manner for some PAHs of interest. EPA should have fully evaluated the utility of studies with these two alternate PAHs in EPA (2010). EPA did not scientifically justify nor appropriately describe the rationale for choosing BaP as the sole index compound.

2c. Please comment on whether the weight of evidence indicating that PAHs, as a chemical class, have a similar mode of carcinogenic action has been adequately described and is scientifically justified.

EPRI Comment: EPA (2010) performed a weight of evidence evaluation, but it appears that this evaluation was not performed in accordance with the Weight of Evidence Narrative required in EPA's Guidelines for Carcinogen Risk Assessment (EPA 2005) for assessing the human carcinogenic potential of agents. Inspection of Figure 6-1 which is entitled "Weight of evidence analysis of [sic] for selection of PAHs to be included in the RPF approach" and evaluation of the accompanying text reveals that EPA did not perform a weight of evidence approach to determine if a given PAH was "carcinogenic to humans," "likely to be carcinogenic to humans," "suggestive evidence of carcinogenic potential," or "not likely to be carcinogenic to humans. " The analysis was restricted to determining if the substance was positive in an assay in which BaP was tested, so EPA determined if the weight of evidence was adequate to conclude that a substance tested positive in one short term system, such as the two-stage mouse bioassay. More importantly, EPA considered a single positive result to be adequate weight of evidence to conclude that the substance should be included in the RPF scheme. Ten RPFs are based on a single positive result and some of these were accompanied by negative results. One stand-alone positive result in a tumorigenicity test or one positive plus one or more negative results provides an inadequate weight of evidence that a PAH should be included in any RPF scheme. In conclusion, EPA's weight of evidence analysis was scientifically inadequate.

EPA has not adequately described and scientifically justified that all PAHs have the same mode of action. EPA (2010) has concluded that all PAHs act via a mutagenic mode of action but that there is considerable uncertainty:

> "In conclusion, there is evidence that an assumption of a similar toxicological action is reasonable for PAHs; however, the carcinogenic process for individual PAHs is likely to be related to some unique combination of multiple molecular events resulting from formation of several reactive species. For these reasons, the use of an RPF approach to estimate cancer risk associated with PAH exposure is considered appropriate. A common mutagenic mode of action for carcinogenic PAHs is hypothesized based on information available for the indicator chemical, benzo[a]pyrene (U.S. EPA, 2005b)."

EPA further states:

> "In the case of PAHs, there are inadequate data to identify a specific mode of action that is applicable across all health endpoints."

In addition to the statement that there is considerable uncertainty with respect to the "unique combination of molecular events" involved with each individual PAH, EPA (2010) has not addressed the considerable data that indicates that the mode of action

for PAHs in mouse skin is not the same as the mode of action of PAHs in human skin. Urano et al. (1995), Graem (1986), and Soballe et al. (1996) all showed with human skin xenografts that mouse skin is sensitive to papilloma formation with a variety of treatments, including PAH treatments, while human skin is not. With the human skin xenografts, tumors were seen in the mouse skin adjacent to the human xenograft, but no tumors were seen in the human skin itself. The fact that the mode of action of PAHs in mouse skin is not the same as the mode of action of the same PAHs in human skin is very important because a great number of the RPFs are derived from mouse skin bioassays.

There are a variety of explanations for this high sensitivity of the murine skin in strains commonly used for the initiation-promotion bioassays. They include:

- Greater dermal penetration than human skin
- Greater metabolic enzyme activity than human skin
- Defective DNA repair genes
- A species-specific mode of action that is not relevant to human skin

The latter scientific finding was summarized succinctly by ICF (2000) in their dermal risk assessment of polycyclic aromatic hydrocarbons (PAHs), which was presented to a Toxicology Excellence in Risk Assessment (TERA) Peer Review Panel. They reported that the mechanism by which chemicals cause promotion of PAH-initiated skin tumorigenesis are specific to mouse skin and do not occur in human skin:

> "Induction of ODC activity is an obligatory precursor step in mouse skin tumor promotion, which is mediated by products of cyclooxygenase (COX) activity (Verma et al. 1977; Yamamoto et al. 1992). In contrast, the induction of ODC in human epidermis is independent of lipoxygenase and COX pathways (Arnold et al. 1992). It is questionable that the promotional mechanisms operative in murine skin are also operative in human skin (Arnold et al. 1990, 1991, 1992, 1993; Ashendel and Boutwell 1979; Boutwell 1978; Cameron et al. 1992; Chida and Kuroki 1984; Fischer et al. 1993; Kennard et al. 1995; Lowe 1981; Lowe et al. 1982; Suda et al. 2000; Verma et al. 1977, 1988; Young et al. 1999)."

Because the mode of action for PAHs in mouse skin is not the same as their mode of action in human skin, it is thus likely that PAH-containing mixtures that cause skin cancer in mouse skin do not cause skin cancer in human skin in vivo. Specifically, coal tar and coal tar creosote have been shown to cause skin cancer in mouse skin when given at high doses and high frequencies. However, these same mixtures have not been shown to cause cancer in human skin despite the fact that many thousands

of workers have been dermally exposed to these two PAH-containing mixtures for decades.

2d. Please comment on whether the assumption that interactions among PAH mixture components do not occur at low levels of exposure typically encountered in the environment has been adequately described and is scientifically justified.

EPRI Comment: EPA (2010) has not adequately described or scientifically justified the presence or absence of interactions among PAH mixture components at levels of exposure encountered in the environment. No quantitative data are presented and only a simple statement of assumption is made. Many examples of interactions of components of PAH-containing mixtures are presented in the comments below. In such experiments, it is not uncommon for the dose to be 10-100 ug of PAH per animal. The doses of PAHs encountered in the environment are similar or higher, not lower. For example, as noted below, the BaP-TE concentration of two typical coal tar samples from manufactured gas plant sites was 20,000 to 23,000 ug/g. It would be usual for a risk assessment to assume that a person could come into contact with coal tar dermally over 1,000 cm^2 of skin surface area per day. With such a common exposure, the dose of BaP-TE would be 4,000 to 4,600 ug total BaP-TE per day. PAH-contaminated soil from any site in America can easily contain 1,000 ug/g of BaP-TE and the concentrations will be higher with the proposed RPFs. A typical soil exposure scenario would be exposure to soil over 3,000 cm^2 or more surface area. With such a common exposure, the dose of BaP-TE would be 600 ug total BaP-TE per day. Given that interactions, specifically inhibitory interactions, have been seen among PAHs at doses similar to those typically encountered in the environment, EPA's assumption that interactions will not occur is not supported by the scientific literature.

Chapter 3. Discussion of Previously Published RPF Approaches

3. Please comment on whether the discussion provides a meaningful background on how RPFs have been derived in the past, and the advantages and disadvantages of previous methods.

EPRI Comment: EPA (2010) does provide a few citations and a comprehensive table of a dozen or so previous RPF schemes. EPA presents little text that critiques any of these approaches or discusses in depth the uncertainties involved in any RPF scheme. In fact, EPA (2010) concludes that all of these approaches are reasonable and yield similar results:

> "In summary, several approaches are available for the determination of RPFs for PAHs. RPF values are proposed in at least one study for a total of 27 PAHs (see Table 3-1). Because these approaches generally rely on similar bioassay data and modeling methods, the resulting RPF values are fairly comparable for most PAHs across studies." [emphasis added]

EPA's conclusion is not consistent with the actual RPF values presented. EPA's Table 3-1 clearly indicates that there is vast variability and uncertainty associated with the RPF derivation process. RPFs vary by 10-100 fold among investigators and sometimes even higher. For instance, the RPFs for chrysene vary from 0.001 to 0.9. For anthanthrene, they vary from 0-.01. Benzo[j]fluoranthene RPFs vary from 0.05 to 0.5, For benzo[a]anthrene, the RPFs vary from 0.004 to 0.15. For indeno[123,cd]pyrene, the RPFs vary from 0.006 to 0.1.

Chapter 4. Evaluation of the Carcinogenicity of Individual PAHs

4a. Please comment on whether the list of 74 PAHs (Table 2-1) included in the initial literature search is complete. Please comment on whether the rationale for the choice of PAHs included in the literature search has been appropriately described. Please identify other databases or resources that should be included.

<u>EPRI Comment:</u> EPA (2010) was well advised to restrict the PAH list to "unsubstituted PAHs with three or more fused aromatic rings containing only carbon and hydrogen atoms, because these are the most widely studied members of the PAH chemical class." EPRI is not aware of any significant mammalian toxicological literature on alkylated PAHs, despite the fact that forensic analyses often use data on alkylated PAHs for diagnostic purposes. EPRI agrees that heterocyclic PACs or PAHs with hydroxyl, sulfhydryl, amino and nitro groups should not be included in the RPF scheme. Such compounds may be present in PAH-containing mixtures and may contribute to the overall toxicological effects of any such mixture, but they are members of different classes of substances and would be expected to behave quite differently than PAHs.

4b. Chapter 4 includes a description of how studies were selected for use in dose-response assessment. Please comment on whether the choices and assumptions in making the selection have been adequately described. Please comment on whether the information in EPA Tables 4-1 through 4-14 provides adequate information to inform how decisions were made. Please comment on whether studies were rejected or included appropriately. Please comment on whether positive and nonpositive studies have been considered appropriately.

EPRI Comment: EPRI has overseen the evaluation of selected studies and EPA's treatment of data from those studies to gain insight into the decisions that were made in the RPF derivation process. Many issues are discussed in the comments below. Major comments include the following. Two of the selection criteria were set arbitrarily and cause useful data to be excluded:

- "Benzo[a]pyrene was tested simultaneously with another PAH"
- "The carcinogenic response observed in either the benzo[a]pyrene- or other PAH-treated animals at the lowest dose level was not saturated (i.e., tumor incidence at the lowest dose was <90%), with the exception of tumor multiplicity findings"

One selection criterion was properly defined but not executed:

- "There were no study quality concerns or potential confounding factors that precluded use (e.g., no concurrent control, different vehicles, strains, etc. were used for the tested PAH and benzo[a]pyrene; use of cocarcinogenic vehicle; PAHs of questionable purity; unexplained mortality in treated or control animals)."

Specifically, when evaluating study quality, EPA (2010) did not take into account the age of studies and the quality of the test article. With studies performed 30-40 years ago, there is a real issue with regard to the identity and purity of the test article. In many of these old studies, PAHs were purified by paper or column chromatography and then identified by melting point or ultraviolet absorption spectroscopy. While these were state of the art methods of the day, there is great uncertainty with regard to both the identity and purity of the material that was tested and then attributed to a specific chemical structure. In some cases no information is provided about the test chemical except to say that it was provided by a named person.

Another comment is that many old studies carry considerable weight because they are the source of more than one, and in some cases, many RPFs. For instance, the Hoffmann and Wynder (1966) study is the sole basis for five RPFs. Such studies should be scrutinized to a greater degree because of their overall importance in the entire scheme. EPRI finds that some of these studies should be rejected for a variety of quality reasons.

Chapter 5. Methods for Dose Response Assessment and RPF Calculation

5a. Please comment on whether the scientific rationale for the dose-response modeling approaches used in the derivation of RPFs is adequately described. Please comment

on whether there are other appropriate modeling approaches for estimating the relative potencies of PAHs. Please describe alternative approaches (e.g., other model forms) that could be considered.

EPRI Comment: EPRI has no comments on this charge question at this time.

Chapter 6. Selection of PAHs for Inclusion in the Relative Potency Approach

6a. Please comment on whether the rationale for the weight-of-evidence evaluation is scientifically justified and adequately described. Please comment on whether the approach adequately considers the available information. Please comment on whether other information (e.g., additional structure-activity) could contribute further to the weight-of-evidence evaluation and how this information could be utilized in the analysis.

EPRI Comment: Carcinogenic potential should be evaluated within the current EPA Cancer Guidelines (EPA 2005) and clearly presented and weighted in the RPF approach. Examples of where this would add clarity and scientific credibility to the proposed approach include the following:

1. EPA indicates PAHs selected for inclusion in the RPF approach were initially chosen based on an "evaluation of whether the available data were adequate to assess the carcinogenicity of each compound." (Chapter 6, page 113, paragraph 1). Based on this, 35 PAHs were identified for further evaluation. EPA should clearly define "carcinogenicity" for this step within the context of the relevance to potential to be carcinogenic to humans with consideration of EPA's 2005 Cancer Guidelines. This step does not appear to consider the likelihood that the PAH is carcinogenic to humans.

2. The proposed RPF approach appears to rely on the 1994 IRIS toxicological assessment for benzo(a)pyrene.

    - EPA states that PAHs included in the RPF weight of evidence "were assumed to be carcinogenic due to toxicological similarity to the indicator compound, benzo[a]pyrene." (page iv and vii).
    - EPA states that "For the purposes of this analysis, PAHs were assumed to be carcinogenic by inferring toxicological similarity to the indicator compound, benzo[a]pyrene." (page 114)

    It appears that EPA is assuming that the weight of evidence for carcinogenic potential for benzo(a)pyrene ("Classification - B2; probable human carcinogen")

completed in 1994 is relevant to all of the PAHs included in the proposed RPF approach. This is a short-cut that should not be taken in an analysis of this significance. The published scientific data do not support this assumption for all 35 PAHs included in the analysis. The rationale should be justified within the context of study data, and the "weight of evidence for the carcinogenic hazard potential" humans should be completed consistent with the 2005 Cancer guidelines for each PAH. A detailed analysis of data for each PAH supporting and demonstrating the assumed "toxicological similarity" would add clarity and credibility to the document. In addition, the benzo(a)pyrene cancer classification was completed in 1994, prior to finalizing the current guidance (2005 Cancer Guidelines). At a minimum it should be updated to be consistent with current guidance and policy before being used as a point of reference for the proposed RPF approach.

3. EPA describes the process used to identify PAHs to be evaluated in using the proposed RPFs as a "weight of evidence approach".

- EPA indicates a "weight of evidence approach" was used to "determine whether the available data (including the calculated RPFs as well as non-positive studies that met selection criteria) were adequate to include each compound in the RPF approach" (Chapter 6, page 113, paragraph 2; addition detail provided in Section 6.2, page 117).
- EPA indicates that "a weight of evidence evaluation was conducted to assess the evidence that each PAH could induce a carcinogenic response" (page 114).
- Section 6.6 Weight of Evidence Evaluation for 35 Individual PAHs.

The RPF weight of evidence approach is different than the "weight of evidence for the carcinogenic hazard potential" evaluation described in the 2005 Cancer Guidelines and does not include an analysis that weighs the evidence that the PAH is actually carcinogenic to humans consistent with these guidelines.

The rationale for each RPF proposed for each PAH should also include a "weight of evidence for the carcinogenic hazard potential" evaluation as described with the 2005 Cancer Guidelines (e.g., "Likely to be Carcinogenic to Humans" or "Suggestive Evidence of Carcinogenic Potential"). This would provide a more scientifically sound rationale for each recommendation, and is likely to result in modifications to the proposed scheme. For example, EPA's Cancer Guidelines state that chemicals classified as "Suggestive Evidence of Carcinogenic Potential" generally do not have adequate data for a dose-response assessment (Section 3. Dose Response Assessment). However, EPA notes in this section that if there is a

"well conducted" study, a quantitative assessment may be completed. Including the classification may identify chemicals where study data may require greater scrutiny before being used to determine the RPF or where data indicate that the proposed RPF should be modified to reflect uncertainty.

6b. The weight-of-evidence analysis does not include data related to Ah-receptor binding, cytotoxicity or tumor promotion. Please comment on whether the scientific rationale for this decision is appropriate. If these data should be considered in the derivation of RPFs, please describe how they should be incorporated into the analysis.

EPRI Comment: It is appropriate to exclude in vitro data on Ah-receptor binding, cytotoxicity or tumor promotion. The presented weight of evidence analysis is deficient because it does not evaluate the probability that a substance poses a carcinogenic risk to humans. Instead, it evaluates the probability that a substance poses a carcinogenic risk to the sensitive skin of the mouse in the screening mouse skin bioassay, to the sensitive lungs of the mouse in the mouse lung adenoma, and the site-of-entry lung tissue in rats into which pellets containing PAHs are implanted. Given that EPA has not performed a weight of evidence analysis in accordance with EPA (2005), the addition of in vitro data on Ah-receptor binding, cytotoxicity or tumor promotion would provide no value. In addition, no RPFs should be derived based on such in vitro data.

Chapter 7. Derivation of RPFs for Selected PAHs

7a. Please comment on the scientific justification for the approach for deriving the final RPFs and the discussion of alternative options for the estimation of the final RPFs. Please comment on the reporting of the range of RPFs as a measure of variability instead of a confidence interval. Please comment on whether the data are adequate to support more (or less) precision in deriving the RPFs.

EPRI Comment: EPRI provides detailed comments below about the derivation of RPFs for selected PAHs. It is pointed out that certain papers should be rejected for RFP derivation based on quality issues, such as lack of information on chemical identify and purity, high mortality, and significant skin toxicity. It is also pointed out that many of the RPFs rated as having "low" confidence are, indeed, suspect and should be removed from the final RPF values. Only RPFs with medium or high confidence ratings should be considered for finalization.

EPA (2010) has chosen to report the final RPF value and the range, but the manner in which the average value is calculated is quite convoluted. It allows for single studies to receive undue weight. It uses the higher of the incidence and multiplicity RPFs when

available instead of the simple mean. EPRI agrees that calculating a statistical mean with confidence limits would erroneously lead the public into thinking that the degree of precision of the calculations is greater than that allowed by the data. However, EPA should re-consider the manner of calculating the final average RPF values as noted in the comments below.

7b. Please comment on whether the scientific rationale for consideration of bioassay data versus cancer-related endpoint data has been adequately described. Please comment on whether the cancer-related endpoint data could be used in a more quantitative manner. Please comment on the justification of the final RPF derived for dibenz[a,c]anthracene. Please comment on the use of tumor multiplicity data in the weight-of-evidence evaluations and for the determination of the RPFs.

<u>EPRI Comment:</u>

(a) Cancer-Related Data: EPA (2010) has derived quantitative RPFs from a variety of cancer-related endpoints. EPA (2010) has not used RPFs derived from cancer-related endpoints to calculate the proposed-RPF except for one substance. EPRI agrees with EPA's decision that such RPFs are not suitable for use in quantitative human health risk assessment. EPRI agrees that using DNA adduct, DNA damage, mutagenicity and cell transformation studies, especially in vitro studies and especially studies with one dose group, is scientifically inappropriate for quantitative dose-response assessment. RPFs should not be derived from such studies, and they should be removed from the RPF document altogether.

(b) Dibenz[a,c]anthracene: EPA (2010) has used RPFs derived from cancer-related endpoints to calculate the proposed-RPF for one substance, dibenz[a,c]anthracene. It is not appropriate to use such values for DBacA, as noted below.

According to EPA, DBacA has no tumorigenicity assays and EPA has derived an RPF of 4 based entirely on DNA adduct, DNA damage, mutagenicity and cell transformation studies. The range of calculated RPFs was 0.04 to 50 and EPA rated the confidence "very low." Of the 14 studies reviewed, 8 were single dose studies and of the 6 multiple dose studies, all were in vitro studies (bacterial mutagenicity, DNA damage, and DNA adduct formation).

EPA (2010) has misreported the data on DBacA. Slaga et al. (1980) studied DBacA and found it to be negative for mouse skin tumors. Slaga et al. (1980) met EPA's study inclusion criteria and is listed on EPA's Table 4-1. Results from this study were used for CH and DBahA. Table 4-1 lists BeP and DBacA as "nonpositive." EPRI confirmed that DBacA and BeP were, indeed, negative results in this study. Neither

negative result is reported in EPA's figures, Figure 6-11 for BeP or Figure 6-22 for DBacA.

EPA also misreports the data for DBacA in the text. It states:

> "There were 15 datasets for dibenz[a,c]anthracene that met selection criteria and included benzo[a]pyrene (Figure 6-22)."
>
> "In at least one study, benzo[k]fluoranthene, benz[l]aceanthrylene, 4 benz[e]aceanthrylene, naphtho[2,3-e]pyrene, dibenz[a,h]anthracene, dibenz[a,c]anthracene, and 5 benz[b,c]aceanthrylene showed positive initiating activity. Nonpositive results were reported for pyrene, perylene, benzo[g,h,i]fluoranthene, fluoranthene, anthanthrene, dibenzo[e,l]pyrene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene, benzo[e]pyrene, anthracene, 2,3-acepyrene, and phenanthrene." [emphasis added]

However, this is incorrect. DBacA was negative in dermal bioassays. The above quotation also fails to take into account the three negative mouse skin bioassay results that EPA notes below:

> "Conflicting results were reported in three dermal initiation bioassays of dibenz[a,c]anthracene in which benzo[a]pyrene was not included."

It is inappropriate in the field of toxicology to use in vitro mutagenicity, DNA damage and other cancer "related" bioassay results to conclude that a substance is carcinogenic to humans when at least four studies reported by EPA have shown that the substance is not tumorigenic in the exquisitely sensitive mouse skin bioassay. Thus, EPA has not considered four in vivo negative studies in the mouse and derived RPFs exclusively from cancer-related endpoints. The RPF for DBacA should be withdrawn given these inadequacies.

7c. Please comment on whether the recommendation to apply the proposed RPFs across all routes of exposure is adequately described. Please comment on whether there is additional scientific information that would inform this recommendation. Please comment on whether the available data are adequate to recommend exposure route- or target organ-specific RPFs.

<u>EPRI Comment:</u> It is not appropriate to apply the proposed RPFs to all routes of exposure. As noted in the comments below, the RPFs based on mouse skin studies estimate the relative potency of PAHs to the mouse skin. The criteria have no relevance to human skin or to humans exposed to other routes of exposure. The

RPFs based on the IP dosing of mice are not relevant to any human route of exposure. No CSF has ever been done for any substance based on tumor response data from experiments in which animals were exposed by the IP route of exposure, because this method has no relevance to human health. The RPFs based on lung implantation studies with surgical administration of PAH-containing pellets are also not relevant to human health. No CSFs have ever been derived for any substance data from any of these animal models and routes of exposure, because the data are not relevant to human health.

Furthermore, Schneider et al. (2002) performed a comparison of relative potency factors derived from various studies using different routes of exposure and found that cross route extrapolation is not scientifically justified: "Evaluation of several studies with various PAH mixtures revealed that the potency ratio between pure BAP and the PAH mixture in the same assay is highly dependent on the exposure pathway and the target organ, therefore potency estimates for PAH mixtures should be derived separately for oral, dermal and inhalation exposure using data from studies with the relevant pathway.

EPA (2010) is aware of Schneider et al.'s results and has noted cross route extrapolation as a significant source of uncertainty in the proposed RPFs. Specifically, EPA (2010) stated:

> "However, Schneider et al. (2002) have cautioned that potency ratios appear to cluster by exposure route and target organ and have suggested that route-specific RPFs be developed. There is also some concern regarding the use of benzo[a]pyrene as an index chemical to estimate lung cancer from PAH mixtures, considering that the lung is relatively insensitive to benzo[a]pyrene-induced tumorigenicity following oral exposure (Gaylor et al., 1998)."

7d. Please comment on whether the scientific rationale for the assignment of an RPF of zero for some PAHs is adequately described. Please comment on whether there are other data that should be considered to assess whether an RPF of zero is appropriate. Please comment on whether the scientific rationale for assigning no RPF based on inadequate data for some PAHs is adequately described. Please comment on whether there are alternative methods for assigning RPFs to these PAHs. Please comment on whether the text provides adequate distinction between PAHs with RPFs of zero and PAHs with no selected RPF and whether this distinction is useful for describing uncertainty in determining the cancer risk associated with PAH exposure.

<u>EPRI Comment:</u> It is helpful to propose RPFs for these three PAHs that are routinely analyzed for in environmental samples and that are routinely included in human health

risk assessments. The assignment of RPFs of 0 indicates that, with medium to high confidence, repeated studies have shown them not to pose carcinogenic risk in any test systems.

7e. The final RPFs are characterized with confidence ratings. Please comment on whether the rationale for the confidence ratings is appropriately described. Please comment on whether there are other approaches for describing confidence using the available data that could be applied in either a qualitative or quantitative manner that would be more useful for risk assessment.

EPRI Comment: The confidence ratings are described by a table that gives a checklist with regard to various factors. The ratings are apparently based on the availability of data. EPA should include other factors in rating the confidence of RPF, such as:

- How many RPF estimates are available;
- The publication date of the studies used;
- The availability of identify confirmation and purity data on each study;
- The relevance of the bioassays used to human health risk assessment;
- The relevance of the route of administration used to human health risk assessment;
- The incidence of background tumor rates;
- The mortality rate of animals in the studies used; and
- The presence of significant toxicity, such as skin lesions, in the studies used.

Chapter 8. Uncertainties and Limitations Associated with the RPF Approach

8. Please comment on whether, overall, the document describes the uncertainties and limitations in the methodology used to derive RPFs in a transparent manner. Please comment on whether the most important uncertainties and limitations are identified. Please comment on whether there is existing information that could be used to evaluate the accuracy or validity of the RPF values to predict the cancer risk associated with exposure to PAH mixtures.

EPRI Comment: There is considerable discussion of uncertainties. In particular, EPA (2010) has rated 14 RPFs as having low or very low confidence. Any RPF that is ranked as having such low confidence should be omitted from the final document,

especially BjA, which has the highest proposed RPF (60) and a low confidence. As noted in the comments below, there are many scientific issues associated with this RPF of 60 that render it inappropriate for use in human health risk assessment.

The document does not discuss the limitations of all of the tumor bioassay studies used for RPF derivation, specifically the fact that all of these, with the exception of the one feeding study (Weyand et al. 2004), were specifically designed for hazard identification purposes and were not and are not intended to serve as the basis for quantitative dose-response modeling.

Most importantly, the document does not present or even discuss any validation exercises that were or could be executed with bioassay results from PAH-containing mixtures, including complex mixtures and mixtures constructed by mixing known amounts of identified PAHs. EPRI has overseen the performance of such validation exercises and presents them in the comments below. All of the validation exercises demonstrate that the proposed RPFs overestimate the actual tumor responses seen in studies in which animals were dosed with PAH-containing mixtures.

II. Validation Exercises and Derivation of Updated Cancer Slope Factor for Benzo[a]pyrene

EPA (2010) refers to studies that attempted to validate the RPFs developed by others, but they have not performed any sort of validation on the RPFs proposed in EPA (2010). Scientifically, no models have any value unless and until they are validated with real world data. EPRI recommends that EPA should check the real world accuracy of the proposed RPFs and perform validation exercises in the documents in which new models are proposed. In the absence of any validation, EPRI has overseen the performance of de novo validations. These exercises validate both the assumption of additivity among PAHs and more importantly they directly validate the RPFs themselves. In addition, this study allows for a modern Cancer Slope Factor (CSF) to be derived for benzo[a]pyrene (BaP) to replace its poor quality and highly outdated CSF.

1. Updated Cancer Slope Factor (CSF) for Benzo[a]pyrene Using EPRI-Funded Feeding Study

EPA (2010) states that BaP is the most highly studied PAH and is the only logical choice for an index chemical for derivation of RPFs. They then cite several “robust animal dose-response studies:”

> "The only PAH for which there are robust animal dose-response data is benzo[a]pyrene (Kroese et al., 2001; Culp et al., 1998, 1996a,b; Thyssen et al., 1981, 1980; Rigdon et al., 1969; Rigdon and Neal, 1969, 1966; Neal and Rigdon, 1967)….
>
> In summary, benzo[a]pyrene is the most appropriate compound to use as an index chemical for carcinogenic PAHs. It is well-studied, with a robust database of both bioassay data and mode of action information. Benzo[a]pyrene is a complete carcinogen with both initiating and promoting properties, is among the most potent PAH carcinogens, and is prevalent in many complex environmental mixtures. No alternative index chemical was identified from the list of target PAHs."

Despite the above, EPA (2010) does not make any statements about the robustness of the current cancer slope factor of 7.3 $(mg/kg/day)^{-1}$, which is the geometric mean of several cancer slope factors derived from two, outdated studies, Neal and Rigdon (1967) and Brune et al. (1981). According to the current IRIS profile for BaP, "The data are considered to be less than optimal, but acceptable." In addition, EPA (2010) states about these studies:

> "These studies were not conducted using standard, modern toxicological methods and have several limitations, including inconsistent dosing protocols; varying ages of the animals; use of benzene as a solvent; small numbers of animals; and evaluation of only a limited number of tissues. These studies do, however, provide useful dose-response information on benzo[a]pyrene carcinogenicity."

EPRI agrees that the "robust" studies that EPA (2010) refers to are the more recent studies, such as the EPRI-funded GLP study conducted at the National Center for Toxicological Research under EPA oversight (Beland and Culp 1996; Culp et al. 1998).

EPA (2010) has proposed an RPF scheme that will update the manner in which benzo[a]pyrene-toxic equivalents (BaP-TE) will be evaluated in human health risk assessments. There is no mention of the outdated CSF for benzo[a]pyrene that is currently listed in EPA's IRIS database except for Table 7-4, in which an example is provided for how RPFs are to be used. In Table 7-4, the value 7.3 $(mg/kg/day)^{-1}$, which is the current IRIS CSF, is listed.

This CSF is outdated. The oral CSF for benzo[a]pyrene (7.3 $[mg/kg\text{-}day]^{-1}$) is the geometric mean of four slope factors derived from two rodent feeding studies: Neal and Rigdon (1967) and Brune et al. (1981). In the first study, CFW mice were dosed with benzo[a]pyrene in their laboratory chow (diet). In the second, Sprague Dawley rats were also dosed with benzo[a]pyrene in their laboratory chow (diet). Both studies are very old, and neither was conducted in accordance with modern National Toxicology Program

(NTP) protocols. There is no reason to continue basing PAH risk assessment on this old CSF value when high-quality data from a newer, guideline-compliant study are available.

As part of extensive toxicological studies on a PAHs and PAH-containing mixtures funded by EPRI, the tumorigenicity of benzo[a]pyrene was studied study at the National Center for Toxicological Research (NCTR) of the Food and Drug Administration (FDA) in a two-year dietary administration study in female B6C3F1 mice (Beland et al. 1998; Culp et al. 1998). Groups of mice were fed 0, 5, 25, and 100 ppm benzo[a]pyrene in their diet. The study was performed in accordance with NTP protocols and Good Laboratory Practices.

Gaylor et al. (2000) derived a CSF using multistage modeling for benzo[a]pyrene based on forestomach tumors, which was the most sensitive toxicological endpoint. The CSF was 1.2 $(mg/kg/day)^{-1}$. EPRI has overseen the calculation of a CSF from the Beland et al. (1998) study using EPA's Benchmark Dose Software (BMDS) in order to use EPA's current approach and the approach used in EPA (2010). Using the BMDS and a benchmark response level (BMR) of 0.10, the CSF was identical to that derived by Gaylor et al. (2000), 1.2 $(mg/kg/day)^{-1}$.

The CSF derived in accordance with EPA methods and procedures from a guideline-compliant study is 1.2 $(mg/kg/day)^{-1}$, compared to EPA's outdated CSF of 7.3 $(mg/kg/day)^{-1}$. EPRI recommends that EPA update the benzo[a]pyrene CSF using this newer and higher quality study. In addition, this CSF should be discussed in the RPF document.

2. Validation of Proposed RPFs Using EPRI-Funded Guideline-Compliant Two-Year Feeding Study Results from the National Center for Toxicological Research

EPA (2010) has issued a proposed set of RPFs without performing any validation exercise to determine if they are predictive of the tumorigenicity of real world mixtures. A perfect study for such a validation exercise is the two-year dietary study performed on two well-characterized coal tar samplesfunded by EPRI. The tumorigenicity of two coal tar mixtures and benzo[a]pyrene was studied at the National Center for Toxicological Research (NCTR) in a two-year dietary administration study in female B6C3F1 mice (Beland et al., 1998; Culp et al., 1998). Groups of mice were fed 0, 5, 25, and 100 ppm benzo[a]pyrene in their diet. Other animals were fed one of two coal tar samples. Mixture 1 was a composite of coal tar from seven coal gasification plant waste sites. Forty-eight animals per group were fed coal tar #1 at doses of 0.0, 0.01, 0.03, 0.1, 0.3, 0.6, and 1.0% in their diet. Mixture 2 was a composite of coal tar from two of the seven waste sites and another site having a high benzo[a]pyrene content. Forty-eight animals per group were fed coal tar #2 at doses of 0.0, 0.03, 0.1, and 0.3% in their diet.

Gaylor et al. (2000) derived CSFs using multistage modeling for benzo[a]pyrene and both coal tar mixtures. The CSF for benzo[a]pyrene was based on forestomach tumors. CSFs were derived for both forestomach tumors and lung tumors in the mice. The coal tar mixtures were more potent in causing lung tumors than in causing forestomach tumors:

Substance/Mixture	Cancer Slope Factor	Endpoint
Benzo[a]pyrene	1.2 $(mg/kg/day)^{-1}$	Forestomach
EPRI Coal tar # 1	0.006 $(mg/kg/day)^{-1}$	Lung
EPRI Coal tar #2	0.013 $(mg/kg/day)^{-1}$	Lung

EPRI has overseen the independent calculation of CSFs from the Beland et al. (1998) study using EPA's Benchmark Dose Software (BMDS) in order to use EPA's current approach and the approach used in EPA (2010). CSFs derived from the BMDS show good model fits, and the results are nearly identical to the results presented by Gaylor et al. (2000):

Substance/Mixture	Cancer Slope Factor	Endpoint
Benzo[a]pyrene	1.2 $(mg/kg/day)^{-1}$	Forestomach
EPRI Coal tar # 1	0.007 $(mg/kg/day)^{-1}$	Lung
EPRI Coal tar #2	0.012 $(mg/kg/day)^{-1}$	Lung

The CSFs for whole coal tars resulting from the study performed at NCTR can serve as the basis for an instructive validation exercise. The validation calculations were performed with both the current IRIS benzo[a]pyrene CSF of 7.3 $(mg/kg/day)^{-1}$ and the new CSF of 1.2 $(mg/kg/day)^{-1}$ because the validation must be done in conjunction with a specified estimate of benzo[a]pyrene's potency.

The validation exercise requires that the PAH content of the tested coal tar mixtures be known. The coal tar samples were characterized with respect to the content of each of the standard PAHs but not the full list of PAHs for which EPA (2010) is proposing RPFs. Culp et al. (1998) documented the concentrations of these standard PAH analytes. However, after benzo[c]fluorene was discovered in a coal tar sample in the early 2000's, EPRI requested META Environmental, Inc. (2001) to separate and quantitate benzo[c]fluorene in coal tar samples that made up the composite samples that were tested by NCTR in 1998. The average benzo[c]fluorene concentration in ten coal tar samples was 629 mg/kg. As a reasonable estimate of the benzo[c]fluorene content of the two coal tar samples used in the two-year dietary study, 629 mg/kg was used as a surrogate concentration. The BaP-TE for the coal tar samples was then calculated using the EPA (1993) RPFs and the RPFs proposed in EPA (2010).

Table 1. Comparison of BaP-TE Values for EPRI Coal Tar Mixture 1 (Culp et al. 1998) Using Current and Proposed RPFs

	EPA (1993) RPF	EPA (2010) Average RPF	Coal Tar Mixture 1 (mg/kg)	BaP-TE (mg/kg)	
				EPA (1993) RPFs	EPA (2010) RPFs
Anthanthrene		0.4		0	0
Anthracene		0	2524	0	0
Benz[a]anthracene	0.1	0.2	2374	237.4	474.8
Benz[b,c]aceanthrylene, 11H-		0.05		0	0
Benzo[b]fluoranthene	0.1	0.8	2097	209.7	1677.6
Benzo[c]fluorene[1]		20	629	0	12580
Benz[e]aceanthrylene		0.8		0	0
Benzo[g,h,i]perylene		0.009	1493	0	13.437
Benz[j]aceanthrylene		60		0	0
Benzo[j]fluoranthene		0.3		0	0
Benzo[k]fluoranthene	0.01	0.03	699	6.99	20.97
Benz[l]aceanthrylene		5		0	0
Chrysene	0.001	0.1	2379	2.379	237.9
Cyclopenta[c,d]pyrene		0.4		0	0
Cyclopenta[d,e,f]chrysene, 4H-		0.3		0	0
Dibenzo[a,e]fluoranthene		0.9		0	0
Dibenzo[a,e]pyrene		0.4		0	0
Dibenz[a,h]anthracene	1	10	267	267	2670
Dibenzo[a,h]pyrene		0.9		0	0
Dibenzo[a,i]pyrene		0.6		0	0
Dibenzo[a,l]pyrene		30		0	0
Fluoranthene		0.08	4965	0	397.2
Indeno[1,2,3-c,d]pyrene	0.1	0.07	1353	135.3	94.71
Naphtho[2,3-e]pyrene		0.3		0	0
Phenanthrene		0	7640	0	0
Pyrene		0	5092	0	0
Dibenz[a,c]anthracene		4		0	0
Benzo[a]pyrene	1	1	1837	1837	1837
Total				**2,696**	**20,004**

Notes:
Data from Culp et al. (1998)
[1] Benzo[c]fluorene data from Meta Environmental (2001): average of concentrations in ten coal tar samples

Table 2. Comparison of BaP-TE Values for EPRI Coal Tar Mixture 2 (Culp et al. 1998) Using Current and Proposed RPFs

	EPA (1993) RPF	EPA (2010) Average RPF	Coal Tar Mixture 1 (mg/kg)	BaP-TE (mg/kg)	
				EPA (1993) RPFs	EPA (2010) RPFs
Anthanthrene		0.4		0	0
Anthracene		0	2900	0	0
Benz[a]anthracene	0.1	0.2	3340	334	668
Benz[b,c]aceanthrylene, 11H-		0.05		0	0
Benzo[b]fluoranthene	0.1	0.8	2890	289	2312
Benzo[c]fluorene[1]		20	629	0	12580
Benz[e]aceanthrylene		0.8		0	0
Benzo[g,h,i]perylene		0.009	2290	0	20.61
Benz[j]aceanthrylene		60		0	0
Benzo[j]fluoranthene		0.3		0	0
Benzo[k]fluoranthene	0.01	0.03	1010	10.1	30.3
Benz[l]aceanthrylene		5		0	0
Chrysene	0.001	0.1	2960	2.96	296
Cyclopenta[c,d]pyrene		0.4		0	0
Cyclopenta[d,e,f]chrysene, 4H-		0.3		0	0
Dibenzo[a,e]fluoranthene		0.9		0	0
Dibenzo[a,e]pyrene		0.4		0	0
Dibenz[a,h]anthracene	1	10	370	370	3700
Dibenzo[a,h]pyrene		0.9		0	0
Dibenzo[a,i]pyrene		0.6		0	0
Dibenzo[a,l]pyrene		30		0	0
Fluoranthene		0.08	6370	0	509.6
Indeno[1,2,3-c,d]pyrene	0.1	0.07	1990	199	139.3
Naphtho[2,3-e]pyrene		0.3		0	0
Phenanthrene		0	10100	0	0
Pyrene		0	7220	0	0
Dibenz[a,c]anthracene		4		0	0
Benzo[a]pyrene	1	1	2760	2760	2760
Total				**3,965**	**23,016**

Notes:
Data from Culp et al. (1998)
[1] Benzo[c]fluorene data from Meta Environmental (2001): average of concentrations in ten coal tar samples

Table 3. Comparison of Risk Estimates for Coal Tar Using the EPRI Derived Coal Tar CSF, the Outdated IRIS Benzo[a]pyrene CSF and Current or Proposed RPFs

	EPRI Coal Tar CSF $(mg/kg/day)^{-1}$	10^6 x Coal Tar CSF[2]	BaP-TE EPA (1993) RPFs	BaP-TE x IRIS CSF[1]	RPFs / Whole Mixture	BaP-TE EPA (2010) RPFs	BaP-TE x IRIS CSF[1]	RPFs / Whole Mixture
Coal Tar #1	0.007	7,000	2,696 ppm	19,681	19,681 / 7,000 = 2.8	20,004 ppm	146,029	146,029 / 7,000 = 21
Coal Tar #2	0.012	12,000	3,965 ppm	28,945	28,945 / 12,000 = 2.4	23,016 ppm	168,017	168,017 / 12,000 = 14

Notes:
[1] IRIS benzo[a]pyrene CSF = 7.3 $(mg/kg/day)^{-1}$
[2] Coal tar concentration x EPRI coal tar CSF

Table 4. Comparison of Risk Estimates for Coal Tar Using the EPRI Derived Coal Tar CSF, the NCTR Benzo[a]pyrene CSF, and Current or Proposed RPFs

	EPRI Coal Tar CSF $(mg/kg/day)^{-1}$	10^6 x Coal Tar CSF[2]	BaP-TE EPA (1993) RPFs	BaP-TE x NCTR CSF[1]	RPFs / Whole Mixture	BaP-TE EPA (2010) RPFs	BaP-TE x NCTR CSF[1]	RPFs / Whole Mixture
Coal Tar #1	0.007	7,000	2,696 ppm	3,235	3,235 / 7,000 = 0.46	20,004 ppm	24,005	24,005 / 7,000 = 3.4
Coal Tar #2	0.012	12,000	3,965 ppm	4,758	4,758 / 12,000 = 0.40	23,016 ppm	27,619	27,619 / 12,000 = 2.3

Notes:
[1] NCTR benzo[a]pyrene CSF = 1.2 $(mg/kg/day)^{-1}$
[2] Coal tar concentration x EPRI coal tar CSF

As shown above, when using the outdated CSF for benzo[a]pyrene, the RPF approach overestimates the true carcinogenic potencies of the coal tar mixtures using either current or proposed RPFs. When the proposed RPFs are used, the overestimation of risk increases from 2 to 3-fold to 14 to 21-fold. It must be noted that this overestimation is the minimal overestimation of potency, because only one additional, non-standard PAH is

incorporated into the estimate of BaP-TE. If the concentrations of the other PAHs for which EPA is proposing RPFs were added in to the calculation, the overestimations would rise considerably.

When using the newly derived CSF based on the 1998 NCTR study of EPRI-provided manufactured gas plant site coal tars, the old EPA (1993) RPFs underestimate coal tar potency. However, the proposed EPA (2010) RPFs still overestimate the tumorigenic potency of coal tar.

As a theoretical exercise, EPRI oversaw the recalculation of the BaP-TE for the two coal tar samples making the conservative assumption that all other PAHs for which EPA is proposing RPFs are present in coal tar at a low level of 100 ppm each. Under this assumption, the results of the validation exercise are as noted below.

Table 5. Comparison of Risk Estimates for Coal Tar Using the EPRI Derived Coal Tar CSF, the Outdated IRIS Benzo[a]pyrene CSF, Proposed RPFs and Assumed Concentrations of 100 ppm for Non-Quantitated PAHs

	EPRI Coal Tar CSF $(mg/kg/day)^{-1}$	10^6 x Coal Tar CSF[2]	BaP-TE EPA (2010) RPFs	BaP-TE x IRIS CSF[1]	RPFs / Whole Mixture
Coal Tar #1	0.007	7,000	30,439 ppm	222,205	222,205 / 7,000 = 32
Coal Tar #2	0.012	12,000	33,451 ppm	244,192	244,192 / 12,000 = 20

Notes:
[1] IRIS CSF = 7.3 $(mg/kg/day)^{-1}$
[2] Coal tar concentration x EPRI coal tar CSF

Table 6. Comparison of Risk Estimates for Coal Tar Using the EPRI Derived Coal Tar CSF, the NCTR Benzo[a]pyrene CSF, Proposed RPFs, and Assumed Concentrations of 100 ppm for Non-Quantitated PAHs

	EPRI Coal Tar CSF $(mg/kg/day)^{-1}$	10^6 x Coal Tar CSF[2]	BaP-TE EPA (2010) RPFs	BaP-TE x NCTR CSF[1]	RPFs / Whole Mixture
Coal Tar #1	0.007	7,000	30,439 ppm	36,527	36,527 / 7,000 = 5.2
Coal Tar #2	0.012	12,000	33,451 ppm	40,141	40,141 / 12,000 = 3.3

Notes:
[1] NCTR CSF = 1.2 $(mg/kg/day)^{-1}$
[2] Coal tar concentration x EPRI coal tar CSF

As shown above, when using the outdated CSF for benzo[a]pyrene, the RPF approach overestimates the true carcinogenic potencies of the coal tar mixtures by 20 to 32-fold using the proposed RPFs and assuming that PAHs not quantitatively identified are present at 100 ppm each. When using the newly derived CSF based on the EPRI-funded NCTR study, the proposed RPFs overestimate coal tar potency by 3 to 5-fold.

3. Validation of Additivity Assumption, Existing Studies Showing PAH Inhibition

EPA (2010) assumes as a basis for deriving RPFs that individual PAH components act in an additive manner. This assumption is not required when toxicological data on whole mixtures are derived. There certainly are reports in the literature of dose additivity, but there are a multitude of scientific studies that show that PAHs inhibit the carcinogenic effect of other PAHs in animal bioassays.

Table 1 in Appendix A lists some of these studies. The intent of this table is to demonstrate that many examples of inhibition can be found in the literature. As noted in Table 2-2 of EPA (2010), some studies have also reported potentiation effects with certain mixtures of PAHs, but these citations are not presented here.

Examples of potentiation can also be found in the literature, but these studies are not reported here.

In fact, the issue of interactions between individual PAHs is very complicated. Experimental results make it very difficult to predict the effect, if any, of selected PAHs in humans. For instance, the effects of benzo(e)pyrene are species-specific. In mouse skin, benzo(e)pyrene inhibits the carcinogenic effects of DMBA but potentiates the effects of benzo(a)pyrene. However, in a hamster embryo cell-mediated assay, benzo(e)pyrene has no effect on benzo(a)pyrene- or DMBA-induced mutagenesis. Similarly, phenanthrene inhibited mouse skin tumor formation caused by dibenz(a,h)anthracene when given in ethyl laurate as vehicle. However, when given in triethylene glycol as vehicle, phenanthrene potentiated the effects of dibenz(a,h)anthracene.

The weight of evidence from the literature on interactions between individual PAHs given as pure chemicals does not support a conclusion that all PAHs act in a dose-additive fashion. EPA should not rely on its assumption of dose-additivity based on the results of controlled experiments in mouse skin, because results in mouse skin cannot easily be extrapolated to humans.

First, mouse skin is known to be more sensitive to the chemical-induced tumorigenesis than any other animal. Also, in humans, PAH-exposures do not occur in the absence of

many other exposures. It is known that many other factors can cause inhibition of mouse skin tumorigenesis. For instance, Slaga (1984, 1989) and Wattenberg (1985) report that anti-inflammatory steroids, vitamin A derivatives, retinoids, protease inhibitors, vitamins E and C, selenium, histamine, flavonoids from foods, and a multitude of other agents can inhibit tumor formation in mouse skin.

In addition, skin tumor formation in mouse skin in a reversible process (Slaga 1983, 1984; Ewing 1988). Thus, many of the agents that have been shown in the laboratory to promote skin tumor formation must be repeatedly administered on a daily basis for the animal's lifetime before skin tumors develop. Occasional exposure to these agents does not promote skin tumor formation.

4. Validation of Proposed RPFs and Assumption of Additivity, Existing Studies of Mixtures

The Schmahl et al. (1977) study is presented by EPA (2010) as supportive of the assumption of additivity of effects among a mixture of PAHs. In fact, this study shows that certain PAHs inhibit the carcinogenic effects of other PAHs known to be carcinogenic in animals. Specifically, EPA (2010) has erroneously reported the results of Schmähl et al. (1977). EPA (2010) states:

> “Additivity has been observed in carcinogenicity studies of complex mixtures of PAHs. Schmähl et al. (1977) evaluated the production of skin tumors following combined dermal treatment with 11 PAHs found as constituents of automobile exhaust. Tumor findings were presented separately for two groups of PAHs. High potency carcinogens (Group 1) included benzo[a]pyrene, dibenz[a,h]anthracene, benz[a]anthracene, and benzo[b]fluoranthene. Lower potency PAHs (Group 2) included anthracene, benzo[e]pyrene, benzo[g,h,i]perylene, chrysene, fluoranthene, phenanthrene, and pyrene. Chronic dermal exposure to PAHs in both groups resulted in an additive response when compared to the tumor response for each group alone.”

Using the proposed RPFs, all three dose groups of PAH Mixture #1 (carcinogenic PAH mixture) would be expected to have 100% cancer incidence if the assumption of additivity is correct and the RPFs are valid estimators of the potency of the three additional PAHs in this mixture. In fact, BaP appears to be responsible for 40-80% of the observed tumorigenicity despite that fact that BaP is responsible for only 11% of the BaP-TE (by experimental design).

PAH Mixture #2 was labeled a noncarcinogenic mixture, but it contains fluoranthene, chrysene, and bengo[ghi]perylene. If EPA’s proposed RPFs are correct and the additivity assumption is correct, the expected tumor incidences based on BaP-TE would be 14%,

56%, 100%, and 100%, but the observed tumor incidences are 1%, 0%, 1%, and 19%. Clearly, the RPFs overestimate comparative potency or the assumption of additivity is not correct, or both. It is noted that Mixture #2 does not contain any BaP, but its BaP-TE using the proposed RPFs is equal in the first two dose groups to dose groups which were administered BaP at the same dose. If the RPFs were correct, the tumor incidence would be expected to be 14% and 56%, but the actual tumor incidences were 1% and 0%. This demonstrates that the RPFs are not correct.

Schmähl et al. (1977) also dosed three groups of animals with Mixtures 1 and 2 simultaneously. The BaP-TE of the first group is 10 ug/treatment. This BaP-TE dose of 9-10 ug/treatment gave the following tumor incidences, as noted in Table 2 in Appendix A.

PAH Mixture 1 (cPAH)	36%
PAH Mixture 2 (ncPAH)	1%
PAH Mixtures 1+2 (cPAH + ncPAH)	52%

Similarly, three groups were doses with total BaP-TE of 27-30 ug/treatment. This BaP-TE dose of 27-30 ug/treatment gave the following tumor incidences, as noted in Table 2 in Appendix A.

PAH Mixture 1 (cPAH)	72%
PAH Mixture 2 (ncPAH)	19%
PAH Mixtures 1+2 (cPAH + ncPAH)	70%

In both of these cases, the results of the mixture experiments do not support an assumption of additivity, and they do not support the proposed RPFs.

An evaluation of the data of Pfeiffer et al. (1977) also can be used to validate the assumption of additivity among PAHs. EPA (2010) erroneously excluded this study from the RPF derivation process and from the summary of experimental results that support or refute its assumption of additivity for the RPF scheme. It has done so, because there was 90% mortality reported in the control group before sacrifice. What EPA fails to report is that the experimental data reported in Pfeiffer et al. (1973) at 56 weeks was almost identical to that seen at 144 weeks. In Pfeiffer et al. (1977) it is stated that the experiment was terminated at 114 weeks because of high mortality in the control group and “because during the preceding weeks no increase in the number of tumour-bearing mice had occurred.” Comparison of data from 56 weeks to that from 114 weeks demonstrates that the data from 58 weeks earlier when mortality was not presumably an issue would yield

essentially the same results as those presented here. The presence or absence of tumors in the control animals does not affect the validity of the following analysis.

In Table 3 of Appendix A, the data for mixtures benzo(a)pyrene and dibenz(a,h)anthracene are presented. In this experiment, Pfeiffer et al. (1977) merely dosed animals with BaP alone, with DahA alone, and then with a binary mixture of the two. If the assumption of additivity is correct, the tumor incidence of the binary mixture would simply be the sum of the two individual results.

The data clearly show that the animal carcinogenicity of the two chemicals is not additive. For instance, the tumor incidence for dose group 6 for BaP was 83% and for DBahA was 69%. The tumor incidence expected for a binary mixture assuming additivity would have been 100%. However, the observed tumor incidence was 79%. It is possible that nonlinearities may occur in the dose-response curve at high doses. Thus, one should perhaps not expect that a chemical giving an X% tumor incidence would strictly add with a chemical giving an X% incidence so that the mixture would yield exactly 2X% incidence. However, the observed results demonstrate that the assumption of additivity does not hold and that inhibition was occurring. In the two highest dose groups, for instance, the addition of dibenz(a,h)anthracene caused the tumor incidence of the mixture to be less than the incidence seen when benzo(a)pyrene alone was administered, thus showing that the presence of other PAHs inhibited the action of BaP.

Table 4 in Appendix A shows the data for the twelve component mixture, where the researchers added a mixture of 10 PAHs (including benz[a]anthracene, fluoranthene, chrysene, and benzo[ghi]perylene, to benzo(a)pyrene and dibenz(a,h)anthracene. Again, additivity was not seen. For instance, the tumor incidence for dose group 6 for BaP was 83%, for DBahA was 69%, and for the ten PAH mixture was 5%. The tumor incidence expected for a tertiary mixture assuming additivity would have been 100%. However, the observed tumor incidence was 82%. In two cases, doses 5 and 6, the addition of dibenz(a,h)anthracene and the mixture of 10 PAHs caused the tumor incidence of the mixture to be less than the incidence seen when benzo(a)pyrene alone was administered, thus showing that the presence of other PAHs inhibited the action of BaP.

In Table 5, Appendix A, the data on the five animal groups are shown along with their BaP-TE doses using the proposed RPFs. Expected tumor incidence was estimated by simply assuming the EPA's proposed RPFs are correct and that the dose-response curve was linear as was assumed by EPA (2010). The expected incidence in all cases exceeded the observed tumor incidence. For instance, the expected tumor incidence for the six dose groups in the "Mixture of 12 PAHs" group was 68%, 92%, 100%, 100%, 100%, and 100%, whereas the observed tumor incidence was 41%, 55%, 61%, 72%, 68%, and 82%.

One can also compare the tumor incidence directly for several different animal groups that received similar doses of BaP-TE assuming the RPFs are correct.

Group	BaP-TE (ug)	Observed Tumor Incidence
BaP	12.5	51%
Mixture of 10	13.2	6%
BaP	25	57%
DahA	23.5	37%
Mixture of 2	27	48%
Mixture of 10	26	4%
Mixture of 12	30	41%

All of the above comparisons demonstrate that either the RPFs are incorrect estimates of relative potency or the assumption of additivity is incorrect, or both.

EPA (2010) also cited Nesnow et al. (1998) as support for the assumption of additivity. First, the cited paper, Nesnow et al. (1998), did not involve any studies of PAH mixtures, as reported by EPA. Second, whatever study EPA is attempting to cite appears not to support an assumption of additivity. EPA (2010) states:

> "The results of the study indicated that greater-than-additive effects were seen at low doses, while less-than-additive effects were observed at high doses. However, the magnitude of the interactions was relatively small (twofold), suggesting that potential interactions are limited in extent."

EPRI suspects that EPA (2010) meant to cite Nesnow et al. (1996) or (1998). The 1996 citation is entitled "Lung tumorigenic interactions of five environmental polycyclic aromatic hydrocarbons: Additivity, synergism, and antagonism." The citation is an abstract only and no data or detailed results are available for review and evaluation. However, the authors' conclusion does not support an assumption of additivity:

> "Comparison of the observed numbers of lung adenomas/mouse vs. the expected numbers of adenomas/mouse [based on additive tumor responses] for each of the 32 dose groups indicated that a majority of these groups deviated from additivity."

In a 1998 publication, Nesnow et al. presented quantitative results for these mixture experiments. Nesnow et al. (1998a) concluded:

> "Comparison of observed lung adenoma formation with that expected from additivity identified both greater than additive and less than additive interactions that were dose related i.e., greater than additive at lower doses and less than additive at higher doses."

Other studies cited by EPA (2010) focus on cancer-related endpoints and do not carry the weight that data from tumorigenicity experiments do. Thus, EPA (2010) has presented insufficient evidence that an assumption of additivity is correct.

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Appendix A

Ancillary Data Tables

Appendix A, Table 1. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
methylcholanthrene	dibenzofluorene	skin tumor	mouse	LaCassagne et al. 1945
dibenz (a,h) anthracene	1,2,5,6-dibenzacridine	skin tumor	mouse	LaCassagne et al. 1945
methylchloranthrene	chrysene	skin tumor	mouse	LaCassagne et al. 1945
dibenz(a,h) anthracene	benz(a)anthracene	injection tumors	mouse	Steiner and Falk 1951
benzo(a)pyrene	1,2,3,4-benzanthracene	skin tumor	mouse	Finzi et al. 1968
benzo(a)pyrene	perylene	skin tumor	mouse	Finzi et al. 1968
DMBA	dibenz(a,c) anthracene	skin tumor	mouse	DiGiovanni et al. 1980; DiGiovanni et al. 1982; Slaga 1983
DMBA	benzo(e)pyrene	skin tumor	mouse	DiGiovanni et al. 1980; DiGiovanni et al. 1982; Slaga 1983; Slaga et al. 1979
DMBA	pyrene	skin tumor	mouse	DiGiovanni et al. 1980; Slaga et al. 1979
DMBA	fluoranthene	skin tumor	mouse	DiGiovanni et al. 1980; Slaga et al. 1979
7,12-DMBA	benz(a)anthracene	breast tumor	rat	Huggins et al. 1964
7,12-DMBA	3,9-DMBA	breast tumor	rat	Huggins et al. 1964
7,12-DMBA	6,8-DMBA	breast tumor	rat	Huggins et al. 1964
7,12-DMBA	chrysene	breast tumor	rat	Huggins et al. 1964
9,10-dimethyl-1,2-benzanthracene	phenanthrene	skin tumor	mouse	Huh and McCarter 1960
20-methylcholanthrene	6,7-dihydro methylcholanthrene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
20-methylcholanthrene	hexahydro methylcholanthrene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
20-methylcholanthrene	perhydro methylcholanthrene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
dibenz(a,h,) anthracene	dihydro-dibenz (a,h)anthracene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
dibenz (a,h) anthracene	decahydro-dibenz (a,h)anthracene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964

Appendix A, Table 1. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
dibenz(a,h) anthracene	perhydro-dibenz (a,h)anthracene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
7,12 DMBA	1-ethynylpyrene	DNA binding	mouse skin	Viaje et al. 1990
benzo(a)pyrene	1-ethynylpyrene	DNA binding	mouse skin	Viaje et al. 1990
7,12 DMBA	1-ethynylpyrene	skin tumor	mouse	Alworth et al. 1991
benzo(a)pyrene	1-ethynylpyrene	skin tumor	mouse	Alworth et al. 1991
dibenz(a,h) anthracene	phenanthrene	skin tumor	mouse	Falk et al. 1964
DMBA	1,2,3,4-dibenzanthracene	skin tumor	mouse	Slaga and Boutwell 1977
dibenz(a,h) anthracene	benzo(e)pyrene	skin tumor	mouse	DiGiovanni et al. 1982
dibenz(a,h) anthracene	dibenz(a,c) anthracene	skin tumor	mouse	DiGiovanni et al. 1982
3 - methyl cholanthrene	dibenz(a,h) anthracene	skin tumor	mouse	DiGiovanni et al. 1982
DMBA	dibenz(a,c) anthracene	skin tumor	mouse	Slaga 1978
benzo(a)pyrene	benzo(a)fluorene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	perylene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	benz(a)carbazole	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	chrysene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	benzo(h) fluoranthene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	benz(m,n,o) fluoranthene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	anthracene + pyrene + phenanthrene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	carcinogenic oxy-neutral air pollution fractions	skin tumor	mouse	Falk et al. 1964
DMBA	benzo(e)pyrene	mutagenicity	hamster embryo cells	Baird et al. 1984
benzo(a)pyrene	benzo(e)pyrene	mutagenicity	*Salmonella*	Hass et al. 1981
benzo(a)pyrene	benzo(e)pyrene	metabolic activation	Syrian hamster embryo cultures	Smolarek and Baird 1984
benzo(a)pyrene	anthracene	skin tumor	mouse	Crabtree 1946
benzo(a)pyrene	naphthalene	skin tumor	mouse	Crabtree 1946

Appendix A, Table 1. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
benzo(a)pyrene	phenanthrene	skin tumor	mouse	Crabtree 1946
20-methylcholanthrene	anthracene	skin tumor	mouse	Crabtree 1946
20-methylcholanthrene	naphthalene	skin tumor	mouse	Crabtree 1946
20-methylcholanthrene	phenanthrene	skin tumor	mouse	Crabtree 1946
20-methylcholanthrene	1,2,5,6-dibenzofluorene	skin tumor	mouse	Wartman et al. 1950
9,10-dimethyl-1,2-benzanthracene	8-methyl-1,2-benzanthracene	skin tumor	mouse	Stranger et al. 1952
9,10-dimethyl-1,2-benzanthracene	1,2,5,6-dibenzofluorene	skin tumor	mouse	Hill et al. 1951
9,10-dimethyl-1,2-benzanthracene	6,8-dimethyl-1,2-benzanthracene	skin tumor	mouse	Hill et al. 1951
benzo(a)pyrene	dibenzo(a,l)pyrene + dibenzo(a,e)pyrene	skin DNA adducts	mouse	Hughes and Phillips 1990
DMBA	benzo(e)pyrene	in vivo DNA adducts	mouse	Smolarek et al. 1987
benzo(a)pyrene	crude oil	mutagenicity	*Salmonella*	Petrilli et al. 1980
benzo(a)pyrene	DMSO extract of crude oil	mutagenicity	*Salmonella*	Petrilli et al. 1980
benzo(a)pyrene	PNA fraction Shale oil	mutagenicity	*Salmonella*	Pelroy and Petersen 1979
benzanthracene	PNA fraction Shale oil	mutagenicity	*Salmonella*	Pelroy and Petersen 1979
dimethyl-benzacridine	PNA fraction Shale oil	mutagenicity	*Salmonella*	Pelroy and Petersen 1979
2-aminoanthracene	PAH fraction of Athabasca tar sand	mutagenicity	*Salmonella*	Shahin and Fournier 1978
9,10-dimethyl-1,2-benzanthracene	1,2,5,6-benzanthracene	skin tumor	mouse	Hill et al. 1952
methylcholanthrene	1,2,5,6-benzanthracene	skin tumor	mouse	Hill et al. 1952
benzo(a)pyrene	high boiling mutagenic COM	mutagenesis	*Salmonella*	Dankovic et al. 1989
benzo(a)pyrene	solvent refined coal liquid (426-454 deg)	mutagenesis	*Salmonella*	Dankovic et al. 1989
benzo(a)pyrene	solvent refined coal liquid (454 deg)	mutagenesis	*Salmonella*	Dankovic et al. 1989
benzo(a)pyrene	4 carcinogenic COM	skin tumors	mouse	Dankovic et al. 1989
benzo(a)pyrene	5 carcinogenic COM	metabolic activation	mouse	Dankovic et al. 1989

Appendix A, Table 1. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
DMBA	3-methyl cholanthrene	breast tumors	rats	Wheatley 1988
benzo(a)pyrene	naphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	1-methylnaphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	2-methylnaphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	1-ethylnaphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	2-ethylnaphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	1,2-dimethyl-naphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	1,5-dimethyl-naphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	2,6-dimethyl-naphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	2,3,5-trimethyl-naphthalene	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	mix of above naphthalenes	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	naphthalene fraction of cigarette smoke condensate	skin tumors	mouse	Schmettz et al. 1978
benzo(a)pyrene	white oil	mutagenicity	*Salmonella*	Hermann et al. 1980
benzo(a)pyrene	unused & used hardening oil	mutagenicity	*Salmonella*	Hermann et al. 1980
benzo(a)pyrene	unused & used crankcase oil	mutagenicity	*Salmonella*	Hermann et al. 1980
benzo(a)pyrene	petroleum distillates	mutagenicity	*Salmonella*	Hermann et al. 1980
benzo(a)pyrene	aromatic fractions of coal-derived oils	mutagenicity	*Salmonella*	Haugen and Peak 1983
benzo(a)pyrene	aromatic fractions of coal-derived oils	metabolic activation	rat liver microsomes	Haugen and Peak 1983
benzo(a)pyrene	aromatic fractions of coal-derived oils	DNA adducts	DNA *in vitro*	Haugen and Peak 1983
3'-methyl-4-dimethylaminoazo-benzene	3-methylcholanthrene	liver tumors	rats	Miller et al. 1958

Appendix A, Table 1. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
3'-methyl-4-dimethylaminoazo-benzene	3,4-benzpyrene	liver tumors	rats	Miller et al. 1958
3'-methyl-4-dimethylaminoazo-benzene	1,2,5,6-dibenzanthracene	liver tumors	rats	Miller et al. 1958
3'-methyl-4-dimethylaminoazo-benzene	1,2-benzanthracene	liver tumors	rats	Miller et al. 1958
2-acetylaminofluorene	3-methylcholanthrene	liver tumors	rats	Miller et al. 1958
benzo(a)pyrene	gasoline & diesel exhaust condensates	BaP-DNA adducts	mouse	Schoket et al. 1989
Dibenz(a,l)pyrene	Diesel exhaust	Skin tumors	mouse	Courter et al. 2008
Benzo(a)pyrene	Urban dust particulate matter	Skin tumors	mouse	Courter et al. 2007
Dibenz(a,l)pyrene	Urban dust particulate matter	Skin tumors	mouse	Courter et al. 2007
Benzo(a)pyrene	Coal tar extract	DNA adducts	Chinese hamster cells	Mahadevan et al. 2007
Dibenz(a,l)pyrene	Coal tar extract	DNA adducts	Chinese hamster cells	Mahadevan et al. 2007
Benzo(a)pyrene	Dibenz[a,h]anthracene	Skin tumors	Mouse	Pfeiffer 1973, 1977

Appendix A, Table 2. Data of Schmahl et al. (1977)

Test Group	Total Dose ug/treatment	BAP-TE Dose ug/treatment[1]	Expected Cancer Incidence[2]	Observed Cancer Incidence
Benzo(a)pyrene	1.0	1.0		14%
	1.7	1.7		28%
	3.0	3.0		56%
PAH Mixture #1 (cPAH)	4.0	9	100%	36%
	6.8	15.4	100%	65%
	12.0	27	100%	72%
PAH Mixture #2 (ncPAH)	65	1	14%	1%
	195	3	56%	0%
	585	9.1	100%	1%
	1755	27.3	100%	19%
PAH Mixtures 1 + 2 (cPAH + ncPAH)	69	10.0	100%	52%
	117	17.1	100%	61%
	207	30	100%	70%

Notes:
[1] EPA (2010) proposed RPFs
[2] Expected incidence was estimated from observed BaP response at nearest BaP-TE dose assuming linear dose-response curve.
BaP-TE = benzo(a)pyrene toxic equivalents
ug = microgram

Appendix A, Table 3. Validation of Assumption of Additivity
Data of Pfeiffer et al. (1977) Two Component Mixture

Dose Group	Cancer Incidence-BaP	Cancer Incidence-D(ah)A	Expected Incidence for Mixture[1]	Observed Incidence
1	9%	37%	46%	48%
2	35%	39%	74%	44%
3	51%	44%	95%	61%
4	57%	56%	100%	68%
5	77%	65%	100%	69%
6	83%	69%	100%	79%

Notes:
[1] Sum of incidence of BaP-dosed animals and D(ah)A-dosed animals.

Appendix A, Table 4. Validation of Assumption of Additivity
Data of Pfeiffer et al. (1977) Twelve Component Mixture

Dose Group	Cancer Incidence-BaP	Cancer Incidence-D(ah)A	Cancer Incidence-10 PAHs	Expected Incidence for Mixture[1]	Observed Incidence
1	9%	37%	6%	52%	41%
2	35%	39%	8%	82%	55%
3	51%	44%	6%	100%	61%
4	57%	56%	4%	100%	72%
5	77%	65%	13%	100%	68%
6	83%	69%	5%	100%	82%

Notes:
[1] Sum of incidence of BaP-dosed animals and D(ah)A-dosed animals.

Appendix A, Table 5. Validation of EPA (2010) RPFs Using Tumor Data of Pfeiffer (1977)

Test Group	Total Dose (ug/treatment)	BaP-TE Dose EPA (2010) RPFs (ug/treatment)	Observed Incidence	Expected Incidence[1]
Benzo[a]pyrene	3.12	3.12	9%	
	6.25	6.25	35%	
	12.5	12.5	51%	
	25	25	57%	
	50	50	77%	
	100	100	83%	
Dibenz[a,h]-anthracene	2.35	23.5	37%	54%
	4.7	47	39%	72%
	9.3	93	44%	77%
	18.7	187	56%	100%
	37.5	375	65%	100%
	75	750	69%	100%
Mixture of 2 PAHs	5.5	27	48%	62%
	11	53	44%	82%
	22	100	61%	83%
	44	210	68%	100%
	88	430	69%	100%
	175	850	79%	100%
Mixture of 10 PAHs	270	3.3	6%	10%
	550	6.6	8%	37%
	1100	13.2	6%	54%
	2200	26	4%	59%
	4400	53	13%	82%
	8800	100	5%	83%
Mixture of 12 PAHs	280	30	41%	68%
	560	60	55%	92%
	1100	120	61%	100%
	2200	240	72%	100%
	4500	480	68%	100%
	9000	960	82%	100%

Notes:
[1] Expected incidence was estimated from observed BaP response at nearest BaP-TE dose assuming linear dose-response curve.
Red indicates RPFs overestimate observed potency.