UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

DATE

EPA-SAB-10-0XX

The Honorable Lisa P. Jackson
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: SAB Review of EPA's "Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures"

Dear Administrator Jackson:

In 1993, EPA developed the document, *Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs),* which recommends a Relative Potency Factor (RPF) approach for assessing PAH mixtures. EPA's RPF approach is a component-based approach to assessing the toxicity of PAH mixtures, which involves an analysis of the toxicity of individual PAHs of the mixture relative to the toxicity of the index compound, benzo[a]pyrene (BaP). EPA's Office of Research and Development (ORD) has developed a draft technical document, *Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures,* hereafter called "PAH Mixtures document", to update the 1993 document by expanding the number of PAHs assessed and including recent studies from the published literature.

ORD asked the SAB to provide recommendations on the scientific soundness and rationale of the PAH Mixtures document in several areas: rationale for recommending an RPF approach, discussion of previously published RPF approaches, evaluation of the carcinogenicity of individual PAHs, methods for dose-response assessment and RPF calculation, selection of PAHs for inclusion in the RPF approach, derivation of RPFs for selected PAHs, and uncertainties and limitations associated with the RPF approach. The SAB convened the PAH Mixtures Review Panel, which held a public meeting from June 21-23, 2010 to provide advice to the Agency. The key points and recommendations of the Panel are detailed in the report. Below is a brief highlight of the major comments and recommendations.

The Panel recognizes the pragmatic need for the RPF approach and based upon the currently available data, agrees with EPA's use of the RPF approach for assessing PAH mixtures. However, the Panel does not find the scientific basis for the RPF approach to be well justified in the document.

The Panel agrees with EPA's selection of benzo[a]pyrene (BaP) as the index compound for the RPF approach. However, the current cancer slope factor for BaP is outdated and in order to estimate the risk of PAH mixtures, an up-to-date cancer slope factor for BaP is essential. The Panel urges the Agency to quickly finalize the BaP assessment.

The Panel has concerns with the methodology used to calculate RPFs. In particular, the Panel has concerns with RPF calculations that are based on single experiments, and also those calculations that are based on studies that only have single-dose data. The Panel is also concerned with extraordinarily high RPF values that were calculated from only a limited number of bioassays. Without sufficient additional information or justification, the Panel cannot recommend developing RPFs for PAHs with these data characteristics. Additionally, when cancer bioassay data are not available, the Panel does not recommend calculating an RPF, even if there are cancer-related endpoint data available (e.g. dibenz[a,c]anthracene).

The Panel recommends that EPA pursue developing a whole mixtures approach for PAHs to replace the RPF approach in the near future (5-10 years). The Agency should set this goal as a strategic initiative, with a specific timeline and benchmarks, that lays the foundation for an underlying concerted research program to achieve this goal. The Panel recommends that the Agency seek support from the National Toxicology Program (NTP) and/or other entities to test a portfolio of 12-15 different complex PAH mixtures, using *in vivo* tumor studies (e.g., skin tumorigenesis studies). These complex PAH mixtures should represent a diverse array of mixtures, but also represent the most important PAH mixture classes of concern to EPA. The Panel believes that, with these data in hand, one could then compare a real world mixture to this portfolio of standardized mixtures and be able to adequately estimate cancer risk

The SAB appreciates the opportunity to provide EPA with advice. We look forward to receiving the Agency's response.

Sincerely,

Dr. Deborah L. Swackhamer
Chair
EPA Science Advisory Board

Dr. Nancy K. Kim
Chair
PAH Mixtures Review Panel

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U.S. Environmental Protection Agency
Science Advisory Board
Polycylic Aromatic Hydrocarbon (PAH) Mixtures Review Panel

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ACRONYMS

BaP	Benzo[a]pyrene
EPA	Environmental Protection Agency
IRIS	Integrated Risk Information System
NCEA	EPA's National Center for Environmental Assessment
ORD	EPA's Office of Research Development
PAH	Polycyclic Aromatic Hydrocarbon
RPF	Relative Potency Factor
SAB	Science Advisory Board

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1. EXECUTIVE SUMMARY

In 1993, EPA developed the document, *Provisional Guidance for Quantitative Risk Assessment of PAHs*, which recommends a Relative Potency Factor (RPF) approach for assessing PAH mixtures. EPA's Office of Research and Development (ORD) has developed a draft technical document, *Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures*, hereafter called "PAH Mixtures document", to update the 1993 document by expanding the number of PAHs assessed and including recent studies from the published literature.

EPA's Office of Research and Development (ORD) requested that the Science Advisory Board (SAB) Polycyclic Aromatic Hydrocarbon (PAH) Mixtures Review Panel review the PAH Mixtures Review document. There were nine charge questions, which focused on an overview of the document, on the specific chapters of the document, and the appendices. These charge questions and responses are detailed in the report and the major recommendations from the Panel are highlighted below.

The Panel recognizes the pragmatic need for the RPF approach and agrees with EPA that, based upon the currently available data, the RPF approach should be used to assess PAH mixtures. However, the Panel does not find the scientific basis for the RPF approach to be well justified in the document.

The Panel agrees with EPA's selection of benzo[a]pyrene (BaP) as the index compound for the RPF approach. However, the current cancer slope factor for BaP is outdated and to assess the risk of PAH mixtures, it is essential to have an up-to-date cancer slope factor for BaP. The Panel urges the Agency to quickly finalize the BaP assessment.

The Panel recommends that EPA pursue developing a whole mixtures approach for PAHs to replace the RPF approach in the near future (5-10 years). The Agency should set this goal as a strategic initiative, with a specific timeline and benchmarks, that lays the foundation for an underlying concerted research program to achieve this goal. The Panel recommends that the Agency seek support from the National Toxicology Program (NTP) and/or other entities to test a portfolio of 12-15 different complex PAH mixtures, using *in vivo* tumor studies (e.g., skin tumorigenesis studies). These complex PAH mixtures should represent a diverse array of mixtures, but also represent the most important PAH mixture classes of concern to EPA. The Panel believes that, with these data in hand, one could then compare a real world mixture to this portfolio of standardized mixtures and be able to adequately estimate cancer risk

Rationale for Recommending an RPF Approach

The PAH Mixtures document presents the rationale for recommending an RPF approach for PAH mixtures. The Panel does not find the scientific basis for the proposed RPF approach to be well justified in the PAH Mixtures document. There are two basic assumptions that are proposed for applying the dose-additivity model used in the RPF approach: that the PAHs in the

mixture act by a similar mechanism and that no significant interactions occur at low, environmentally relevant doses. The document itself cites data that call into question both of the underlying assumptions of the document. The document discusses a number of other uncertainties, some of which cannot currently be validated or dismissed, that further undermine the logical and scientific basis for the assumptions on which the RPF method is based. However, it is not clear that the first assumption is required for the RPF method, since it is based on the outcomes of cancer bioassays, not on the underlying mechanism(s). The second assumption could be tested by directly comparing a surrogate mixture of key PAHs with RPFs to the results of cancer bioassays of real world complex mixtures such as coal tar and directly to benzo[a]pyrene (BaP) as a single agent.

As a practical matter, the Panel recommends that EPA continue to use the RPF approach until sufficient data are available on surrogate complex PAH mixtures to replace the RPF approach. Additional historical perspective, in particular a summary of EPA's previous discussions about implementing a whole mixtures approach, should be included in the revised document since it is an important component in, and justification for the Agency's decision to pursue the RPF method. The Panel agrees with EPA's decision to update the 1993 approach by increasing the number of compounds in the approach, and including the most recent data in calculating and expanding the RPF values for PAHs.

The document uses benzo[a]pyrene as the index compound for the RPF approach. The Panel finds that the choice of BaP as the index chemical is well justified and is appropriately described for this RPF approach. BaP is the best studied PAH and meets the criteria for the index compound for an RPF assessment. During the meeting, the Agency noted that a revised Integrated Risk Information System (IRIS) assessment of BaP is undergoing parallel review that will likely lead to a revised cancer slope factor (CSF) and values for oral, dermal and inhalation exposures. A good estimate of the CSF for BaP is central to the validity of the RPF method.

The document presents the assumption that PAHs, as a chemical class, have a similar mode of carcinogenic action. The Panel does not believe that this assumption is well justified. Although evidence suggests that a subset of closely related PAHs have "similar" modes of action for specific steps in the overall mechanism, these compounds may each act via different precise mechanisms at a more detailed level, and may therefore weaken the support for this assumption. Most importantly, the RPF method may not require this assumption since it is based on the ultimate endpoint, cancer. Thus, the mechanistic underpinnings should be de-emphasized as a rationale for RPF and a stronger argument should be made for emphasizing actual cancer bioassay data to directly compare PAHs alone and in mixtures. Mechanistic information on BaP could be referenced from other comprehensive sources such as IRIS and International Agency for Research on Cancer (IARC) monographs or other recent literature reviews.

The document discusses the assumption that interactions among PAH mixture components do not occur at low levels of exposure typically encountered in the environment. The Panel does not find this assumption to be scientifically well justified. As discussed in the document, coal tar behaved very differently in *in vivo* carcinogenesis assays than would be

predicted from studies with BaP as a single agent, or what would likely be predicted from a RPF approach based on BaP as a single agent. Also, as discussed in the document, the complex and unpredictable results of simple binary combinations of PAHs also undercuts both scientific assumptions of the RPF approach. However, in the absence of data that support a specific interaction (additive, sub- or super-additive, etc.), a default assumption of additivity is a reasonable assumption for the purposes of the RPF analysis.

Discussion of Previously Published RPF Values

The PAH Mixtures document presents a background on how RPFs have been derived in the past. The Panel believes that the document adequately summarizes the previous RPF approaches, but could be improved by providing more quantitative information, and editing the table to use a standardized approach for reporting values (same significant figures, scale, etc.).

Evaluation of the Carcinogenicity of Individual PAHs

The document discusses the development of a database of primary literature and the criteria used to include or exclude studies. Based upon the initial literature search, a list of 74 PAHs were evaluated. The Panel finds that the list of 74 PAHs is reasonable in view of the criteria of having three or more fused rings and not containing heteroatoms or alkyl substituents. Nonetheless, the possible availability of bioassay data on two recently synthesized and identified environmental PAHs, naphtha[1,2-a]pyrene and naphtha[1,2-e]pyrene, should be investigated. Additionally, a recently published IARC Monograph on PAHs, Volume 92, should be considered as an additional resource (IARC, 2010).

The Panel believes that quality scores should be assigned to individual studies. The Panel recommends including information on sample size, dosing, mortality (prior to tumor development), defined test compound purity, and whether or not the data utilized are derived from tumor incidence or multiplicity. The Panel does not believe that a PAH should be excluded solely if statistical significance is not achieved.

The PAH Mixtures document stipulates that BaP must be tested concurrently with the PAH being considered. This restriction raises the concern that quality tumorigenicity studies may be dismissed. The Panel recommends that EPA consider exploring a “daisy-chain” approach, where a PAH that was tested with BaP could serve as a surrogate for BaP in studies where BaP was not tested concurrently. This may allow for additional quality studies to be included. The Panel recommends that this be examined especially in those instances where limited tumor data were used to establish a RPF value. However, in considering this alternative approach, EPA should also take into account factors that could potentially outweigh the benefits in the establishment of a RPF for a specific PAH, such as cross-study and cross-laboratory comparability issues.

Methods for Dose-Response Assessment and RPF Calculation

The document presents the selection of dose-response data and methods for dose-response assessment and RPF calculation. The Panel believes that for quantal data, the multi-stage cancer model should be used, parameterized with the degree of the polynomial to equal the number of dose groups (g) minus 2 (i.e., g-2). When this model is not adequate, a higher degree polynomial may be considered i.e., (g-1)-polynomial function, to calculate the benchmark dose (BMD). For continuous data, a polynomial model or nonlinear model should be used to calculate the BMD. The selected benchmark response (BMR) should be based on the low response region (i.e., 10% for quantal data and 1 standard deviation (SD) from the control mean for continuous data). Deviations from these strategies should be explained in the document. While other nonlinear models may be used for the quantal data, the multi-stage cancer model is considered sufficiently flexible to accommodate the dose-response shapes in the available data.

The Panel finds that the strategy of using study-specific dose-response data for BaP with another PAH is advantageous since downstream calculations are intra-study and avoid comparisons without accounting for study effects/characteristics. It should be noted that the estimates of the BaP slope across studies are very different; the range of the estimates can be more than 1,000 fold different. This supports the idea of using study-specific data for calculating the RPFs.

The Panel believes that it is correct to base the derivation of the RPFs on the unbiased estimates derived from the BMDs, rather than the lower confidence limit on the benchmark dose (BMDL), in order to obtain an unbiased estimate of the total exposure for a mixture (expressed as the total BaP equivalent dose). The Panel does not believe that any alternative approaches are necessary.

The Panel believes that when multiple doses are available for dose-response modeling, all the data should be used with a sufficiently flexible model, i.e., the multi-stage cancer model. The Panel is concerned about using high-BMR values to calculate the RPFs in single-dose studies. In a single-dose study, a one-stage model can be fit, which will exactly predict the observed mean response. In this case, the ratio of slopes for calculating the RPF is not dependent on the BMR. However, with the single-dose studies, there is no way to verify the prediction where data are not available. The Panel recommends that when single-dose studies are used to calculate the RPF, the impact on the RPF calculation should be described.

Selection of PAHs for Inclusion in the Relative Potency Approach

The document describes the selection of PAHs for inclusion in the RPF approach. The Panel finds that the method for selecting the PAHs appears to be scientifically justified, but several issues are incompletely considered. The Panel recommends that a list of quality criteria should be defined and articulated (e.g., methodologically sound, such as inclusion of an adequate control group, sample size, dose, number of PAHs measured, purity of the compounds considered) prior to the weight of the evidence evaluation (e.g., prior to its entrance into Figure

6-1). This information should be illustrated in tabular form or individual graphs. Only studies of sufficient quality (defined a priori) should be considered in the weight of evidence evaluation. The Panel also recommends that once a study is considered to be of sufficient quality to be included in the weight of evidence evaluation, the variability of the study characteristics should be considered prior to the calculation of the RPF, rather than after.

The weight-of-evidence analysis in the document does not include data related to Ah-receptor binding. The Panel finds that the rationale for the omission of Ah-receptor data is well justified. The Panel also agrees with EPA that once information on tumor formation is demonstrated, then the additional information on cytotoxicity and tumor promotion is not needed. However, the PAH Mixtures document should justify the reasons for omission of these data.

The analysis presented in the document uses an RPF detection limit as a means of comparing positive and nonpositive (or negative) bioassays. The Panel recommends that the description of the RPF detection limit be made clearer, particularly whether the detection limit is or is not a post hoc power calculation. If it is a post hoc power calculation, this information would have been more useful prior to calculating the RPF. It is unclear why the detection limits for cancer-related endpoints were not calculated.

Graphic arrays of the calculated RPFs (Figures 6-2 through 6-35) are presented in the document as a means of representing the variability in RPFs. The Panel finds that Figures 6-2 through 6-35 provide a good summary of the individual studies considered as well as the variability of individual RPF estimates across studies. However, the Panel recommends that the studies used to estimate the final RPF be clearly identified in the Figures.

The Panel also recommends that the studies be shown as point estimates coupled with some type of information on variability (e.g., standard error, SD, confidence interval, range). The information on variability is viewed as key to help the reader interpret the study findings.

Derivation of RPFs for Selected PAHs

The document describes various methods (e.g. prioritization of studies) and different approaches for deriving final RPFs (e.g. arithmetic mean). The Panel finds that the use of an arithmetic mean to estimate the final RPFs is appropriate, as is presenting a range, instead of a confidence interval. The Panel does have several reservations regarding the RPF calculation approach. The Panel is concerned about calculating RPFs based upon a single experiment as well as calculating RPFs using studies where there was only a single-dose level of BaP and/or the PAH being evaluated. The Panel is also concerned about calculating the arithmetic mean for PAHs that have markedly divergent individual RPFs. Without sufficient additional justification, the Panel can not recommend calculating RPFs for PAHs with these data characteristics.

The Panel strongly believes that use of cancer bioassay data is essential for determining the RPF for a given PAH. Cancer-related endpoint data are useful as supporting data but the Panel does not recommend the use of only cancer-related endpoint data for determining the RPF.

Therefore, the Panel does not recommend calculating an RPF for dibenz[a,c]anthracene and recommends that it be removed from Table 7.2 until further bioassay data becomes available.

The Panel is concerned about combining RPFs calculated from tumor multiplicity data with RPFs calculated from tumor incidence data in calculating final RPFs. RPF values should not be averaged from these two different measures without sufficient justification for using the multiplicity data. The Panel recommends that tumor multiplicity data should only be used when dose-response data are available to allow accurate assessment of relative differences between the compounds being compared.

The approach described in the document averages RPFs across all routes of exposure. The Panel agrees with this approach and does not believe that there would be much value in providing route- or target organ-specific RPFs at the present time, because there are not sufficient data to do so.

Although the Panel agrees with the decision to not calculate separate RPF values for different routes of exposure, the route of exposure may be an issue of concern for generating RPF values for compounds where the available data are only via non-physiological routes. Without additional supporting data, the Panel does not recommend developing RPFs for these PAHs since route of exposure can play an important role in bioavailability and toxicokinetics that may alter the relative potency of the test compound as compared to BaP, when tested via a more standard route of exposure.

The document describes the scientific rationale of assigning an RPF of zero for some PAHs. The Panel generally finds that the scientific rationale presented in the document for assignment of an RPF of zero, the assignment of no RPF, and the distinction between them is appropriate. The Panel recommends that a consistent approach be adopted for using RPFs of zero for all compounds for which final RPFs are calculated.

The document characterizes final RPFs with confidence ratings. In general, the Panel believes that the confidence ratings are a good idea. However, the confidence ratings don't appear to give any indication of the overall quality of the data being assessed and used for the RPF calculation. The Panel strongly believes that there needs to be some measure of the quality of the individual studies used to generate the RPFs.

Uncertainties and Limitations Associated with the RPF Approach

The document discusses the uncertainties and limitations associated with using the RPF approach for PAH mixtures risk assessment. The Panel finds that the uncertainties in the methodology of deriving RPFs are well described. The major methodological uncertainties are clearly defined and discussed such that there is little doubt about the methods that were used and the limitations of the final RPF values reported.

In evaluating the average RPF values, the quality of the source material should be evaluated rather than giving equal weight to each in calculating average RPF values. Some type of weighting scheme needs to be developed for RPFs based on the quantity and quality of existing data.

More data dealing with the comparisons of the RPF approach and estimates of cancer risk derived from complex mixtures are needed, which would reduce some of the uncertainties associated with the RPF approach described in the document.

The cancer slope factor for BaP is multiplied by the RPFs in order to obtain cancer unit risk factors for each of the PAHs. Hence, the cancer unit risk factor for BaP is critical to the calculation of the cancer risk estimate for a mixture using the RPF approach. Because of the relatively large uncertainty in the cancer unit risk factor for BaP, this value needs to be updated before reliable estimates of cancer risk can be derived for mixtures of PAHs.

Since RPFs generally vary as dose increases, RPFs based on a single dose, especially at high doses, are quite uncertain and should not be used until additional data become available. The question arises of the relevance of high doses in animal studies to the much lower doses experienced by humans. This question is not discussed adequately in the document.

The state of a single PAH administered to animals in bioassays may be different from the state of the same PAHs in mixtures where they may not be easily desorbed from solid particles. The bioavailability to humans for PAHs in a mixture needs to be compared to the bioavailability in animal bioassay experiments that utilize purified PAH compounds. Cancer risk estimates based on the RPF values and total concentration of PAH in mixtures may be overestimated.

The composition for each individual mixture must be adequately determined, otherwise additional uncertainty is added to the RPF approach. Completely characterizing mixtures is difficult and this limitation and uncertainty should be discussed.

Appendices

The appendices in the document contain information to allow independent verification of the calculated RPFs. The Panel finds the appendices to be generally useful for verifying the calculations of the RPFs. The Panel recommends reorganizing the appendices by chemical (with each identified in the Table of Contents). This would include the corresponding BaP data for each study within each chemical section which may be repeated across PAHs.

The Panel finds the plots from the BMD software output to be useful but it should be noted that the linear extrapolation to the origin is based on BMDLs instead of BMDs. The calculation of the multi-stage cancer slope factor is also given based on the BMDL instead of the BMD. The Panel recommends that slope factors based on BMDs be added to these appendices.

2. INTRODUCTION

In 1993, EPA developed the document, *Provisional Guidance for Quantitative Risk Assessment of PAHs*, which recommends a Relative Potency Factor (RPF) approach for assessing PAH mixtures. EPA's Office of Research and Development (ORD) has developed a draft technical document, *Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures*, hereafter called "PAH Mixtures document", to update the 1993 document by expanding the number of PAHs assessed and including recent studies from the published literature.

PAHs are a class of chemicals that have variously been defined to include organic compounds containing either two or more, or three or more, fused rings made up of hydrogen and carbon atoms (WHO, 1998). The number of chemicals that comprise the PAH class is not known, but hundreds of PAHs are thought to be present in complex mixtures (WHO, 1998). PAHs do not occur in the environment as isolated entities; they primarily occur in complex mixtures generated from the incomplete combustion or pyrolysis of substances containing hydrocarbons. Some of the complex mixtures containing PAHs that are typically found in the environment include coal tar, manufactured gas plant (MGP) residues, coke oven emissions, diesel and gasoline exhaust, and coal plant emissions. Many PAHs are demonstrated tumorigenic agents in animal bioassays and are active in cancer–related *in vivo* or *in vitro* tests.

EPA's PAH Mixtures document presents a component-based approach to assessing the toxicity of PAH mixtures, which involves an analysis of the toxicity of components of the mixture and provides an approach for estimating cancer risk for PAH mixtures by summing doses of component PAHs after scaling the doses relative to the potency of the selected index PAH, benzo[a]pyrene (BaP). The cancer risk is then estimated using the dose-response curve for the index PAH.

The PAH Mixtures document is limited in focus to analyzing only unsubstituted PAHs with three or more fused aromatic hydrocarbon rings, because they are the most widely studied members of the PAH chemical class. The analysis evaluated 74 PAHs and final non-zero RPFs were calculated for 24 of the PAHs.

The Panel met through a public teleconference call on June 8, 2010 for a briefing on EPA's draft PAH Mixtures document and to review the charge questions presented by the Agency. The Panel then met in a public face-to-face meeting on June 21 – 23, 2010 in Washington, DC, to review the Agency's draft PAH Mixtures document and to deliberate on the charge questions. There were nine charge questions, which focused on an overview of the document, on the specific chapters of the document, and the appendices. These charge questions are presented below along with the responses from the Panel.

3. RESPONSE TO EPA CHARGE QUESTIONS

3.1. General Charge Questions

1a. 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.

Overall the Panel finds the PAH Mixtures document to be logical, clear, and concise. However, the Panel does not believe that the scientific basis for the RPF approach is well justified. The Panel recommends that EPA begin developing a comparative/surrogate mixtures approach to replace the RPF approach within the next 5-10 years.

It is recommended that the Agency should set this goal as a strategic initiative, with a specific timeline and benchmarks, that lays the foundation for an underlying concerted research program to achieve this goal.

The Agency should seek support from the National Toxicology Program (NTP) or other entities to test a portfolio of 12-15 different complex mixtures, in *in vivo* tumor studies (e.g., skin tumorigenesis studies). These mixtures should represent a diverse array of mixtures but also represent the most important mixture classes of concern to EPA (based on the level of health concerns and/or extent of exposure) such as coal tar, manufactured gas plant (MGP) residues, coke oven emissions, diesel and gasoline exhaust, coal plant emissions, etc. The Panel believes that, with these data in hand, one could then compare a real world mixture to this portfolio of standardized mixtures and be able to adequately estimate risk.

These mixtures could also be compared to a surrogate mixture (e.g., a mixture representing the ca. two dozen compounds being assessed in the RPF method) as well as BaP as a single agent. This would provide a direct validation of the RPF method and link these results to previous data on real world samples for which RPF compound values are known.

In parallel with the bioassay testing, the Agency should support research to develop a suite of short-term assays and biomarkers that accurately reflect the carcinogenic potential of or an *in vivo* exposure to these mixtures. These assays and biomarkers could be used as indicators both in animal studies and human epidemiology studies.

However, until sufficient cancer bioassay is available, the Panel recommends that EPA continue to use the RPF approach for PAH mixtures and to finalize the document based upon the Panel's comments and recommendations.

1b. Please comment on whether the report provides adequate context for how the proposed RPF approach could be used in a PAH mixtures risk assessment.

The Panel finds that the PAH Mixtures document does not provide an adequate context for how the proposed RPF approach could be using in a PAH mixtures risk assessment. The Panel recommends that more discussion is needed to provide this context, including moving relevant portions from Chapter 7 into earlier sections of the document.

3.2. Charge Question 2 - Chapter 2 – Rationale for Recommending an RPF Approach

Chapter 2 presents the rationale for recommending an RPF approach. In an RPF approach, doses of component chemicals that act in a toxicologically similar manner are added together, after scaling the doses relative to the potency of an index chemical. Benzo[a]pyrene (B[a]P) is selected as the index compound for this RPF approach. The RPF approach involves two key assumptions related to the application of a dose-additivity model: (1) PAH components in the mixture act in a similar toxicological manner; and (2) interactions among PAH mixture components do not occur at low levels of exposure typically encountered in the environment.

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.

At the meeting, the Panel discussed this issue in considerable detail, and concluded that this charge question actually represents two distinct questions: first whether, based on available literature, there is a sound scientific foundation for use of the single-agent relative potency factor (RPF) approach, particularly with respect to the two core assumptions of this rationale that were proposed in the PAH Mixtures document; and second, whether there is a reasonable practical consideration in using the RPF approach at this time, independent of the scientific foundation and underlying assumptions. The rationale for this dichotomy is outlined below.

With regard to the first question, the Panel concludes that the scientific basis for the proposed RPF approach is not well justified in the current document. There are two basic assumptions that are proposed in the document as the basis for considering the RPF approach specifically for PAHs: first, that the chemicals of comparison are all assumed to act by a similar mechanism as the reference compound (i.e., benzo[a]pyrene - BaP), allowing one to model them relative to each other based on this reference compound; and second, that their effects are additive by assuming no significant interactions at low, environmentally relevant doses.

The Panel considered the PAH Mixtures document, the studies cited within, as well as other data. The document discusses studies that call into question both of the underlying core assumptions, and further elaborates on a number of other uncertainties, some of which cannot currently be validated or dismissed, that further undermine the logical and scientific basis for the assumptions on which the RPF method is based. These are discussed in more detail in response

to charge questions 2c. and 2d. below, but are briefly summarized here. It is not clear that the first assumption – i.e., that the other PAHs under consideration all act by a similar mechanism as BaP - is required as a foundation for the RPF method, since for these particular PAHs the method is based on the outcomes of cancer bioassays, rather than the underlying mechanism(s). There are also results, some of which are discussed in the document, that call into question the second assumption – i.e., that there are no significant low-level interactions of PAHs in a mixture beyond simple additivity, and therefore that the effects (cancer risks) of a mixture of agents are the simple sum of the individual risks. This should be tested by a direct comparison of a surrogate mixture of key compounds compared directly to BaP as a single agent and a real world complex mixture such as coal tar in a cancer bioassay, but results to date suggest that these PAH mixtures may, in fact, produce cancer risks that are different than simple additivity might predict.

Despite these concerns about the underlying scientific justification for the RPF method and the logic of the two core assumptions, the Panel concludes that there is adequate practical justification for continuing to use this approach in the near term to assess cancer risk of PAH mixtures in the absence of a good alternative. In particular, although this Panel and previous expert panels have strongly suggested that the EPA move toward a whole mixtures-based approach, the fact remains that the regulatory and scientific communities do not have sufficient information to adopt a whole mixtures approach for risk assessment at this time. Therefore, the Panel recommends the continued use of the component-based RPF approach as the most practical choice but recommends that this should be pursued in parallel with continued development of one or more whole mixtures-based approaches that could eventually replace it.

Given these conclusions, the Panel has several recommendations for revising the document and moving forward with the RPF approach. First, additional historical perspective should be included in the revised document, since it is an important component in, and justification for the agency's practical decision to pursue the RPF method. In particular, a summary of the previous discussions about moving to a whole mixtures approach, and the Agency's own evaluation of the significant data gaps that currently preclude them from doing so, should be included in the second chapter. The Panel agrees with the Agency that in order to continue with the RPF method, it is important to expand the number of compounds that are used from the 1993 guidance, and for the most part the candidate compounds for this expanded list are appropriate (see Chapter 4 discussion). The Panel also agrees that it is important to include more recent data for these compounds (since 1993) in calculating and expanding the RPF values for PAHs, since many of the values used in the current RPF method are based on older data. In this regard the agency noted that a revised IRIS assessment of BaP is undergoing parallel review which will likely lead to a revised cancer slope factor (CSF) as well as separate values for oral, dermal and inhalation BaP exposures. An up-to-date estmate of the CSF for BaP is central to the validity of the RPF method since this is the index compound.

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.

The choice of BaP as the index compound is well justified and is appropriately described for this RPF approach. It is the best studied PAH and meets the criteria for an index compound for an RPF assessment. However, it should be noted that the first core assumption of this document, that the other PAHs under consideration act via a similar mechanism, by definition, can lead to a choice of only those PAHs or polycyclic aromatic compounds (PACs) that are thought to act in this manner, and therefore may exclude PAHs or PACs that act via other mechanisms, or affect the behavior of the comparison compounds, and therefore contribute to cancer risk but are not included in the RPF calculation. As discussed below, the RPF method does not require this assumption, and therefore one could include any PAH for which cancer bioassay data are available.

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.

There is some evidence that a subset of closely related PAHs have "similar" modes of action for specific steps in the overall mechanism as described in the document. This is not unexpected since the compounds in question have already been defined in large part by their comparison to BaP. However, although these compounds are "similar" at a certain level, available data indicate that they each act via different precise mechanisms when examined at a more detailed level, and therefore may weaken the support for this assumption. For example, even though many PAHs are metabolized to reactive intermediates that then form DNA adducts at guanine residues, their potency for conversion of DNA adducts to mutations varies among compounds. Moreover, the pattern of guanine mutations within specific DNA sequences varies among these adducts. By definition, these adducts are therefore acting by slightly different mechanisms at this level. Since cancer risk can be related to mutation rate and to specific mutations within certain DNA sequences, this will result in different risks even though these compounds share mechanisms at a basic level.

Additionally, there are hundreds of other PAHs and PACs that may not act by these mechanisms and that likely, particularly in complex mixtures, contribute in positive or negative ways to the overall carcinogenicity of the mixture. These compounds should also be considered in the RPF method if good tumorigenesis data are available.

Also of importance, other PAHs in a mixture may alter the risk for known PAHs in that mixture in more complicated ways that also involve different mechanisms. For example, through mass action a complex mixture may contain total PAHs that collectively overwhelm the levels of an individual PAH such as BaP, perhaps by 1000:1 or greater. These may collectively interfere with the overall metabolism of BaP, or ratios of specific metabolites, or the capacity to repair DNA adducts from BaP, etc., such that one cannot predict the cancer risk from BaP solely

from its concentration in the mixture. Therefore, this assumption is not scientifically well justified.

In addition, there is a question as to whether similar modes of action are sufficient to predict *in vivo* carcinogenicity. As discussed in the PAH Mixtures document (e.g., page 35, section 2.6), mutagenicity, genotoxicity and similar short-term assays are relatively poor predictors of in vivo carcinogenesis. Yet a basic assumption of the document is that this mechanistic information is sufficient to predict their relative carcinogenicity. There are PAHs that are positive in short-term in vitro assays but negative or weak in *in vivo* tumor assays, and vice versa, further undercutting this basic assumption.

The document also discusses the role of the Ah receptor (AhR) in detail as another potential unifying mechanism for carcinogenic PAHs, but elsewhere also acknowledges that interaction with and activation of the AhR is not a good indicator of promotion or in vivo tumorigenesis for PAHs (as opposed to dioxins). The Panel agrees with this latter assessment, and therefore recommends removing this discussion and consideration of this mechanism.

Taken together, these points argue that this basic assumption of the RPF model is not well justified based on available data. More importantly, the RPF method may not require this assumption since it is based on the ultimate endpoint, cancer. In fact, the RPF method is completely independent of, and does not require any mechanistic understanding so long as there are good tumor data that can generate a slope for an RPF comparison to BaP. Thus, the mechanistic underpinnings should be de-emphasized as a rationale for RPF and a stronger argument should be made for emphasizing actual cancer bioassay data to directly compare PAHs alone and in mixtures. Mechanistic information on BaP could be referenced from other comprehensive sources such as IRIS and International Agency for Research on Cancer (IARC) monographs, or other recent literature reviews. Because of the lack of predictive power for data from short-term assays and the lack of correlation between these mechanistically based assays and tumor outcome, these should not be used in the RPF approach.

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.

The assumption that there are not significant interactions among PAHs in complex mixtures at low doses is not scientifically well justified. As discussed in the document (page 23, lines 11-19) coal tar behaved very differently in *in vivo* carcinogenesis assays than would be predicted from studies with BaP as a single agent, or what would likely be predicted from a RPF approach based on BaP as a single agent. Likewise, as discussed in the document (page 39, lines 3-12 and Table 2-2), the complex and unpredictable results to date of simple binary combinations of PAHs that do not follow simple additivity also undercuts both scientific assumptions of the RPF approach. However, in the absence of consistent data that support a specific type of interaction (additive, sub- or super-additive, etc.) that could be used for a variety

of PAH mixtures, a default assumption of additivity is a reasonable assumption for the purposes of the RPF analysis.

It should be noted, however, that complex mixtures such as coal tar, MGP residues, creosote, diesel exhaust and other PAH mixtures contain hundreds of other compounds, not included in this RPF assessment, that likely contribute to the overall biological effects of the mixtures. Other contributing mechanisms may include: induction or suppression of specific metabolic pathways; competition for metabolism through mass action at active sites; epigenetic effects; promotion and progression effects; endocrine disruption, neurological and immunological effects that contribute to cancer risk; and other classes of potentially potent carcinogens including substituted PAHs, volatile organic compounds (VOCs), metals, and other compounds. Collectively, these mechanisms may contribute in complicated ways to the overall cancer risk of a complex mixture, further reinforcing the recommendation to move in a concerted way from a component-based RPF approach to a whole mixtures-based approach.

3.3. Charge Question 3 - Chapter 3 - Discussion of Previously Published RPF Approaches

This chapter presents a discussion of previously published RPF approaches. Due to the evolution of the state of the science and an increased understanding of PAH toxicology, EPA is reevaluating the RPF approach for PAHs in this analysis.

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.

This chapter adequately summarizes the previous RPF approaches, but could be improved by providing more quantitative information, and editing the table to use a standardized approach for reporting values (same significant figures, scale, etc.).

3.4. Charge Question 4 - Chapter 4 - Evaluation of the Carcinogenicity of Individual PAHs

This chapter discusses the development of a database of primary literature on PAH carcinogenicity and cancer-related endpoints and the criteria used to include or exclude studies from the database.

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.

Chapter 4 of the PAH Mixtures document details the basis for the selection criteria that was used to develop the database related to PAH carcinogenicity and cancer-related endpoints. The list of 74 PAHs provided in Table 2-1 is believed by the Panel to be reasonable in view of the criteria of having three or more fused rings and not containing heteroatoms, alkyl or nitro substituents. The development of the database of primary literature on PAH carcinogenicity and cancer-related endpoints and the criteria used to include or exclude studies from the database are described in detail within this chapter. The database appears adequate, with the recommendation that a recently published IARC Monograph on PAHs, Volume 92, be included as an additional resource (IARC, 2010).

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 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.

The basis for selection of which studies were used in dose-response assessment is clearly delineated. The information in Tables 4-1 through 4-14 does provide adequate information related to whether certain studies were rejected or included in this document. Positive and nonpositive studies are given appropriate consideration. The choices and assumptions in making the selection have been adequately described in this chapter.

4c. The methodology for the choice of studies to use in the derivation of RPFs includes studies where at least one PAH was tested at the same time as B[a]P. Studies where individual PAHs were tested without concurrent testing of B[a]P were not included in the quantification of RPFs. Please comment on the scientific rationale for this approach. Please comment on whether the advantages and disadvantages of excluding certain data from the derivation of RPFs have been adequately described.

Chapter 4 of the document stipulates that BaP had to be tested concurrently for inclusion of a study on the carcinogenicity or other cancer-related endpoints of one or more of these 74 PAH. This restriction raises a concern that quality carcinogenicity studies might be dismissed. The Panel recommends that EPA consider whether a PAH other than BaP, with a RPF that has a comparatively narrow range, might be able to serve as the surrogate for the BaP index compound in those instances where BaP was not included in a bioassay. This approach offers the possibility that additional quality studies could be to be included in the development of a RPF for a given PAH. The Panel recommends that this be examined especially in those instances where limited tumor data were used to establish a RPF value. However, in considering this alternative approach, EPA should also take into account factors that could potentially outweigh the benefits in the establishment of a RPF for a specific PAH, such as cross-study and cross-laboratory comparability issues.

The Panel has a few recommendations that relate to the evaluation of the carcinogenicity studies for individual PAHs. These recommendations include providing some quality assessment to individual studies, such as a tabulation of various studies with included information on: 1) sample size, 2) dosing, 3) mortality (prior to tumor development), 4) defined test compound purity and 5) whether or not the data utilized are derived from tumor incidence or multiplicity.

In addition, it would be beneficial to incorporate or reiterate some of the discussion about alternatives for ranking RPFs provided in Appendix G into the discussions on individual PAHs in Chapter 4 as well as in Chapter 6. For example, the Panel considers the discussion about the influence of the route of administration on the RPF calculations to be particularly informative.

3.5. Charge Question 5 - Chapter 5 - Methods for Dose-Response Assessment and RPF Calculation

This chapter describes the selection of dose-response data and methods for dose-response assessment and RPF calculation from the selected datasets. The methodology for estimation of the RPFs varied depending on the characteristics of the datasets, however, the general equation was the ratio of the slope of the dose-response curve for the subject PAH to the slope of the dose-response curve for B[a]P.

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.

The modeling approaches described in Chapter 5 of this document for multi-dose studies are based on whether the data are quantal (binary) or continuous. The quantal endpoints considered in this document include: tumor incidence or incidence of cancer-related endpoints including frequency of mutations per number of cells interrogated. The continuous endpoints include: tumor counts (number of tumors per animal) or cancer-related endpoints of a continuous-variable nature (e.g., number of sister chromatid exchanges, number of morphologically transformed colonies).

When modeling quantal data, the mean model is for the probability of response (e.g., tumor incidence) and is generally assumed to follow a sigmoid-shape. Commonly used models that could be used include the logistic, probit, multi-stage, and Gompertz models among others. Since the multi-stage cancer model has a biological basis, it is the standard model used for cancer incidence and is considered sufficiently flexible to accommodate the dose-response data for these PAHs. Specifically, the multi-stage cancer model for the probability of a tumor is parameterized based on the number of dose groups (g) with the polynomial assumed to equal g-2:

$$\mu = \beta_0 + (1-\beta_0)\left[1-\exp(\beta_1 x + \beta_2 x^2 + ... + \beta_{g-2} x^{g-2}\right].$$

It should be noted that a model for data with g dose groups will exactly track the sample means (here, sample proportions) if the degree of the polynomial is g-1. However, a variation of this general model, typically used in risk assessment, assumes a monotonic relationship and constrains all parameters to be non-negative. The Benchmark Dose (BMD) Software used in the document, makes such an assumption as the default analysis. With quantal data, assumed to be independent across and within-dose groups, it is generally assumed that the data are binomially distributed with binomial variance (i.e., with n subjects evaluated at a dose group, the variance in the number "responding" is assumed to be $n\mu(1-\mu)$). Alternatively, the data may follow hyper- or hypo-binomial variability, i.e., greater than or less than binomial variability. These assumptions are not specified in the document and should be. The BMD Software used to estimate unknown model parameters uses a maximum likelihood estimation criterion and standard iterative algorithms for estimation. However, these distributional assumptions and the parameterization of the multi-stage cancer model should be clearly stated in the document. It is not clear whether the assumption of binomial variability was verified; the assumption of binomial variability should be verified and the document should include information about the verification. Instead, the model checking was based on the goodness-of-fit of the mean model and did not assess the assumptions regarding variability.

For continuous endpoints, a nonlinear dose-response shape may be expected from the data. However, the analysis plan for continuous endpoints is to use a linear model (i.e., a linear function). The justification for using the linear model for the multi-dose continuous data is insufficient and additional justification should be added. Although the linear model is the simplest model, there are other models such as the Hill model or polynomial model that are commonly used. An explanation for the use of the linear model is the number of dose groups is small.

The modeling strategy for the continuous endpoints should include polynomial models or nonlinear models (e.g., the Hill model) that are flexible enough to fit the data and would also adequately approximate a linear relationship. In some cases, the variance in response is assumed to be constant over the dose range of observed data. A least-squares (or nonlinear least squares) criterion is used to estimate unknown model parameters. In contrast, the sample variance may change with the mean. For example, the responses in the low-dose region may have lower variance than that observed as the dose (and response) increase. Such data may be estimated using a quasi-likelihood estimation criterion.

For the continuous data included in this document, the assumption about whether the variance changes across the dose groups is not addressed and the potential for a nonlinear shape is not allowed. Only a linear model was used to estimate the mean response. A goodness-of-fit criterion was used; if the model did not provide adequate fit, high-dose groups were sequentially eliminated in an effort to achieve adequate fit. This strategy is arbitrary and should be avoided.

A more flexible model should be used instead that accommodates the nonlinearity of the data.

Selection of Benchmark Response (BMR)

Since the RPFs are going to be used to estimate cancer risks at generally low environmental exposures, the calculation of RPFs should be applicable to the low-dose range, preferably excess risks ≤0.10 for quantal data. Similarly for continuous data, the calculation of RPFs should preferably be based on changes in the mean of less than or equal to one standard deviation (and certainly less than two standard deviations) in order to remain in the low-dose region of interest. For normally-distributed data, a change in the mean from the control mean of two standard deviations will result in approximately 50% of the subjects in the abnormal range. The RPF can increase or decrease substantially as dose (incidence or response) changes.

The analysis strategy described in Chapter 5 (with the suggested changes included) should be specifically followed. Deviations from the planned analysis strategy should be clearly explained.

To illustrate the use of a nonlinear model, the *in vitro* clastogenicity dose-response data of Tong et al (1981) (Table C-19, page C-85 of PAH Mixtures document) is reanalyzed. For convenience, the data table is reproduced in Table 1. The data clearly follow a nonlinear relationship, which is particularly evident, considering the two highest concentrations of benz(a)anthracene (BaA) which have similar responses with a log change in concentration.

Table 1: Data from Tong et al, 1981 for sister chromatid exchange summary data (Record number: 21710; Table C-1, page C-85). The BMR was set to the control mean from the predicted Hill model + SD of the control group. The BMDs are estimated from the Hill model using the specified BMR.

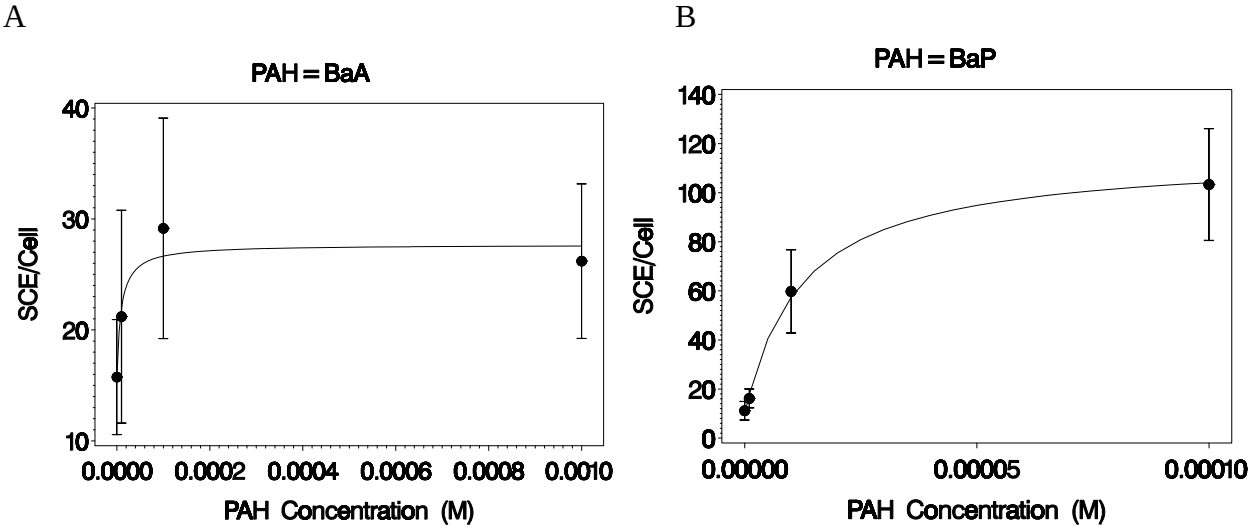
PAH	Concentration (M)	Mean Sister Chromatid Exchange/cell	Standard Deviation (SD)	Benchmark Response (BMR)	Benchmark Dose (BMD) (M)
Control	0	11.15	3.81	13.7	4×10^{-7}
BaP	10^{-6}	16.15	3.83		
BaP	10^{-5}	59.75	16.96		
BaP	10^{-4}	103.3	22.75		
Control	0	15.75	5.18	20.9	7×10^{-6}
BaA	10^{-5}	21.2	9.59		
BaA	10^{-4}	29.15	9.93		

Instead of fitting a linear model to these data, a 3-parameter Hill model is selected, which can accommodate an asymptotic response for large concentrations, i.e.,

$$\mu = \alpha + \frac{\gamma x}{x + \theta},$$

where x is the concentration of the PAH, α is the response for the control group, γ is the range of response such that $\alpha+\gamma$ is the asymptote for large x. Since only sample means and standard deviations are available at each concentration level, a weighted analysis is imposed, with weights set to the inverse of the sample standard deviation at each concentration. Unknown parameters are estimated using a weighted least squares criterion in a Gauss-Newton iterative algorithm using PROC NLIN in SAS (version 9.2). The resulting predicted models for BaP and BaA are provided in Figure 1. Using all of the data, a Hill model adequately fits the observed sample means for both PAHs.

Figure 1: Observed sample means and predicted response from a Hill model. Sample means are denoted with dots and +/- one standard deviation from each mean is denoted by the error bars.



The specified BMR for continuous data is one standard deviation (SD) above the control mean as predicted from the Hill model (shown in Table 1). For BaP, the estimated BMD_{1SD} is 4×10^{-7} and for BaA, the estimate is 7×10^{-6}. However, in Table E-14 (page E-31), the BMR and BMD values are blank and the point estimate responses are 92 and 13 for BaP and BaA, respectively; and the point estimate dose is $1x10^{-4}$ for both compounds. It is not clear how the point estimate responses were calculated. This is an example where the described analysis plan does not seem to be followed without any explanation of why it was not followed.

5b. For each individual dataset considered in the assessment, the B[a]P dose-response was calculated from the study-specific data. Please comment on whether this approach has been appropriately described. If there are additional approaches using the available data that should be considered, please describe how the approach could lead to a better estimate of cancer risk.

The strategy of using study-specific data for the BaP dose-response with PAH dose-response is advantageous since downstream calculations are intra-study and avoid comparisons without accounting for study effects.

It should be noted that the estimates of BaP slope across studies with different characteristics are very different. The range of the estimates can be more than 1,000 fold. This supports the idea of using study-specific estimates for calculating the RPFs.

5c. The point of departure for slope estimation that has been used for the derivation of RPFs is the benchmark dose (BMD) estimate rather than the lower confidence limit on the benchmark dose (BMDL). Please comment on whether this approach is scientifically justified and adequately described. Please comment on whether alternative approaches should be considered.

It is correct to base the derivation of the RPFs on the unbiased estimate derived from the BMD, rather than the lower confidence limit on the benchmark dose (BMDL), in order to obtain an unbiased estimate of the total exposure for a mixture (expressed as the total BaP equivalent dose). Due to chance experimental variation, some of the RPFs will be overestimated and some will be underestimated. These biases will tend to cancel each other for the total exposure of a mixture. On the other hand, when the study sizes are similar, the BMDLs between the BaP and PAH may be stable. But when the two studies have different precision, the ratio of BMDLs is tenuous. Therefore, the ratio of BMDs is advisable.

5d. Please comment on the methodology used for the RPF calculations for multidose and single dose datasets. Please comment on whether the process for calculating RPFs from the various datasets is scientifically justified and adequately described. Please comment on the utilization of high response levels in some instances as the point of comparison. Please describe alternative approaches that could lead to a better estimate of cancer risk that should be considered using the available data. Please comment on whether the considerations for RPF calculation as outlined in Sections 5.6 and 5.7 are scientifically justified and adequately described.

When multiple doses are available for dose-response modeling, all of the data should be used with a sufficiently flexible model, e.g., the multi-stage cancer model or a polynomial model for continuous endpoints. An example of such an analysis strategy is given in 5a above. In the Appendix, there are cases where single-dose data were used when multiple doses were available; this should be explained.

Generally, the Panel is concerned about using high-BMR values to calculate the RPFs in single-dose studies. If the dose-response curves were parallel across PAHs, then the choice of

BMR would not impact the estimation of a relative potency factor. However, as discussed in earlier chapters, it is generally assumed that the chemicals are not dilutions of one another, so their dose-response curves will generally not be parallel. Thus, the choice of the BMR should be in the low dose-region. However, in some special cases, the RPF calculation is not dependent on the response level. For example, consider the data from a BaP single-dose study and multi-dose comparison PAH for benzo[k]fluoranthene (BkF) (LaVoie et al, 1982). For convenience, the data from Table C-1, page C-4 of the PAH Mixtures document are reproduced below in Table 2.

Table 2: Data from LaVoie et al, 1982 for dermal bioassay data (Record number: 630; Table C-1, page C-4) – primarily squamous cell papilloma in female mice. The data include a single-dose study for BaP and multiple-dose study for the PAH, BkF.

PAH	Dose (μg/mouse)	Number of Animals in Group	Number of Animals with Tumors	% Tumor-bearing animals
Control	0	20	0	0
BaP	30	20	17	85
BkF	30	20	1	5
BkF	100	20	5	25
BkF	1000	20	15	75

Suppose a one-stage model is used for analysis of the single-point BaP study, i.e.,

$$\mu = \beta_0 + (1-\beta_0)\left[1-\exp(-\beta_1 x)\right]$$

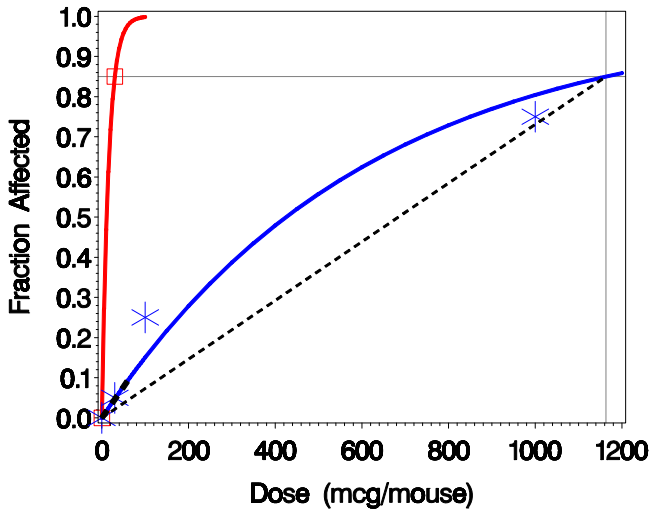
where β_0 =0, x is the dose of BaP, and β_1 is the unknown parameter associated with the slope. Assuming a zero background response rate (i.e., β_0 =0), the BMD(10) is estimated as $BMD(10) = -\log(0.9)/\beta_1$ and the BMD(85) is estimated as $BMD(85) = -\log(0.15)/\beta_1$. Since there are four dose groups for BkF, a multi-stage model is used, parameterized with linear and quadratic terms (i.e., g-2= 2 for a second-degree model):

$$\mu = \beta_0 + (1-\beta_0)\left[1-\exp(-\beta_1 x-\beta_2 x^2)\right]$$

where x is the dose of BkF and again we assume β_0 =0. However, in the PAH Mixtures document, β_2 was set to zero and the one-stage model was used due to convergence problems.

Therefore the same parameterization is used for both BaP and BkF. The fitted dose-response curves are provided in Figure 2. Notice the predicted response from the single-dose study is the sample mean (here, observed sample proportion).

Figure 2: Single-dose data for BaP (red) and multi-dose data for BkF (LaVoie et al, 1982).



When the one-stage model is used for both chemicals, the choice of BMR is not relevant in the calculation of the RPF. Consider the following algebraic manipulations to demonstrate for a general BMR=μ_0 and for a general j^{th} PAH:

$$RPF = \frac{\mu_0 / BMD(\mu_0)_j}{\mu_0 / BMD(\mu_0)_{BaP}} = \frac{BMD(\mu_0)_{BaP}}{BMD(\mu_0)_j}$$
$$= \frac{-\log(1-\mu_0)/\beta_{BaP}}{-\log(1-\mu_0)/\beta_j}$$
$$= \frac{\beta_j}{\beta_{BaP}}$$

Thus, the RPF is not a function of the BMR when a one-stage model is used for both the BaP and comparison PAH. To illustrate from the LaVoie (1982) data, the results for a BMR of 10% and 85% (the observed response from BaP are given in Table 3. The resulting RPFs are identical.

Table 3: Illustration with BaP single dose study and multi-dose comparison PAH, here BkF from LaVoie et al, 1982.

LaVoie et al 1982 data	BMD10 estimates (μg)	Slope = 0.1/BMD10	BMD85 estimates (μg)	Slope = 0.85/BMD85
BaP	1.7	0.060	30	0.028
BkF	64.6	0.0015	1163	0.0007
RPF = $\frac{\text{slope PAH}}{\text{slope BaP}}$		0.025		0.025

This illustration demonstrates that in a single-dose study, a one-stage model can be fit, which will exactly predict the observed mean response. In this case, the ratio of slopes for calculating the RPF is not dependent on the BMR. However, with the single-dose studies, there is no way to verify the prediction where data are not available. Therefore the result is based on a lack of information rather than evidence that both the BaP and PAH dose-response data are adequately approximated with one-stage models.

Although the use of single-dose study data may be helpful in informing the risk assessment, these studies are clearly less informative than multi-dose studies. When single-dose studies are used to calculate the RPF, it would be helpful to describe the impact on the RPF calculation. For example, in Table 7-1 it would be helpful to include the number of studies per RPF calculation based on a one-dose study.

In section 5.7, we recommend the use of a (g-1)-degree polynomial in the multi-stage model (page 111, lines 31-36) instead of reducing the degree of the polynomial. This model will exactly track the observed sample means.

3.6 Charge Question 6 - Chapter 6 - Selection of PAHs for Inclusion in the Relative Potency Approach

This chapter describes the selection of PAHs for inclusion in the RPF approach. The evaluation focuses on whether the available data were adequate to assess the carcinogenic potential of each compound. If the data were not considered adequate, then the PAH was excluded.

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.

The Panel believes that the method for selecting the PAH appears to be scientifically justified, but has recommendations about several issues that are incompletely considered. These issues include: (1) the quality of the individual studies considered and (2) the variability of the study characteristics prior to their inclusion in a summary RPF.

Regarding the quality of individual studies considered, the Panel recommends that a list of quality criteria should be defined and articulated (e.g., methodologically robust, such as inclusion of an adequate control group, sample size, dose, number of PAHs measured, purity of the compounds considered) prior to the weight of the evidence evaluation. This information should be illustrated in the form of tables or individual graphs. Only studies of sufficient quality (defined *a priori*) should be considered in the weight of evidence evaluation.

Regarding the variability of study characteristics, the Panel recommends that once a study is considered to have sufficient quality to be included in the weight of evidence evaluation, the variability of the study characteristics should be considered, prior to the calculation of the RPF. For example, the yes/no criteria in the weight of evidence evaluation in the document does not consider consistency within a study. Instead, the Agency could clearly articulate the quality criteria (e.g., expand and articulate the characteristics listed in Table 7.1), and then only use studies with adequate quality to calculate RPFs.

The Panel believes that tumorigenic profiles of PAHs depend on the route and dose of administration. If tumor data are to be used for RPF calculations, then the following issues should be considered: choice of animal model used, doses administered, route of administration, frequency of administration, exposure duration, location of tumors, types of tumors (papillomas, adenomas, carcinomas etc.), and stage of tumors (benign, malignant). The pertinent information should be incorporated. This additional narrative will help readers understand whether the spectrum of tumors observed in laboratory animal models corresponds to the same tumor category or tumor site in humans and helps in the characterization of risk.

Some Panel members believe that additional information could go into the weight-of-evidence evaluation such as requiring a minimum number of positive studies before a PAH is considered carcinogenic. These Panel members also believe that when there are one or more negative tumor bioassay studies with RPF detection limits lower than RPFs reported for positive studies, a PAH is declared carcinogenic only when the number of positive studies is far greater than the number of negative studies (e.g, 2 to 1) or that adequate scientific reasoning for the results of the negative studies is given. Other Panel members disagree with this viewpoint and argue that it is difficult to point out the right ratio of positive to negative studies to consider a particular PAH compound carcinogenic and believe that study quality must outweigh negative or positive evaluations.

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.

The Panel finds that the rationale for omission of Ah-receptor data is well justified. Additional information is not necessary. The Panel also agrees with EPA's decision that once information demonstrating tumor formation is obtained, additional information on cytotoxicity and tumor promotion is not necessary. However, the document should clearly state the reasons for the omission of these data.

6c. The analysis uses an RPF detection limit as a means of comparing positive and nonpositive (or negative) bioassays. Please comment on whether this method is scientifically justified and adequately described.

The Panel has some recommendations about the RPF detection limits. The Panel assumes that the RPF detection limit is a post hoc power calculation; however, the description is not clear in the document. If it is a post hoc power calculation, this information would have been more useful prior to calculating the RPF. One possible remedy is to first develop a criteria list that a study must meet before it is even considered for inclusion in the RPF, regardless of whether is it statistically significant. Then, to calculate the RPF, the sample size of each study should be considered (e.g, weigh each individual study RPF to derive the overall RPF).

The Panel is unclear why the RPF detection limit was not calculated for studies of cancer-related endpoints (for example, in Figure 6-18, there are 9 such studies). At a minimum, a brief notation of the RPF detection limit should be included in the figures.

6d. Graphic arrays of the calculated RPFs (Figures 6-2 through 6-35) are presented as a means of representing the variability in RPFs from different data sources, the weight-of-evidence for carcinogenic potential, and the basis for the selected RPF. Please comment on whether the figures are informative and adequately described. Please comment on whether there is other information that should be included in the figures. Please comment on whether the narratives are informative and complete.

The Panel finds that Figures 6-2 through 6-35 provide a good summary of the individual studies considered and the variability of individual RPF estimates across studies. However, they would be much more informative if they clearly indicated which studies were used to estimate the final RPF and the Panel recommends that this be done.

With respect to the presentation of RPFs for individual studies, the Panel proposes that rather than graphically displaying the RPF for each individual study as a bar, it can be shown as a point estimate coupled with information on variability (e.g., standard error, standard deviation, confidence interval, and range). The information on variability in the study is viewed as key, to help the reader interpret the study findings.

Consensus has not been achieved among the Panel members regarding presentation of RPF data from both positive and negative studies. Some Panel members agree with EPA's position of only including positive studies. Other Panel members believe that where conflicting results exist, the narrative should focus on contrasting negative and positive studies and point out possible reasons for conflicting results. If a PAH is declared carcinogenic under such a scenario, then it should be mentioned clearly why negative studies are being discounted. These Panel members also suggest enhancing the graphical display of studies to include: 1) RPF detection limits for positive studies; 2) RPFs for negative studies (unless the RPF was zero); 3) RPF detection limits for cancer-related endpoint studies.

Some Panel members believe that the narratives should offer some discussion of when RPFs of bioassay data and cancer-related endpoint data significantly differ (i.e., BghiP, BjAc, FA and DBaeF). Additionally, the Panel proposes that for ease of reading and to ensure completeness, it might be useful to have narratives set up in a way similar to structured abstracts in scientific journals. The Panel also suggests integrating information provided in Appendix G into the narratives that correspond to Figures 6-2 through 6-35.

3.7 Charge Question 7 - Chapter 7 - Derivation of RPFs for Selected PAHs

This chapter describes various methods (e.g. prioritization of studies) and different approaches for deriving final RPFs (e.g., arithmetic mean). Final RPFs were derived by averaging the individual study RPFs (across all exposure routes) calculated from bioassay data for PAHs that had at least one RPF based on a bioassay. The exception was dibenz[a,c]anthracene, where the RPF was calculated from cancer-related endpoint data.

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.

The Panel believes that the use of an arithmetic mean to estimate the final RPFs is appropriate in most cases. The Panel also believes that presenting the range instead of a confidence interval is also appropriate. The Panel does have reservations regarding several aspects of the RPF calculation approach. First, the Panel has concerns regarding calculating RPFs based upon a single experiment (e.g., 11H-benz[b,c]aceanthrylene, benzo[g,h,i]-perylene, benzo[e]aceanthrylene, benz[j]ace-anthrylene, dibenzo[a,h]pyrene, indeno-[1,2,3-c,d]pyrene and naphtho[2,3-e]pyrene). Second, there is concern regarding the use of data for calculating RPF values in which there was only a single-dose level of BaP and/or the other PAH being evaluated (e.g., benz(a)-anthracene, 11H-benz[b,c]-aceanthrylene, benzo[e]aceanthrylene, naphtho[2,3-e]pyrene and fluoranthene). Finally, there is concern about calculating the arithmetic mean for PAHs that have markedly divergent individual RPFs (e.g., benzo(c)fluorene). Without

additional information and justification, the Panel cannot recommend calculating RPFs for PAHs with these data characteristics. In addition, given the concerns discussed about the use of arithmetic mean above, the Agency is encouraged to continue evaluating other methods, such as using a geometric mean instead of an arithmetic mean. Where sufficient data are available, the use of a geometric mean would give less weight to outlier values. The Panel believes that calculating RPFs to one significant figure is appropriate.

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.

The Panel believes that the scientific rationale for considering bioassay data versus cancer-related endpoint data has been adequately described. The Panel strongly believes that the use of cancer bioassay data is essential for determining the RPF for a given PAH. Cancer-related endpoint data are useful as supporting data, but the Panel does not recommend the use of only cancer-related endpoint data for determining the RPF. As such, the Panel does not have recommendations on how to use cancer-related endpoint data in a more quantitative manner. The Panel does not recommend calculating an RPF for dibenz[a,c]anthracene and recommends that it be removed from Table 7.2 until further bioassay data become available.

The Panel recommends that additional information and justification be provided for the inclusion or exclusion of cancer bioassay data for PAHs that did not give significant tumor responses in well-designed studies. One suggestion is to include the IARC classification for those PAHs where a classification exists in Table 7.1 or perhaps in Table 7.3. The Panel believes that there is a need for some additional measure of the quality of individual studies used in determining the final RPF values. This is important in addition to the confidence ratings provided in Table 7.3 (see also further discussion below). The Panel also strongly believes that more cancer bioassay data with mixtures would be extremely helpful in further validating the RPF approach.

Tumor multiplicity (continuous data; average number of tumors per mouse) and tumor incidence (quantal data; percentage of mice with tumors) represent different measures of tumorgenicity/carcinogenicity. In the document, RPFs calculated from tumor multiplicity data are being combined with other RPFs calculated from tumor incidence data to calculate final RPFs. An example of the problem is benzo(c)fluorene. The divergent RPFs used to calculate the final RPF value for benzo(c)fluorene in Table 7.1 come from averaging a study where multiplicity data were used (RPF of 50) and one where incidence data were used (RPF of 1). RPF values should not be averaged from these two different measures without sufficient justification for using the multiplicity data. The Panel believes that tumor multiplicity data should only be used when dose-response data are available to allow accurate assessment of relative differences between the compounds being compared. Therefore, the Panel recommends

that, where possible, the Agency should primarily use tumor incidence data to calculate final RPFs. Multiplicity data can be used when there is adequate dose-response data to allow for an accurate quantitative comparison.

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.

The Panel does not believe that there would be much value in providing route- or target organ-specific RPFs at the present time, because a significant proportion of the studies used to calculate the final RPFs involved dermal application/carcinogenesis (approximately 60% of the studies involve dermal application to mice and >90% of the studies were conducted in mice). Additional studies and data using different routes of exposure and tumor data from other organ sites would be necessary to calculate such RPFs. Although the Panel agrees with the decision to not calculate separate RPF values for different routes of exposure, the route of exposure may be an issue of concern for generating RPF values for compounds where the available data are only via non-physiological routes (e.g., benzo[g,h,i]-perylene, lung implantation in rat only; benzo[j]aceanthrylene, intra-peritoneal only; fluoranthrene, intra-peritoneal only; indeno[1,2,3-e]pyrene, lung inplantation in rat only). Additional studies may be warranted in these cases in a dermal or oral tumor study, since the route of exposure can play an important role in bioavailability and toxicokinetics that may alter the relative potency of the test compound as compared to BaP, when tested via a more standard route of exposure. A sensitivity analysis sould be performed to determine, in those cases where there are data from several routes of exposure, whether these alternative routes cause a particular bias or greater variability in the RPF values. It is interesting to note in this regard, that some compounds, such as benzo[c]fluorene, demonstrate widely divergent RPFs in studies using different routes of exposure (in this case, oral versus interperitoneal, with values of 1 and 50) (see also dibenz[a,h]anthracene and dibenzo[a,l]pyrene). Without additional supporting data, the Panel does not recommend developing RPFs for compounds with data only from studies using non-physiological routes of exposure.

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.

The Panel generally believes that the scientific rationale presented in the document for assignment of an RPF of zero, the assignment of no RPF and the distinction between them is appropriate. The Panel does have concern regarding the quality of the data used to assign an

RPF of zero for some studies and also regarding the inconsistent use of studies with RPFs of zero in calculating the final RPFs. The Panel recommends that a consistent approach be adopted for using RPFs of zero for all compounds for which final RPFs are calculated. In addition, the Panel recommends that the Agency continue to evaluate how RPFs of zero are calculated as well as the rationale for assigning no selected RPF values. In addition, the Panel recommends the Agency continually evaluate how the current usage of zero RPFs may bias the calculation of final RPF values.

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.

In general, the Panel believes that characterizing the final RPFs with confidence ratings are a good idea. However, the confidence ratings do not appear to give any indication of the overall quality of the data being assessed and used for the RPF calculation. Based on the information provided in Table 7.3, confidence ratings appear to be related to the number of studies used, data from more than one route of exposure, presence of non-cancer endpoint supporting data to calculate the RPFs, etc. The Panel strongly believes that there needs to be some measure of the quality of the individual studies used to generate the RPFs. In this context, quality refers to study characteristics such as sample size and statistical power, presence or absence of non-lethal toxicity, unusual mortality and other potential confounding factors. Also, the Panel makes several recommendations for calculating RPFs; depending on EPA's final RPF approach, these recommendations may be useful in developing confidence ratings.

Chapter 7 also includes a description of how the RPF method is used to calculate relative cancer risk from exposure to PAH mixtures (section 7.3). In addition, there is a section (section 7.4) dealing with the use of age-dependent adjustment factors (ADAFs) to adjust for differences in susceptibility during early life (i.e., <16 years of age). The Panel believes that these two sections are extremely important to the overall presentation of the document and are somehow lost by inclusion at the end of Chapter 7. It is strongly recommended that the information on cancer risk assessment (sections 7.3 and 7.4) be moved to the beginning of the document either as a separate section or in the Executive Summary.

3.8 Charge Question 8 - Chapter 8 - Uncertainties and Limitations Associated with the RPF Approach

This chapter discusses the uncertainties and limitations associated with using the RPF approach for PAH mixtures risk assessment. Many of the general uncertainties related to chemical-specific risk assessment are also applicable to the proposed RPF approach for PAHs. In addition, uncertainties exist regarding the selection of data and dose-response assessment methodology, the selection of PAHs for inclusion in the analysis, the derivation of the final RPF,

the assumption of a common mode of action and dose additivity, and the extrapolation of RPFs across exposure routes.

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.

The uncertainties in the methodology of deriving RPFs are described quite well in the PAH Mixtures document. The major methodological uncertainties are clearly defined and discussed so that there is little doubt about the methods that were used and the limitations of the final RPF values reported.

In evaluating the average RPF values, the quality of the source material should be evaluated rather than giving equal weight to each in calculating average RPF values. Some type of weighting scheme needs to be developed for RPFs based on the quantity and quality of existing data.

Existence of a common mode of action is not necessary in order to apply the RPF approach. The discussion of the mode of action in the document should be reduced considerably by utilizing brief references to relevant literature that discusses the current knowledge of the three mechanisms of metabolic activation of PAHs. There is growing evidence that PAHs and other related compounds in complex mixtures, such as coal tar / MGP residue, can act by other non-genotoxic and mutagenic mechanisms. Such mechanisms include acting as endocrine disruptors, epigenetic agents, by causing immunologic and neurologic effects, and other non-genotoxic effects that may contribute to cancer risk. Genotoxicity and mutagenicity are but one of many ways that environmental agents can contribute to cancer risk. PAHs in mixtures can also affect each others' metabolism and toxicokinetics in complex and poorly predicted ways; for example, by induction or suppression of specific metabolic enzymes and pathways, by competition for active site metabolism of key enzymes, by altering cell proliferation and differentiation, and other factors that affect metabolism, distribution, toxicokinetics, potency, and the dose-response curve. Although the individual doses of specific PAHs in a complex mixture may be small, their cumulative amount may be sufficient to interact in these non-additive manners that are not described by the simple mechanisms assumed for BaP and similar PAHs described in the document.

More data dealing with the comparisons of the RPF approach and estimates of cancer risk derived from complex mixtures are needed, which would reduce some of the uncertainties associated with the RPF approach described in the document. The feasibility of directly studying complex mixtures is illustrated by the limited pair of existing data sets. Chronic bioassays in mice for two synthesized coal tar mixtures were conducted at the National Center for Toxicological Research, Food and Drug Administration (Culp et al., 1998). The RPF approach applied to these data were reported in the Electric Power Research Institute (EPRI) public comments (Rohr, 2010). Comparisons of cancer risk observed in the chronic animal bioassays

for the two coal tar mixtures were within a factor of two to four (lower) of the cancer risks based on the RPF approach. Albeit for only two mixtures, this is an encouraging result for use of the RPF approach. Additional comparisons such as those submitted by EPRI should be added to the document as it provides very useful information about the RPF approach. Statistical variation of cancer risk estimates between chronic animal bioassays on the order of three to four is expected (Gaylor et al., 2000). More data dealing with the comparisons of the RPF approach and estimates of cancer from tested mixtures are needed.

Additional mixtures of PAHs need to be studied in chronic animal bioassays in order to compare the observed cancer risk of a mixture with the risk estimated from the RPF approach. Section 3.1 of the PAH Mixtures document discusses the availability of several studies on mixtures that provide data for comparing cancer risk estimates using the RPF approach with direct estimates of risk from the mixtures. Unfortunately, no quantitative information was presented in the document to indicate the potential size of uncertainty for the RPF approach. This quantitative information needs to be added to the document in order to evaluate the accuracy and precision of the RPF approach from existing examples.

The cancer slope factor for BaP is multiplied by the RPFs in order to obtain cancer unit risk factors for each of the PAHs. Hence, the cancer unit risk factor for BaP is critical to the calculation of the cancer risk estimate for a mixture using the RPF approach. Based on old studies, the upper limit of the cancer unit risk factor for lifetime oral exposure to BaP is 7.3×10^{-3} per μg/ kg per day listed in the EPA Integrated Risk Information System (IRIS), 1994. Based on a Good Laboratory Practice (GLP) study the upper limit of the cancer unit risk factor for BaP is 1.2×10^{-3} per μg/kg per day (Gaylor et al., 2000). Because of the relatively large uncertainty in the cancer unit risk factor for BaP, this value needs to be updated before reliable estimates of cancer risk can be derived for mixtures of PAHs.

Extending the classes of PAH should be considered by incorporating other PAH derivatives, e.g., PACs that occur in mixtures, particularly where bioassays exist such as for nitro-aromatics and alkylated PAHs.

Since RPFs generally vary as dose increases, RPFs based on a single dose, especially at high doses, are quite uncertain and should not be used until additional data become available. The question arises of the relevance of high doses in animal studies to the much lower doses experienced by humans. This question is not discussed adequately in the document.

The state of a single PAH administered to animals in bioassays may be different from the state of the same PAHs in mixtures where they may not be easily desorbed from solid particles. The bioavailability to humans for PAHs in a mixture needs to be compared to the bioavailability in animal bioassay experiments that utilize purified PAH compounds. Cancer risk estimates based on the RPF values and total concentration of PAH in mixtures may be overestimated.

Using measured concentrations of PAHs in mixtures, sensitivity analyses can indicate which uncertainties in individual RPFs have a significant impact on the total BaP equivalents for

a mixture. EPA should consider adding this to the document, perhaps by using the mixtures discussed in the EPRI comments.

More PAHs could be included, where concurrent data on BaP were not collected, by calculating the RPF of the PAH to a second PAH and calculating the RPF of this second PAH to BaP. Then, the RPF of the PAH to BaP is the product of these two intermediate RPFs. Although less direct and potentially less accurate than the concurrent bioassays that include the BaP reference-based RPF method, this approach could prove useful for identifying additional PAH candidates for inclusion in a secondary RPF data set. The Panel recommends that this be examined especially in those instances where limited tumor data were used to establish a RPF value. However, in considering this alternative approach, EPA should also take into account factors that could potentially outweigh the benefits in the establishment of a RPF for a specific PAH, such as cross-study and cross-laboratory comparability issues.

The composition for each individual mixture must be adequately determined, otherwise uncertainty is added to the RPF approach. Completely characterizing mixtures is difficult, and this limitation and uncertainty should be discussed. For example, different PAHs may have different effects on the induction phase I and/or phase II enzymes that might affect the metabolic activation or deactivation of other potentially highly tumorigenic PAHs, i.e., a non-additive effect as mentioned in the PAH Mixtures document. Various PAHs may inhibit each other. Mixtures may or may not contain substances that act as promoters of tumorigenesis rather than as genotoxic initiators. Without adequately characterizing mixtures, these effects may not be considered.

3.9 Charge Question 9 - Appendices

9. Please comment on whether the information in the Appendices is adequate to allow independent verification of the calculated RPFs. If not, please comment on what additional information would be useful.

The appendices are generally useful for verifying the calculations of the RPFs. However, it would be helpful to reorganize the appendices by chemical (with each identified in the Table of Contents). This would include the corresponding BaP data for each study within each chemical section which may be repeated across PAHs.

The plots from the Benchmark Dose Software output are useful but it should be noted that the linear extrapolation to the origin is based on BMDLs instead of BMDs. The calculation of the multi-stage cancer slope factor is also given based on the BMDL instead of the BMD. The Panel recommends that the slope factors be added to these appendices based on the BMD – which is the approach taken in the document.

4. REFERENCES

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