Comments on Docket ID No. EPA-HQ-ORD-2010-0047, Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures

Submitted by the Following:

American Coke and Coal Chemicals Institute
American Petroleum Institute
Asphalt Institute
Association of American Railroads
National Petrochemical & Refiners Association
Pavement Coatings Technology Council

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Abbreviations and Acronyms

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

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

Executive Summary

The American Coke and Coal Chemicals Institute, the American Petroleum Institute, the Asphalt Institute, the Association of American Railroads, the National Petrochemical & Refiners Association and the Pavement Coatings Technology Council have reviewed the Docket ID No. EPA–HQ–ORD–2010–0047, Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures and provide the following comments. Detailed scientific comments are presented first. Responses to the EPA Charge Questions are then presented in Appendix A. Finally, technical details that support the scientific comments are documented in Appendices B -D.

The U.S. Environmental Protection Agency (EPA) has not effectively documented the basic scientific principles underlying the RPF approach. According to IRIS document (EPA 2010) "The EPA RPF approach involves two key assumptions (1) a similar toxicological action of PAH components in the mixture and (2) interactions among PAH mixture components do not occur at low levels of exposure typically encountered in the environment" (p. iv). However, upon review of the IRIS assessment, we conclude that EPA did not provide adequate scientific evidence or quantitative data to support the above hypotheses (i.e., the similar mode of action of PAHs).

Our assessment supports comments submitted by the National Aeronautics and Space Administration (NASA)[1] to EPA on 10/28/2009. In those comments NASA states a "Review of the current draft found extensive discussion of mode of action but little to no substantiation for EPA's actual determination of the primary mode of carcinogenic action". The commenters share NASA's concerns that "The current draft actively narrows data use to only those experiments performed at the same lab and does not consider the range of available data, especially for a diverse group of chemicals, such as PAH mixtures. Of particular concern is the EPA approach to limit the use to only 'positive results', a concern that NASA previously identified in its review of the draft TCE risk assessment under IRIS. Overall, this limitation of data raises the potential for skewed results, the appearance of "cherry picking" data for a desired results and would exclude much of the literature or data sources used consistently in other EPA risk assessments"

Subsequently, comments submitted by the Department of Defense[2] to this EPA on 10/28/2009 echo similar concerns "... if EPA believes that there are specific mutations required for the carcinogenicity, it would seem hard to assert that a stochastic process is occurring, which is one of the assumptions for response additivity"

In addition to the above points, we note the following:

- EPA has not performed a Weight of Evidence Evaluation as called for in its Guidelines for Carcinogen Risk Assessment (EPA, 2005). The weight of evidence assessment presented ignored the question of human carcinogenic risk and instead determined if the substance was positive in any short term assay in which benzo[a]pyrene was tested. EPA considered a single positive result to be adequate weight of evidence to conclude that a PAH should be included in the RPF scheme. EPA's weight of evidence analysis was scientifically inadequate, and EPA should not derive RPFs for PAHs unless a formal

weight of evidence evaluation has resulted in a weight of evidence narrative that there is, at a minimum, suggestive evidence of carcinogenic potential in humans.

- EPA assumed that all PAHs act by a similar mode of action. Specifically, EPA stated that a common mutagenic mode of action for carcinogenic PAHs is hypothesized based on information available for the indicator chemical, benzo[a]pyrene (U.S. EPA, 2005b). However, after stating this, EPA contradicts their own assumption by stating "the carcinogenic process for individual PAHs is likely to be related to some unique combination of multiple molecular events resulting from formation of several reactive species." Unique action argues against the same mode of action. EPA's support for their proposed mode of action assumption is that many PAHs are metabolized similarly and many PAHs form DNA adducts. However, PAHs that are metabolically activated and form DNA adducts in human skin do not cause tumors in human skin as they do in mouse skin. The mode of action for PAHs is complex and EPA has not demonstrated that the mode of action is the same for all PAHs for which RPFs were derived.

- EPA makes an assumption of dose additivity however, little information was presented to support this assumption. Subsequently, a great deal of scientific data on PAH antagonistic interactions was not addressed. Some antagonism data contradicting this key assumption is presented in the detailed comments below.

- The criteria EPA used in developing the RPF approach excluded valuable data and at times EPA failed to follow the set criteria. For example, EPA selected benzo[a]pyrene as the index chemical and excluded literature with other index PAHs. As well, no studies were included unless benzo[a]pyrene positive controls were run in the experiment concurrently. However, certain exceptions were made that are not adequately justified. Another criteria, regarding how data were excluded if the tumor incidence was 90% or greater at the lowest dose tested, was selectively applied by EPA. EPA also stated that studies were excluded from the RPF approach if the purity of the test chemical was in question, but a majority of the studies contained no information about the identity and purity of the test chemicals. These and other scientific topics are discussed in the detailed comments below.

- There were also several technical problems identified that are discussed below. For example, data from several studies that exceeded the maximum tolerated dose were used for RPF derivation. However, EPA should have excluded such data. As well, EPA's RPFs were in many cases based on a single test result, and many of these derived from a small number of scientific studies of questionable quality. Another issue identified by the commenters was the use of slope factors derived from single data points, versus the use of slope factors derived from multidose dose-response curves.

- EPA also failed to validate the derived RPFs using cancer response data from real world complex mixtures. As noted in "Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures (EPA, 2000)," data from whole mixtures are preferable to data from mixture components. The commenters have performed validation exercises

that demonstrate that EPA's RPFs overestimate the carcinogenic risk observed when the interactions between components is inherently taken into account.

[1] Wennerberg, L. S., "PAH mixtures NASA comments 10-28-09", aspub.epa.gov/eims/eimscomm.getfile?p_download_id=494551

[2] Department of Defense Comments on the Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures ,oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=494554

I. Scientific Recommendations

The undersigned have reviewed the Docket ID No. EPA–HQ–ORD–2010–0047, Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures and provide the following detailed comments. Some of these comments have also been discussed briefly in the responses to the charge questions.

1. Assumption that PAHs Pose Carcinogenic Risk to Humans

1.1 Hazard Assessment of Individual PAHs in Humans

Recommendation: The U.S. Environmental Protection Agency (EPA) should ensure there is correlation between cancer classifications and RPF values. There is insufficient human evidence for all 27 PAHs classified by EPA or International Agency for Research on Cancer (IARC) with the exception of benzo[a]pyrene (BaP). BaP is the only PAH that IARC has classified as Group 1. It has an EPA-proposed RPF of 1, by definition. However, several PAHs that IARC has not classified have RPFs that exceed BaP's. In addition, several PAHs that IARC has classified as only "possibly" carcinogenic in humans have RPFs that exceed 1.

EPA's Integrated Risk Information System (IRIS) weight-of-evidence classifications for individual PAHs are restricted to seven PAHs. However, IARC (2010) has classified 60 PAH compounds as to their potential to cause cancer in humans, including BaP and the 27 PAHs with proposed RPFs in EPA (2010). The following table summarizes both the EPA and the IARC classifications.

As noted in the table, there is insufficient human evidence for all 27 PAHs classified by one or both of these organizations with the exception of BaP.

Table 1. Carcinogenic Classifications of Individual PAHs

PAH	Proposed RPF	IARC Classification	EPA Classification
Anthanthrene	0.4	3	NC
Anthracene	0	3	D
Benz[a]anthracene	0.2	2B	B2
Benz[b,c]aceanthrylene, 11H-	0.05	3	NC
Benzo[a]pyrene	1	1	B2
Benzo[b]fluoranthene	0.8	2B	B2
Benzo[c]fluorene	20	3	NC
Benz[e]aceanthrylene	0.8	NC	NC
Benzo[g,h,i]perylene	0.009	3	D
Benz[j]aceanthrylene	60	2B	NC
Benzo[j]fluoranthene	0.3	2B	NC

Table 1. Carcinogenic Classifications of Individual PAHs

PAH	Proposed RPF	IARC Classification	EPA Classification
Benzo[k]fluoranthene	0.03	2B	B2
Benz[l]aceanthrylene	5	3	NC
Chrysene	0.1	2B	B2
Cyclopenta[c,d]pyrene	0.4	2A	NC
Cyclopenta[d,e,f]chrysene, 4H-	0.3	3	NC
Dibenzo[a,e]fluoranthene	0.9	3	NC
Dibenzo[a,e]pyrene	0.4	3	NC
Dibenz[a,h]anthracene	10	2A	B2
Dibenzo[a,h]pyrene	0.9	2B	NC
Dibenzo[a,i]pyrene	0.6	2B	NC
Dibenzo[a,l]pyrene	30	2A	NC
Fluoranthene	0.08	3	D
Indeno[1,2,3-c,d]pyrene	0.07	2B	B2
Naphtho[2,3-e]pyrene	0.3	3	NC
Phenanthrene	0	3	D
Pyrene	0	3	D

Notes:
NC = not classified by Agency
IARC Classification:
- Group 1: The agent is carcinogenic to humans
- Group 2A: The agent is probably carcinogenic to humans
- Group 2B: The agent is possibly carcinogenic to humans
- Group 3: The agent is not classifiable as to its carcinogenicity to humans

EPA Classification:
- A: Known human carcinogen
- B1: Probable human carcinogen - indicates sufficient evidence in animals and limited evidence in humans
- B2: Probable human carcinogen – indicates sufficient evidence in animals and inadequate or no evidence in humans
- C: Possible human carcinogen
- D: Not classified as to human carcinogenicity based on no human data and inadequate animal data

1.2 EPA (2010) "Weight of Evidence" Evaluation

Recommendation: The rationale for each RPF proposed for each PAH should also include a "weight of evidence for the carcinogenic hazard potential" evaluation as described with the 2005 Cancer Guidelines (e.g., "*Likely to be Carcinogenic to Humans*" or "*Suggestive Evidence of Carcinogenic Potential*"). Examples of where this would add clarity and scientific credibility to the proposed approach include the following:

EPA indicates PAHs selected for inclusion in the RPF approach were initially chosen based on an "evaluation of whether the available data were adequate to assess the carcinogenicity of each compound." (Chapter 6, page 113, paragraph 1). Based on this, 35 PAHs were identified for further evaluation. EPA should clearly define "carcinogenicity" for this step within the context of the relevant potential to be carcinogenic to humans with consideration of EPA's 2005 Cancer Guidelines.

For example, EPA's Cancer Guidelines state that chemicals classified as "*Suggestive Evidence of Carcinogenic Potential"* generally do not have adequate data for a dose-response assessment (Section 3. Dose Response Assessment). However, EPA notes in this section that if there is a "well conducted" study, a quantitative assessment may be completed. Including the classification may identify chemicals where study data may require greater scrutiny before being used to determine the RPF or where data indicate that the proposed RPF should be modified to reflect uncertainty

2. BaP as Index Chemical

Recommendation: EPA should update the toxicology risk assessment for BaP prior to finalizing the PAH mixtures IRIS. At a minimum it should be consistent with current EPA 2005 Cancer Guidelines before being used as a point of reference for the proposed RPF approach.

The proposed RPF approach appears to rely on the 1994 IRIS toxicological assessment for benzo(a)pyrene. EPA states that PAHs included in the RPF weight of evidence "were assumed to be carcinogenic due to toxicological similarity to the indicator compound, benzo[a]pyrene." (page iv and vii). Data do not support this assumption for all 35 PAHs included in the analysis. The rationale should be justified within the context of study data and the "weight of evidence for the carcinogenic hazard potential" to humans should be completed and consistent with the 2005 Cancer guidelines for each PAH. A detailed analysis of data for each PAH supporting and demonstrating the assumed "toxicological similarity" would add clarity and credibility to the document.

3. Cancer Slope Factor (CSF) for Benzo[a]pyrene

Recommendation: EPA should update the current CSF of 7.3 $(mg/kg/day)^{-1}$ using the Beland and Culp (1998) and Culp et al. (1998) data as summarized by Gaylor et al. (2000).

The BaP CSF is the geometric mean of several cancer slope factors derived from two, outdated studies, Neal and Rigdon (1967) and Brune et al. (1981). According to the current IRIS profile for BaP, "The data are considered to be less than optimal, but acceptable." In addition, EPA (2010) states: "These studies were not conducted using standard, modern toxicological methods and have several limitations, including inconsistent dosing protocols; varying ages of the animals; use of benzene as a solvent; small numbers of animals; and evaluation of only a limited number of tissues."

There are several more recent studies available such as a Good Laboratory Practices (GLP) study conducted at the National Center for Toxicological Research under EPA oversight

(Beland and Culp 1998; Culp et al. 1998). As well Gaylor et al. (2000) derived a CSF using multistage modeling for benzo[a]pyrene based on forestomach tumors, which was the most sensitive toxicological endpoint. The CSF was 1.2 $(mg/kg/day)^{-1}$.

4. Omission of Studies Not Including BaP

Recommendation: EPA should include studies which contain pertinent PAH toxicology data regardless of whether the studied PAH was tested in conjunction with BaP.

One of EPA's selection criteria for inclusion of a scientific study in the RPF analysis was: "Benzo[a]pyrene was tested simultaneously with another PAH." Several papers excluded because of this criterion which could provide useful information are discussed below for illustrative purposes.

4.1 Wood et al. (1980)

[Wood, A.W., W. Levin, R.L. Chang, et al. 1980. Mutagenicity and tumor-initiating activity of cyclopenta[c,d]pyrene and structurally related compounds. Cancer Res 40:642–649.]

In Wood et al. (1980) the tumorigenicity of CPcdP, BaA and CH were studied. Also in the paper is a study of BaP. This study has utility despite that BaP was not concurrently tested with the PAHs, because: (a) CPcdP was run concurrently with BaP in the paper's Table 1 experiment, and (b) one can determine the relative potencies relative to another PAH, such as chrysene, for which EPA assigns a RPF of 1.0.

Table 2. Effect of Wood et al. (1980) Data on RPF Derivation

	EPA RPFs with Chrysene 1	Wood et al. (1980) Incidence (2.5 umol)	Wood et al. (1980) Multiplicity (2.5 umol)
CH	1	1	1
BaA	2	0.4	0.2
CPcdP	4	0.4	0.3

As noted above, EPA's proposed RPFs conclude that the relative potency of these PAHs is CPcdP > BaA > CH. However, Wood et al. (1980)'s relative potency is CH > BaA, CPcdP. Clearly, this study yielded different results from the other papers that EPA relied on to derive RPFs for these three PAHs.

4.2 Cavalieri et al. (1989)

[Cavalieri, E.L., E.G. Rogan, S. Higginbotham, et al. 1989. Tumor-initiating activity in mouse skin and carcinogenicity in rat mammary gland of dibenzo[a]pyrenes: the very potent environmental carcinogen dibenzo[a,l]pyrene. J Cancer Res Clin Oncol 115:67–72.]

In Cavaleri et al. (1989), a tumorigenesis study with five PAHs of interest but not BaP. Both incidence and multiplicity results are provided. EPA (2010) has rated dibenzo[a,h]pyrene very similar in potency to BaP with a RPF of 0.9. Accordingly, all of the PAHs included in the study can be normalized to dibenzo[a,h]pyrene to determine if the results from Cavaleri et al. (1989) are similar to the EPA proposed RPFs. As noted below, the results for several PAHs are not wholly inconsistent with EPA's RPFs, but the results for dibenzo[a,l]pyrene (see bold font) are inconsistent with the EPA's reported results. If results from Cavaleri et al. (1989) were used in the RPF derivation, the mean RPF would drop considerably from the proposed value of 30. The results for AA were also inconsistent with the EPA-derived RPF of 0.4.

Table 3. Effect of Cavalieri et al. (1989) Data on RPF Derivation

PAH	EPA RPF	EPA RPF assuming DB[a,h]P were 1.0	Cavaleri et al. (1989) Incidence	Cavaleri et al. (1989) Multiplicity
AA	**0.4**	**0.4**	**0.03**	**0**
DB[a,l]P	**30**	**33**	**1.3**	**0.7**
DB[a,h]P	0.9	1	1	1
DB[a,i]P	0.6	0.7	0.8	0.5
DB[a,e]P	0.4	0.4	0.2	0.04

4.3 Van Duuren et al. (1970)

[Van Duuren, B.L., A. Sivak, B.M. Goldschmidt, et al. 1970. Initiating activity of aromatic hydrocarbons in two-stage carcinogenesis. J Natl Cancer Inst 44:1167–1173.]

In Van Duuren et al. (1970), tumorigenesis was studied with four PAHs of interest but not BaP. The PAHs studied include DBacA, BaA, BghiP, and CH. EPA's relative potency order is BaA > CH > BghiP > DBacA. Van Duuren et al. (1970) showed a different order of relative potency: DBacA > BaA > BghiP and in another experiment: CH > BaA > DBacA. It is also important to note that EPA did not derive a RPF for DBacA because no animal tumorigenicity studies were identified that passed EPA's inclusion criteria. This study could have been used to derive a RPF for this PAH.

4.4 Chang et al. (1982)

[Chang, R.L., W. Levin, A.W. Wood, et al. 1982. Tumorigenicity of bay-region diol-epoxides and other benzo-ring derivatives of dibenzo[a,h]pyrene and dibenzo[a,i]pyrene on mouse skin and in newborn mice. Cancer Res 42:25–29.]

In Chang et al. (1982), tumorigenesis was studied with two PAHs of interest but not BaP. The PAHs are DBahP and DBaiP. These two PAHs are ones that have EPA-derived RPFs based on a single study (Hoffmann and Wynder 1966). EPA's RPFs are 0.9 for DBahP and 0.6 for DBaiP from Hoffmann and Wynder's study. Thus, DBahP is classified as 1.5 times more potent than DBaiP.

Chang et al. (1982) provides three dose groups for the mouse skin assay for the two PAHs and one intraperitoneal (IP) dose group in the newborn mouse model. At the lowest dose, this study would indicate that DBahP is 2 times more potent than DBaiP using incidence data and 1.9 times more potent using multiplicity data. These results are similar to EPA's results from the Hoffman and Wynder (1966) data and would be useful to present.

5. Protocol Issues

Recommendation: EPA should derive RFPs separately for each route of exposure based on the available scientific data. However, EPA (2010) should not establish RFPs based on IP and lung implantation studies because they have no relevance to human health.

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

5.1 Mouse Skin Assay

EPA (2010) has relied extensively on screening models for the source of the data that it used for quantitative dose-response assessment and in particular it has relied extensively on screening level mouse skin tumorigenicity studies quantitatively for RPF derivation. However, according to the National Research Council (NRC 1993), the typical two-year rodent carcinogenesis bioassay was designed to be a qualitative screening tool and was not designed for quantitative dose-response modeling. Specifically, NRC (1993) states: "The long-term animal bioassay for carcinogenicity was developed during the 1960s and early 1970s primarily as a *qualitative* screen for carcinogenic potential. Long-term animal bioassays are now used regularly to determine whether chemical agents are capable of inducing cancer in exposed animals. The bioassays are also commonly used as a basis for making qualitative inferences about the likelihood that an agent poses a carcinogenic hazard for humans as well (IARC, 1991)."

McKee et al. (1990) compared the dose-response curves two materials (10 assays of benzo(a)pyrene and 12 assays of catalytically cracked clarified oil) and showed they were not parallel. They concluded that if one is attempting to derive relative potency estimates between and among PAHs, one should determine the slopes of the dose-response curves of both the reference material and the test material. "If the experimentally determined dose-response curves are not parallel, then the relative potency estimations will be dose-specific (i.e., the ratio of the relative potencies of the two material will vary with dose)."

McKee et al. (1990) also showed that variations occur from dermal bioassay to dermal bioassay for a single constituent (e.g., benzo(a)pyrene) that were much greater than variations observed within a given assay. This variability may be attributable to a number of things and

has the potential to significantly contribute to experimental error. Factors known to affect the response in dermal bioassays include: application frequency (shown for coal liquids which are severe dermal irritants); different vehicles have be associated with differences in tumor responses in benzo(a)pyrene assays; housing conditions (such as number of animals per cage, type of caging, and the temperature); and changes in animal sensitivity appear to occur over time.

McKee et al. (1990) concluded: "Considerable uncertainty would be associated with any risk estimate based on an extrapolation from experimental data obtained in epidermal carcinogenesis studies." EPA (1988) has also noted that background tumor rate and skin toxicity must both be carefully considered during the design and execution of any carcinogenesis study via the dermal route.

5.2 A/J Mouse Lung Adenoma Model

Another screening model that EPA (2010) used was the Strain A mouse lung adenoma assay in which mice with extremely high spontaneous lung adenoma rates are employed. In this screening assay, mice derived from Strain A, such as the A/J mouse, are given a high, IP dose at the maximally tolerated dose level, and animals are observed for increases in adenoma rates over the already high background rates.

Such an assay should not be used for quantitative dose-response assessment for several reasons. The Strain A mice have high spontaneous rates of lung adenoma formation and are very sensitive to carcinogenic agents. Humans have no similar tumor type, and the IP route of administration has no relevance to human health.

According to Robinson et al. (1986), the A/J mouse strain, which was used for many of the RPF derivations, is the most sensitive strain available for this type of assay: "…the A/J strain is the most susceptible model for lung adenomas." When they tested four mouse strains in their laboratory for lung adenoma formation with several known animal carcinogens, they confirmed that the A/J mouse strain was more sensitive than any other tested strain.

Adkins et al. (1986) reported that the background pulmonary adenoma rate in control Strain A/J mice was 41.7% and that this spontaneous tumor rate was similar to the rates reported in the literature. Adkins et al. (1986) also reported inconsistency in the results of the assay in their hands. In Stoner's laboratory over ten years Stoner (1991), the historical spontaneous tumor rate in control animals varied from 25% to 32%.

Also, the mouse lung adenoma bioassay is carried out at extremely high doses that can cause toxicity, including mortality. According to Stoner (1991), the Strain A lung tumor bioassay calls for animals to be dosed at "the maximum single dose that all five mice tolerate (survive) for a period of two weeks after receiving six i.p. injections (three injections per week)." After the maximum tolerated dose (MTD) is established, the assay should be carried out with three dose levels, the MTD, 50% MTD and 20% MTD with 30 mice per group. EPA (2010) used both multiplicity and incidence data from such studies, although according to Stoner (1991),

only tumor multiplicity should be used to determine if a chemical is carcinogenic in Strain A mice.

In addition, the Strain A lung adenoma bioassay does not predict carcinogenicity well. As stated in Maronpot (1991): "Fifty-nine chemicals that had completed National Cancer Institute rat and mouse two-year carcinogenicity tests were tested in the Strain A mouse pulmonary tumor assay…. The Strain A results were generally not predictive of the 2-year rat and mouse carcinogenicity test results."." Based on such data, toxicologists have concluded that this bioassay is a not a reliable predictor of carcinogenicity in humans. Clayson (1988) has stated that "a number of bioassay systems for the identification of carcinogens are no longer considered reliable indicators of possible carcinogenicity for humans. These include the lung adenoma test in Strain A mice (8), the induction of bladder tumors in the presence of urinary calculus (9), and bladder implantation (10,11)."

Similarly, Stoner (1991) also noted that this bioassay is not routinely used for carcinogenesis testing stating: "In the past few years, the Strain A mouse lung tumor bioassay has come into disfavor as a routine test for chemical carcinogens. This stems largely from the fact that, in a National Cancer Institute sponsored study, the assay failed to detect the carcinogenic activity of numerous compounds that were active in 2-year rodent bioassays…"

5.3 Lung Implantation Model

EPA (2010) derived RPFs for eight PAHs based on data from two lung implantation studies from a single researcher. The papers are cited as Deutsch-Wenzel et al. (1983) and Wenzel-Hartung et al. (1990). The implantation technique used in the study does not appear to reflect the normal exposure pattern for humans. While it is possible for PAHs to be inhaled into the human lung, the relevance of using the implantation technique noted in the study (i.e. injecting a hot mixture of PAH-beeswax-triotanoin directly into the lung) is not evident. Deutsch-Wenzel et al. (1983) noted that the "… the implantation of pellets containing environmental carcinogens as an experimental model for studying pulmonary carcinogenesis also has disadvantages..."

EPA's Guidelines for Carcinogen Risk Assessment (EPA 2005) state that route of exposure is an important consideration when assessing scientific data and making weight-of-evidence judgments about the potential of substances to cause cancer in humans. EPA (2010) acknowledges that route of exposure and site-of-entry tumor formation are important issues and notes in the document that ""… cross-route extrapolation of RPFs is a significant source of uncertainty in this approach."

5.4 Numerical Methodology

Recommendation: EPA should not use the highest average RPFs from multiple target organs in order to determine a final RPF, as this introduces a bias to the calculation.

EPA (2010) states that the range and average RPF for each PAH was calculated using the following strategy. First, within a given animal study group (i.e. the same sex, route of

administration and reference), if RPFs were calculated for multiple target organs, the highest of the RPFs was chosen for inclusion in calculations. Then, if applicable, the higher of RPF values calculated using incidence and multiplicity data for the study group was chosen. Lastly, RPFs were arithmetically averaged across the various study groups to one significant figure.

This method appears to bias the calculation of the RPFs by choosing only the highest value. EPA (2010) addressed the issue by performing a regression analysis relating paired incidence and multiplicity RPFs, which resulted in an r^2 value of 0.76. EPA concluded that the relationship between incidence and multiplicity RPFs was adequate to justify using the higher of the two for a given animal study group. However, there is little scientific justification in favor of using the higher of RPFs from incidence and multiplicity data. Consequently, by using a neutral numerical methodology, either incidence would be given more weight based on the greater historical use of incidence data or the mean value from the two metrics would be used.

Of 23 PAHs assigned non-zero RPFs based on in vivo bioassay data, 12 were based on more than two study groups (Table 7-1 in EPA 2010). In order to test the bias of the EPA method, RPFs for these 12 PAHs were recalculated according to the following method:

- For a given route of administration and reference, average incidence and multiplicity RPFs for the same sex and target organ
- Average RPFs for males and females for the same target organ
- Average RPFs for all target organs
- Average across routes of administration and references to one significant figure

Using this methodology, RPFs for benzo[k]fluoranthene, chrysene, cyclopenta[c,d]pyrene, and dibenzo[a,l]pyrene remained the same, and RPFs for the other eight PAHs were slightly lower than reported. As mentioned above, EPA (2010) determined that incidence and multiplicity RPFs were similar for a given study group. In addition, only four PAHs had RPFs based on studies in which data for multiple sexes or target organs for a single study group were reported. Thus, the reduction in RPF using the new method above was generally small. The exception is benzo[c]fluorene, which dropped from the proposed value of 20 to 10. This large reduction is a result of the order-of-magnitude difference between incidence and multiplicity RPFs calculated from the Weyand et al. (2004) oral bioassay, in which the incidence RPF was 5 while the multiplicity RPF (calculated from a point estimate) was 50.

Table 4. Recalculation of Select RPFs Using Alternative Methodology

Analyte	EPA (2010) Range RPFs	EPA (2010) Average RPF	Recalculation[1]	Change in RPF
Benz[a]anthracene	0.02–0.4	0.2	0.1	Lower
Benzo[b]fluoranthene	0.1–2	0.8	0.6	Lower
Benzo[c]fluorene	1–50	20	10	Lower
Benz[e]aceanthrylene	0.6–0.9	0.8	0.7	Lower
Benzo[j]fluoranthene	0.01–1	0.3	0.2	Lower
Benzo[k]fluoranthene	0.03–0.03	0.03	0.03	Same
Benz[l]aceanthrylene	4–7	5	4	Lower
Chrysene	0.04–0.2	0.1	0.1	Same
Cyclopenta[c,d]pyrene	0.07–1	0.4	0.4	Same
Dibenz[a,h]anthracene	1–40	10	9	Lower
Dibenzo[a,l]pyrene	10–40	30	30	Same
Fluoranthene	0.009–0.2	0.08	0.07	Lower

Notes:
[1] See text. RPFs are rounded to one significant figure per EPA (2010) procedures.

5.5 Transparency of Data Used

Recommendation: EPA should clearly document which RPFs are averaged to derive the final RPF values.

EPA (2010) presents all derived RPFs in chemical-specific histogram figures, but not all of the RPFs shown in those figures are averaged to derive the final RPF presented in Table 1 of EPA 2010.

For instance, benzo(c)fluorene is reported as 20. Figure 6-9 (EPA 2010) show 4 RPFs from one study, Weyand et al. (2004). According to Appendix E tables, the values are:

Oral multiplicity	48.9	IP multiplicity	0.56
Oral incidence	5.48	IP incidence	1.05

The total average of these four RPFs is 14.00. The combined average of the oral average and the IP average is 14.01. However, EPA did not use all of the data when deriving the final proposed RPF. They instead used the higher of the two oral values and the higher of the two IP values. This is misleading and EPA should note on the histograms which RPFs are actually averaged to derive the final RPF values.

5.6 Dependence of RPFs of "Low" Confidence or Few Studies

Recommendation: EPA should not finalize a RPF for any PAH that receives a *"low confidence or very low confidence rating."*

According to EPA (2010), "Once a final RPF was derived for a given PAH, the resulting value was assigned a relative confidence rating of *high, medium,* or *low confidence*. The relative confidence rating characterized the nature of the database upon which the final RPF was based. Confidence rankings were based on the robustness of the database. For final RPFs based on tumor bioassay data, confidence ratings considered both the available tumor bioassays and the availability of supporting data for cancer-related endpoints. The most important factors that were considered included the availability of in vivo data and whether multiple exposure routes were represented. Other database characteristics that were considered included the availability of more than one in vivo study, and whether effects were evident in more than one sex or species. *Very low relative confidence* was reserved for final RPFs based on cancer-related endpoint data only (e.g., dibenz[a,c]anthracene). An RPF of zero was only applied if the data implied *high* or *medium relative confidence*."

EPA (2010) has rated 12 RPFs as low confidence and one as very low confidence. Ten of the low confidences RPFs were derived from data from a single publication each. One publication, (Hoffmann and Wynder 1966) was solely responsible for five RPFs and another publication (Deutsch-Wenzel et al. 1983) was responsible for two RPFs. Thus, five specific publications (Mass et al. 1993; Rice et al. 1988; Hoffmann and Wynder 1966; Deutsch-Wenzel et al. 1983; and Nesnow et al. 1984) are solely responsible for ten RPFs.

Table 5. PAHs and RPFs with Low Confidence Ratings

PAH	Relative Confidence	Number of Datasets	References (Positive Studies Only)
Benz[b,c]aceanthrylene, 11H-	Low	1	Rice et al. (1988)
Benz[e]aceanthrylene	Low	2	Nesnow et al. (1984)
Benzo[g,h,i]perylene	Low	1	Deutsch-Wenzel et al. (1983)
Benz[j]aceanthrylene	Low	1	Mass et al. (1993)
Benz[l]aceanthrylene	Low	2	Nesnow et al. (1984)
Cyclopenta[d,e,f]chrysene, 4H-	Low	2	Rice et al. (1985), Rice et al. (1988)
Dibenzo[a,e]fluoranthene	Low	2	Hoffmann and Wynder (1966)
Dibenzo[a,e]pyrene	Low	2	Hoffmann and Wynder (1966)
Dibenzo[a,h]pyrene	Low	1	Hoffmann and Wynder (1966)
Dibenzo[a,i]pyrene	Low	2	Hoffmann and Wynder (1966)
Fluoranthene	Low	5	Busby et al. (1984), LaVoie et al. (1994), Busby et al. (1989)
Indeno[1,2,3-c,d]pyrene	Low	1	Deutsch-Wenzel et al. (1983)
Naphtho[2,3-e]pyrene	Low	1	Hoffmann and Wynder (1966)

Table 5. PAHs and RPFs with Low Confidence Ratings

PAH	Relative Confidence	Number of Datasets	References (Positive Studies Only)
Dibenz[a,c]anthracene	Very Low	14	Cancer-related endpoints: Philips et al. (1979), Andrews et al. (1978), Rossman et al. (1991), Baker et al. (1980), McCann et al. (1975), Kaden et al. (1979), Martin et al. (1978), Grover and Sims (1968), Hermann (1981), Bryla and Weyand (1992), DiPaolo et al. (1969), Mersch-Sundermann et al. (1992), Pahlman and Pelkonen (1987), Huberman and Sachs (1976)

The uncertainty of each RPF derived from a single publication is extremely high, especially when the publications use outdated methods and procedures, and have methodological issues. Many of these publications should be rejected for use in RPF derivation for various reasons listed below.

- Hoffmann and Wynder (1966) is an outdated publication that has significant shortcomings. The study had high mortality in many of the animal groups, even early in the duration of the experiment. Finally, EPA (2010) calculated incidence rates for results from this paper using surviving animals at the date of first tumor rather than the total study group number as was used for most other incidence calculations.
- Deutsch-Wenzel et al. (1983) is the sole basis for two RPFs. However it used an unconventional animal lung implantation assay in rats that does not appear to be relevant to normal human exposure patterns. As well, the author's noted that "BghiP showed only a weak effect when applied as an intrapulmonary implant. It cannot be excluded that the effect observed in the lung implantation test originates from the impurities of the BghiP administered."
- Mass et al. (1993) exhibited a high mortality rate, ranging from 22-33%, and the mortality could not be assessed with relation to the control because the initial number of control animals was not reported in the paper. Given the criterion in EPA (2010) to exclude studies based upon unexplained mortality, data from Mass et al. (1993) should be excluded from RPF derivation.
- The RPFs for cyclopenta[d,e,f]chrysene, 4H- and cyclopenta[d,e,f]chrysene, 4H- were derived from two publications (Rice et al. 1985; and Rice et al. 1988). In both cases, it was stated that the test substances were synthesized by a specific method, but in neither case do the methods characterize the identity of the constituent.
- Nesnow et al. (1984) does not pass EPA's stated study inclusion criterion, in that papers with substances of questionable purity should be excluded from the analysis. It has not been proven that the cited synthetic route produces benz[l]aceanthrylene and benz[e]aceanthrylene. Because of these chemicals rarity, their identity and purity are highly uncertain. In addition, Nesnow et al. (1984) reported that their BaP dose group

had incidence and multiplicity rates that were “low compared to previously published reports.” Similarly for tumor multiplicity, the observed multiplicity was 1.4 tumors/mouse for males and 1.5 tumors/mouse for females compared to a historical range of 1.5-8.4 tumors/mouse.

5.7 Point Estimate Methodology and Benchmark Dose Model Fits

Recommendation: EPA should document the criteria for defining a “poor fit” or a “good fit” of the data in the Benchmark Dose Modeling.

When Benchmark Dose Modeling of multi-dose results provided a “poor fit,” EPA’s protocol required that “point estimates” from single dose groups be used for RPF derivation instead of slope factors using modeling of the entire dataset. This protocol is noted in Section 5.4 of EPA 2010. However, EPA does not provide criteria for defining a “good fit” of the data. The Benchmark Dose Software generates three goodness-of-fit metrics to guide in the selection of the optimal model. There is no identified criterion to judge “fitness,” and EPA’s decision strategy is not documented. Tables in EPA’s Appendix E report in some cases that there was “no model fit,” but the criteria for making this determination are not provided, nor are the BMDS outputs.

5.7.1 Benchmark Dose Model Fit Validation

EPA (2010) used the Benchmark Dose Modeling Software (BMDS) to calculate benchmark doses (BMDs). In many cases, the point estimate approach instead of the BMD approach because EPA (2010) reported that a good fit to the data was not found. A validation exercise was undertaken by the undersigned to determine if the lack of a “good fit” was due to EPA’s protocol, which used the multistage model for quantal data sets and the linear model for continuous data sets. Consistent with EPA guidance on model selection, all relevant fit criteria were evaluated including visual inspection of the dose-response curve, the range of BMD estimates among candidate models, inspection of the maximum scaled residual (particularly among the low dose group), p-value of the chi-square test, AIC values, and hypothesis test results (p-values) for continuous datasets (i.e., Tests 1 to 4). This validation found that EPA excluded acceptable model fits with other models available in the BMDS and instead used the point estimate approach. Use of this approach ignores a full dose-response curve for those experiments with multiple dose groups. In several cases this affected the calculated RPFs. Some examples of validated model fits to determine if it was possible to derive RPFs based on BMDs noted below and BMDS outputs are presented in Appendix C.

5.7.2 Mass et al. (1993)

EPA (2010) used the point estimate approach for modeling BaP and BjAC data from Mass et al. (1993) because they stated that there was “no model fit.” A validation exercise of BMDS modeling found that the data for BaP acceptably fit three models, including Hill, exponential 5, and linear. The average BMD at BMR=0.1 from the three models was 40.3 mg/kg/day. For BjAC, BMDS revealed acceptable fits for four models, including exponential 4, linear, polynomial, and power. The average BMD at BMR=0.1 from the four models was 4 mg/kg/day. The RPF for BjAC using the BMDS modeling would be 10, instead of EPA’s RPF

of 59. This is a significant difference in RPF. By using the full dataset and comparing two full dose-response curves, the RPF is 10. When EPA defaulted to comparing two points on the dose-response curves, the derived RPF was 59.

5.7.3 Nesnow et al. (1984)

BlAC and Male Mouse

EPA (2010) used BMDS modeling with the three highest doses omitted to obtain an adequate fit for the Multistage-cancer model for the male BlAC data from Nesnow et al. (1984). A validation exercise of BMDS modeling of this data set found that the scaled residuals were very high (i.e., absolute value > 2.0) when all six dose groups (including control) were included, but when the single highest dose group (1,000 nmol) was omitted per EPA criteria, all models provided an adequate fit and similar BMD estimates. Therefore, no additional dose groups were omitted. Among the six best fitting models with maximum scaled results less than 0.5, the BMD at the 67% effect level (BMR = 0.67) ranged from 54.6 to 56.5 nmol (i.e., ratio = 1.0). Furthermore, the AIC was the same (AIC = 39), suggesting that there is minimal model uncertainty. Following EPA guidance, the BMD is determined by the mean of the values for those models with the lowest (and equal) AIC. The BMD at BMR=0.67 is 56 nmol, which is similar to EPA's BMD of 50 nmol. The RPF using the modeling would be 3.6 instead of EPA's RPF of 4.

BlAC and Female Mouse

EPA (2010) used BMDS modeling with the three highest doses omitted to obtain an adequate fit for the multistage-cancer model for the female BlAC data from Nesnow et al. (1984). A validation exercise the modeling yielded a good fit with the loglogistic model using all dose groups. This model yielded a maximum scaled residual =of1.3 (i.e., absolute value ≤ 2.0), chi-square p-value = 0.4 (i.e., p > 0.10), and the lowest AIC from among nine models considered. The BMD at the 51% effect level (BMR=0.51) was 26 nmol, which was similar to EPA's BMD of 30 nmol. The RPF using the modeling would be 5.6 instead of EPA's 6.67.

BeAC and Male Mouse

EPA (2010) used the point estimate approach for modeling the male BeAC data from Nesnow et al. (1984) because they stated that there was "no model fit." A validation exercise of the BMDS modeling found an acceptable fit for seven models, all of which had low maximum scaled residuals, high p-values for the chi-square test, and comparable AIC values. The BMD at the 67% effect level ranged from 341 to 393 nmol (i.e., ratio = 1.2). Following EPA guidance, the BMD is determined by the mean of the values for those models with the lowest (and equal) AIC. Five models had the same minimum AIC of 106 and identical BMD values of 393 nmol. Therefore, the BMD at BMR=0.67 was 393 nmol. The RPF using the modeling would be 0.5 instead of EPA's 0.71.

BeAC and Female Mouse

EPA (2010) used BMDS modeling with the two highest doses omitted to obtain an adequate fit for the multistage-cancer model for the female BeAC data from Nesnow et al. (1984). A validation exercise of the BMDS modeling found an acceptable fit for eight models, all of which had low maximum scaled residuals, high p-values for the chi-square test, and comparable AIC values. The BMD was relatively consistent among these models, ranging from 297 to 537 nmol (ratio = 1.8). The loglogistic model, with the lowest AIC of 126, yielded a BMD at BMR=0.51 of 297 nmol. The RPF using the modeling would be 0.67 instead of EPA's 0.88.

5.7.4 Habs et al. (1980)

EPA (2010) used the point estimate approach for modeling BaP data from Habs et al. (1980) because they stated that there was "no model fit." A validation exercise of the BMDS modeling found an acceptable fit for eight models when the highest dose was omitted per EPA criteria. The best fits were with the gamma, loglogistic, logprobit, and Weibull models, because they had the lowest maximum scaled residuals, low AICs, and the highest p-values on the chi-squared test. Thus, the full dose-response curve could have been used for RPF derivation.

5.7.5 LaVoie et al. (1982)

EPA (2010) used the point estimate approach for modeling BbF and BjF data from LaVoie et al. (1982), because they stated that there was "no model fit." A validation exercise of the BMDS modeling found a model fit for both PAHs with the loglogistic model. With the highest dose removed from the analysis, the model fit was acceptable with eight models for BbF and with seven models for BjF. Thus, the full dose-response curve could have been used for RPF derivation.

5.7.6 Rice et al. (1988)

EPA (2010) used the point estimate approach for modeling CH and BbcAC data from Rice et al. (1988) because they stated that there was "no model fit." A validation exercise of the BMDS modeling found that the data for CH provide an acceptable fit for the loglogistic model. With the highest dose omitted, BDMS provides an acceptable fit for eight models including the multistage model. The BMD for CH varied from 0.09 to 0.1 umol/animal among the model results showing acceptable fit. For BbcAC, an acceptable fit was found with the loglogistic model. Thus, the full dose-response curve could have been used for RPF derivation for these two PAHs.

5.7.7 Busby et al. (1984)

EPA (2010) used the point estimate approach for modeling BaP and FA data from Busby et al. (1984) because they stated that there was "no model fit." A validation exercise of the BMDS modeling found for both the male and female groups that BaP data was acceptably fit by the Exponential 4 and Polynomial models. For FA, BMDS showed an acceptable fit for six models including the linear model in males. For females, there was an acceptable fit for six models

including the linear model. Thus, the full dose-response curve could have been used for RPF derivation for these two PAHs.

5.7.8 Nesnow et al. (1998)

EPA (2010) used the point estimate approach for modeling BaP, CPcdP, and DBalP data from Nesnow et al. (1998) because they stated that there was “no model fit.” A validation exercise of the BMDS modeling found that BaP data acceptably fit four models, CPcdP data acceptably fit four models, and DBalP data acceptably fit six models. Thus, the full dose-response curve could have been used for RPF derivation for these three PAHs.

5.7.9 Conclusion

As noted above, EPA (2010) has ignored many full data sets and defaulted to a point estimate approach that relied on a single data point. Regardless of the type of assay, full data sets should be used to define the slope of the dose-response curve. This is particularly important with mouse skin assay results. If data from mouse skin tumor assays are going to be used quantitatively to derive RPFs, there is uncertainty introduced, and it is critical that a full dose-response curve be used in every case. McKee et al. (1990) compared the dose-response curves of two materials (10 assays of benzo(a)pyrene and 12 assays of catalytically cracked clarified oil) and showed they were not parallel. They concluded that if one is attempting to derive relative potency estimates between and among PAHs, one should determine the slopes of the dose-response curves of both the reference material and the test material. “If the experimentally determined dose-response curves are not parallel, then the relative potency estimations will be dose-specific (i.e., the ratio of the relative potencies of the two materials will vary with dose).”

5.8 Exclusion of Data from Studies Showing Tumor Incidence of 90% or Higher

Recommendation: EPA should justify why exclusion of all studies showing tumor incidence of 90% or greater is justified. Further, EPA should explain why some such studies were excluded and some were not. EPA should also justify and document the use of multiplicity of tumors for quantitative dose-response estimates when tumor incidence was 90% or greater.

According to EPA (2010), tumor incidence data were deemed unusable if tumor incidence at the lowest dose level (or the only dose level) was greater than or equal to 90However, EPA should explain why all such data should be excluded and EPA should explain why it selectively invoked this rule. For instance, if the tumor incidence at dose X was 90% in BaP and 1% at the same dose for PAHx, that study would indicate that PAHx is much less potent than BaP. Similarly, if the tumor incidence of BaP at dose X was 1% and 90% at the same dose for PAHx, that study would indicate that PAHx is much more potent than BaP. The scientific validity of the EPA’s criteria should be validated.

In several cases, EPA (2010) did exclude some datasets based on this criterion. Datasets excluded include those from Hoffman and Wynder (1966), Slaga et al. (1978), Cavalieri et al. (1981), El-Bayoumy et al. (1982), Busby et al. (1984), Rice et al. (1985), Cavalieri et al.

(1991), and Weyand et al. (1992). Use of this criterion truncated the available datasets for benz[a]anthracene, benzo[j]fluoranthene, chrysene, cyclopenta[c,d]pyrene, 4H-cyclopenta[d,e,f]chrysene, dibenzo[a,h]pyrene, dibenzo[a,l]pyrene, and fluoranthene. Furthermore, EPA should explain why they did *not* use the criterion for data on benzo[c]fluorene from Weyand et al. (2004). Incidence data from an intraperitoneal study in Weyand et al. (2004) were included in the RPF calculations for benzo[c]fluorene even though the tumor incidence of benzo[a]pyrene at the only dose level, 100 mg/kg, was 90% and the tumor incidence for benzo[c]fluorene was 92%.

As noted above, EPA included the use of tumor incidence data in quite a few cases when tumor incidence is 90% or greater, but in the same cases, EPA made use of the multiplicity data. It is not clear that multiplicity of tumors is informative in these cases.

5.9 Role of Cancer-related Endpoints

Recommendation: EPA should not use derived RPFs from cancer-related endpoints when deriving a final RPF value, as was the case in generating the RPF for dibenz[a,c]anthracene.

EPA (2010) has derived quantitative RPFs from a variety of cancer-related endpoints. However, using DNA adduct, DNA damage, mutagenicity and cell transformation studies, is inappropriate for quantitative dose-response assessment and the use of RPFs derived from cancer-related are not suitable for use in quantitative human health risk assessment. Consequently, EPA has not used RPFs derived from cancer-related endpoints to calculate the final RPF except for one substance, dibenz[a,c]anthracene.

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

Slaga et al. (1980) studied DBacA and found it to be negative for mouse skin tumors. This study met EPA’s inclusion criteria and is listed on EPA’s Table 4-1. Results from this study were used for CH and DBahA. Table 4-1 lists BeP and DBacA as “nonpositive.” However, neither negative result is reported in EPA’s figures, Figure 6-11 for BeP or Figure 6-22 for DBacA.

As well, EPA 2010 states “There were 15 datasets for dibenz[a,c]anthracene that met selection criteria and included benzo[a]pyrene (Figure 6-22).” “In at least one study, benzo[k]fluoranthene, benz[l]aceanthrylene, 4 benz[e]aceanthrylene, naphtho[2,3-e]pyrene, dibenz[a,h]anthracene, dibenz[a,c]anthracene, and 5 benz[b,c]aceanthrylene showed positive initiating activity. Nonpositive results were reported for pyrene, perylene, benzo[g,h,i]fluoranthene, fluoranthene, anthanthrene, dibenzo[e,l]pyrene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene, benzo[e]pyrene, anthracene, 2,3-acepyrene, and phenanthrene.” However, DBacA was negative in dermal bioassays and the above quotation fails to take into account the three negative mouse skin bioassay results that EPA

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

It is unjustified to use *in vitro* mutagenicity, DNA damage and other cancer “related” bioassay results to conclude that a substance is carcinogenic to humans when at least four studies reported by EPA have shown that the substance is *not* tumorigenic in the mouse skin bioassay. Thus, EPA has ignored four *in vivo* negative studies in the mouse and derived RPFs exclusively from cancer-related endpoints.

5.10 Concurrent BaP Control

Recommendation: EPA should be diligent about following any criteria it sets to determine studies for inclusion in the RFP approach, and it should apply its criteria equally across all data sets.

According to EPA (2010), the first criterion for study selection was that “benzo[a]pyrene was tested simultaneously with another PAH.” Simultaneity was defined as testing in the same laboratory with the same protocol at the same time. Thus, for most studies in EPA’s Tables 4-1 through 4-5, which summarize bioassays involving benzo[a]pyrene and at least one other PAH, studies with comments indicating that reviewers could not be sure whether benzo[a]pyrene was tested concurrently with another PAH failed to meet selection criteria. However, when calculating RPFs, EPA made exceptions to its criterion for Slaga et al. (1980) and Rice et al. (1988):

> “In a number of reports, it appears that bioassays were done in batches and reported in a single publication. In these cases, it appears that benzo[a]pyrene treatment may not have been undertaken concurrently with all of the compounds in the report. For some of these studies (Horton and Christian, 1974; Bingham and Falk, 1969), there are differences in the choice of vehicle or promoter, or other issues that argue against using the benzo[a]pyrene data for direct comparison. In several other studies, however (Rice et al., 1988; Slaga et al., 1980; Van Duuren and Goldschmidt, 1976; Wynder and Hoffmann, 1959), the protocols (including vehicle and promoting agent) appear to have been the same.”

Slaga et al. (1980) reported the results of dermal initiation experiments in mice in several different tables. While there was only one protocol described in the materials and methods section, implying that all work was performed the same way in the same laboratory, results for benzo[a]pyrene, chrysene, and dibenz[a,h]anthracene were each reported in a separate table with a separate control. Whether the results for the three PAHs meet EPA selection criteria then depends on a subjective interpretation of testing simultaneity – the same time or the same approximate time period. The PAHs were tested in three different batches and were compared to different controls which, implies that the batches were considered separate and/or were not tested at the exact same time. Such a circumstance would preclude use of the study data for the calculation of RPFs for chrysene and dibenz[a,h]anthracene.

Rice et al. (1988) reported the results of dermal initiation experiments in mice in a single table. However, the results state that 11H-benz[b,c]aceanthrylene (designated 1,12-MBA) was

evaluated in a different bioassay than chrysene and 4H-cyclopenta[d,e,f]chrysene (designated 4,5-MC). In Table 1 of Rice et al. (1988), separate acetone controls were reported for each bioassay, and benzo[a]pyrene was only reported with 11H-benz[b,c]aceanthrylene. As with Slaga et al. (1980), results for chrysene and 4H-cyclopenta[d,e,f]chrysene may not strictly meet EPA selection criteria and should not be used to calculate RPFs if they were tested in a different batch than benzo[a]pyrene and compared to a different control, which implies that they were not tested at the same time as benzo[a]pyrene.

5.11 Suitability of Data Sets for Dose-Response Modeling

Recommendation: EPA should only use data sets for RPF derivation which are suitable for dose-response slope factor derivation.

EPA (2010) derived the RPFs by comparing the tumorigenic potency of two PAHs in the form of a ratio of two slopes of two dose-response curves from screening levels bioassays, such as the mouse two-stage skin model. Out of 43 data sets, 16 (37%) had multiple doses for both BaP and the PAH of interest. In these cases, assuming that there were "good model fits," two dose-response curve slopes could be compared. However, out of these 43 data sets, 12 (28%) had a single BaP dose group and a single dose group for one or more PAHs of interest. 15 (35%) had multiple dose groups for PAHs of interest but only a single dose group for BaP. So, fully 63% of the data sets EPA used for RPF derivation were unsuitable for dose-response slope factor derivation. In these cases either one or both of the slope factors was derived using a single data point and assuming that the dose-response curve was linear to the origin, regardless of whether the tumor incidence at that single dose was 1%, 50% or 85%.

Taking EPA's actual protocol into account, a review of the data sets finds that of the 43 data sets:

- 18 (42%) used a single BaP dose and single doses for other PAHs
- 13 (30%) used a single BaP dose and a dose-response curve for other PAHs
- 12 (28%) used a dose-response curve for BaP dose and a dose-response curve for other PAHs

Thus, EPA based 72% of the RPFs on ratios of "slopes" that were derived using a single data point for one or both of the PAHs being compared.

Table 6. Number of Dose Groups for EPA Data Sets

Study	Number of BaP Doses	Number of Doses for Other PAHs	Study	Number of BaP Doses	Number of Doses for Other PAHs
Habs et al. 1980	3	3	Rice et al. 1985	1	1
Cavalieri et al. 1983	3	3	Cavalieri et al. 1991	3	3
Hoffmann and Wynder 1966	2	2	Cavalieri et al. 1991	3	3

Table 6. Number of Dose Groups for EPA Data Sets

Study	Number of BaP Doses	Number of Doses for Other PAHs	Study	Number of BaP Doses	Number of Doses for Other PAHs
Cavalieri et al. 1977	1	1	Wood et al. 1980	2	2
LaVoie et al. 1982	1	3	Hoffmann et al. 1972	1	1
Hecht et al. 1974	1	1	Nesnow et al. 1984	1	1
Nesnow et al. 1984	1	4	Busby et al. 1989	1	2
Slaga et al. 1980	1	1	LaVoie et al. 1987	1	1
Raveh et al. 1982	5	3	LaVoie et al. 1994	1	2
Hoffmann and Wynder 1966	1	3	Nesnow et al. 1998	5	5
Cavalieri et al. 1981b	1	3	Wislocki et al. 1986	1	2
Rice et al. 1988	1	3	Busby et al. 1989	1	2
Cavalieri et al. 1983	3	3	LaVoie et al. 1994	1	2
Cavalieri et al. 1981b	3	3	Wislocki et al. 1986	1	3
LaVoie et al. 1982	1	3	Busby et al. 1984	2	2
Hecht et al. 1974	1	1	Nesnow et al. 1998	5	5
Slaga et al. 1980	1	1	Mass et al. 1993	3	3
Raveh et al. 1982	5	3	Weyand et al. 2004	1	1
Cavalieri et al. 1981	1	3			
Slaga et al. 1978	1	1	Deutsch-Wenzel et al. 1983	3	3
Weyand et al. 1992	1	3	Wenzel-Hartung et al. 1990	3	3
El-Bayoumy et al. 1982	1	1	Weyand et al. 2004	1	2

Notes:
Red = data sets evaluated with one dose of BaP and one dose of other PAHs (Total of 12)
Blue = data sets evaluated with one dose of BaP and more than one dose of other PAHs (Total of 15)
Black = data sets evaluated with more than one dose of BaP and more than one dose of other PAHs (Total of 16)

6. Exceedance of Maximum Tolerated Dose

Recommendation: EPA should exclude data sets data from experiments performed above the maximum tolerated dose (MTD) from quantitative dose-response assessments.

Many of the studies were carried out at doses that exceeded the MTD. In accordance with EPA (2005) policy, data from experiments performed above the MTD should be excluded from quantitative dose-response assessment. Specifically, EPA (2005) states:

> *"In general, while effects seen at the highest dose tested are assumed to be appropriate for assessment, it is necessary that the experimental conditions be scrutinized.* Animal studies are conducted at high doses in order to provide statistical power, the highest dose

> being one that is minimally toxic (maximum tolerated dose or MTD). …If adequate data demonstrate that the effects are solely the result of excessive toxicity rather than carcinogenicity of the tested agent *per se*, then the effects may be regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the agent. This is a matter of expert judgment, with consideration given to all of the data available about the agent, including effects in other toxicity studies, structure-activity relationships, and effects on growth control and differentiation."

Lastly, for dermal carcinogenesis studies, EPA (1988) has defined the Maximum Tolerated Dose as a dose that does not cause a "marked inflammatory response or ulcerative lesion." EPA (1988) specifically stated:

> "It was recommended that a dose level that incites a marked inflammatory response of ulcerative lesion that is clearly related to application of the compound, should not be used for an MTD."
>
> "Microscopic lesions of inflammation, spongiosis, degeneration, dermal edema, and possibly others, must be evaluated carefully in the selection of the MTD. If, in the opinion of the pathologist, the severity of such lesions might lead to destruction of the functional integrity of the epidermis, these lesions would indicate selecting a lower dose for the MTD."

6.1 Mortality

Clearly, mortality is not consistent with the MTD. In a few of the studies, mortality was very high. EPA should have excluded at least some of the studies with high mortality, especially when the elevated mortality was early in the experiment. Instead of excluding datasets with high mortality, EPA (2010) biased the tumor incidence high by decreasing the size of the animal group to include only animals that were alive at the time of the first tumor. If a small fraction of animals died for reasons unrelated to PAH administration, then this would be a reasonable statistical approach. However, when mortality is significant compared to control group mortality or is unusually high, the data should be excluded entirely from use in deriving RPFs. In fact, EPA (2010) specifically listed "unexplained mortality in treated or control animals" as a criterion for excluding studies from the RPF derivation exercise.

Table 7 presents mortality rates for two papers, Hoffmann and Wynder (1966) and Mass et al. (1993), that contain data solely responsible for six RPFs. Mortality in experiments conducted by Hoffmann and Wynder (1966) was very high for many test groups even at the appearance of the first tumor. In several groups in the complete carcinogenicity bioassay, no animals survived to the end of experiment. EPA (2010) reported in Table E-1 that there was significant mortality in the dibenzo[a,e]pyrene-treated animals in the complete carcinogenicity bioassay: "Toxicity resulted in significant mortality unrelated to tumor induction." EPA (2010) also acknowledged in the section pertaining to dibenzo[a,e]pyrene that "the complete carcinogenicity bioassay was confounded by significant toxicity-related mortality unrelated to tumors." In fact, the mortality was 100% in one 0.05% dose group and 75% in another 0.05% dose group (Table 7). Instead of omitting the study, EPA merged the two low dose groups,

which yielded a pooled mortality of 88%, and used the data anyway to calculate an RPF. This procedure is suspect and does not diminish the high uncertainty surrounding the RPF for this substance due to high experimental mortality. Mortality in the initiation bioassay was also high for the control group, which suggests a noncarcinogenic cause of mortality.

As well the mortality data in Mass et al. (1993) appears high but could not be assessed with relation to the controls because the initial number of control animals was not reported in the paper. Given the criterion in EPA (2010) to exclude studies based upon unexplained mortality, data from neither Hoffmann and Wynder (1966) nor Mass et al. (1993) should be used to derive RPFs for any PAH.

Table 7. High Mortality Rates in Studies Used to Calculate RPFs in EPA (2010)

Reference	Exposure Route	PAH[1]	Dose Level	Units	Initial Number of Animals	Mortality at Appearance of First Tumor (%)	Final Number of Animals	Mortality Overall (%)	Length of Experiment
Hoffmann and Wynder 1966	Dermal-CC	Control			20	NA	12	40%	15 months
	Dermal-CC	DBaeP	0.05	%	20	70%	0	100%	15 months
	Dermal-CC	DBaeP	0.05	%	20	30%	5	75%	15 months
	Dermal-CC	DBaeP	0.1	%	20	55%	4	80%	15 months
	Dermal-CC	DBahP	0.05	%	20	15%	0	100%	15 months
	Dermal-CC	DBahP	0.1	%	20	10%	0	100%	15 months
	Dermal-CC	DBaiP	0.05	%	20	5%	2	90%	15 months
	Dermal-CC	DBaiP	0.1	%	20	5%	2	90%	15 months
	Dermal-CC	DBaeF	0.05	%	20	10%	0	100%	15 months
	Dermal-CC	DBaeF	0.1	%	20	10%	0	100%	15 months
Hoffmann and Wynder 1966	Dermal-Init	Control			30	0%	26	13%	6 months
	Dermal-Init	DBaeP	0.25	mg	28	4%	26	7%	6 months
	Dermal-Init	DBahP	0.25	mg	29	0%	26	10%	6 months
	Dermal-Init	DBaiP	0.25	mg	30	0%	27	10%	6 months
	Dermal-Init	DBaeF	0.25	mg	30	0%	28	7%	6 months
	Dermal-Init	AA	0.25	mg	30	10%	25	17%	6 months
	Dermal-Init	BghiP	0.25	mg	30	10%	27	10%	6 months
	Dermal-Init	N23eP	0.25	mg	30	0%	27	10%	6 months
	Dermal-Init	I123cdP	0.25	mg	30	0%	29	3%	6 months

Table 7. High Mortality Rates in Studies Used to Calculate RPFs in EPA (2010)

Reference	Exposure Route	PAH[1]	Dose Level	Units	Initial Number of Animals	Mortality at Appearance of First Tumor (%)	Final Number of Animals	Mortality Overall (%)	Length of Experiment
Mass et al. 1993[2]	IP	Control			NS	NS	34	NC	8 months
	IP	BjAC	20	mg/kg	18	NS	12	33%	8 months
	IP	BjAC	50	mg/kg	18	NS	13	28%	8 months
	IP	BjAC	100	mg/kg	18	NS	14	22%	8 months

Notes:

[1] LaCassagne et al. (1968) and Cavalieri et al. (1991) state that the compound thought to be DBalP prior to 1968 was actually DBaeF.

[2] Initial group size was 27; nine animals (three each at three times) were killed for DNA adduct analysis. Incidence data were not used to calculate RPFs due to saturation (tumor incidence greater than or equal to 90% at the lowest dose.)

AA = anthanthrene
Dermal-CC = dermal, complete carcinogenicity
Dermal-Init = dermal, initiation
IP = intraperitoneal
mg = milligram
mg/kg = milligrams per kilogram
NA = not applicable
NC = not calculable
NS = not stated in text
PAH = polycyclic aromatic hydrocarbon
RPF = relative potency factor
EPA = United States Environmental Protection Agency
Yellow highlight = mortality greater than or equal to 10%

BghiP = benzo[g,h,i]perylene
BjAC = benz[j]aceanthrylene
DBaeP = dibenzo[a,e]pyrene
DBahP = dibenzo[a,h]pyrene
DBaiP = dibenzo[a,i]pyrene
DBaeF = dibenzo[a,e]fluoranthene
N23eP = naphtho[2,3-e]pyrene
I123cdP = indeno[1,2,3-cd]pyrene

6.2 Significant Skin Toxicity

Most of the studies in which PAHs were tested in mouse skin assays did not address the structural integrity of the skin. Thus, there is no way to determine if the MTD was exceeded or not. However, several studies did specifically note that the MTD was exceeded. All results from such studies should be omitted from the RPF derivation process. In addition, EPA (2002) has stated that skin toxicity in a mouse skin assay is an indication that the MTD has been exceeded. Specifically, EPA (2002) stated: "Production of papillomas by dermally administered phenol (in the absence of an initiator) was observed only at a concentration that caused ulceration, and hence was above the MTD." More importantly, EPA (1988) has defined the Maximum Tolerated Dose in dermal carcinogenesis studies as a dose that does not cause a "marked inflammatory response or ulcerative lesion." EPA (1988) specifically stated:

> "It was recommended that a dose level that incites a marked inflammatory response of ulcerative lesion that is clearly related to application of the compound, should not be used for an MTD."

> "Microscopic lesions of inflammation, spongiosis, degeneration, dermal edema, and possibly others, must be evaluated carefully in the selection of the MTD. If, in the opinion

of the pathologist, the severity of such lesions might lead to destruction of the functional integrity of the epidermis, these lesions would indicate selecting a lower dose for the MTD."

Additionally, McKee et al. (1990) reported that when substances that are severe dermal irritants, such as coal liquids, are tested in mouse skin, the test article application frequency affected both time to tumor onset and tumor incidence. Examples of some studies that note the significance of skin toxicity are listed below

6.2.1 Cyclopenta[c,d]pyrene (Cavalieri et al. 1981b)

"CPAP was virtually inactive, while CPEP elicited skin tumors at the three dose levels with a response inversely proportional to the treatment dose. These results can be attributed to the relatively high toxicity of CPEP, since this compound, especially at the high dose, produced ulcerative skin lesions at the site of treatment."

6.2.2 Dibenzo[a,l]pyrene (Cavalieri et al. 1991)

"The mice treated with DB[a,/]P developed erythemas after the first TPA treatment due to the toxicity of the *DB[a,[]P.* The severity of the reaction was directly proportional to the initiating dose of DB[a,/]P. Due to this skin reaction, promotion of all mice was stopped until the fourth experimental week. It was then resumed and continued twice-weekly for 12 weeks. The mice initiated with DB[a,/]P or DB[a,/]P 11,12-dihydrodiol exhibited erythemas similar to those described above. The number of skin tumors was charted weekly."

"Fig. 4. Time course of tumor appearance in mouse skin expressed as percentage of tumor-bearing mice. Because erythemas developed in mice treated with DB[a,/]P and DB[a,/]P 11,12-dihydrodiol, promotion of all groups was not begun until the third experimental week."

"For both DB[a,/]P and DB[a,/]P 11,12-dihydrodiol at doses ranging from 4 to 100 nmol, tumorigenic activity in mouse skin was inversely proportional to the dose (Table II and Figures 4-6). At the high dose, DB[a,/]P elicited tumors earlier than DMBA; however, the tumor multiplicity of DMBA was much higher than that of DB[a,/]P. At the low dose, DB[a,/]P and DMBA exhibited similar tumor-initiating activity. These results indicate that toxicity interfered with the initiation of tumors by DB[a,/]P, and experiments with lower initiating doses are necessary to learn which compound has the higher tumorigenic potency."

6.2.3 Benzo[j]fluoranthene (Weyand et al. 1992)

"Initiation commenced during the early part of the second telogen phase of the hair cycle (50-55 days old). Each of the non-alternant PAH evaluated in this study were administered at a total initiation dose ranging from 0.3 to 2.0/umol per mouse. Solutions of these hydrocarbons were applied topically in 100/uL acetone every other day to the shaved backs of the mice using a biopipette. Evidence of skin irritation required that at certain

doses the number of applications to be limited. For a total initiation dose of 2.0 umol, five applications of a 4.0 mM solution of each non-alternant hydrocarbon was applied. Ten applications of a 1.0 mM solution of each non-alternant hydrocarbon was used for providing a total initiating dose of 1.0/umol. Skin irritation was noted for several of the treated mice at this dose, particularly those receiving 4F-B[j]F. Because of skin irritation observed in a few mice receiving 0.4 mM 4F-B[j]F, only seven applications were made to the mice receiving a total initiating dose of 0.3 umol of each non-alternant hydrocarbon."

6.2.4 Dibenzo[a,e]pyrene (Hoffmann and Wynder 1966)

EPA (2010) reported in Table E-1 that there was significant mortality in the dibenzo[a,e]pyrene-treated animals. Specifically, EPA (2010) stated: "Toxicity resulted in significant mortality unrelated to tumor induction." In fact, the mortality was 100% in one 0.05% dose group and 75% in another 0.05% dose group. Instead of omitting the entire study as would be called for with the EPA (2010) criterion to omit studies with 90% or greater mortality in the low dose group, EPA merged the two low dose groups, which yielded a pooled mortality of 88%. This practice introduces significant uncertainty in the RPF for this substance.

7. Identity and Purity of Test Article

Recommendation: EPA should exclude studies from the RPF approach if the identity and purity of the test article cannot be verified.

Many of the papers inadequately address the identity and purity of the test articles used. According to EPA (2010), studies in which the purity of the PAHs was "questionable," should have been excluded from the analysis. There is considerable uncertainty surrounding the relevance of any study used for RPF derivation unless there is certainty of the test article identity. EPA did not specifically state that there needed to be a positive identity of the test object, but clearly identity is important. A few examples are listed below:

7.1 Benzo[c]fluorene

RPFs for benzo[c]fluorene are derived from Weyand et al. (2004), which states: "7*H*-Benzo[*c*]fluorene was synthesized by the method previously reported and purity was ≥98% as determined by high-performance liquid chromatography (HPLC) analysis (23)" The paper provides no discussion on the manner in which the purity of the test article was actually determined and no information on confirmation of the test article identity.

Weyand et al. (2004) provides no discussion of the melting point of the synthesized compound, the NMR spectrum, or the mass spectrum. In fact, no information is presented to confirm the identity of the compound that was synthesized. Purity was tested by HPLC, but HPLC can only confirm that the area under the curve (AUC) of the major peak was 98% of the total AUC of all discernable peaks *under the specific column, solvent, flow and temperature conditions of the analysis*. HPLC cannot confirm the identity of the substance tested. There is no mention of isolating the HPLC peak and confirming the substance's identity by mass spectrometry or other methods.

7.2 Benz[l]aceanthrylene and benz[e]aceanthrylene

RPFs for benz[l]aceanthrylene and benz[e]aceanthrylene are derived from Nesnow et al. (1984), which states: "B[e]A and B[l]A were synthesized according to the method of Sangaiah et al. [10]." The paper provides no citation to a synthesis method, no discussion on the purity of the test article, and no information on confirmation of the test article identity. In addition, Johnsen et al. (1997) studied benz[l]aceanthrylene and stated: "The CP-PAH [3H]B[j]A, B[j]A and B[l]A were synthesized and purified according to published methods (27), and kindly provided by Dr S.Nesnow United States Environmental Protection Agency, NC, USA." Again, no information was provided on purity and positive identification. Citation (27) is the same Sangaiah et al. (1983) paper.

Nesnow et al. (1984) provides no discussion of the melting point of the synthesized compound, the NMR spectrum, or the mass spectrum. In fact, no information is presented to confirm the identity of the compound that was synthesized. No testing of purity was performed.

The paper of Sangaiah et al. (1983) was obtained. The synthesis of both benz[l]aceanthrylene and benz[e]aceanthrylene were described, and the authors presented melting points, UV spectral maxima, and NMR spectral features. No information on purity was except for melting point. As above, the melting points and spectral features describe the two materials that they produced, but there is no confirmation in the paper that the two PAHs of interest were, in fact, the synthetic products. It appears that this study does not pass EPA's stated study inclusion criterion that papers with substances of questionable purity should be excluded from the analysis. It has not been proven that the cited synthetic route produces benz[l]aceanthrylene and benz[e]aceanthrylene.

7.3 Benz[j]aceanthrylene

RPFs for benz[j]aceanthrylene are derived from Mass et al. (1993), which states that B[j]A was "synthesized by Dr. Avram Gold, University of North Carolina School of Public Health." The paper provides no citation to a synthesis method, no discussion on the purity of the test article, and no information on confirmation of the test article identity. In addition, Johnsen et al. (1997) studied benz[j]aceanthrylene and stated: "The CP-PAH [3H]B[j]A, B[j]A and B[l]A were synthesized and purified according to published methods (27), and kindly provided by Dr S.Nesnow United States Environmental Protection Agency, NC, USA." Again, no information was provided on purity and positive identification. Citation (27) is the Sangaiah et al. (1983) paper.

7.4 Dibenzo[a,l]pyrene

RPFs for dibenzo[a,l]pyrene are derived from Cavalieri et al. (1991) and Nesnow et al. (1998). Cavalieri, et al. (1991) states: "*B[a]P* and DMBA were available in our laboratory, whereas DB[a,/]P was obtained from the National Cancer Institute Chemical Carcinogen Repository (Bethesda, MD)." The paper provides no citation to a synthesis method and no information on confirmation of the test article identity. It is stated that the purity of the PAHs was determined to be 99+% by HPLC methods, but, as noted above, HPLC cannot confirm identity, and there

no mention of co-chromatography of authentic standards. Data from Cavalieri, et al. (1991) and Nesnow et al. (1998) on dibenzo[a,l]pyrene should be rejected in accordance with EPA's study inclusion criteria.

7.5 Cyclopenta[d,e,f]chrysene, 4H-

The RPF for cyclopenta[d,e,f]chrysene, 4H- was derived from two papers by the same laboratory (Rice et al. 1985; and Rice et al. 1988) stated that cyclopenta[d,e,f]chrysene, 4H- was synthesized by the method of Lee-Ruff et al. (1984). The Rice papers make no mention of any determination of identity. Rice et al. (1985) states that purity was tested by gas chromatography, and Rice et al. (1988) states that purity was tested by HPLC. In neither case do the methods characterize the identity of the constituent.

Lee-Ruff et al. (1984) report a synthesis of cyclopenta[d,e,f]chrysene, 4H- and the residue was cleaned up on preparative thin layer chromatography. The material that was eluted was characterized by melting point, IR, NMR and UV. The melting point was reported as 174-176 degrees C, whereas the literature value was reported as 171-173 degrees C. No tests of purity or identity were performed, and as discussed above, the publication of spectral information does nothing to confirm the identity or the purity of the substance produced by the synthesis.

Data from (Rice et al. 1985; Rice et al. 1988) on cyclopenta[d,e,f]chrysene, 4H- should be rejected in accordance with EPA's study inclusion criteria.

7.6 Benz[b,c]aceanthrylene, 11H-

The RPF for benz[b,c]aceanthrylene, 11H-was derived from a single paper (Rice et al. (1988). Rice et al. (1988) stated that the benz[b,c]aceanthrylene, 11H- was synthesized by the method of Rice and Hussain (1988). Purity was determined by HPLC analysis. However, no mention was made of substance identity.

Rice et al. (1988) cites their synthesis as "Rice, J.E. and Hussain, N. (1988) *J. Org. Chem.,* in press." No such scientific article was ever published in the *Journal of Organic Chemistry.* ' librarian searched this journal and other journals and did find any papers by these authors that describe the synthesis of benz[b,c]aceanthrylene, 11H- .

Nesnow et al. (1998) states: "DB[a,l]P and B[j]A were obtained from Midwest Research Institute (Kansas City, KA)." There is no mention in this paper about tests of purity or identity of the test articles.

Because there is no information at all on the identity this PAH, both papers should be rejected as not meeting EPA's study inclusion criteria.

7.7 Dibenzo[a,e]fluoranthene

The RPF for dibenzo[a,e]fluoranthene is based on a single paper by Hoffmann and Wynder (1966). There is considerable uncertainty surrounding the identity of this substance. According to Table 4-1 of EPA (2010), the authors mis-identified the substance as dibenzo[a,l]pyrene:

> "Paper in German. Paper reports compound as DBalP; LaCassagne et al. (1968) state that it is actually DBaeF."

EPA is relying on results from a 1966 paper in which the authors reported tumorigenicity data for dibenzo[a,l]pyrene. However, EPA designates the data to dibenzo[a,e]fluoranthene because another author states the true identity of the substance is not dibenzo[a,l]pyrene.

8. Substance-Specific Comments

Recommendation: EPA should clearly identify the uncertainties associated with a derived RPF (i.e. RPF is based on a single study or based on only on non-cancer endpoints).

In some cases, major issues may be identified with regard to the RPF(s) for individual substance, perhaps due to scientific issues with regard to the selected scientific papers or the particulars of EPA's modeling treatment of data for that substance.

8.1 Benzo[g,h,i]perylene

The RPF for benzo[g,h,i]perylene is based on a single result from Deutsch-Wenzel, et al. (1983). The study authors did not give strong weight to their weak finding in this study as noted below:

> "BghiP showed only a weak effect when applied as an intrapulmonary implant. It cannot be excluded that the effect observed in the lung implantation test originates from the impurities of the BghiP administered."

In addition, when IARC (1983) evaluated this study, they noted that the authors of the study reported that the weak effect observed after intrapulmonary administration of the test compound might have been caused by impurities in the benzo[g,h,i]perylene used. Thus, it is inappropriate to conclude that this is a positive study. Further, IARC (1983) evaluated additional information on benzo[g,h,i]perylene, all of which was negative. According to IARC (1983), there is inadequate data to permit an evaluation of the carcinogenicity of benzo[g,h,i])perylene to experimental animals and humans. No carcinogenic effects were observed when benzo[g,h,i]perylene was tested in two mouse-skin studies with female mice. It also gave negative results in three mouse-skin initiation-promotion studies. In two subcutaneous injection studies in mice, no injection site tumors were observed. The results of a test using intrapulmonary injection in rats were inadequate for evaluation (the Deutsch-Wenzel et al. 1983 study).

Deutsch-Wenzel, et al. (1983) calculated their own RPFs from their study, and the 95th confidence interval for the RPF for benzo[g,h,i]perylene was 0.00 to 0.06. Thus, the authors had very low confidence that a non-zero RPF could be derived from their experiment. It is also noted that in 1993 EPA reviewed this same dataset for benzo[g,h,i]perylene and concluded that an RPF for benzo[g,h,i]perylene could not be derived. The 1983 study has the same limitations today as it did in 1993.

8.2 Dibenz[a,c]anthracene

Dibenz[a,c]anthracene was mistakenly stated as having no tumorigenicity assays, and EPA (2010) derived an RPF of 4 based entirely on DNA adduct, DNA damage, mutagenicity and cell transformation studies. The range of calculated RPFs was 0.04 to 50 and EPA rated the confidence "very low." Of the 14 studies reviewed, 8 were single dose studies and of the 6 multiple dose studies, all were *in vitro* studies (bacterial mutagenicity, DNA damage, and DNA adduct formation).

As noted above, EPA (2010) has incorrectly reported data on DBacA, because there was at least one mouse skin bioassay with a concurrent BaP control (Slaga et al. (1980)) and three negative mouse skin bioassays without concurrent BaP controls. Slaga et al. (1980) studied DBacA and found it to be negative for mouse skin tumors. Slaga et al. (1980) met EPA's study inclusion criteria and is listed on Table 4-1. Results from this study were used for CH and DBahA. Table 4-1 lists BeP and DBacA as "nonpositive." However, DBacA and BeP did have negative results in this study. Neither negative result is reported in EPA's figures, Figure 6-11 for BeP or Figure 6-22 for DBacA.

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

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

However, this is incorrect because DBacA was negative in dermal bioassays. The above quotation also fails to take into account the three negative mouse skin bioassay results that EPA notes as "Conflicting results were reported in three dermal initiation bioassays of dibenz[a,c]anthracene in which benzo[a]pyrene was not included."

It is unjustified to use *in vitro* mutagenicity, DNA damage and other cancer "related" bioassay results to conclude that a substance is carcinogenic to humans when at least four studies reported by EPA have shown that the substance is *not* tumorigenic in the exquisitely

sensitive mouse skin bioassay. Thus, EPA has ignored four *in vivo* negative studies in the mouse and derived RPFs exclusively from cancer-related endpoints.

8.3 Cyclopenta[c,d]pyrene

Cavalieri et al. (1981) is the source of one RPF derivation for cyclopenta[c,d]pyrene. EPA (2010) did not follow its own protocol when assessing data from this study. For multi-dose studies, EPA (2010) derived RPFs using data the following statistical criteria:

> "Statistical analyses were performed on tumor bioassay data to determine whether the tumor incidence or multiplicity observed at a particular dose represented a statistically significant increase over controls. If statistical analyses were not described in the original report, incidence data were analyzed using Fisher's exact test and the Cochran-Armitage trend test. Positive findings were indicated by a significant ($p < 0.05$) difference for at least one dose group by comparison to control (in Fisher's exact or an equivalent test) or a significant dose-response trend (Cochran-Armitage or equivalent) for multidose studies."

EPA (2010) determined during data assessment that in the dermal initiation experiment conducted by Cavalieri et al. (1981), the lowest and highest of three doses of cyclopenta[c,d]pyrene were not statistically significantly elevated over controls, nor was the trend significant. However, the middle dose was borderline significant (i.e. $p=0.05$ rather than $p<0.05$), and EPA (2010) derived an RPF for cyclopenta[c,d]pyrene based on a point estimate at that middle dose. However, the data from both the lower and higher doses were not statistically significantly different from control data. Thus, Cavalieri et al. (1981) should not be used to derive a RPF for cyclopenta[c,d]pyrene, since the data do not meet EPA (2010) statistical qualifications.

9. Whole Mixtures Approach vs. Component Approach

Recommendation: EPA should use cancer slope factors which are derived from whole mixtures instead of RPFs to assess human risk.

EPA (2010) reports that EPA favors toxicity evaluations on whole mixtures, as noted below.

> *"The Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000) indicates that approaches based on whole mixtures are preferred to component approaches, such as the RPF approach. Risk assessment approaches based on toxicity evaluations of whole mixtures inherently address specific interactions among PAHs and account for the toxicity of unidentified components of PAH mixtures. They also do not require assumptions regarding the toxicity of individual components (e.g., dose additivity or response additivity)."

As noted in Appendix B, a validation of the proposed RPFs against CSFs derived from whole mixture studies demonstrates that the tumorigenicity of the mixture is less than the tumorigenicity predicted by the application of the RPFs.

10. Age-Dependent Adjustment Factors

Recommendation: EPA should not use age-dependent adjustment factors in this PAH RPF approach.

EPA (2010) uses the RPF document as another platform to present the age-dependent adjustment factors (ADAFs) however, the specific ADAFs proposed by EPA are scientifically inappropriate for use with PAHs. In EPA (2005), EPA provides guidance for assessing early-life exposures to carcinogens and derives default ADAFs of 3 for 2-16 year-old children and 10 for 0-2 year-old children for all chemical substances that have a mutagenic mode of action. According to EPA (2005), "Default adjustment factors are meant to be used only when no chemical-specific data are available to assess directly cancer susceptibility from early-life exposure to a carcinogen acting through a mutagenic mode of action."

The default adjustment factors were derived based on scientific data on 12 chemicals described by EPA as having mutagenic modes of action. Of the 12 substances, one is a PAH occurring in the environment, benzo[a]pyrene, and two are experimental PAHs that do not occur in nature, dibenz[a,h]anthracene and 3-methylchloanthrene.

Given that the Supplemental Guidance in 2005 clearly states that chemical-specific ADAFs are superior to generic ones, and EPA (2005) provides chemical-specific ADAFs for three PAHs, the RPF document (EPA 2010) should focus on PAH-specific ADAFs and not generic ones that were calculated for nine chemicals that are not PAHs, such as vinyl chloride. Further, because RPFs derived by EPA (2010) have not taken in consideration all tumorigenicity test results for dibenz[a,h]anthracene and 3-methylchloanthrene, PAH-specific ADAFs for use with BaP and BaP-TE should employ only data for BaP.

11. Bioavailability

Recommendation: EPA's RPF approach should take into consideration differential bioavailability of PAHs in human risk assessment.

PAHs are highly bound to environmental matrices and are not highly bioavailable to humans. RPFs have been derived using studies in which PAHs were administered via IP injections in oily vehicles, lung implants that are designed to maintain high localized doses, and mouse skin painting in solvent vehicles. EPA (2010) has derived RPFs for many PAHs that are instructed to be used to derive a BaP-toxic equivalent (BaP-TE) concentration that takes into account a large number of PAHs present in an environmental sample. Then, human health risk assessment will be performed with the single BaP-TE concentration as if the entire summed concentration behaves all like BaP. The RPFs and the manner in which they are intended to be implemented have not taken into consideration differential bioavailability.

EPA's Guidelines for Carcinogen Risk Assessment (EPA 2005) states that bioavailability is a major issue when considering a substance's carcinogenic potential. Specifically, EPA (2010) states:

"Physicochemical properties affect an agent's absorption, tissue distribution (bioavailability), biotransformation, and degradation in the body and are important determinants of hazard potential (and dose-response analysis)."

"Determining bioavailability via different routes of exposure by analyzing uptake processes under various exposure conditions. This analysis supports identification of hazards for untested routes. In addition, use of physicochemical data (e.g., octanol-water partition coefficient information) can support an inference about the likelihood of dermal absorption (Flynn, 1990)."

When using the EPA (1993) RPFs, the issue of differential bioavailability is present but less pronounced. This is because the six PAHs with RPFs under the 1993 scheme are similar in chemical properties to BaP and might be logically expected to have similar bioavailability in, for instance, soil or sediment. However, the current proposed RPFs include RPFs for smaller PAHs, such as FA, and many larger PAHs, such as AA, BghiP. DBaeF. DBaeP, DBahP, DBaiP, DBalP, DBelP, and N23eP. These PAHs are significantly larger and heavier than BaP and would be expected to have lower bioavailabilities from soil and sediment. With lower bioavailability, the use of RPFs that do not take such an important factor into account will overestimate tissue doses and thus overestimate human health risk.

Table 8. Select Physical and Chemical Properties of PAHs with EPA (2010) RPFs

PAH	Molecular Weight (g/mol)	Number of Aromatic Rings	Log Kow	Koc (L/kg)
Benzo[a]pyrene	252	5	6.13	5.87E+05
Anthanthrene	276	6	NA	NA
Anthracene	178	3	4.45	1.64E+04
Benz[a]anthracene	228	4	5.76	1.77E+05
Benz[b,c]aceanthrylene, 11-H	240	4	NA	NA
Benzo[b]fluoranthene	252	4	5.78	5.99E+05
Benzo[c]fluorene	216	3	NA	NA
Benz[e]aceanthrylene	252	4	NA	NA
Benzo[g,h,i]perylene	276	6	6.63	1.95E+06
Benz[j]aceanthrylene	252	4	NA	NA
Benzo[j]fluoranthene	252	4	6.11	5.99E+05
Benzo[k]fluoranthene	252	4	6.11	5.87E+05
Benz[l]aceanthrylene	252	4	NA	NA
Chrysene	228	4	5.81	1.81E+05
Cyclopenta[c,d]pyrene	226	4	NA	NA
Cyclopenta[d,e,f]chrysene, 4H-	240	4	NA	NA
Dibenz[a,c]anthracene	278	5	NA	NA
Dibenzo[a,e]fluoranthene	302	5	NA	NA
Dibenzo[a,e]pyrene	302	6	7.71	6.48E+06

Table 8. Select Physical and Chemical Properties of PAHs with EPA (2010) RPFs

PAH	Molecular Weight (g/mol)	Number of Aromatic Rings	Log Kow	Koc (L/kg)
Dibenz[a,h]anthracene	278	5	6.75	1.91E+06
Dibenzo[a,h]pyrene	302	6	7.28	6.35E+06
Dibenzo[a,i]pyrene	302	6	7.28	6.35E+06
Dibenzo[a,l]pyrene	302	6	7.71	6.48E+06
Fluoranthene	202	3	5.16	5.55E+04
Indeno[1,2,3-cd]pyrene	276	5	6.70	1.95E+06
Naphtho[2,3-e]pyrene	302	6	NA	NA
Phenanthrene	178	3	4.46	1.67E+04
Pyrene	202	4	4.88	5.43E+04

Notes:
Data from EPA's Estimation Program Interface (EPI) Suite via the Risk Assessment Information System (RAIS; http://rais.ornl.gov/home/about.html).
NA = value not available
Kow = octanol-water partition coefficient
Koc = organic carbon partition coefficient

12. Factual Errors

Recommendation: EPA should review the EPA 2010 document for factual errors.

Errors identified include the following:

a. Benz[j]aceanthrylene: EPA's Table 4-2 incorrectly reports that Mass et al. (1993) reiterates data presented in Nesnow et al. (1998).

b. Nesnow et al. (1998) states that the background tumor multiplicity was 0.6 tumors/animal, but the BMD modeling was done using a control rate of 0.67 tumors/animal according to EPA's Appendix C tables. Figure 2 in Nesnow et al. (1998) mis-identified DBA and DB[a,l]P on the graph, making it difficult for reviewers to compare EPA's tabular data to the data presented in the original paper.

c. Table 4-1 does not list that benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene, anthanthrene, and naphtho[2,3-e]pyrene were all nonpositive results in Hoffmann and Wynder (1966). EPA also reports in this table that "DBahP incidence ≥90% at lowest dose." This is an error. The incidence at the lowest dose was 85%.The table also reports only data for papillomas, but the study provided data on papillomas and epitheliomas.

d. EPA's Table 4-1 lists the Nesnow et al. (1984)'s study was performed for 30 weeks; however, the results were also reported at 15 weeks.

e. Hoffman and Wynder (1966) Data Used: Data in EPA (2010) Appendix C tables were checked against the original data tables from the published report and several errors were found in the data EPA used to derive RPFs. These errors include:

   Complete carcinogenesis study: DBaeP has two low dose (0.05%) study groups, each with 20 animals. One group had 100% mortality, and the other had 75% mortality. Instead of omitting the entire study for RPF derivation or omitting the 100% mortality group, EPA merged the data for the two groups to obtain a pooled low dose group.

   Initiation/Promotion Study: EPA (2010) incorrectly states that the control group had 7% tumor incidence based on 2 animals with tumors. This is an error. The 2 refers to BaP, not to the control group, and it represents the number of BaP-treated animals that developed epitheliomas.

f. Figure 6-11 for BeP and Figure 6-22 for DBacA do not show the nonpositive bioassay from Slaga et al. (1980), which met EPA's study inclusion criteria and is listed on EPA's Table 4-1.

   EPA also incorrectly reports the data for DBacA in the text. EPA also erroneously states:

   "There were 15 datasets for dibenz[a,c]anthracene that met selection criteria and included benzo[a]pyrene (Figure 6-22)."

   "In at least one study, benzo[k]fluoranthene, benz[l]aceanthrylene, 4 benz[e]aceanthrylene, naphtho[2,3-e]pyrene, dibenz[a,h]anthracene, dibenz[a,c]anthracene, and 5 benz[b,c]aceanthrylene showed positive initiating activity. Nonpositive results were reported for pyrene, perylene, benzo[g,h,i]fluoranthene, fluoranthene, anthanthrene, dibenzo[e,l]pyrene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene, benzo[e]pyrene, anthracene, 2,3-acepyrene, and phenanthrene."

g. Results of Schmähl et al. (1977) are incorrectly reported. EPA (2010) states:

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

   This was not reported by Schmähl et al. (1977) found nor what Clement Associates (1988) reported concerning the same study. Clement Associates (1988) did find a reasonable additive result for Mixture 1 with 1993-vintage RPFs, but *inhibition,* not *additivity* was seen

with Mixture 2. With the proposed RPFs, inhibition is seen in both cases, and there is no support for the additivity assumption.

13. Documentation

Recommendation: EPA should provide greater utility and transparency of the data for review by the public.

EPA (2010) has provided a large amount of data that does to a great extent allow peer reviewers to evaluate in detail how EPA derived its RPFs. However, there are several issues to need to be discussed that do not allow the document to be fully reviewed.

13.1 Use of Graphical Data

According to EPA (2010), data that were presented graphically and not tabularly, were used by EPA. Specifically, EPA (2010) states: "For studies that reported results graphically, individual data points were extracted using digitizing software." Because this software is not identified, a reviewer cannot check the accuracy of the digitization.

13.2 Use of Undocumented Data

Nesnow et al. (1998) provides data for RPFs for BbF, CPcdP, DBahA, and DBalP. Although the citation is given as Nesnow et al. (1998), the data used by EPA was, in fact, listed as "Data provided by S. Nesnow." Specifically, numerical data for tumor multiplicity were provided that were only graphically presented in the publication. More importantly, EPA used tumor incidence data on these substances that were not presented or discussed at all in the publication. The data were provided by the author, but they are not provided in a peer-reviewed journal that is accessible to the public.

13.3 Calculation of Incidence

In most cases, tumor incidence was calculated based on the number of total treated animals. However, EPA (2010) calculated incidence in a different manner for the data of Hoffmann and Wynder (1966) and a few other papers. Specifically, EPA (2010) states:

> "For a few PAHs tested in older dermal bioassays, the authors reported mortality prior to the appearance of the first tumor. For these data sets, an assumption was made that the number of animals at risk for tumor development was equal to the total number of animals alive at the time of the appearance of the first tumor. This approach ensures that the incidence is not underestimated by including animals that did not survive long enough to develop tumors. As this assumption applied to a small number of RPFs (specifically, individual RPFs for chrysene, dibenzo[a,e]pyrene, dibenzo[a,e]fluoranthene, and dibenzo[a,h]pyrene calculated from data reported by Hecht et al. [1974] and Hoffmann and Wynder [1966]), it had little impact on the overall analysis."

As noted above, Hoffmann and Wynder (1966) is the sole source of data for the RPFs for dibenzo[a,e]pyrene, dibenzo[a,e]fluoranthene, and dibenzo[a,h]pyrene, so the use of a different way of calculating incidence does not have “little impact on the overall analysis.” In fact, it has a major impact on the overall analysis, because this is the only analysis done for these three substances.

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Appendix A

Comments on Charge Questions

Appendix B

Validation Exercises

Appendix C

Benchmark Dose Modeling Results, Spot Checking of EPA (2010) Conclusions

Appendix D

Benchmark Dose Modeling Results, Cancer Slope Factor Derivations, Benzo[a]pyrene and Coal Tar Mixtures

Appendix A. Comments on Charge Questions

The commenters have reviewed the "Proposed Draft Charge to External Reviewers for the Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures for the IRIS Program, February 2010" and provide the following comments on some of those charge questions.

General Charge Questions

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

   Comment: EPA (2010) has summarized a large number of studies on PAHs but it has not presented a logical, clear and concise report. Specifically, the document:

   - Does not address the scientific appropriateness of using animal bioassay results from tests that were designed for hazard identification and not quantitative dose-response modeling.
   - EPA has not performed a weight-of-evidence assessment in accordance with EPA (2005) criteria.
   - EPA has derived RPFs for many PAHs that are relative to an outdated Cancer Slope Factor (CSF) for benzo[a]pyrene (BaP).
   - EPA has not adequately assessed the data quality of the studies, such as whether there is a reasonable degree of certainty about the identity and purity of the test substance studied in the individual scientific reports.
   - EPA has derived dose-response curve slopes for a majority of the PAH data sets with data in which only one dose group was tested, thus generating slope factors with extremely high uncertainties.
   - EPA has excluded information in studies that compared the tumorigenic potency of multiple PAHs unless BaP was concurrently tested.
   - EPA has used its Benchmark Dose Modeling Software (BMDS) for dose-response modeling, but only tested the model fits to one model, multistage for tumor incidence results and linear for tumor multiplicity results.

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

Comment: The EPA (2010) draft implies that the RPFs will be used to assess each PAH using a CSF for BaP, but the CSF for BaP is not discussed in detail except in an example calculation and is based on an outdated BaP CSF. The CSF for BaP needs to be updated with current, robust animal toxicology studies.

Chapter 2. Rationale for Recommending an RPF Approach

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

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

- There are toxicity data available on numerous PAH-containing mixtures.
- Epidemiological studies and health surveys have been performed over the years on workers exposed to PAH-containing mixtures.
- Chemical analyses of the mixtures that have been well studied are more comprehensive than the analyses of typical environmental media for which the RPF approach is intended for use.

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.

Comment: EPA (2010) states that BaP is the logical choice to be the index PAH for a PAH RPF approach. However, many studies have used 3-methylchloanthrene (3-MC) or 7,12-dimethyl-benz[a]anthracene (DMBA) as positive controls instead of or in addition to BaP. EPA should fully evaluate the utility of studies with these two alternate PAHs.

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.

Comment: EPA has not adequately described and scientifically justified that all PAHs have the same mode of action and in EPA (2010), Section 2.4 it asserts that there may be many modes of action for PAHs. EPA should review the assumption that PAHs, as a chemical class, have a similar mode of action using EPA's 2005 Cancer Guidelines section on determining a mode of action for carcinogenesis and present the data in accord with their MOA framework.

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.

Comment: EPA (2010) has not adequately described or scientifically justified the presence or absence of interactions among PAH mixture components at levels of exposure encountered in the environment. Specifically inhibitory interactions, have been seen among PAHs at doses similar to those typically encountered in the environment, EPA's assumption that interactions will not occur is not supported by the scientific literature.

Chapter 3. Discussion of Previously Published RPF Approaches

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

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

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

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

Chapter 4. Evaluation of the Carcinogenicity of Individual PAHs

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

Comment: Restricting the PAH list to include only "unsubstituted PAHs with three or more fused aromatic rings containing only carbon and hydrogen atoms," is scientifically justified as these are the most widely studied members of the PAH chemical class."

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.

Comment: EPA should review and refine the selection criteria. Specifically, EPA required that "Benzo[a]pyrene was tested simultaneously with another PAH" and that "The carcinogenic response observed in either the benzo[a]pyrene- or other PAH-treated animals at the lowest dose level was not saturated (i.e., tumor incidence at the lowest dose was <90%), with the exception of tumor multiplicity findings". The use of these two criteria eliminated numerous relevant studies from consideration.

Chapter 5. Methods for Dose Response Assessment and RPF Calculation

5a. Please comment on whether the scientific rationale for the dose-response modeling approaches used in the derivation of RPFs is adequately described. Please comment on whether there are other appropriate modeling approaches for estimating the relative potencies of PAHs. Please describe alternative approaches (e.g., other model forms) that could be considered.

Comment: EPA (2010) does not provide criteria for defining a "good fit" of the data. When Benchmark Dose Modeling of multi-dose results provided a "poor fit," EPA's protocol required that "point estimates" from single dose groups be used for RPF derivation instead of slope factors using modeling of the entire dataset. The Benchmark Dose Software generates three goodness-of-fit metrics to guide in the selection of the optimal model however, EPA's judgment of "fitness" were not documented. Tables in EPA's Appendix E report in some cases that there was "no model fit," but the criteria for making this determination are not provided, nor are the Benchmark Dose Modeling outputs.

Consequently, when EPA defaulted to the "point estimate" approach because of a "poor model fit," EPA defaulted to a "point estimate" approach that required a slope factor be developed from a dose-response "curve" defined by a single dose group. EPA's modeling approach as executed has a major impact on the RPF derivation process because only 28% of the data sets were modeled using slope factors based on more than one data point for both PAHs. Most of the data sets used slopes derived from a single data point, which introduces a significant uncertainty. Specifically, of the 43 data sets listed in EPA's tables:

- 18 (42%) used a single BaP dose and single doses for other PAHs
- 13 (30%) used a single BaP dose and a dose-response curve for other PAHs
- 12 (28%) used a dose-response curve for BaP dose and a dose-response curve for other PAHs

Thus, EPA based 72% of the RPFs on ratios of "slopes" that were derived using a single data point for one or both of the PAHs being compared.

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.

Comment: EPA presented all of the quantitative data in EPA's Appendix C tables, but the key summary Tables listed here could be improved by adding additional data about dose groups. Specifically, some data sets were marked "no model fit" which resulted in the use of the reported point estimate approach. In other cases, the point estimate approach was used without any reference to modeling.

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.

Comment: EPA's decision to base the ratios on the estimate of the BMD rather than the BMDL is valid.

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

6a. Please comment on whether the rationale for the weight-of-evidence evaluation is scientifically justified and adequately described. Please comment on whether the approach adequately considers the available information. Please comment on whether other information (e.g.,

additional structure-activity) could contribute further to the weight-of-evidence evaluation and how this information could be utilized in the analysis.

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

1. EPA indicates PAHs selected for inclusion in the RPF approach were initially chosen based on an "evaluation of whether the available data were adequate to assess the carcinogenicity of each compound." (Chapter 6, page 113, paragraph 1). Based on this, 35 PAHs were identified for further evaluation. EPA should clearly define "carcinogenicity" for this step within the context of the relevance to potential to be carcinogenic to humans with consideration of EPA's 2005 Cancer Guidelines.

2. The proposed RPF approach appears to rely on the 1994 IRIS toxicological assessment for benzo(a)pyrene and seems to assume that the weight of evidence for carcinogenic potential for benzo(a)pyrene ("Classification - B2; probable human carcinogen") is relevant to all of the PAHs included in the proposed RPF approach. The rationale should be justified within the context of study data and the "weight of evidence for the carcinogenic hazard potential" to humans should be consistent with the 2005 Cancer guidelines for each PAH.

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.

Comment: The presented weight of evidence analysis appears to be inconsistent by not including data related to AhrR binding. In section 2.6 of the EPA (2010) discusses that the receptor binding correlates better than mutagenicity with the cancer bioassays.

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.

Comment: The graphic arrays provide misleading information and should be improved. EPA (2010) presents all derived RPFs in the graphical arrays, but not all of these RPFs are averaged to derive the final RPF presented in Table 1, as noted in the draft document

benzo(c)fluorene is reported as 20. Figure 6-9 show 4 RPFs from one study, Weyand et al. (2004). According to Appendix E tables, the values are:

Oral multiplicity	48.9	IP multiplicity	0.56
Oral incidence	5.48	IP incidence	1.05

The average of these four RPFs is 14.00. The average of the oral average and the IP average is 14.01. However, EPA did not use all of the data when deriving the final proposed RPF. Instead, the approach uses the higher of the two oral values and the higher of the two IP values. EPA should note on the graphical arrays which RPFs are actually averaged to derive the values presented in Table 1 of EPA (2010).

Chapter 7. Derivation of RPFs for Selected PAHs

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.

Comment: It is not appropriate to apply the proposed RPFs to all routes of exposure. RFPs should be derived separately for each route of exposure based on the available scientific data. Specifically, EPA (2010) references IP and lung implantation studies for use when deriving the RPF however RPFs based on the IP dosing of mice or on lung implantation studies with surgical administration of PAH-containing pellets have no relevance to human health.

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

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

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

relatively insensitive to benzo[a]pyrene-induced tumorigenicity following oral exposure (Gaylor et al., 1998)."

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

Comment: EPA (2010) states that PAHs with adequate evidence to suggest they have little or no carcinogenic potential are assigned an RPF of 0. It would be useful to have the RPF rating correlate with cancer weight of evidence descriptors and as such the assignment of RPFs of 0 would indicate that those PAHs do not pose carcinogenic risk to humans.

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.

Comment: EPA should include other factors in rating the confidence of RPF, such as:

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

Chapter 8. Uncertainties and Limitations Associated with the RPF Approach

8. Please comment on whether, overall, the document describes the uncertainties and limitations in the methodology used to derive RPFs in a transparent manner. Please comment on whether the most important uncertainties and limitations are identified. Please comment on whether

there is existing information that could be used to evaluate the accuracy or validity of the RPF values to predict the cancer risk associated with exposure to PAH mixtures.

Comment: RPFs that are ranked as having low or very low confidence should be omitted from the final document. The document should provide examples of validation that demonstrate if the propose RPFs significantly overestimates or underestimates the actual tumor responses seen in studies in which animals were doses with PAH-containing mixtures.

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.

   Comment: Much useful information is presented in the appendices. However, EPA should provide BMDS output for model runs for which it determined that there was not a good model fit plus the criteria for making said determination.

Appendix B. Validation Exercises

Recommendation: EPA should perform an independent validation of RPFs derived using the proposed approach.

EPA (2010) refers to studies that attempted to validate the RPFs developed by others, but they have not performed any sort of validation on the RPFs proposed in EPA (2010). Scientifically, no models have any value unless and until they are validated with real world data. EPA has a duty to check the real world accuracy of the proposed RPFs and perform validation exercises in the documents in which new models are proposed. *De novo* validations have been performed, and these exercises directly test the validity of both the assumption of additivity among PAHs and more importantly they directly test the validity of the RPFs themselves. As shown below, the exercises demonstrate that the 2010-proposed RPFs are *not* valid when compared to data from real world mixtures.

1. Validation of Proposed RPFs Using Guideline-Compliant Two-Year Feeding Study Results from NCTR

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

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

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

CSFs have been independently calculated from the Beland et al. (1998) study using EPA's Benchmark Dose Software (BMDS) in order to use EPA's current approach and the approach used in EPA (2010). The BMDS model results are documented in Appendix D. CSFs derived from the BMDS show good model fits, and the results are nearly identical to the results presented by Gaylor et al. (2000):

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

As shown above, when using the outdated CSF for benzo[a]pyrene, the RPF approach overestimates the true carcinogenic potencies of the coal tar mixtures using either current or proposed RPFs. When the proposed RPFs are used, the overestimation of risk increases from 2 to 3-fold to 14 to 21-fold. It must be noted that this overestimation is the minimal overestimation of potency, because only one additional, non-standard PAH is incorporated into the estimate of BaP-TE. If the concentrations of the other PAHs for which EPA is proposing RPFs were added in to the calculation, the overestimations would rise considerably.

When using the newly derived CSF based on the 1998 NCTR study, the old EPA (1993) RPFs underestimate coal tar potency. However, the proposed EPA (2010) RPFs still overestimate the tumorigenic potency of coal tar.

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

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

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

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

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

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

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

As shown above, when using the outdated CSF for benzo[a]pyrene, the RPF approach overestimates the true carcinogenic potencies of the coal tar mixtures by 20 to 32-fold using the proposed RPFs and assuming that PAHs not quantitatively identified are present at 100 ppm each. When using the newly derived CSF based on the 1998 NCTR study, the proposed RPFs overestimate coal tar potency by 3 to 5-fold.

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

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

Table B-7 lists some of these studies. The intent of this table is to demonstrate that many examples of inhibition can be found in the literature. Examples of potentiation can also be found in the literature, but these studies are not reported here.

Table B-7. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
methylcholanthrene	dibenzofluorene	skin tumor	mouse	LaCassagne et al. 1945
dibenz (a,h) anthracene	1,2,5,6-dibenzacridine	skin tumor	mouse	LaCassagne et al. 1945
methylchloranthrene	chrysene	skin tumor	mouse	LaCassagne et al. 1945
dibenz(a,h) anthracene	benz(a)anthracene	injection tumors	mouse	Steiner and Falk 1951
benzo(a)pyrene	1,2,3,4-benzanthracene	skin tumor	mouse	Finzi et al. 1968
benzo(a)pyrene	perylene	skin tumor	mouse	Finzi et al. 1968
DMBA	dibenz(a,c) anthracene	skin tumor	mouse	DiGiovanni et al. 1980; DiGiovanni et al. 1982; Slaga 1983
DMBA	benzo(e)pyrene	skin tumor	mouse	DiGiovanni et al. 1980; DiGiovanni et al. 1982; Slaga 1983; Slaga et al. 1979
DMBA	pyrene	skin tumor	mouse	DiGiovanni et al. 1980; Slaga et al. 1979
DMBA	fluoranthene	skin tumor	mouse	DiGiovanni et al. 1980; Slaga et al. 1979
7,12-DMBA	benz(a)anthracene	breast tumor	rat	Huggins et al. 1964

Table B-7. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
7,12-DMBA	3,9-DMBA	breast tumor	rat	Huggins et al. 1964
7,12-DMBA	6,8-DMBA	breast tumor	rat	Huggins et al. 1964
7,12-DMBA	chrysene	breast tumor	rat	Huggins et al. 1964
9,10-dimethyl-1,2-benzanthracene	phenanthrene	skin tumor	mouse	Huh and McCarter 1960
20-methylcholanthrene	6,7-dihydro methylcholanthrene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
20-methylcholanthrene	hexahydro methylcholanthrene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
20-methylcholanthrene	perhydro methylcholanthrene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
dibenz(a,h,) anthracene	dihydro-dibenz (a,h)anthracene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
dibenz (a,h) anthracene	decahydro-dibenz (a,h)anthracene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
dibenz(a,h) anthracene	perhydro-dibenz (a,h)anthracene	subcut. tumor	mouse	Kotin et al. 1956; Falk et al. 1964
7,12 DMBA	1-ethynylpyrene	DNA binding	mouse skin	Viaje et al. 1990
benzo(a)pyrene	1-ethynylpyrene	DNA binding	mouse skin	Viaje et al. 1990
7,12 DMBA	1-ethynylpyrene	skin tumor	mouse	Alworth et al. 1991
benzo(a)pyrene	1-ethynylpyrene	skin tumor	mouse	Alworth et al. 1991
dibenz(a,h) anthracene	phenanthrene	skin tumor	mouse	Falk et al. 1964
DMBA	1,2,3,4-dibenzanthracene	skin tumor	mouse	Slaga and Boutwell 1977
dibenz(a,h) anthracene	benzo(e)pyrene	skin tumor	mouse	DiGiovanni et al. 1982
dibenz(a,h) anthracene	dibenz(a,c) anthracene	skin tumor	mouse	DiGiovanni et al. 1982
3 - methyl cholanthrene	dibenz(a,h) anthracene	skin tumor	mouse	DiGiovanni et al. 1982
DMBA	dibenz(a,c) anthracene	skin tumor	mouse	Slaga 1978
benzo(a)pyrene	benzo(a)fluorene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	perylene	skin tumor	mouse	Falk et al. 1964

Table B-7. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
benzo(a)pyrene	benz(a)carbazole	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	chrysene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	benzo(h) fluoranthene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	benz(m,n,o) fluoranthene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	anthracene + pyrene + phenanthrene	skin tumor	mouse	Falk et al. 1964
benzo(a)pyrene	carcinogenic oxy-neutral air pollution fractions	skin tumor	mouse	Falk et al. 1964
DMBA	benzo(e)pyrene	mutagenicity	hamster embryo cells	Baird et al. 1984
benzo(a)pyrene	benzo(e)pyrene	mutagenicity	*Salmonella*	Hass et al. 1981
benzo(a)pyrene	benzo(e)pyrene	metabolic activation	Syrian hamster embryo cultures	Smolarek and Baird 1984
benzo(a)pyrene	anthracene	skin tumor	mouse	Crabtree 1946
benzo(a)pyrene	naphthalene	skin tumor	mouse	Crabtree 1946
benzo(a)pyrene	phenanthrene	skin tumor	mouse	Crabtree 1946
20-methylcholanthrene	anthracene	skin tumor	mouse	Crabtree 1946
20-methylcholanthrene	naphthalene	skin tumor	mouse	Crabtree 1946
20-methylcholanthrene	phenanthrene	skin tumor	mouse	Crabtree 1946
20-methylcholanthrene	1,2,5,6-dibenzofluorene	skin tumor	mouse	Wartman et al. 1950
9,10-dimethyl-1,2-benzanthracene	8-methyl-1,2-benzanthracene	skin tumor	mouse	Stanger et al. 1952
9,10-dimethyl-1,2-benzanthracene	1,2,5,6-dibenzofluorene	skin tumor	mouse	Hill et al. 1951
9,10-dimethyl-1,2-benzanthracene	6,8-dimethyl-1,2-benzanthracene	skin tumor	mouse	Hill et al. 1951
benzo(a)pyrene	dibenzo(a,l)pyrene + dibenzo(a,e)pyrene	skin DNA adducts	mouse	Hughes and Phillips 1990
DMBA	benzo(e)pyrene	in vivo DNA adducts	mouse	Smolarek et al. 1987
benzo(a)pyrene	crude oil	mutagenicity	*Salmonella*	Petrilli et al. 1980

Table B-7. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
benzo(a)pyrene	DMSO extract of crude oil	mutagenicity	*Salmonella*	Petrilli et al. 1980
benzo(a)pyrene	PNA fraction Shale oil	mutagenicity	*Salmonella*	Pelroy and Petersen 1979
benzanthracene	PNA fraction Shale oil	mutagenicity	*Salmonella*	Pelroy and Petersen 1979
dimethyl-benzacridine	PNA fraction Shale oil	mutagenicity	*Salmonella*	Pelroy and Petersen 1979
2-aminoanthracene	PAH fraction of Athabasca tar sand	mutagenicity	*Salmonella*	Shahin and Fournier 1978
9,10-dimethyl-1,2-benzanthracene	1,2,5,6-benzanthracene	skin tumor	mouse	Hill et al. 1952
methylcholanthrene	1,2,5,6-benzanthracene	skin tumor	mouse	Hill et al. 1952
benzo(a)pyrene	high boiling mutagenic COM	mutagenesis	*Salmonella*	Dankovic et al. 1989
benzo(a)pyrene	solvent refined coal liquid (426-454 deg)	mutagenesis	*Salmonella*	Dankovic et al. 1989
benzo(a)pyrene	solvent refined coal liquid (454 deg)	mutagenesis	*Salmonella*	Dankovic et al. 1989
benzo(a)pyrene	4 carcinogenic COM	skin tumors	mouse	Dankovic et al. 1989
benzo(a)pyrene	5 carcinogenic COM	metabolic activation	mouse	Dankovic et al. 1989
DMBA	3-methyl cholanthrene	breast tumors	rats	Wheatley 1988
benzo(a)pyrene	naphthalene	skin tumors	mouse	Schmetlz et al. 1978
benzo(a)pyrene	1-methylnaphthalene	skin tumors	mouse	Schmetlz et al. 1978
benzo(a)pyrene	2-methylnaphthalene	skin tumors	mouse	Schmetlz et al. 1978
benzo(a)pyrene	1-ethylnaphthalene	skin tumors	mouse	Schmetlz et al. 1978
benzo(a)pyrene	2-ethylnaphthalene	skin tumors	mouse	Schmetlz et al. 1978
benzo(a)pyrene	1,2-dimethyl-naphthalene	skin tumors	mouse	Schmetlz et al. 1978

Table B-7. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

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

Table B-7. Inhibition of Laboratory PAH Carcinogenesis by other PAHs

Animal Carcinogen	Inhibitor	Effect	Species	Reference
benzo(a)pyrene	gasoline & diesel exhaust condensates	BaP-DNA adducts	mouse	Schoket et al. 1989
Dibenz(a,l)pyrene	Diesel exhaust	Skin tumors	mouse	Courter et al. 2008
Benzo(a)pyrene	Urban dust particulate matter	Skin tumors	mouse	Courter et al. 2007a,b
Dibenz(a,l)pyrene	Urban dust particulate matter	Skin tumors	mouse	Courter et al. 2007a,b
Benzo(a)pyrene	Coal tar extract	DNA adducts	Chinese hamster cells	Mahadevan et al. 2007
Dibenz(a,l)pyrene	Coal tar extract	DNA adducts	Chinese hamster cells	Mahadevan et al. 2007
Benzo(a)pyrene	Dibenz[a,h]anthracene	Skin tumors	Mouse	Pfeiffer 1973, 1977

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

The weight of evidence from the literature on interactions between individual PAHs given as pure chemicals does not support a conclusion that all PAHs act in a dose-additive fashion.

3. Validation of Assumption of Additivity, Existing Studies

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

> "Additivity has been observed in carcinogenicity studies of complex mixtures of PAHs. Schmähl et al. (1977) evaluated the production of skin tumors following combined dermal treatment with 11 PAHs found as constituents of automobile exhaust. Tumor findings were presented separately for two groups of PAHs. High potency carcinogens (Group 1)

> included benzo[a]pyrene, dibenz[a,h]anthracene, benz[a]anthracene, and benzo[b]fluoranthene. Lower potency PAHs (Group 2) included anthracene, benzo[e]pyrene, benzo[g,h,i]perylene, chrysene, fluoranthene, phenanthrene, and pyrene. Chronic dermal exposure to PAHs in both groups resulted in an additive response when compared to the tumor response for each group alone."

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

PAH Mixture #2 was labeled a noncarcinogenic mixture, but it contains fluoranthene, chrysene, and benzo[ghi]perylene. If EPA's proposed RPFs are correct and the additivity assumption is correct, the expected tumor incidences based on BaP-TE would be 14%, 56%, 100%, and 100%, but the observed tumor incidences are 1%, 0%, 1%, and 19%. Clearly, the RPFs overestimate comparative potency or the assumption of additivity is not correct, or *both.* It is noted that Mixture #2 does not contain any BaP, but its BaP-TE using the proposed RPFs is equal in the first two dose groups to dose groups which were administered BaP at the same dose. If the RPFs were correct, the tumor incidence would be expected to be 14% and 56%, but the actual tumor incidences were 1% and 0%. This demonstrates that the RPFs are not correct.

Table B-8. Data of Schmahl et al. (1977)

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

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

Schmähl et al. (1977) also dosed three groups of animals with Mixtures 1 and 2 simultaneously. The BaP-TE of the first group is 10 ug/treatment. This BaP-TE dose of 9-10 ug/treatment gave the following tumor incidences, as noted in Table B-8.

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

Similarly, three groups were doses with total BaP-TE of 27-30 ug/treatment. This BaP-TE dose of 27-30 ug/treatment gave the following tumor incidences, as noted in Table B-8.

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

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

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

In Table B-9, the data for mixtures benzo(a)pyrene and dibenz(a,h)anthracene are presented. In this experiment, Pfeiffer et al. (1977) merely dosed animals with BaP alone, with DahA alone, and then with a binary mixture of the two. If the assumption of additivity is correct, the tumor incidence of the binary mixture would simply be the sum of the two individual results.

The data clearly show that the animal carcinogenicity of the two chemicals is not additive. It is possible that nonlinearities may occur in the dose-response curve at high doses. Thus, one should perhaps not expect that a chemical giving a 50% tumor incidence would strictly add with a chemical giving a 50% incidence so that the mixture would yield exactly 100% incidence. However, the observed results demonstrate that the assumption of additivity does not hold and that inhibition was occurring. In the two highest dose groups, for instance, the addition of dibenz(a,h)anthracene caused the tumor incidence of the mixture to be less than the incidence seen when benzo(a)pyrene alone was administered, thus showing that the presence of other PAHs inhibited the action of BaP.

Table B-9. Validation of Assumption of Additivity Data of Pfeiffer et al. (1977) Two Component Mixture

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

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

Table B-10 shows the data for the twelve component mixture, where the researchers added a mixture of 10 PAHs (including benz[a]anthracene, fluoranthene, chrysene, and benzo[ghi]perylene, to benzo(a)pyrene and dibenz(a,h)anthracene. Again, additivity was not seen. In two cases, doses 5 and 6, the addition of dibenz(a,h)anthracene and the mixture of 10 PAHs caused the tumor incidence of the mixture to be less than the incidence seen when benzo(a)pyrene alone was administered, thus showing that the presence of other PAHs inhibited the action of BaP.

Table B-10. Validation of Assumption of Additivity Data of Pfeiffer et al. (1977) Twelve Component Mixture

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

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

In Table B-11, the data on the five animal groups are shown along with their BaP-TE doses using the proposed RPFs. As noted in the table, the expected incidence in all cases exceeded the observed tumor incidence. Expected tumor incidence was estimated by simply assuming

the EPA's proposed RPFs are correct and that the dose-response curve was linear as was assumed by EPA (2010).

Table B-11. Validation of EPA (2010) RPFs Using Tumor Data of Pfeiffer (1977)

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

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

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

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

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

In a 1998 publication, Nesnow et al. presented quantitative results for other mixture experiments. Nesnow et al. (1998a) concluded “Comparison of observed lung adenoma formation with that expected from additivity identified both greater than additive and less than additive interactions that were dose related i.e., greater than additive at lower doses and less than additive at higher doses.” Thus, EPA (2010) has presented little to no evidence that an assumption of additivity is correct.

Appendix C – Benchmark Dose Modeling

EPA's Benchmark Dose Software (BMDS) version 2.1.1 (www.epa.gov/ncea/bmds/index.html) was used to assess dose-response relationships for a variety of relevant health endpoints reported in various studies in the published literature. Compared to the use of NOAEL/LOAELs and cancer potency estimates, the benchmark dose (BMD) approach provides a more quantitative alternative for determining the point of departure (POD) to calculate the toxicity value used in cancer and noncancer risk assessments (EPA 2000). Specifically, the POD is applied in one of two ways, consistent with EPA (2005) guidance : (1) a linear extrapolation method that yields a cancer slope factor (CSF) calculated as the ratio between the chosen benchmark response rate (BMR) or POD and the one-side 95% lower confidence limit of the benchmark dose (BMDL) converted to a human-equivalent external dose (HED); and (2) a nonlinear approach that yields a reference dose (RfD) calculated as the ratio between the benchmark dose (BMD) at the chosen BMR/POD and the product of several extrapolation/adjustment factors.

BMDS can accommodate categorical (including dichotomous) and continuous response variables. A toxicity study yields categorical data when responses are grouped into one or more categories, such as the severity of effect (e.g., mild, moderate, or severe histological change). Dichotomous data can be viewed as a special case in which there is one effect category and the possible response is binary (e.g., effect or no effect) (EPA 2000). For BMD modeling of dichotomous data, both the number of animals showing response and the total number of subjects in the group are included in the input file along with the dose. Categorical regression models can also be used to derive a BMD by estimating the probability of effects of different levels of severity (EPA 2000). Continuous data are reported as a measurement of the effect, such as body weights, or number of tumors per animal in control and exposed groups. The input file consists of either individual results or the number of subjects, mean response, and variance in response for each dose group.

BMDS generates goodness-of-fit metrics to guide in the selection of the optimal model. EPA's technical guidance for BMDS (EPA 2000) provides additional criteria for selecting the optimal models for the final determination of the BMD and BMDL.

References

EPA. 2000. Benchmark Dose Technical Guidance Document, Draft. Risk Assessment Forum. EPA/630/R-00/001. October. Available online: http://www.epa.gov/nceawww1/pdfs/bmds/BMD-External_10_13_2000.pdf.

EPA. 2005. Guidelines for Carcinogen Risk Assessment. EPA/630/P:-03/001F.

Table C1. Mass et al. (1993), benzo[a]pyrene, A/J mouse. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness-of-Fit							POD Benchmark Response (BMR) (5)	Dose (mg/kg)		Notes
					p-value for Maximum Likelihood Tests (2)					Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Mass, 1993	BaP	A/J Mice	lung tumors	Hill	<.0001	<.0001	<.0001	NA	198	-4E-07	-4E-07	1 SD	41.0	29.1	Best Fit: Hill model has the lowest scaled residuals and the lowest AIC
Mass, 1993	BaP	A/J Mice	lung tumors	Exponential 2	< 0.0001	< 0.0001	< 0.0001	0.0147	203	**2.3**	**2.3**	1 SD	61.4	55.0	
Mass, 1993	BaP	A/J Mice	lung tumors	Exponential 3	< 0.0001	< 0.0001	< 0.0001	0.0147	203	**2.3**	**2.3**	1 SD	61.4	55.0	
Mass, 1993	BaP	A/J Mice	lung tumors	Exponential 4	< 0.0001	< 0.0001	< 0.0001	0.052	200	1.0	-1.5	1 SD	36.84	24.13	
Mass, 1993	BaP	A/J Mice	lung tumors	Exponential 5	< 0.0001	< 0.0001	< 0.0001	N/A	198	7.5E-08	-4E-07	1 SD	42.2	29.9	Best Fit: Exponential 5 model has the lowest scaled residuals and the lowest AIC
Mass, 1993	BaP	A/J Mice	lung tumors	Linear	<.0001	<.0001	<.0001	0.15	198	1.1	-1.5	1 SD	37.7	30.6	Acceptable Fit
Mass, 1993	BaP	A/J Mice	lung tumors	Polynomial	<.0001	<.0001	<.0001	0.052	200	0.99	-1.6	1 SD	36.70	24.04	
Mass, 1993	BaP	A/J Mice	lung tumors	Power	<.0001	<.0001	<.0001	0.05	200	1.2	-1.4	1 SD	39.9	30.7	

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.

Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.

Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.

Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.

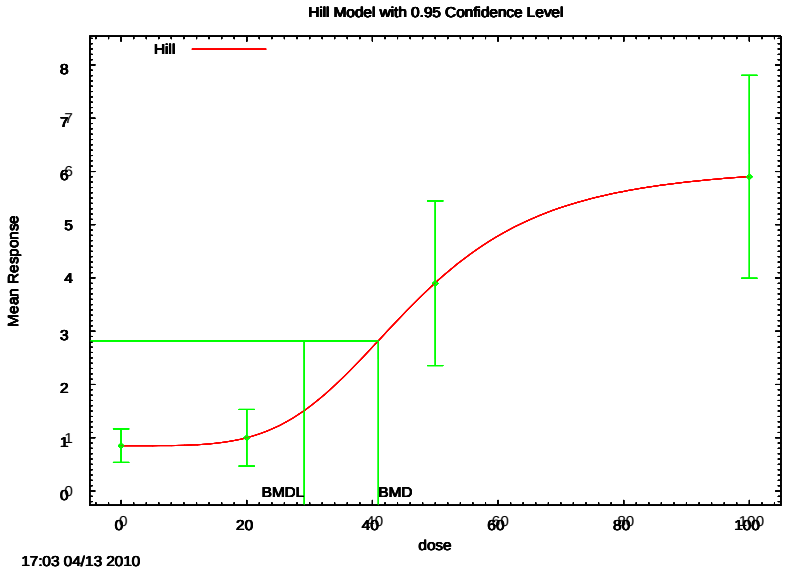
Test 4. (non-constant variance model). Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.

3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.

References

Mass, M.J., A. Abu-Shakra, B.C. Roop et al. 1996. Benzo[b]fluoranthene: tumorigenicity in strain A/J mouse lungs, DNA adducts and mutations in the Ki-ras oncogene. Carcinogenesis 17:1701–1704.

A: Hill_Mass93_BaP_Cont_Hill



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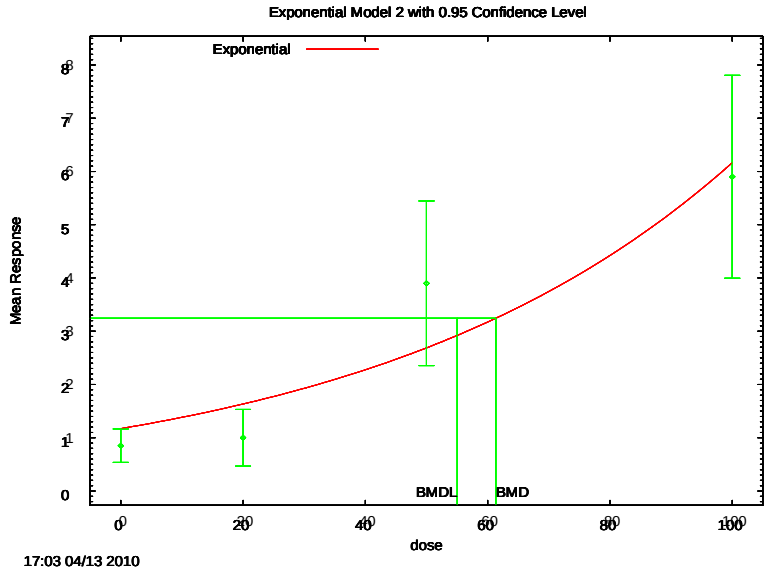

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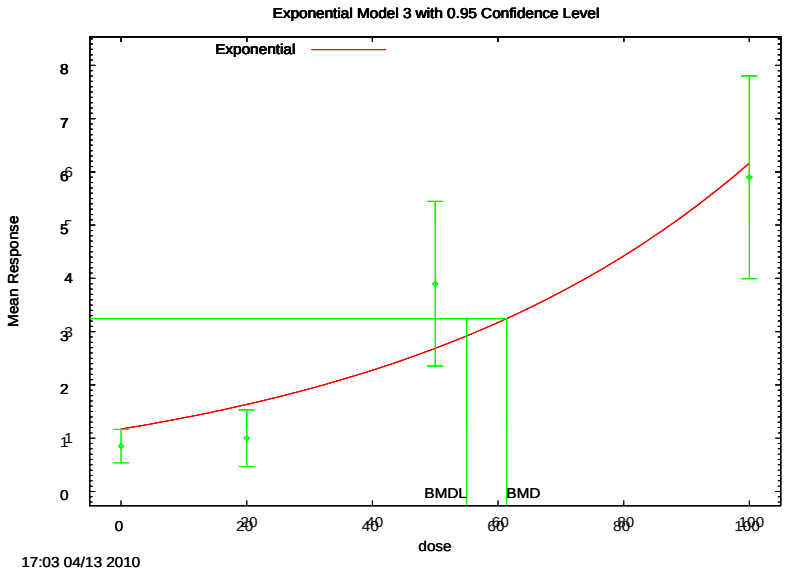

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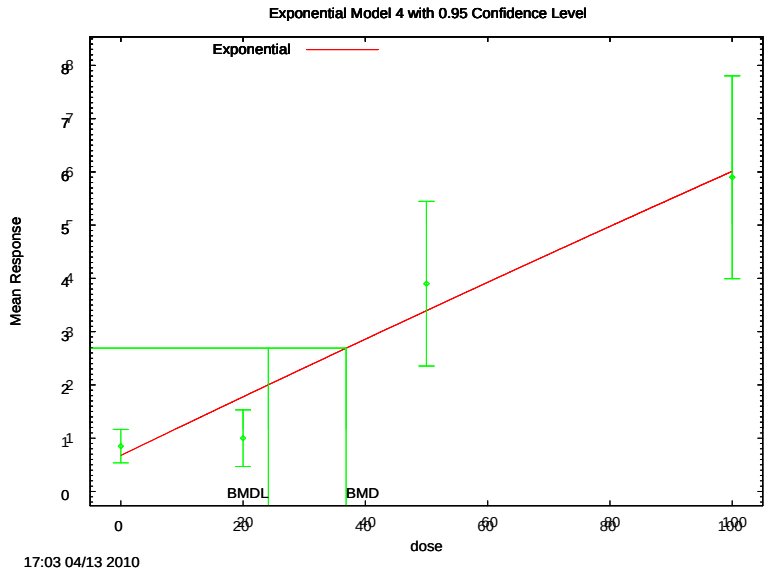

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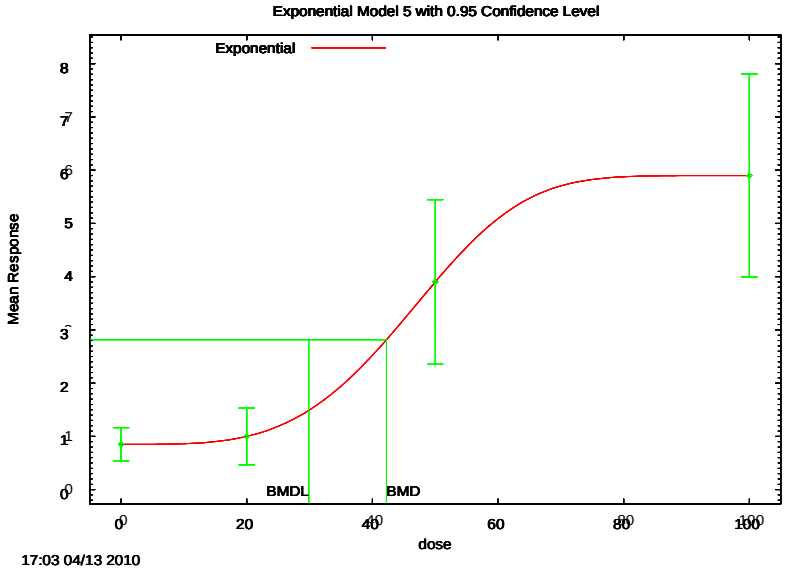

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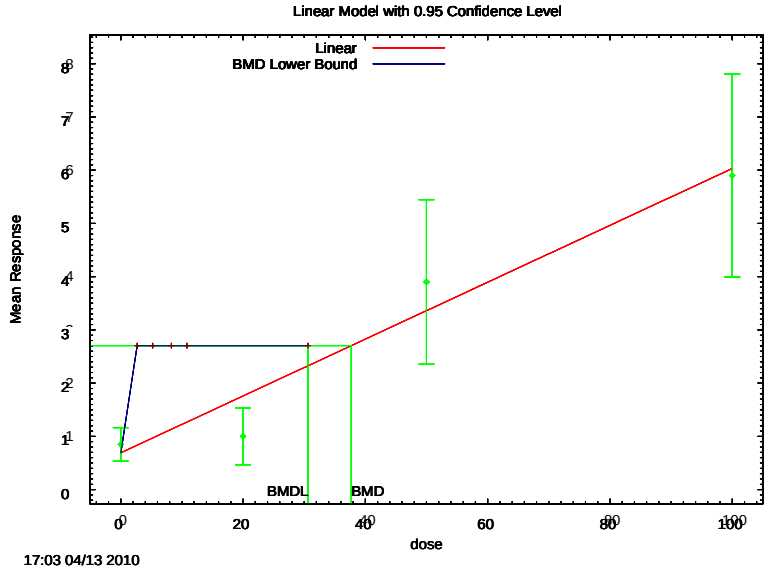

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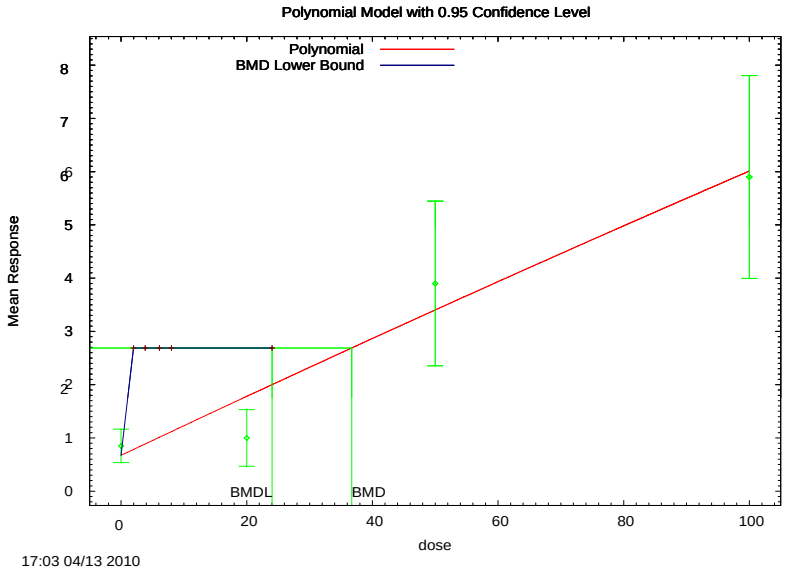

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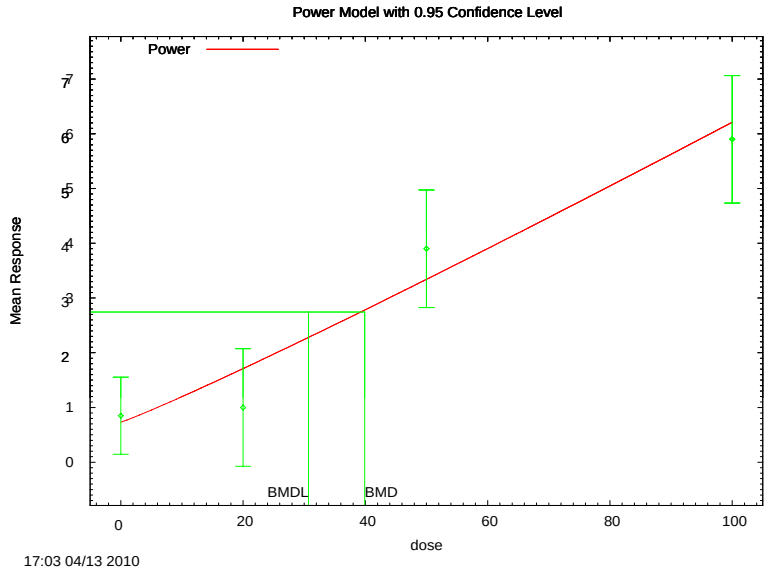

Table C-2. Mass et al. (1993), benz[j]aceanthrylene, A/J mouse. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results. (6)

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness-of-Fit							POD Benchmark Response (BMR) (5)	Dose (mg/kg)		Notes
					p-value for Maximum Likelihood Tests (2)				AIC (3)	Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4		Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Busby, 1984 without highest dose (100 mg/kg)	BjA	A/J Mice	lung tumors	Hill								1 SD			Hill model not applicable
Busby, 1984 without highest dose (100 mg/kg)	BjA	A/J Mice	lung tumors	Exponential 2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	412	**4.5**	**4.5**	1 SD	18.4	16.4	
Busby, 1984 without highest dose (100 mg/kg)	BjA	A/J Mice	lung tumors	Exponential 3	< 0.0001	< 0.0001	< 0.0001	< 0.0001	412	4.0E-08	**4.5**	1 SD	18.4	16.4	
Busby, 1984 without highest dose (100 mg/kg)	BjA	A/J Mice	lung tumors	Exponential 4	< 0.0001	< 0.0001	< 0.0001	N/A	356	1.6E-08	-1.2E-07	1 SD	3.8	3.1	Best Fit: Exponential 4 model has lowest max scaled residuals and low AIC
Busby, 1984 without highest dose (100 mg/kg)	BjA	A/J Mice	lung tumors	Exponential 5	< 0.0001	< 0.0001	< 0.0001	N/A	524	0.83	**3.9**	1 SD	17.1	9.2	
Busby, 1984 without highest dose (100 mg/kg)	BjA	A/J Mice	lung tumors	Linear	<.0001	<.0001	<.0001	3.5E-01	355	-1.7E-13	0.83	1 SD	4.2	3.6	Acceptable Fit
Busby, 1984 without highest dose (100 mg/kg)	BjA	A/J Mice	lung tumors	Polynomial	<.0001	<.0001	<.0001	NA	356	-0.30	-1.3E-12	1 SD	3.8	3.1	Best Fit: Polynomial model has lowest max scaled residuals and low AIC
Busby, 1984 without highest dose (100 mg/kg)	BjA	A/J Mice	lung tumors	Power	<.0001	<.0001	<.0001	3.5E-01	355	0	0.83	1 SD	4.2	3.6	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.
   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.
   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.
   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.
   Test 4. (non-constant variance model) Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.
6. Highest dose level of 100 mg/kg was omitted.

References

Mass, M.J., A. Abu-Shakra, B.C. Roop et al. 1996. Benzo[b]fluoranthene: tumorigenicity in strain A/J mouse lungs, DNA adducts and mutations in the Ki-ras oncogene. Carcinogenesis 17:1701–1704.

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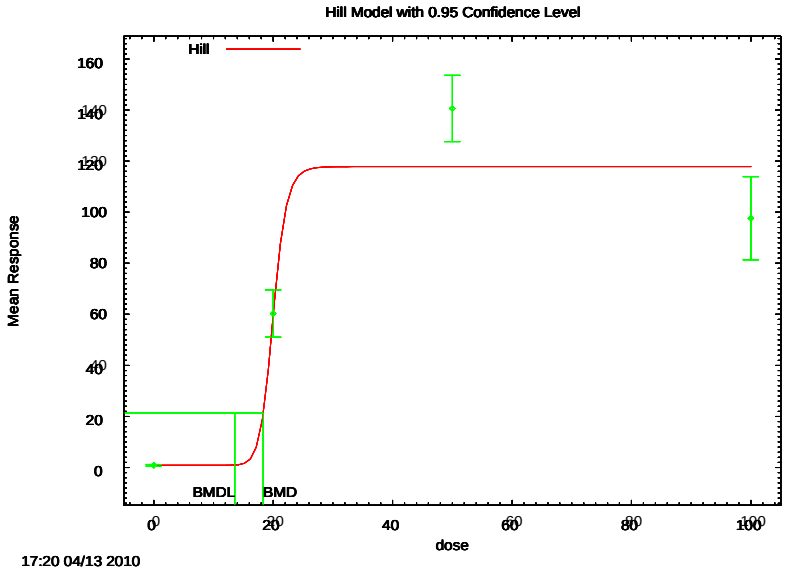

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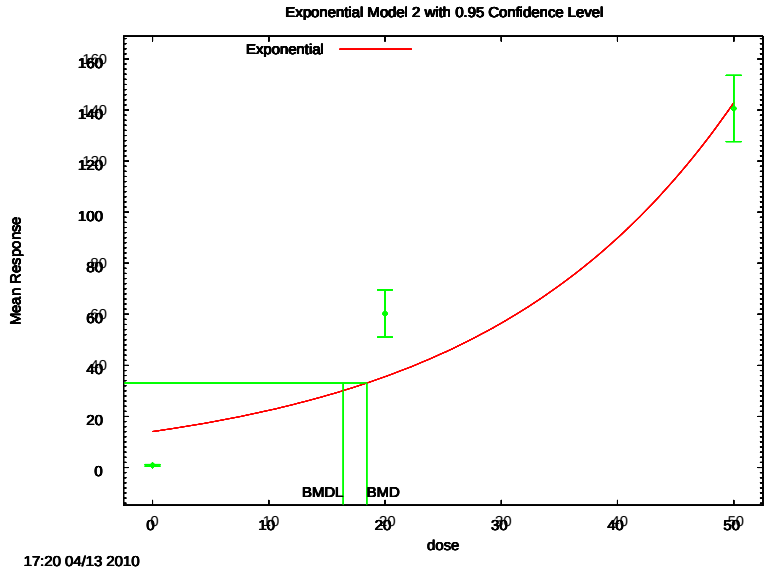

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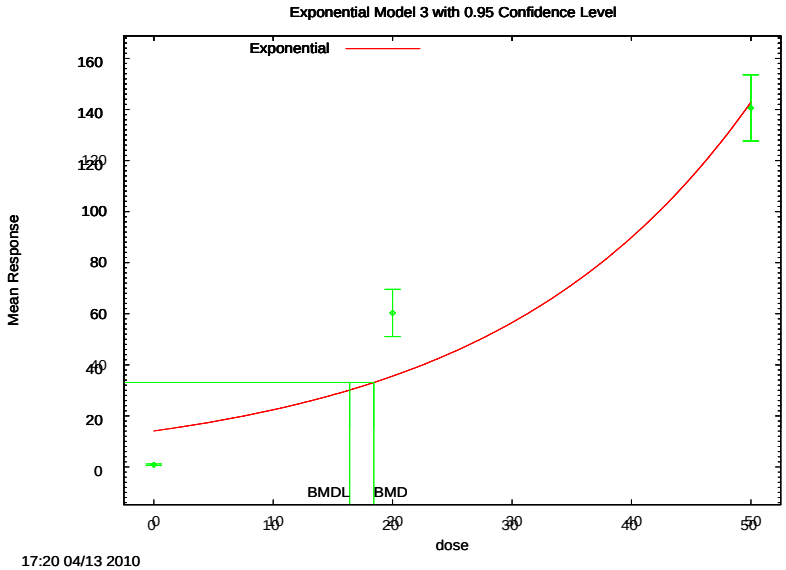

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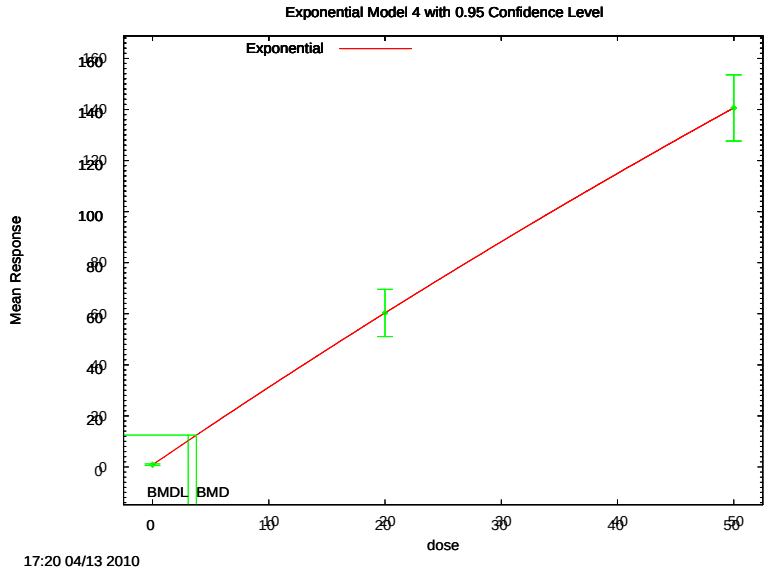

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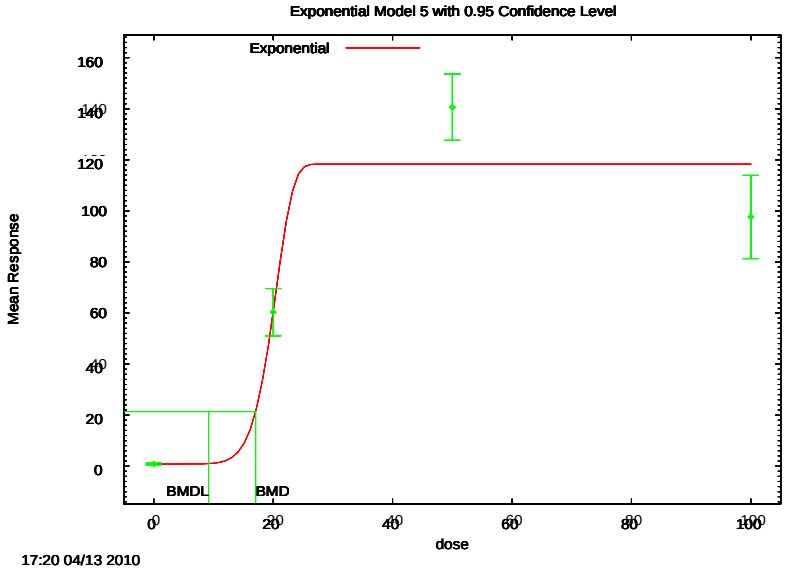

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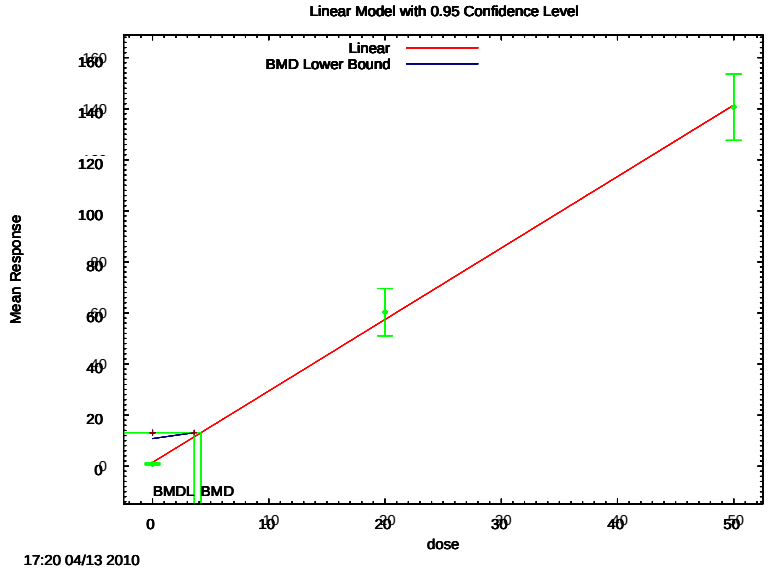

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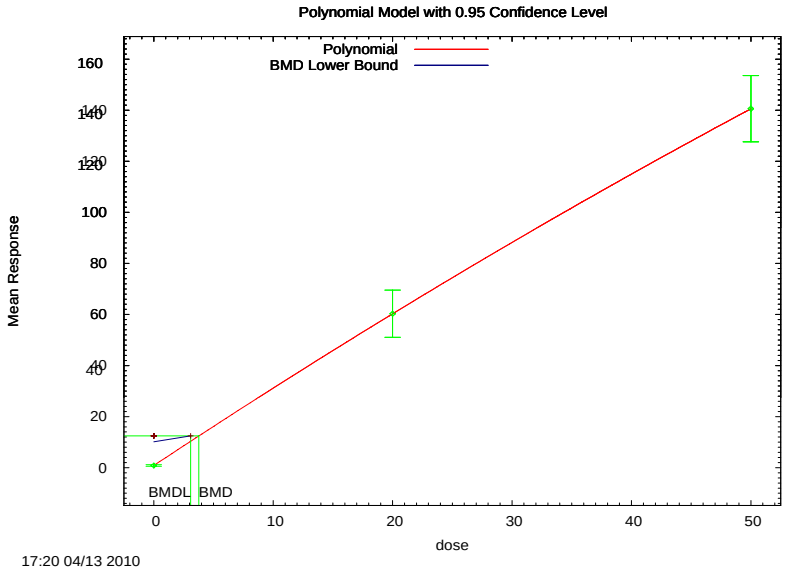


H: Power_Mass93_BjA_3doses_Cont_Pow

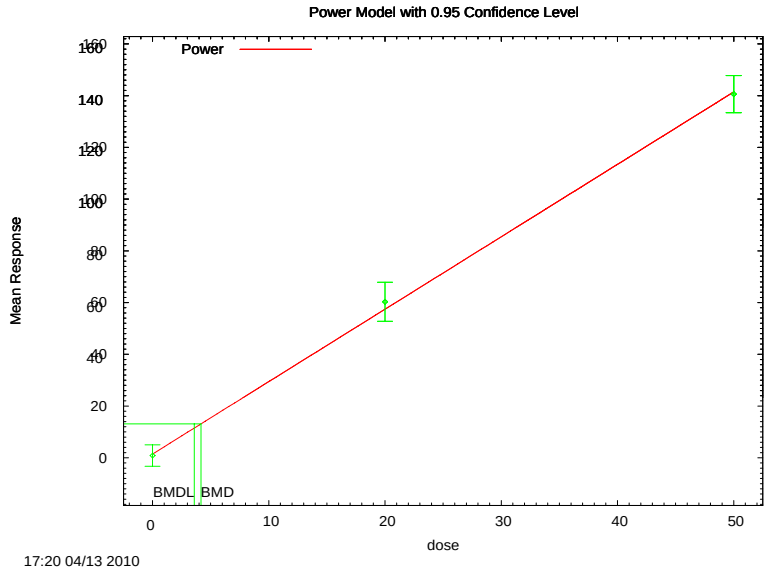


Table C-3. Nesnow et al. (1984), benz[l]aceanthrylene, male mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% and 67% effect levels applied to study results. (5)

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (nmol)		Alternative POD (% Effect)	Dose (nmol)		Notes
							Residuals (4)								
		Animal	Tissue		p-value for Chi-Square Test (2)	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL		BMD	BMDL	
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	Gamma	1.0	39	0	0.030	10%	17.0	3.6	67%	56	38	Acceptable Fit
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	Logistic	0.59	41	-0.80	-1.0	10%	21.2	13.0	67%	58	49	Acceptable Fit
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	LogLogistic	1.0	39	0	0.21	10%	24.4	8.7	67%	55	41	Acceptable Fit
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	LogProbit	1.0	39	0	0.093	10%	22.5	7.2	67%	55	40	Acceptable Fit
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	Multistage	1.0	39	0	0.0030	10%	10.2	3.6	67%	56	38	Acceptable Fit
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	Multistage-Cancer	1.0	39	0	0.0030	10%	10.2	3.6	67%	56	38	Best Fit: Multi-Stage model has the lowest maxmimum scaled residual, low AIC, and highest p-value
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	Probit	0.57	41	-0.78	-0.89	10%	19.4	12.3	67%	61	52	Acceptable Fit
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	Weibull	1.0	39	0	0.0080	10%	13.3	3.6	67%	56	38	Acceptable Fit
Nesnow, 1984 without highest dose (1000 nmol)	BlAC	male mouse	Papilloma	Quantal-Linear	0.91	38	0	-0.71	10%	4.7	3.4	67%	49	35	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

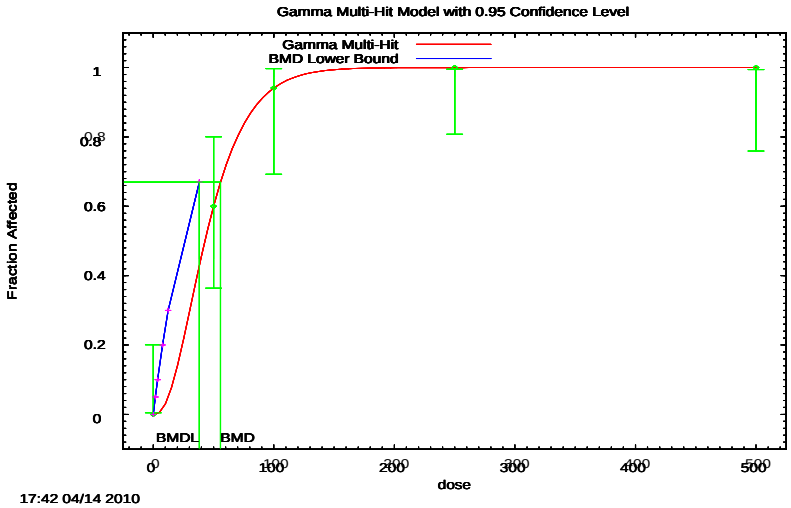
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. Highest dose of 1000 nmol was omitted.

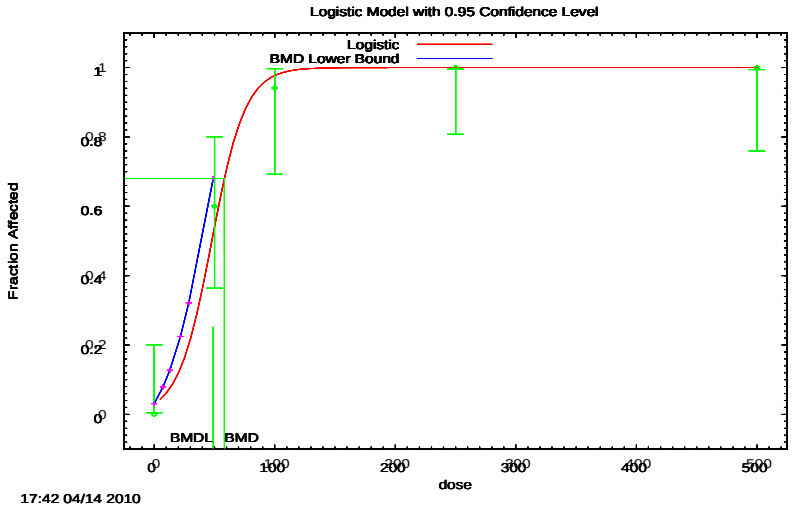
References

Nesnow, S., A. Gold, R. Sangaiah et al. 1984. Mouse skin tumor-initiating activity of benz[e]aceanthrylene and benz[l]aceanthrylene in Sencar mice. Cancer Lett 22:263–268.

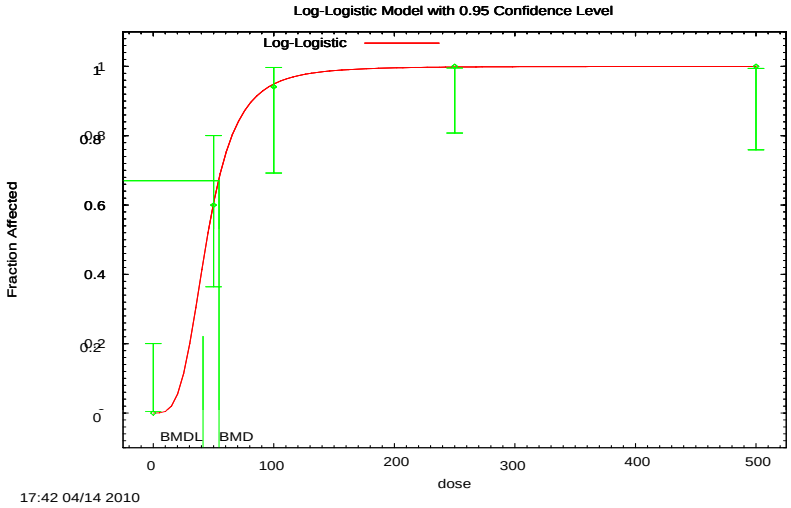
A: Gamma_Nesnow84_BAIC_M_5doses_Dich_Gamma67



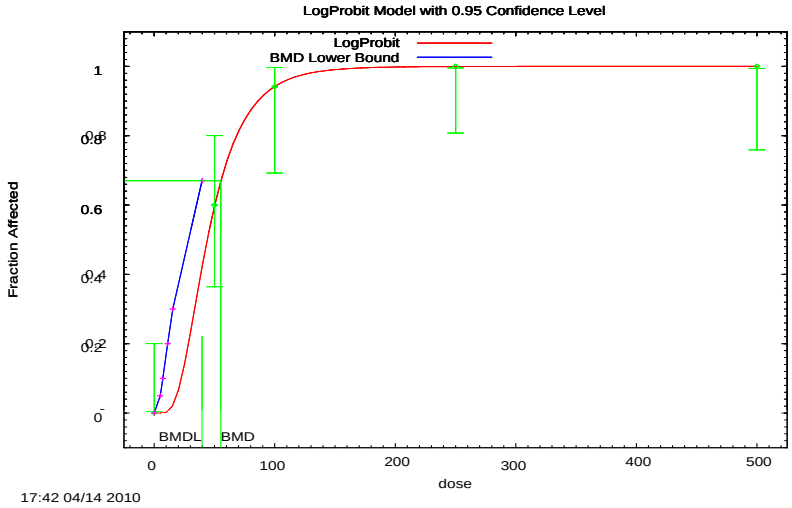
B: Logistic_Nesnow84_BAIC_M_5doses_Dich_Logist67



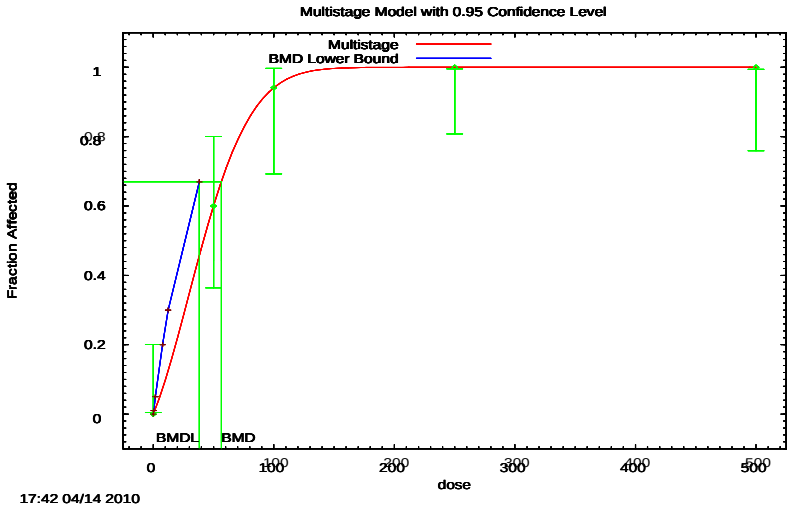
C: LogLogistic_Nesnow84_BAIC_M_5doses_Dich_LogLogist67



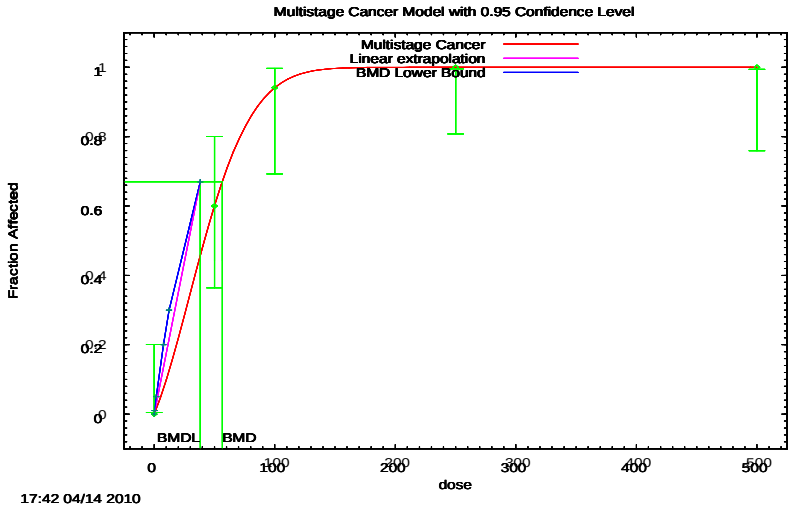
D: LogProbit_Nesnow84_BAIC_M_5doses_Dich_LogProb67



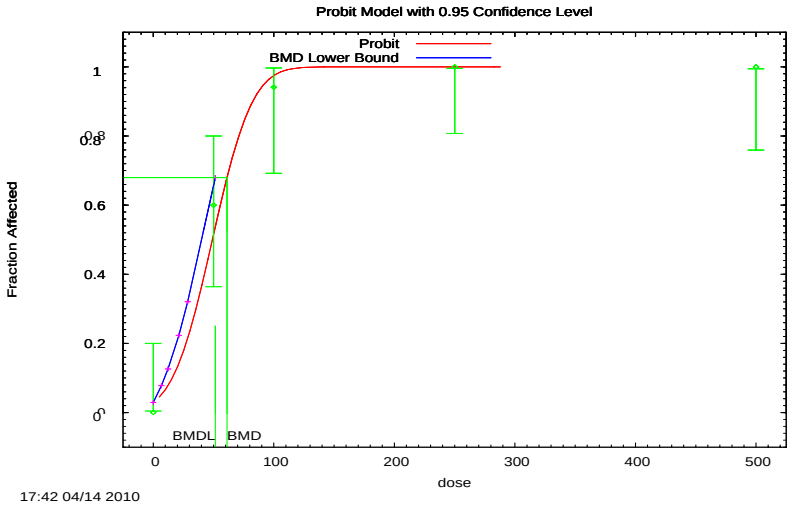
E: Multistage_Nesnow84_BAIC_M_5doses_Dich_Multi67



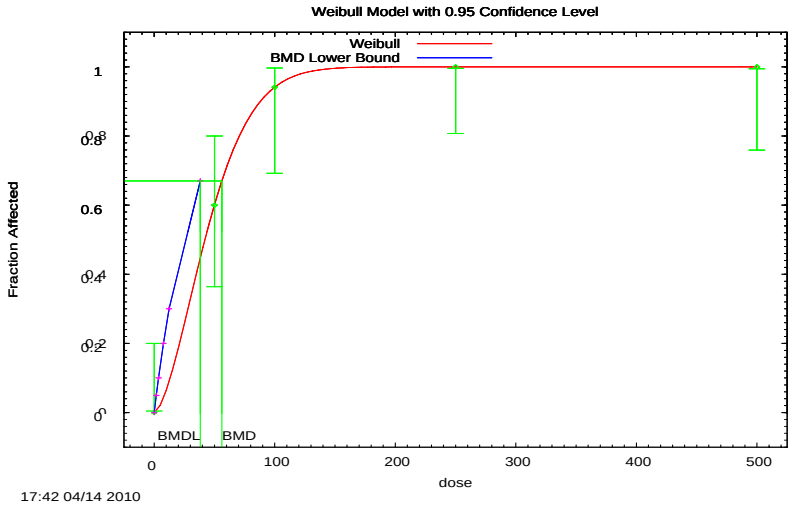
F: Multistage-Cancer_Nesnow84_BAIC_M_5doses_Dich_MultiCanc67



G: Probit_Nesnow84_BAIC_M_5doses_Dich_Probit67



H: Weibull_Nesnow84_BAIC_M_5doses_Dich_Weibull67



I: Quantal-Linear_Nesnow84_BAIC_M_5doses_Dich_QntLin67

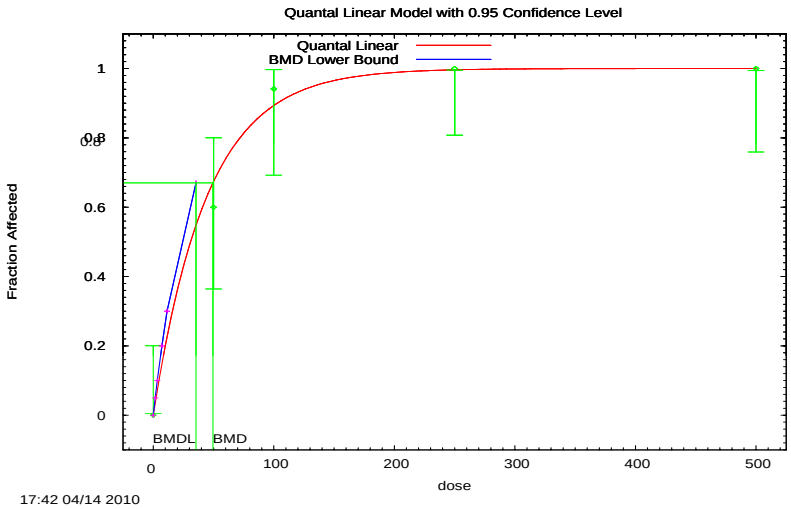


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Table C-4. Nesnow et al. (1984), benz[l]aceanthrylene, female mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% and 51% effect levels applied to study results.

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (nmol)		Alternative POD (% Effect)	Dose (nmol)		Notes
							Residuals (4)								
		Animal	Tissue		p-value for Chi-Square Test (2)	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL		BMD	BMDL	
Nesnow, 1984	BlAC	female mouse	Papilloma	Gamma	**0**	80	-0.61	**-5.1**	10%	8.4	6.2	51%	56.9	42.1	
Nesnow, 1984	BlAC	female mouse	Papilloma	Logistic	**0**	91	**-2.85**	**-3.9**	10%	20.5	14.8	51%	104	75.3	
Nesnow, 1984	BlAC	female mouse	Papilloma	LogLogistic	0.41	72	0.0080	1.3	10%	3.9	1.2	51%	26.2	11.6	Acceptable Fit: LogLogistic model has the lowest maxmimum scaled residuals, lowest AIC, and highest p-value
Nesnow, 1984	BlAC	female mouse	Papilloma	LogProbit	**0.025**	74	-0.12	**-2.5**	10%	11.3	8.0	51%	41.9	29.7	
Nesnow, 1984	BlAC	female mouse	Papilloma	Multistage	**0**	80	-0.61	**-5.1**	10%	8.4	6.2	51%	56.9	42.1	
Nesnow, 1984	BlAC	female mouse	Papilloma	Multistage-Cancer	**0**	80	-0.61	**-5.1**	10%	8.4	6.2	51%	56.9	42.1	
Nesnow, 1984	BlAC	female mouse	Papilloma	Probit	**0**	95	1.0	**-3.3**	10%	26.1	19.8	51%	138	105	
Nesnow, 1984	BlAC	female mouse	Papilloma	Weibull	**0**	80	-0.61	**-5.1**	10%	8.4	6.2	51%	56.9	42.1	
Nesnow, 1984	BlAC	female mouse	Papilloma	Quantal-Linear	**0**	80	-0.61	**-5.1**	10%	8.4	6.2	51%	56.9	42.1	

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

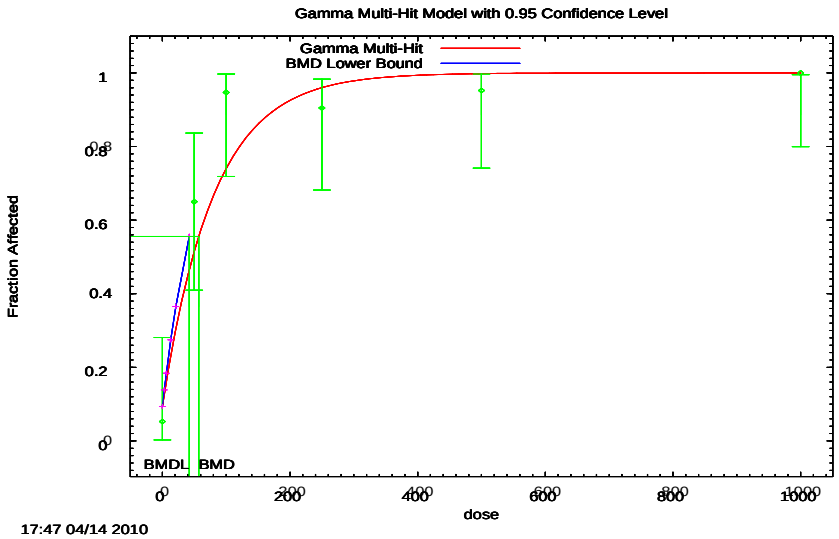
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.

References

Nesnow, S., A. Gold, R. Sangaiah et al. 1984. Mouse skin tumor-initiating activity of benz[e]aceanthrylene and benz[l]aceanthrylene in Sencar mice. Cancer Lett 22:263–268.

A: Gamma_Nesnow84_BAIC_F_Dich_Gamma51



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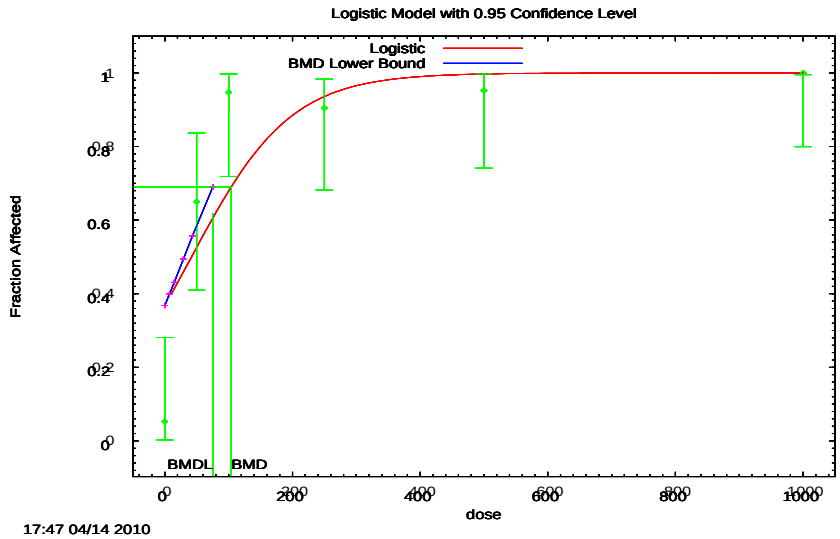
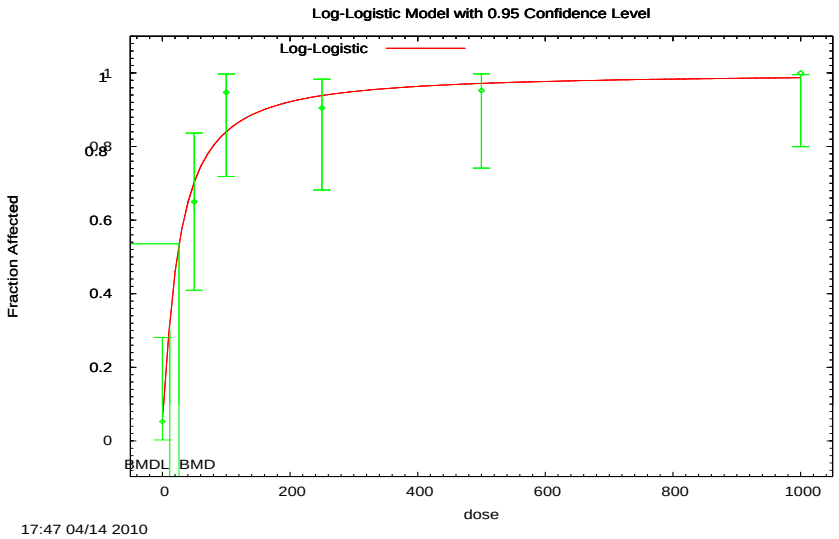


C: LogLogistic_Nesnow84_BAIC_F_Dich_LogLogist51



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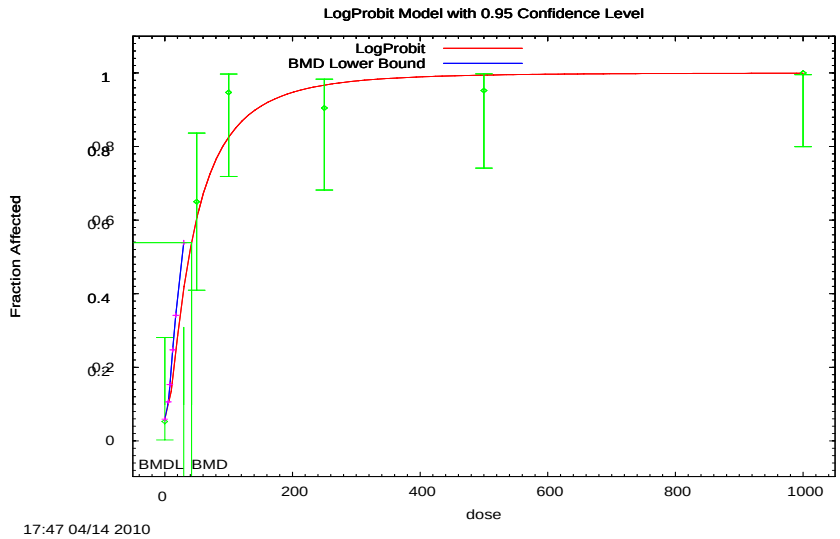
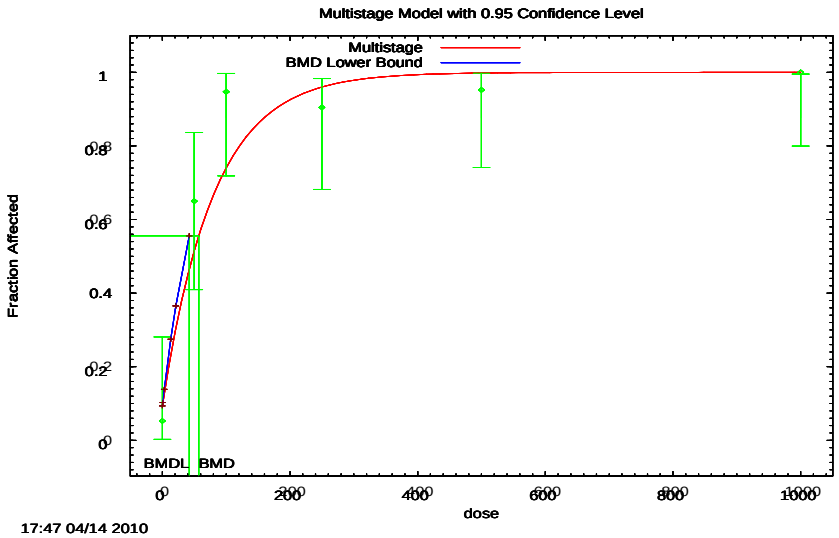
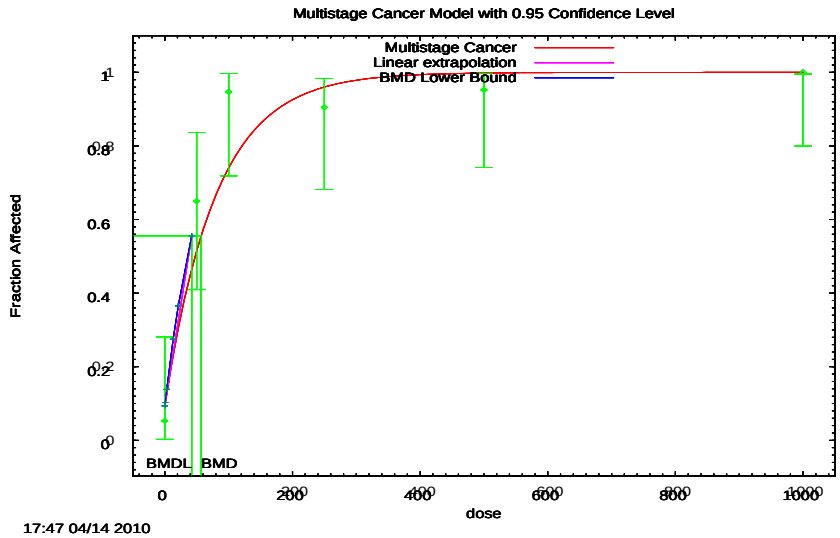


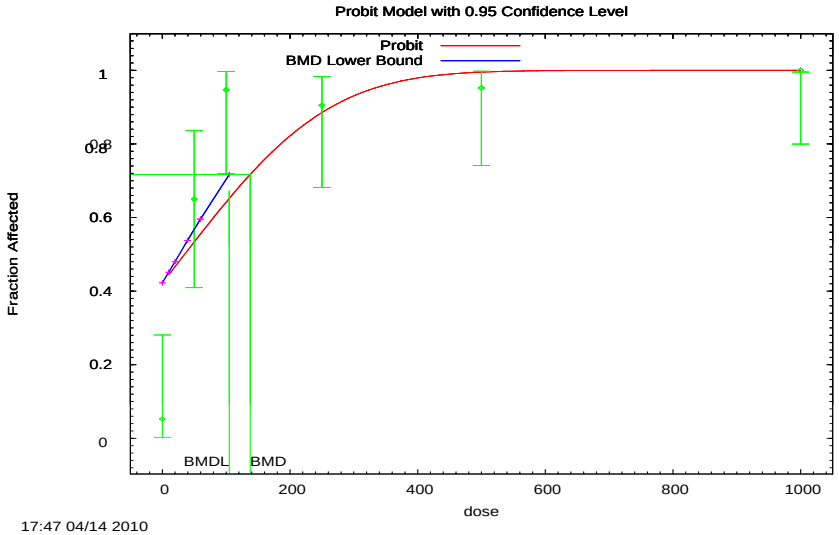
E: Multistage_Nesnow84_BAIC_F_Dich_Multi51



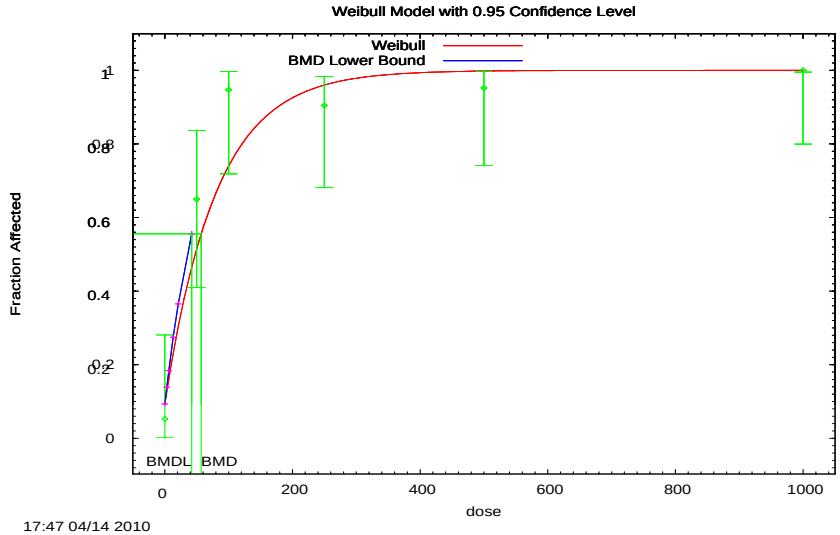
F: Multistage-Cancer_Nesnow84_BAIC_F_Dich_MultiCanc51



G: Probit_Nesnow84_BAIC_F_Dich_Probit51



H: Weibull_Nesnow84_BAIC_F_Dich_Weibull51



I: Quantal-Linear_Nesnow84_BAIC_F_Dich_QntLin51

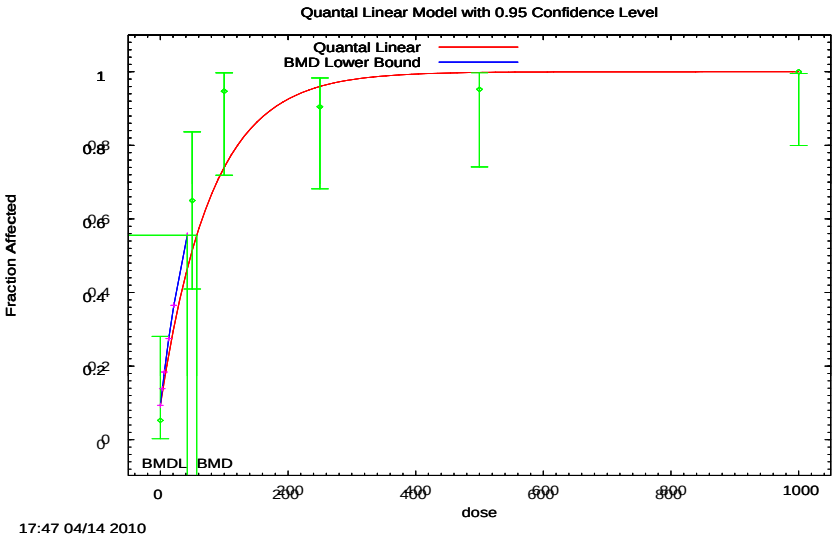


Table C-5. Nesnow et al. (1984), benz[e]aceanthrylene, male mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% and 67% effect levels applied to study results.

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (nmol)		Alternative POD (% Effect)	Dose (nmol)		Notes
							Residuals (4)								
		Animal	Tissue		p-value for Chi-Square Test (2)	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL		BMD	BMDL	
Nesnow, 1984	BeAC	male mouse	Papilloma	Gamma	0.76	106	0.90	-0.93	10%	37.3	29.0	67%	393	306	Acceptable Fit
Nesnow, 1984	BeAC	male mouse	Papilloma	Logistic	**0.025**	119	-0.36	-1.9	10%	105.8	81.7	67%	517	412	
Nesnow, 1984	BeAC	male mouse	Papilloma	LogLogistic	0.85	107	0.69	-0.89	10%	35.6	16.7	67%	357	257	Best Fit: LogLogistic model has the lowest maxmimum scaled residuals, low AIC, and highest p-value.
Nesnow, 1984	BeAC	male mouse	Papilloma	LogProbit	0.33	110	1.68	1.7	10%	61.0	44.8	67%	341	251	Acceptable Fit
Nesnow, 1984	BeAC	male mouse	Papilloma	Multistage	0.76	106	0.90	-0.93	10%	37.3	29.0	67%	393	306	Acceptable Fit
Nesnow, 1984	BeAC	male mouse	Papilloma	Multistage-Cancer	0.76	106	0.90	-0.93	10%	37.3	29.0	67%	393	306	Acceptable Fit
Nesnow, 1984	BeAC	male mouse	Papilloma	Probit	**0.020**	120	-0.4	2.0	10%	107.5	85.5	67%	565	464	
Nesnow, 1984	BeAC	male mouse	Papilloma	Weibull	0.76	106	0.90	-0.93	10%	37.3	29.0	67%	393	306	Acceptable Fit
Nesnow, 1984	BeAC	male mouse	Papilloma	Quantal-Linear	0.76	106	0.90	-0.93	10%	37.3	29.0	67%	393	306	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

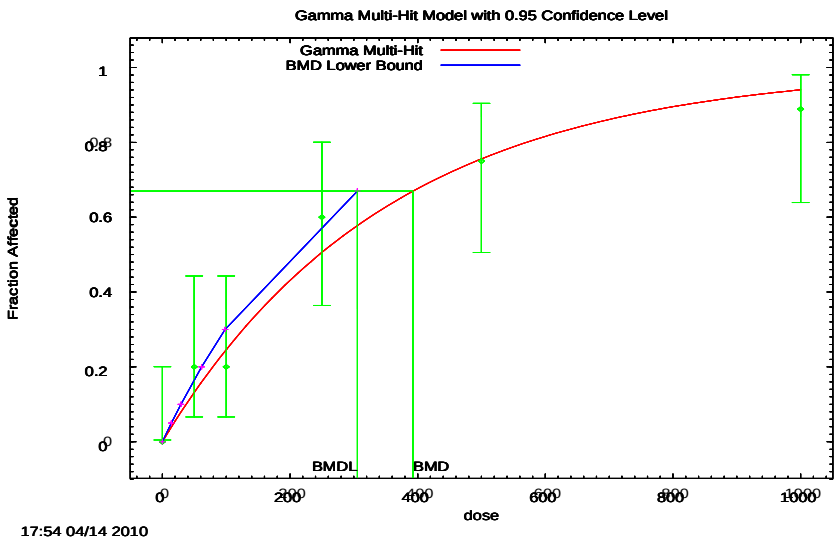
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.

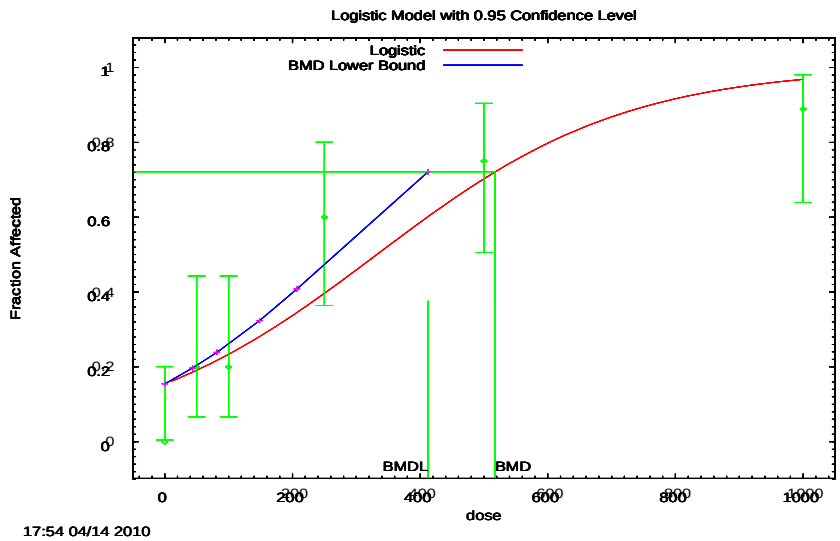
References

Nesnow, S., A. Gold, R. Sangaiah et al. 1984. Mouse skin tumor-initiating activity of benz[e]aceanthrylene and benz[l]aceanthrylene in Sencar mice. Cancer Lett 22:263–268.

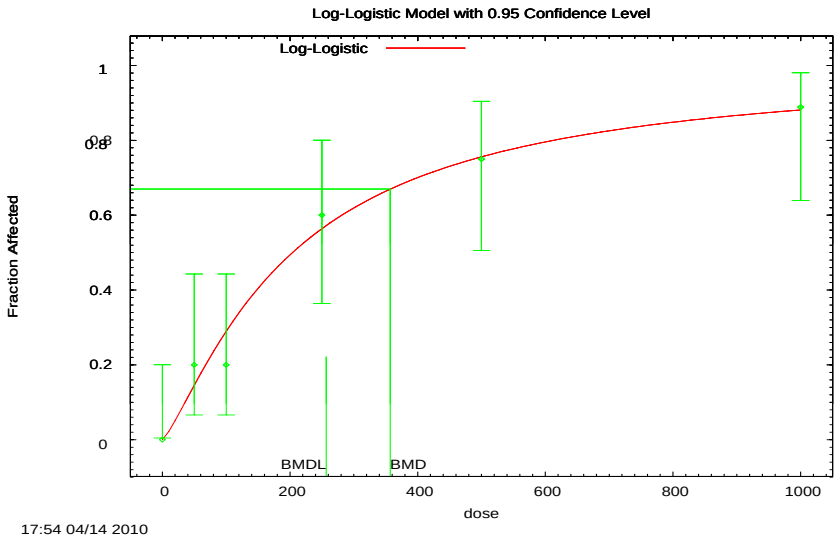
A: Gamma_Nesnow84_BeAC_M_Dich_Gamma67



B: Logistic_Nesnow84_BeAC_M_Dich_Logist67



C: LogLogistic_Nesnow84_BeAC_M_Dich_LogLogist67



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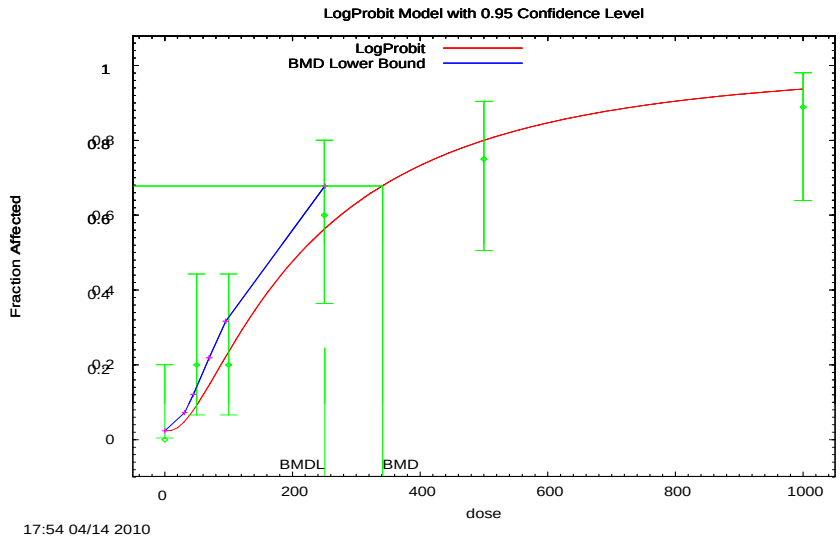

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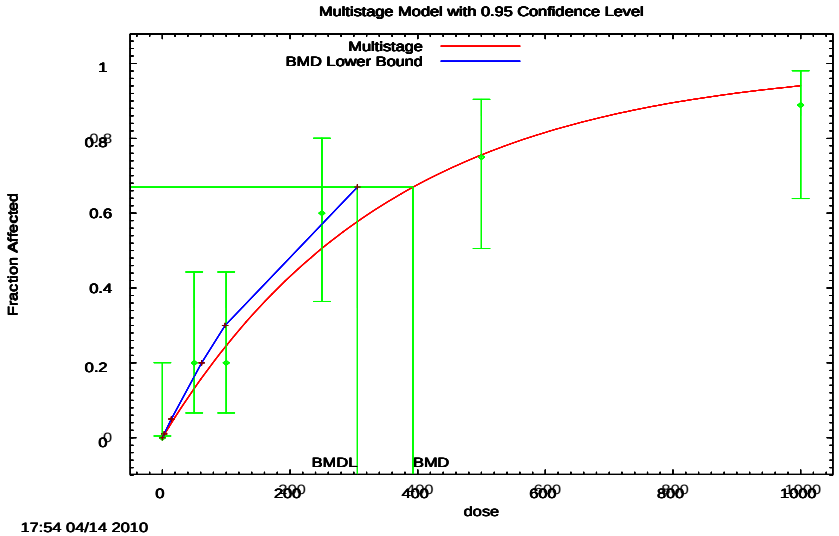

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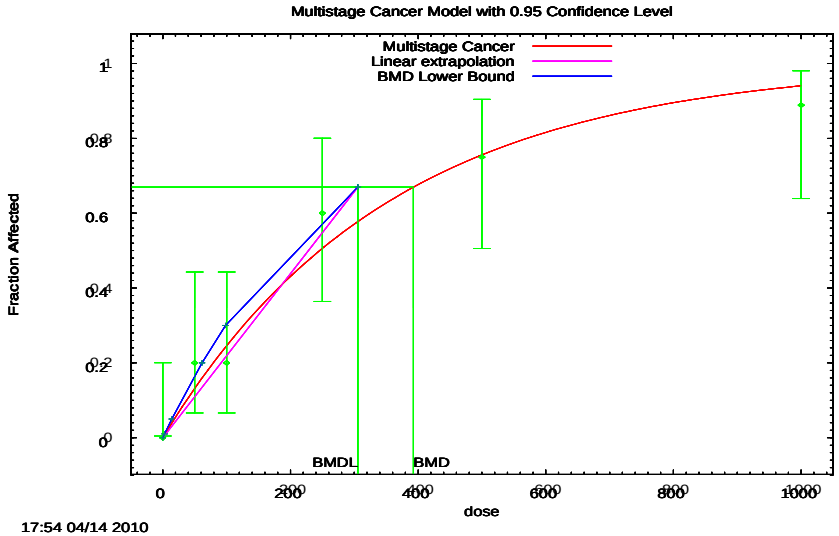

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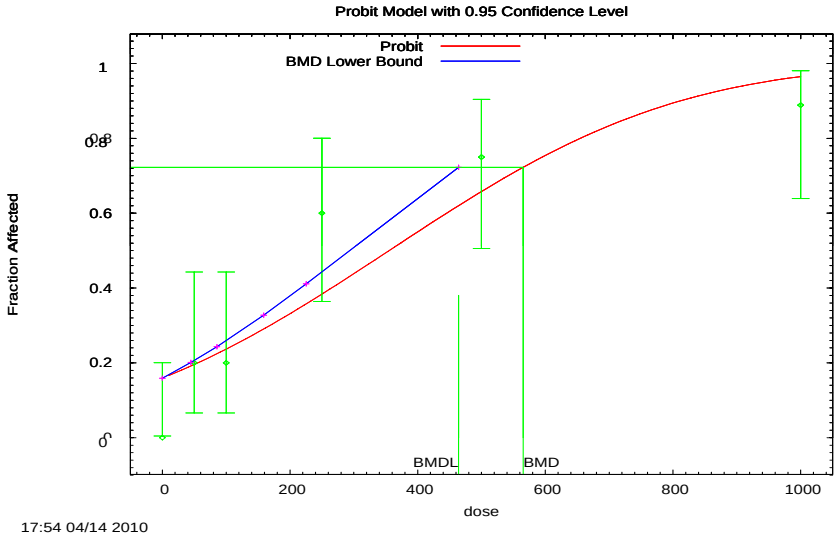

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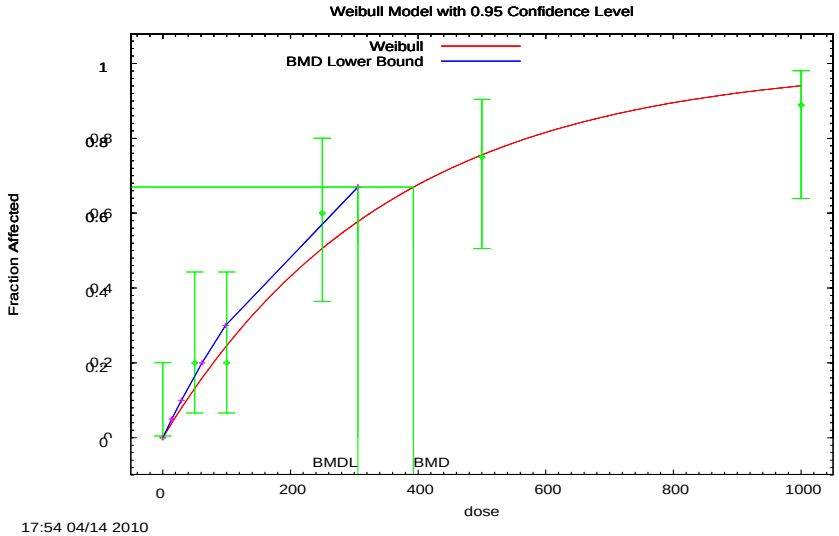


I: Quantal-Linear_Nesnow84_BeAC_M_Dich_QntLin67

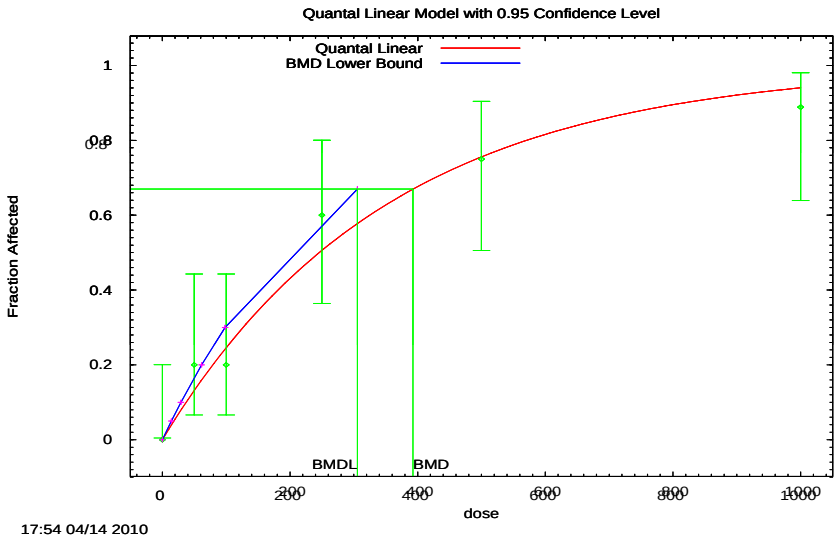


Table C-6. Nesnow et al. (1984), benz[e]aceanthrylene, female mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% and 51% effect levels applied to study results.

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (nmol)		Alternative POD (% Effect)	Dose (nmol)		Notes
							Residuals (4)								
		Animal	Tissue		p-value for Chi-Square Test (2)	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL		BMD	BMDL	
Nesnow, 1984	BeAC	female mouse	Papilloma	Gamma	0.20	127	0.19	-1.8	10%	56.6	40.3	51%	383	273	Acceptable Fit
Nesnow, 1984	BeAC	female mouse	Papilloma	Logistic	0.12	129	1.16	-1.5	10%	130	99.9	51%	530	416	Acceptable Fit
Nesnow, 1984	BeAC	female mouse	Papilloma	LogLogistic	0.24	126	0.0070	-1.9	10%	31.8	20.3	51%	297	190	Best Fit: Log-logistic model has the lowest maxmimum scaled residuals, low AIC, and highest p-value.
Nesnow, 1984	BeAC	female mouse	Papilloma	LogProbit	**0.08**	129	1.40	-2.0	10%	108	71.8	51%	399	265	
Nesnow, 1984	BeAC	female mouse	Papilloma	Multistage	0.20	127	0.19	-1.8	10%	56.6	40.3	51%	383	273	Acceptable Fit
Nesnow, 1984	BeAC	female mouse	Papilloma	Multistage-Cancer	0.20	127	0.19	-1.8	10%	56.6	40.3	51%	383	273	Acceptable Fit
Nesnow, 1984	BeAC	female mouse	Papilloma	Probit	0.13	129	1.2	-1.5	10%	126	99.7	51%	537	431	Acceptable Fit
Nesnow, 1984	BeAC	female mouse	Papilloma	Weibull	0.20	127	0.19	-1.8	10%	56.6	40.3	51%	383	273	Acceptable Fit
Nesnow, 1984	BeAC	female mouse	Papilloma	Quantal-Linear	0.20	127	0.19	-1.8	10%	56.6	40.3	51%	383	273	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

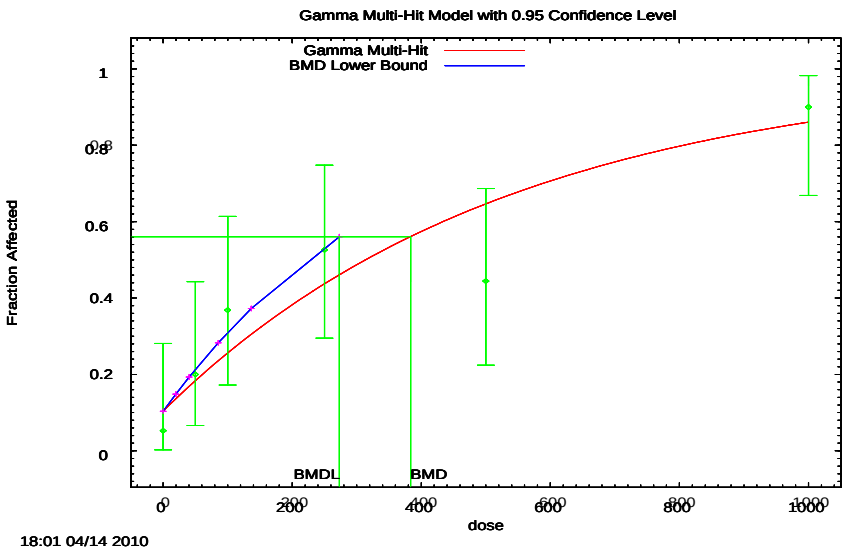
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.

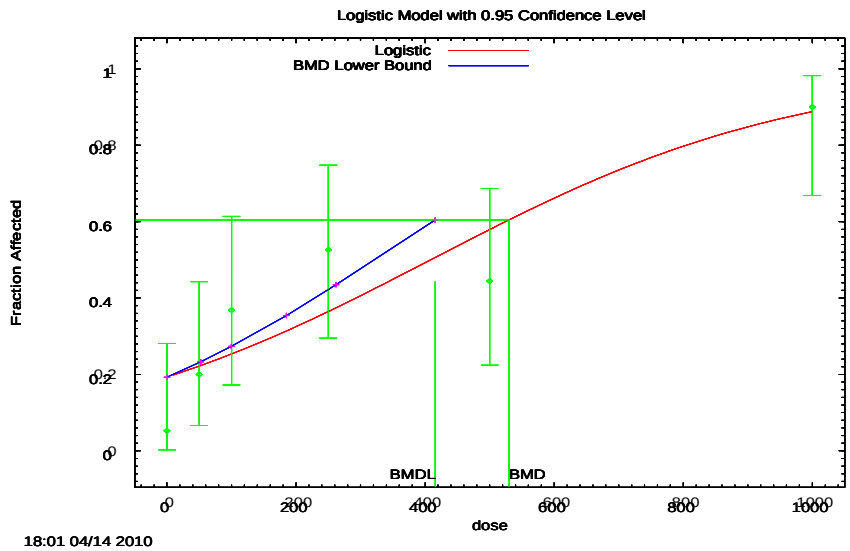
References

Nesnow, S., A. Gold, R. Sangaiah et al. 1984. Mouse skin tumor-initiating activity of benz[e]aceanthrylene and benz[l]aceanthrylene in Sencar mice. Cancer Lett 22:263–268.

A: Gamma_Nesnow84_BeAC_F_Dich_Gamma51



B: Logistic_Nesnow84_BeAC_F_Dich_Logist51



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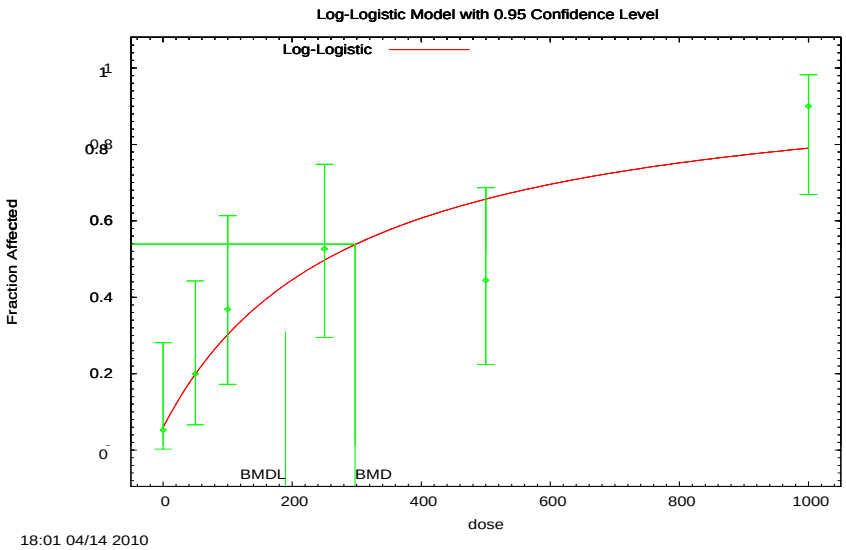
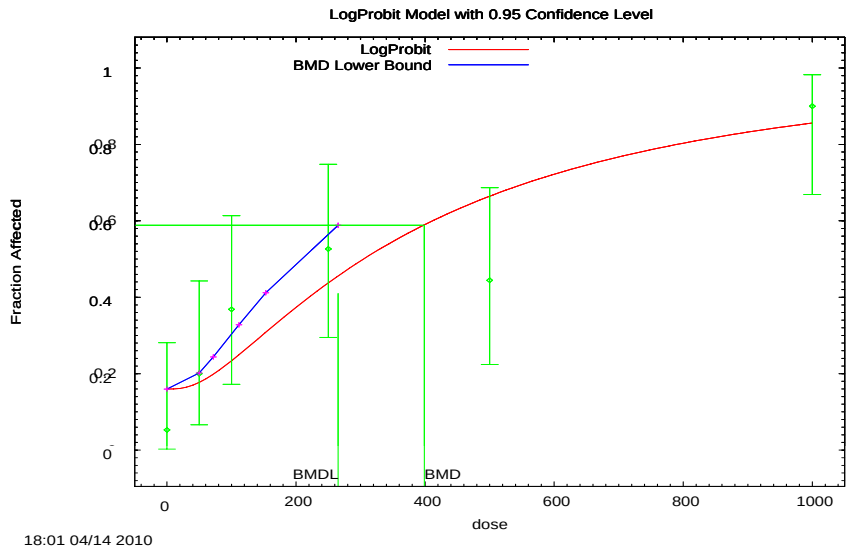
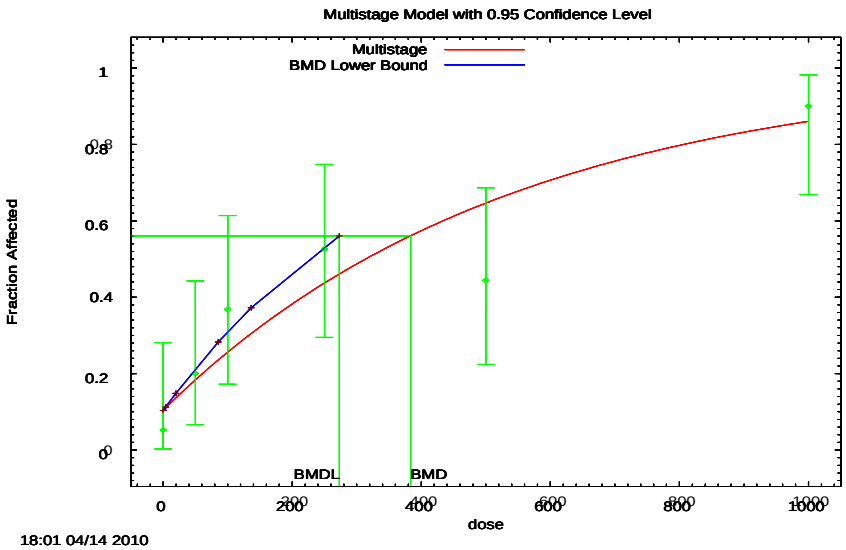


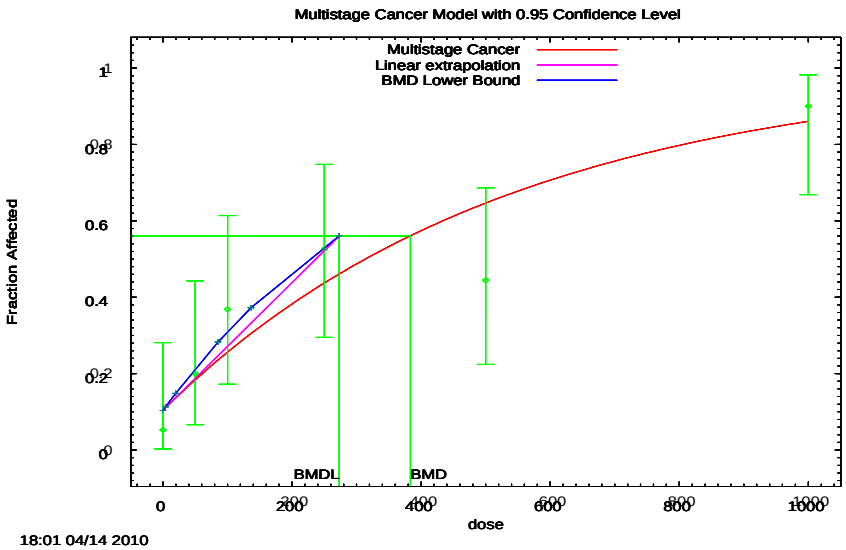
D: LogProbit_Nesnow84_BeAC_F_Dich_LogProb51



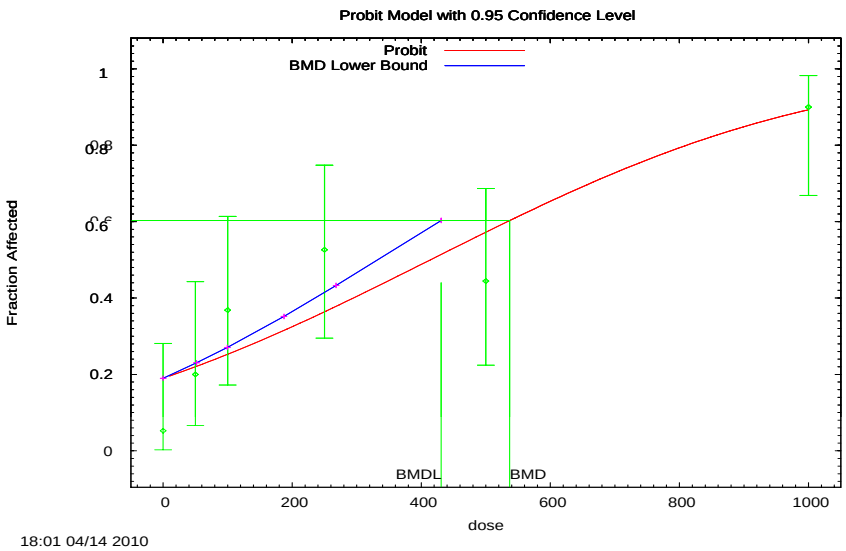
E: Multistage_Nesnow84_BeAC_F_Dich_Multi51



F: Multistage-Cancer_Nesnow84_BeAC_F_Dich_MultiCanc51



G: Probit_Nesnow84_BeAC_F_Dich_Probit51



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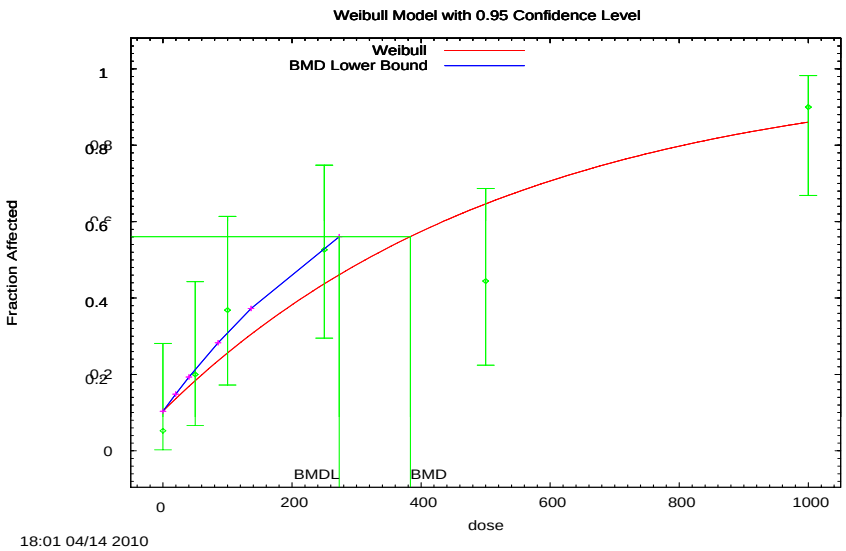

I: Quantal-Linear_Nesnow84_BeAC_F_Dich_QntLin51

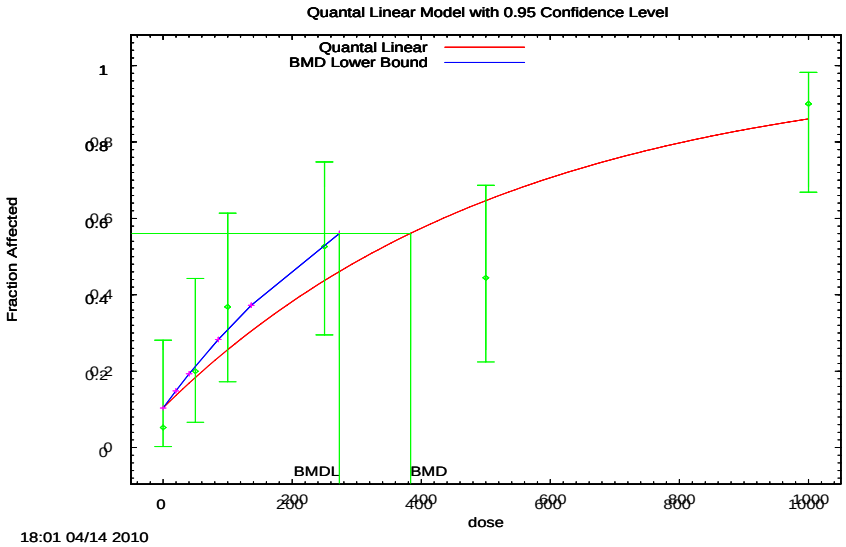

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Table C-7. Habs et al. (1980), benzo[a]pyrene, female mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (µg)		Notes
					p-value for Chi-Square Test (2)	AIC (3)	Residuals (4)					
		Animal	Tissue				Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	Gamma	1.0	85	0	0	10%	1.31	0.78	Best Fit: Gamma, LogLogistic, LogProbit, and Weibull models have the lowest maxmimum scaled residuals, low AIC, and highest p-values.
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	Logistic	0.48	85	0.36	-0.58	10%	1.30	0.96	Acceptable Fit
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	LogLogistic	1.0	85	0	0	10%	1.31	0.84	Best Fit; Gamma, LogLogistic, LogProbit, and Weibull models have the lowest maxmimum scaled residuals, low AIC, and highest p-values.
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	LogProbit	1.0	85	0	0	10%	1.35	0.90	Best Fit: Gamma, LogLogistic, LogProbit, and Weibull models have the lowest maxmimum scaled residuals, low AIC, and highest p-values.
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	Multistage	0.53	84	-0.92	-0.92	10%	0.91	0.48	Acceptable Fit
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	Multistage-Cancer	0.53	84	-0.92	-0.92	10%	0.91	0.48	Acceptable Fit
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	Probit	0.69	85	0.20	-0.33	10%	1.27	0.91	Acceptable Fit
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	Weibull	1.0	85	0	0	10%	1.24	0.72	Best Fit: Gamma, LogLogistic, LogProbit, and Weibull models have the lowest maxmimum scaled residuals, low AIC, and highest p-values.
Habs, 1980 without highest dose (4.6 µg/animal)	BaP	female mouse	Sum of Papilloma, carcinoma, sarcoma	Quantal-Linear	**0.056**	89	0	-1.8	10%	0.36	0.27	

Abbreviations

AIC = Akaike's Information Criterion

POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

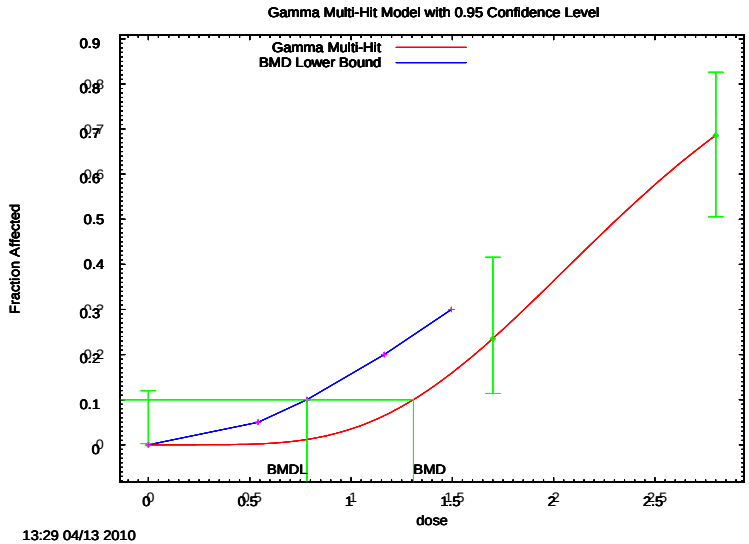
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. Highest dose group of 4.6 ug/animal was omitted in order to obtain a model fit with maximum residuals less than 2 and greater than -2.

References

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

A: Gamma_Habs1980_BaP_3doses_Dich_Gamma



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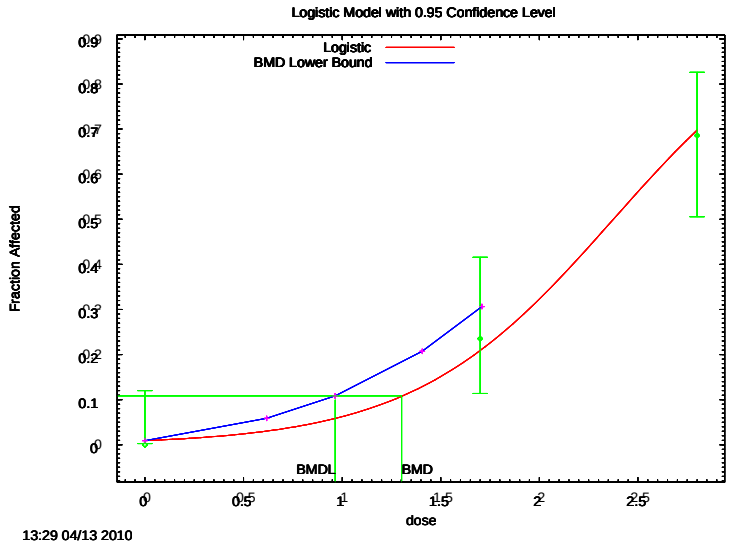

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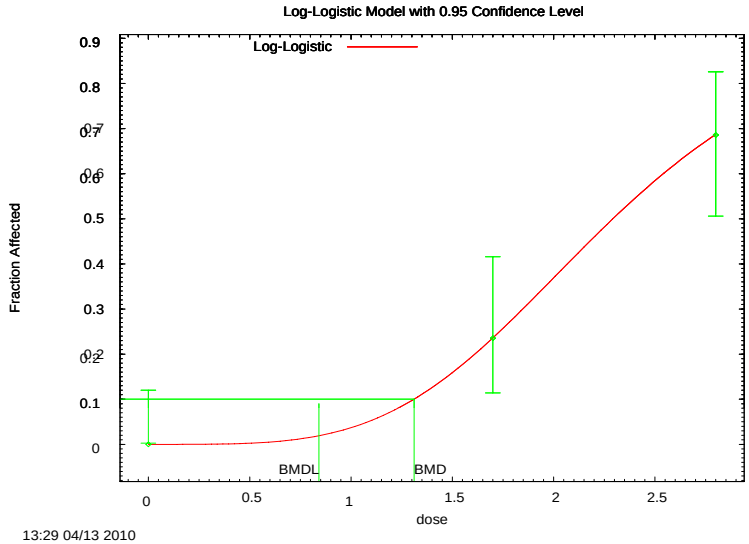

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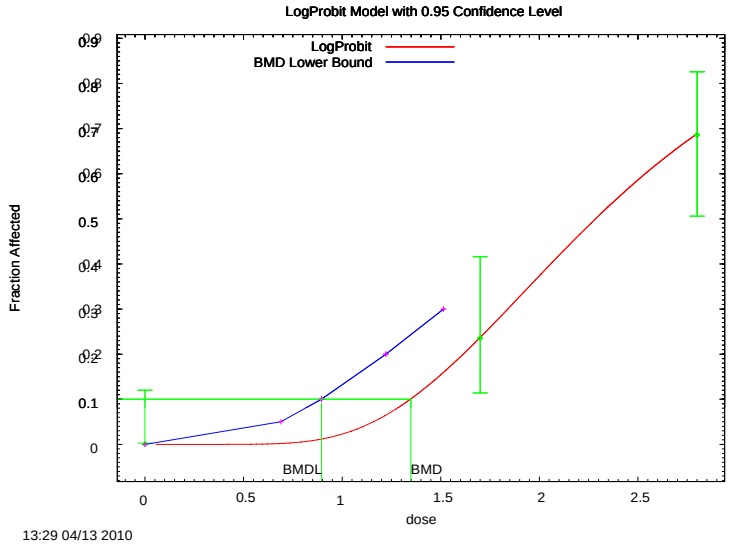
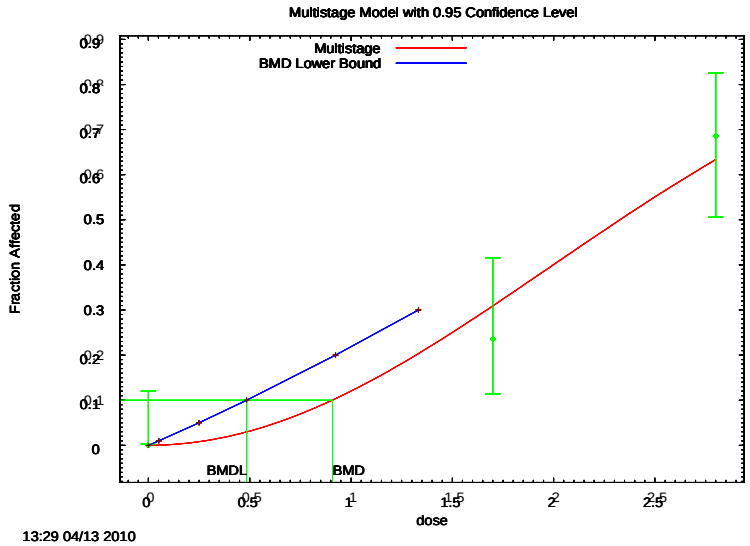


E: Multistage_Habs1980_BaP_3doses_Dich_Multi



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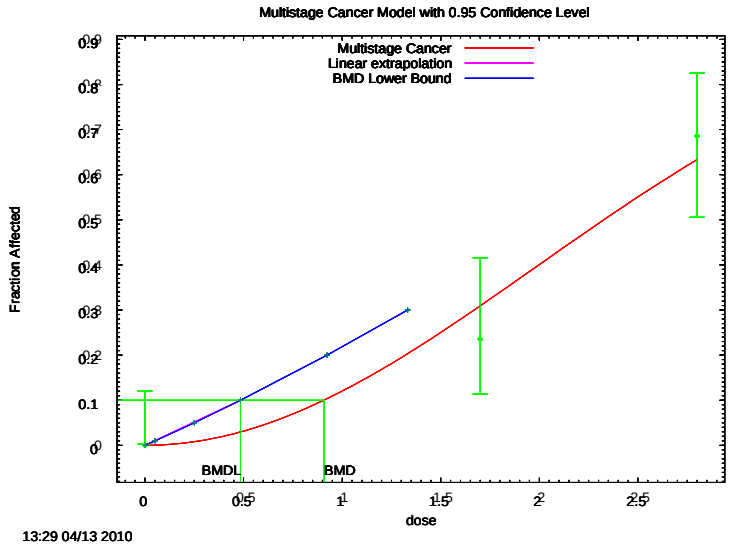

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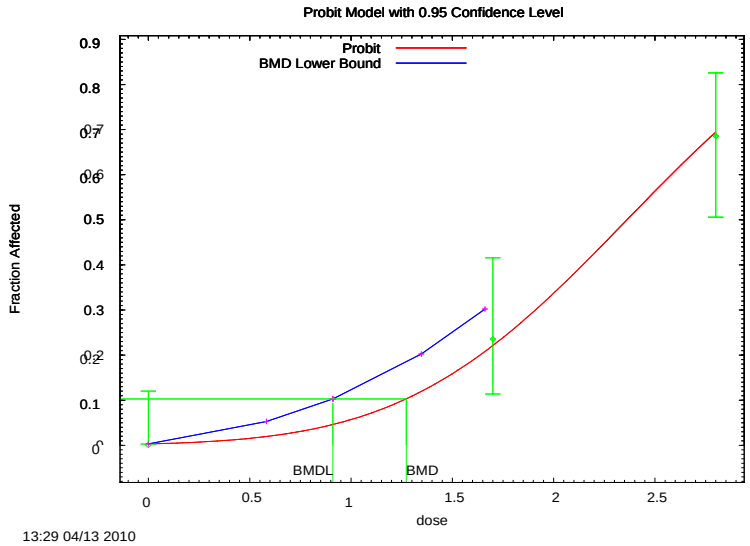
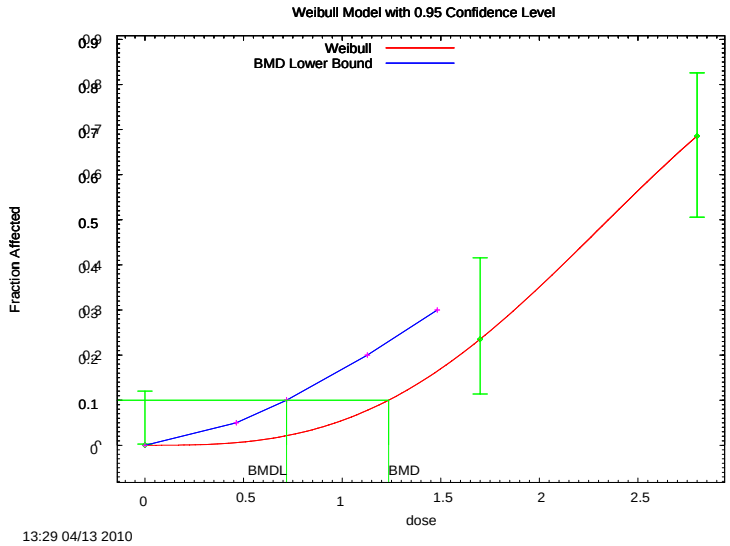


H: Weibull_Habs1980_BaP_3doses_Dich_Weibull



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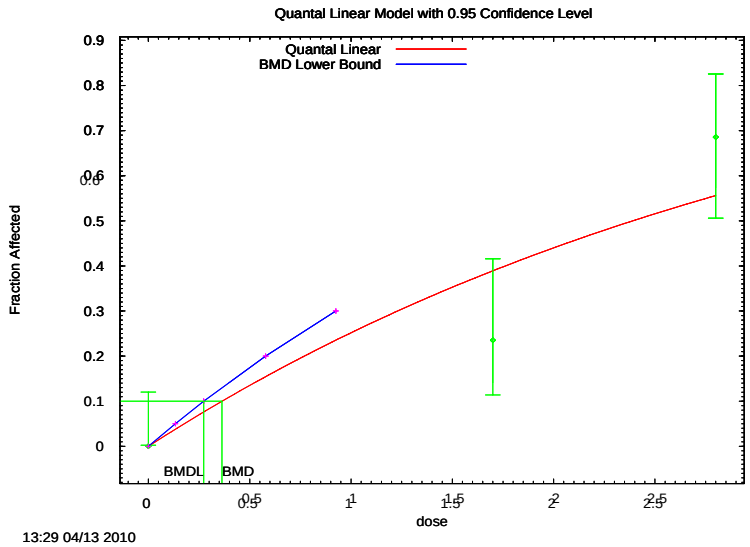

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Table C-8. LaVoie et al. (1982), benzo[b]fluoranthene, female mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (µg)		Notes
		Animal	Tissue		p-value for Chi-Square Test (2)	AIC (3)	Residuals (4)			BMD	BMDL	
							Scaled Residual of Interest	Maximum Scaled Residual				
LaVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	Gamma	0.33	59	0	1.2	10%	2.7	1.9	Acceptable Fit
LaVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	Logistic	**0.0076**	68	2.0	2.0	10%	7	5.2	
LaVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	LogLogistic	0.74	57	0	0.55	10%	1.7	1.0	Best Fit: LogLogistic model has the lowest maxmimum scaled residuals, lowest AIC, and highest p-value.
LaVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	LogProbit	0.18	60	0	-1.3	10%	4	3.2	Acceptable Fit
LaVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	Multistage	0.33	59	0	1.2	10%	2.7	1.9	Acceptable Fit
LaVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	Multistage-Cancer	0.33	59	0	1.2	10%	2.7	1.9	Acceptable Fit
LaVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	Probit	**0.0083**	67	2.0	2.0	10%	7	5.0	
aVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	Weibull	0.33	59	0	1.2	10%	2.7	1.9	Acceptable Fit
LaVoie, 1982 without highest dose (100 µg/mouse)	BbF	female mouse	Primarily squamous cell papilloma	Quantal-Linear	0.33	59	0	1.2	10%	2.7	1.9	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion

POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

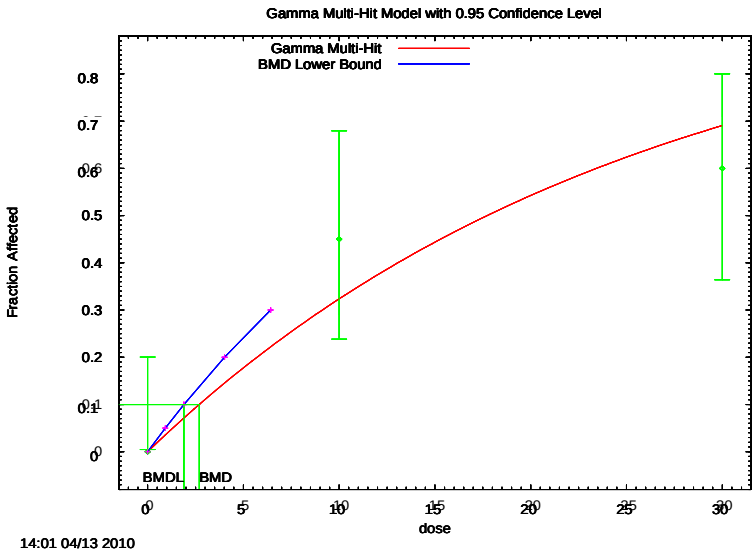
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. Highest dose of 100 µg/mouse was omitted to improve model fits.

References

LaVoie, E.J., S. Amin, S.S. Hecht et al. 1982. Tumour initiating activity of dihydrodiols of benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene. Carcinogenesis 3:49–52.

A: Gamma_LaVoie82_BbF_3doses_Dich_Gamma



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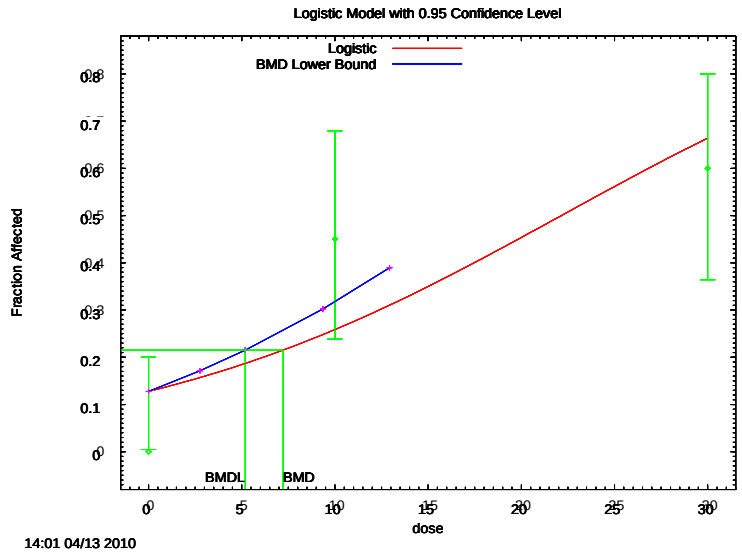

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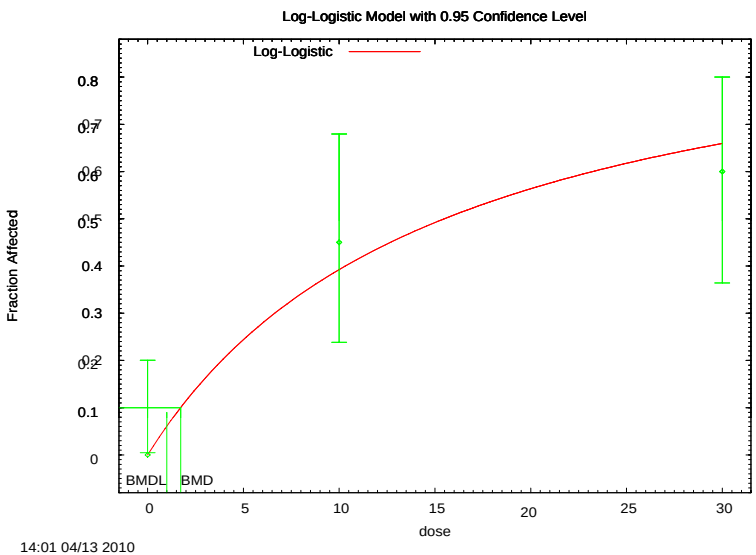

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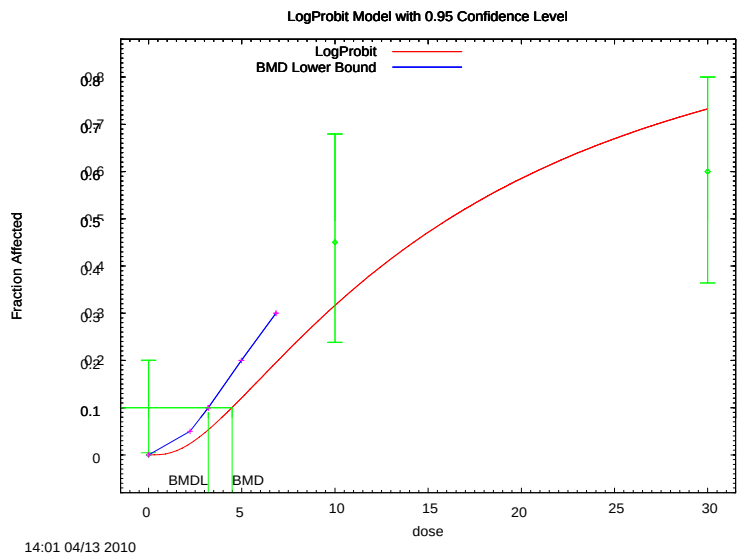

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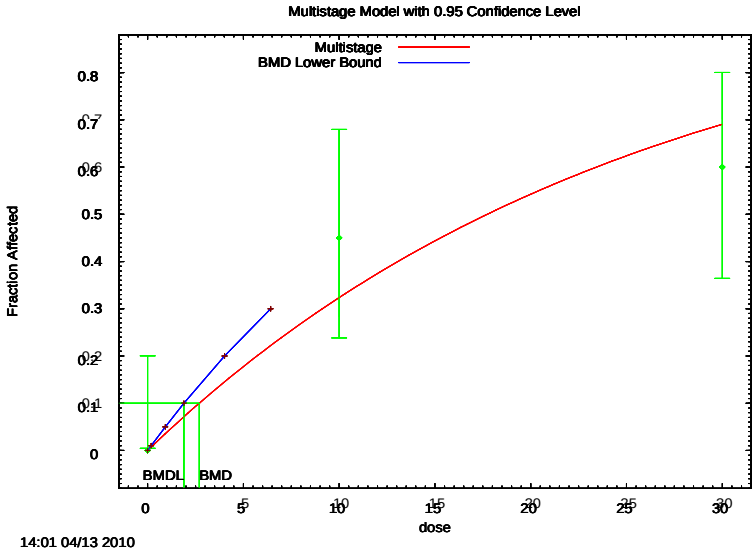

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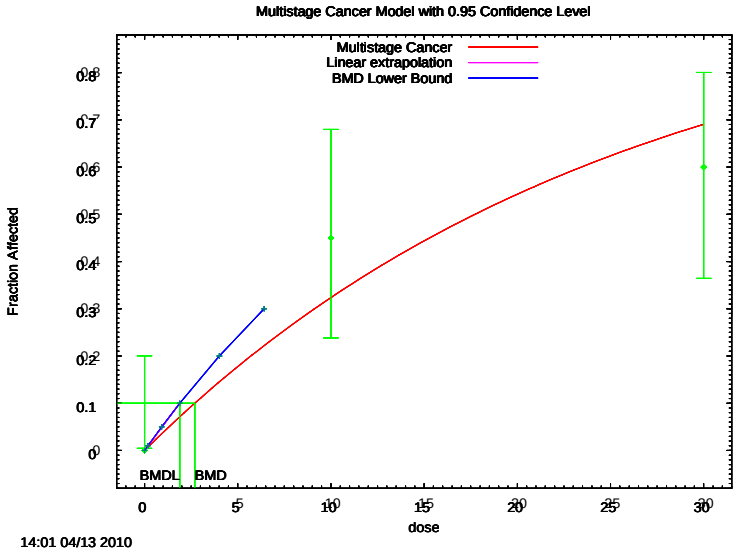

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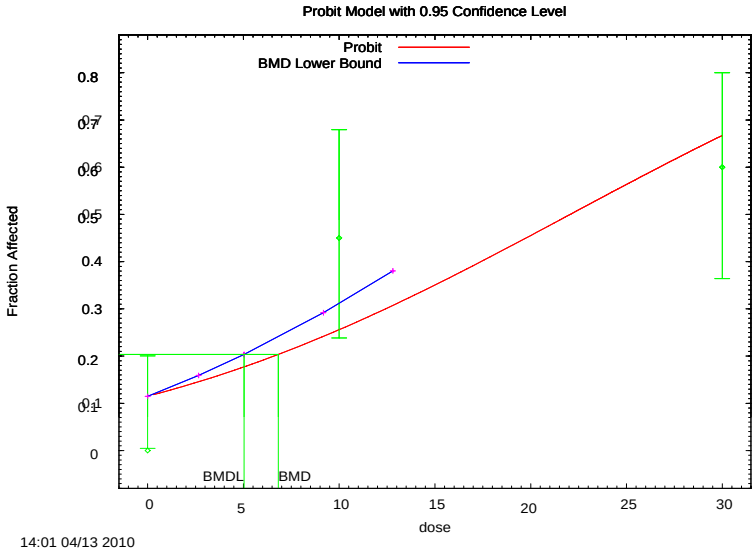

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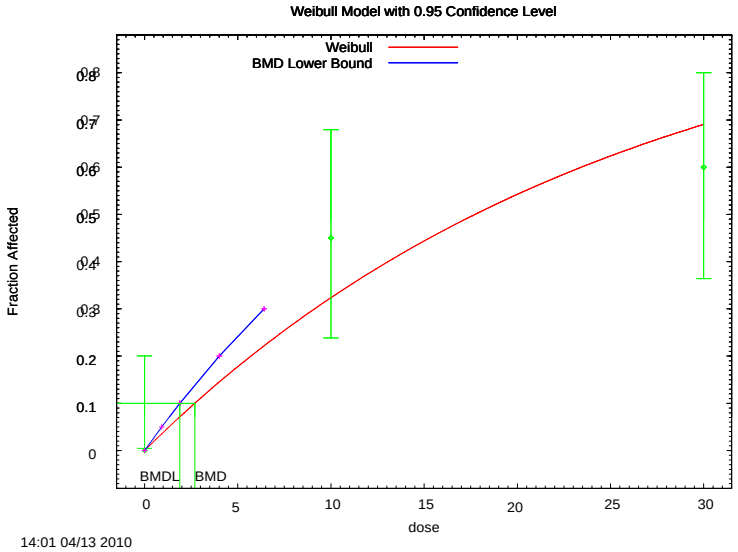

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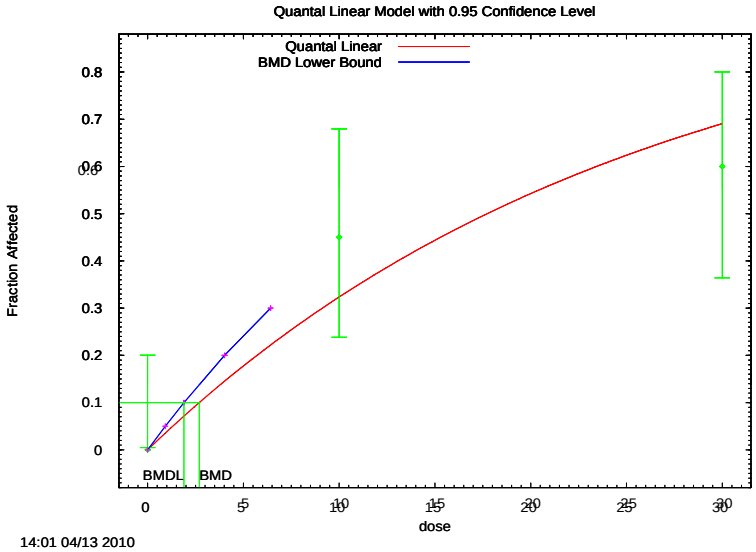

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Table C-9. LaVoie et al. (1982), benzo[j]fluoranthene, female mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (µg)		Notes
		Animal	Tissue		p-value for Chi-Square Test (2)	AIC (3)	Residuals (4)			BMD	BMDL	
							Scaled Residual of Interest	Maximum Scaled Residual				
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	Gamma	0.74	55	0	0.65	10%	11.6	7.9	Acceptable Fit
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	Logistic	**0.042**	62	1.5	1.5	10%	30.5	21.8	
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	LogLogistic	0.97	54	0	0.17	10%	8.5	4.9	Acceptable Fit: LogLogistic model has the lowest maxmimum scaled residuals, lowest AIC, and highest p-value.
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	LogProbit	0.38	56	1.0	1.0	10%	18.9	13.4	Acceptable Fit
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	Multistage	0.74	55	0	0.65	10%	11.6	7.9	Acceptable Fit
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	Multistage-Cancer	0.74	55	0	0.65	10%	11.6	7.9	Acceptable Fit
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	Probit	**0.048**	61	1.5	1.5	10%	28.3	20.6	
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	Weibull	0.74	55	0	0.65	10%	11.6	7.9	Acceptable Fit
LaVoie, 1982 without highest dose (1000 µg/mouse)	BjF	female mouse	Primarily squamous cell papilloma	Quantal-Linear	0.74	55	0	0.65	10%	11.6	7.9	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion

POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

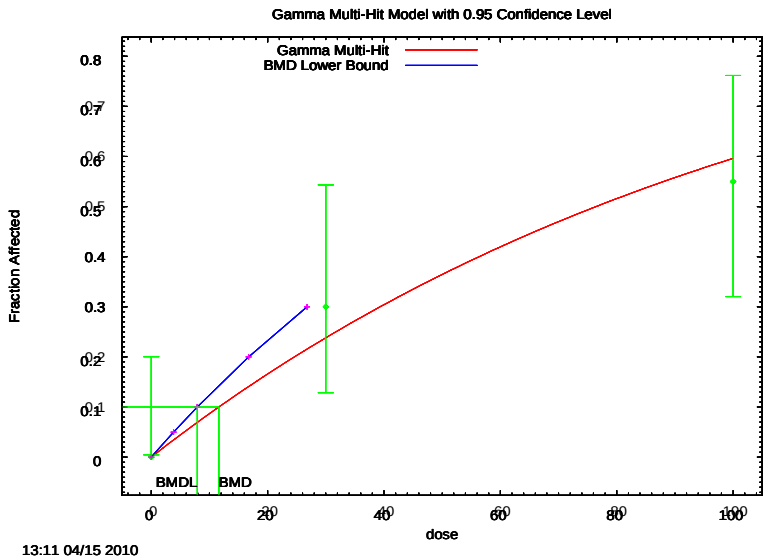
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. Highest dose of 1000 µg/mouse was omitted to improve model fits.

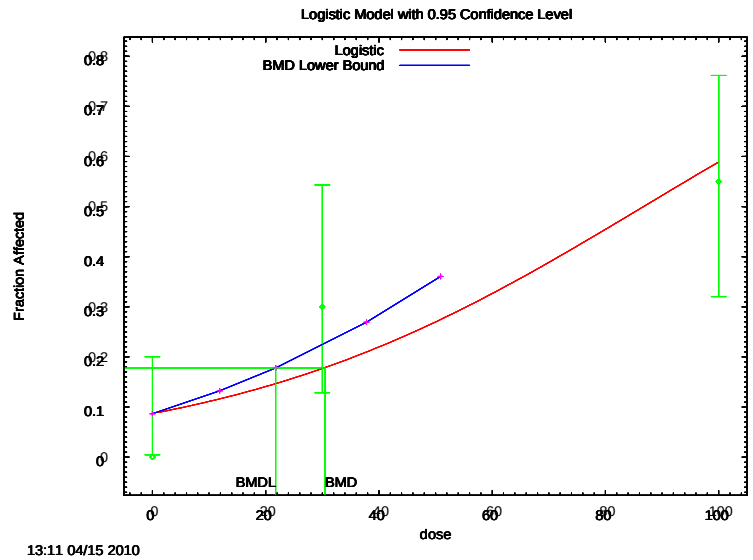
References

LaVoie, E.J., S. Amin, S.S. Hecht et al. 1982. Tumour initiating activity of dihydrodiols of benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene. Carcinogenesis 3:49–52.

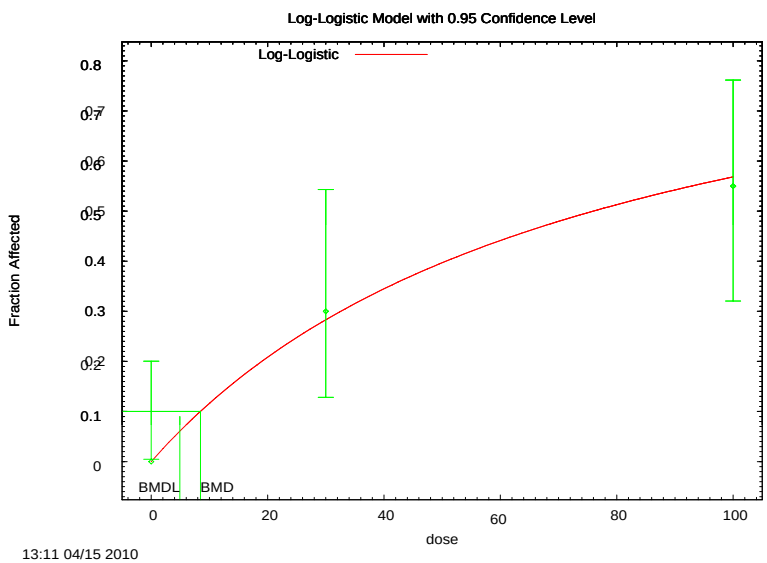
A: Gamma_LaVoie82_BjF_3doses_Dich_Gamma



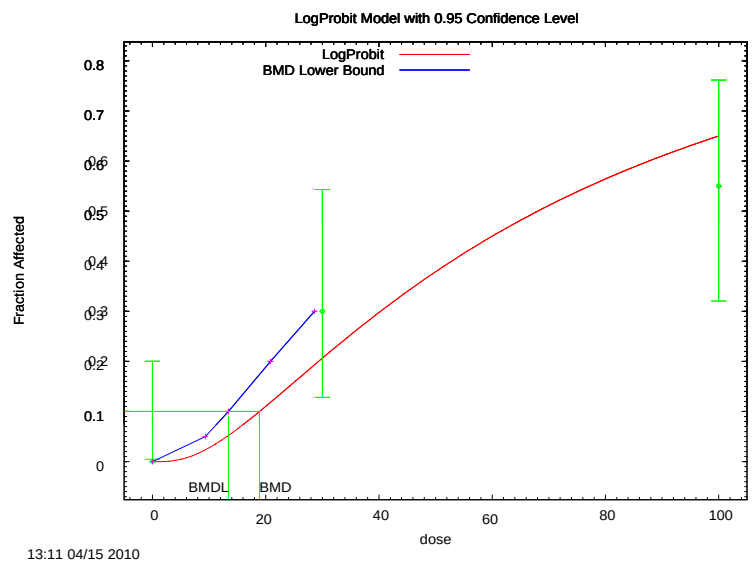
B: Logistic_LaVoie82_BjF_3doses_Dich_Logist



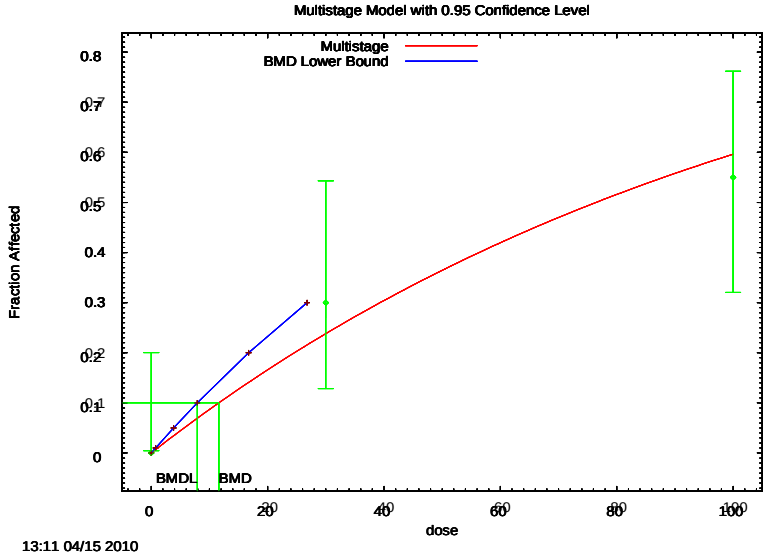
C: LogLogistic_LaVoie82_BjF_3doses_Dich_LogLogist



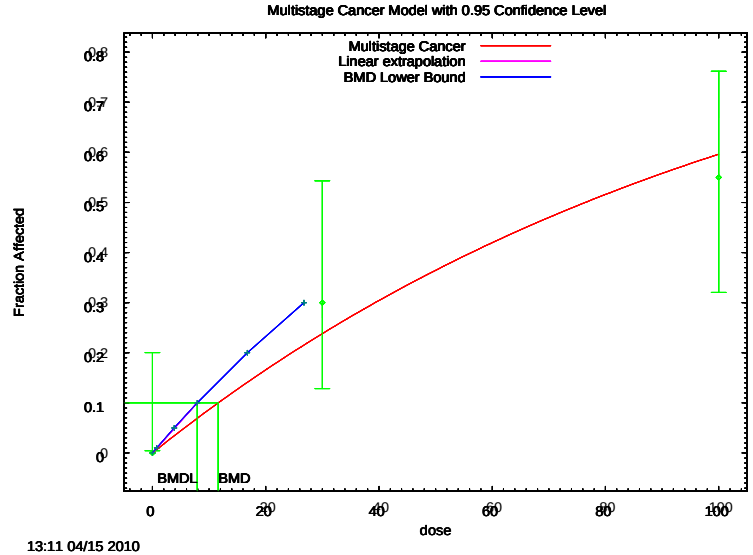
D: LogProbit_LaVoie82_BjF_3doses_Dich_LogProb



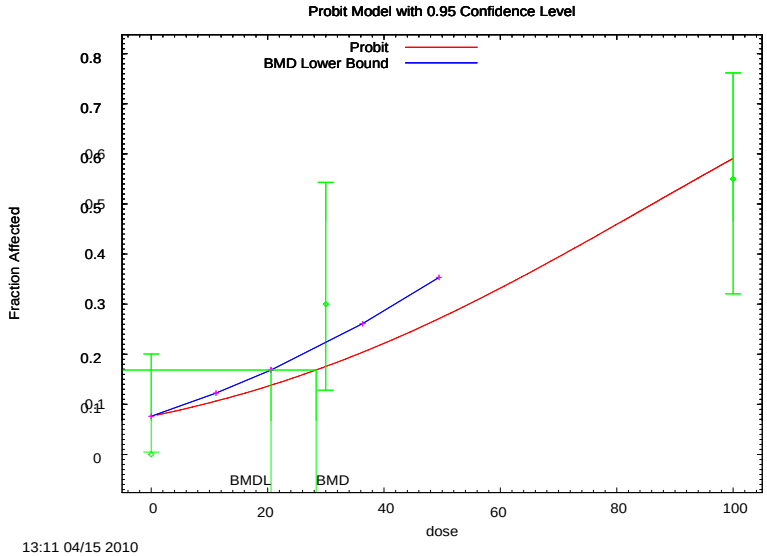
E: Multistage_LaVoie82_BjF_3doses_Dich_Multi



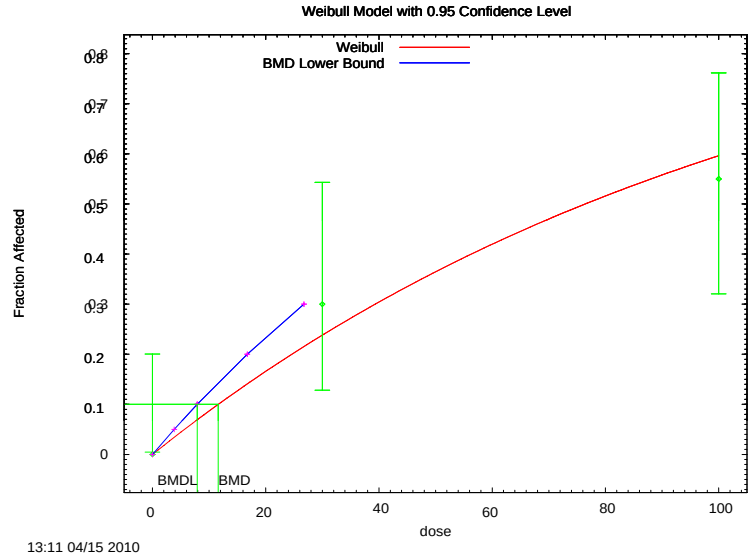
F: Multistage-Cancer_LaVoie82_BjF_3doses_Dich_MultiCanc



G: Probit_LaVoie82_BjF_3doses_Dich_Probit



H: Weibull_LaVoie82_BjF_3doses_Dich_Weibull



I: Quantal-Linear_LaVoie82_BjF_3doses_Dich_QntLin

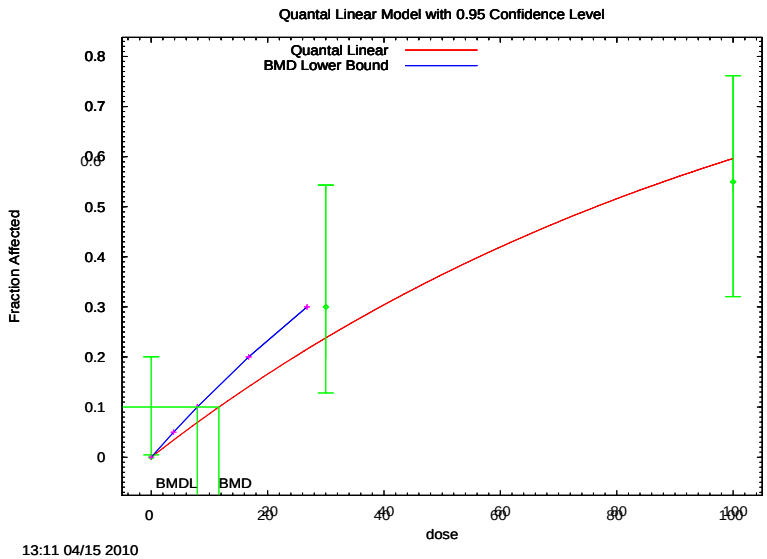


Table C-10. Rice et al. (1988), chrysene, female mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (µmol)		Notes
					p-value for Chi-Square Test (2)	AIC (3)	Residuals (4)					
		Animal	Tissue				Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	Gamma	NA	49	0	0	10%	0.10	0.040	Acceptable Fit
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	Logistic	1	48	0.21	-0.2	10%	0.10	0.069	Acceptable Fit
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	LogLogistic	NA	49	0	0	10%	0.11	0.06	Acceptable Fit
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	LogProbit	NA	49	0	0	10%	0.11	0.060	Acceptable Fit
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	Multistage	NA	49	0	0	10%	0.095	0.034	Acceptable Fit
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	Multistage-Cancer	NA	49	0	0	10%	0.095	0.034	Acceptable Fit
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	Probit	1	47	0.13	-0.1	10%	0.10	0.066	Acceptable Fit
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	Weibull	NA	49	0	0	10%	0.097	0.038	Acceptable Fit
Rice, 1988 without highest dose (1.5 µmol)	CH	female mouse	Unspecified tumor	Quantal-Linear	**0.074**	51	0.24	-1.4	10%	0.033	0.023	

Abbreviations

AIC = Akaike's Information Criterion

POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

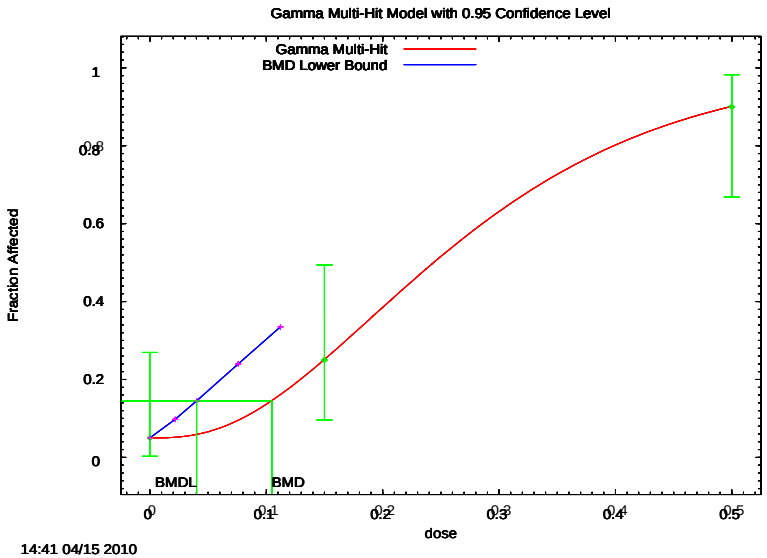
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. Highest dose of 1.5 µmol omitted to improve model fits.

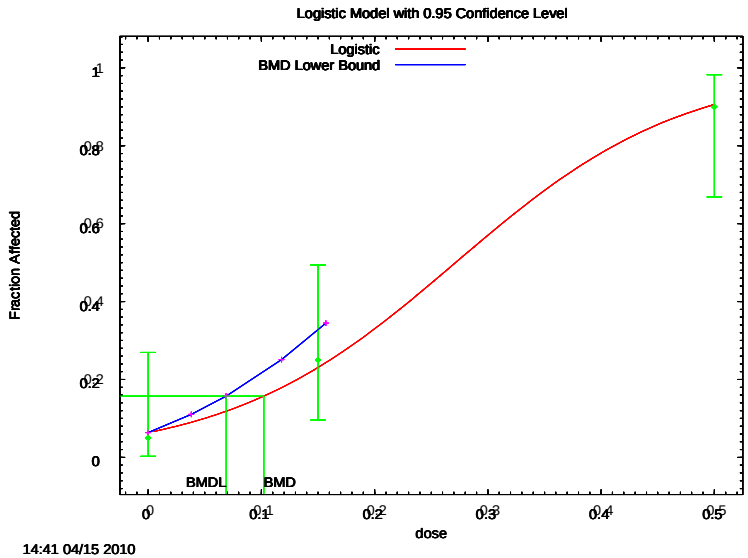
References

Rice, J.E., K. Jordan, P. Little et al. 1988. Comparative tumor-initiating activity of methylene-bridged and bay-region methylated derivatives of benz[a]anthracene and chrysene. Carcinogenesis 9:2275–2278.

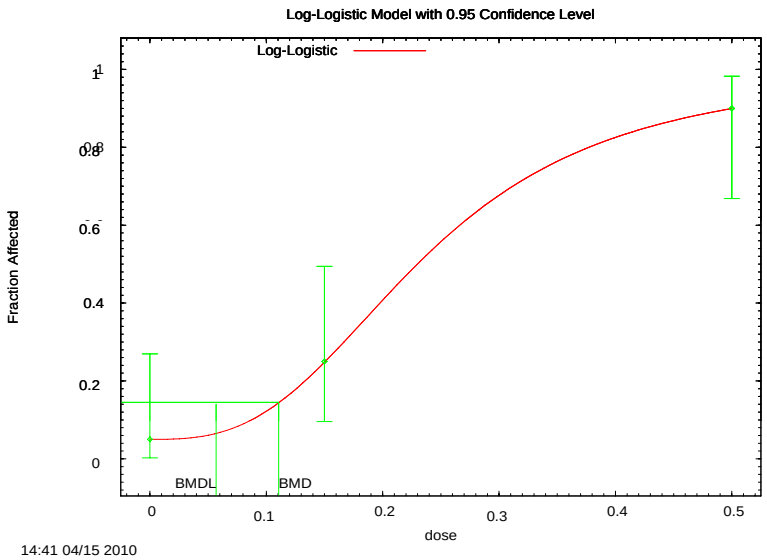
A: Gamma_Rice88_CH_3doses_Dich_Gamma



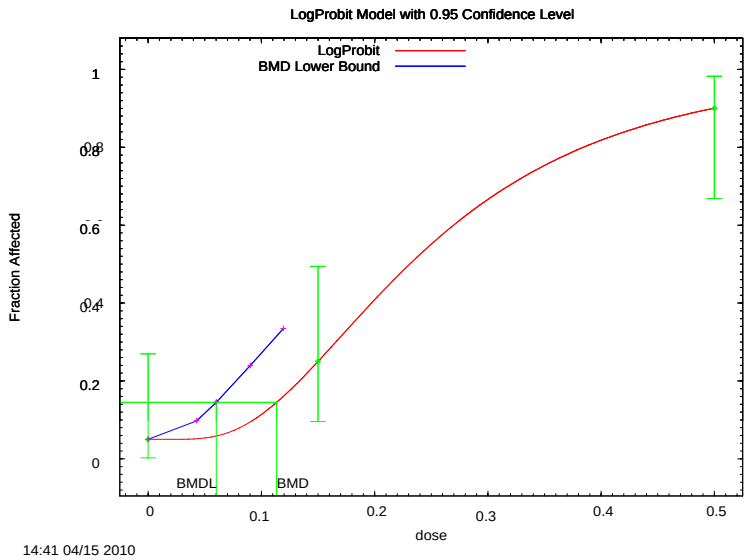
B: Logistic_Rice88_CH_3doses_Dich_Logist



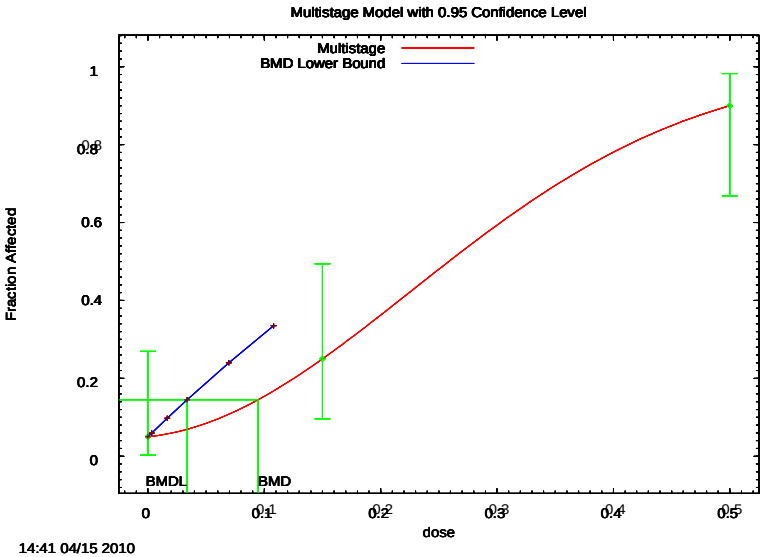
C: LogLogistic_Rice88_CH_3doses_Dich_LogLogist



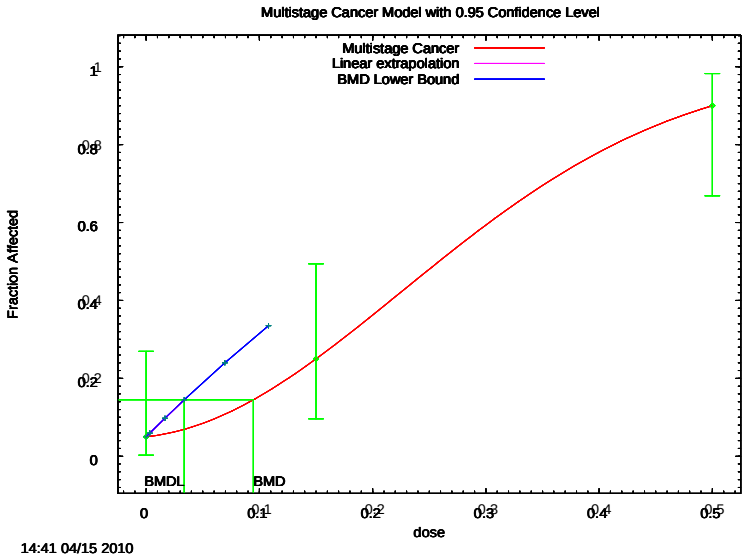
D: LogProbit_Rice88_CH_3doses_Dich_LogProb



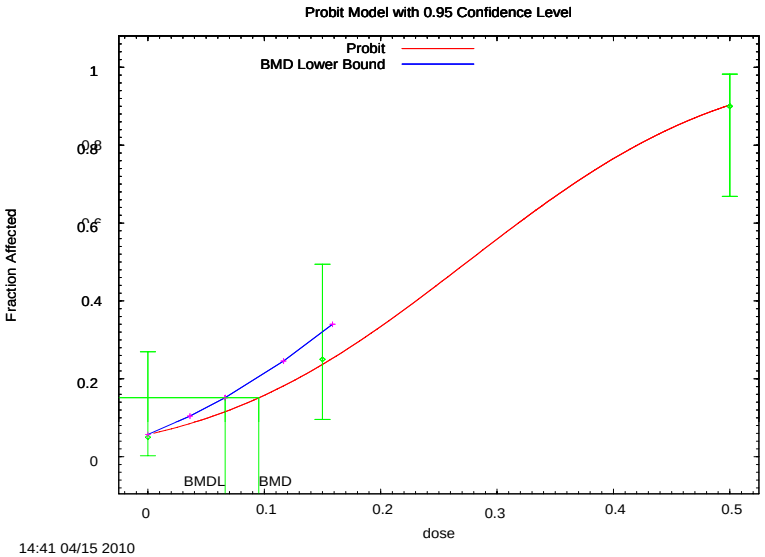
E: Multistage_Rice88_CH_3doses_Dich_Multi



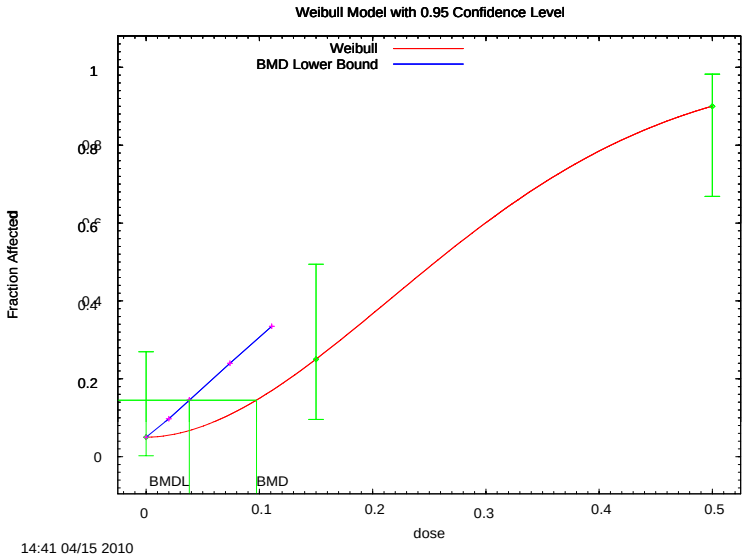
F: Multistage-Cancer_Rice88_CH_3doses_Dich_MultiCanc



G: Probit_Rice88_CH_3doses_Dich_Probit



H: Weibull_Rice88_CH_3doses_Dich_Weibull



I: Quantal-Linear_Rice88_CH_3doses_Dich_QntLin

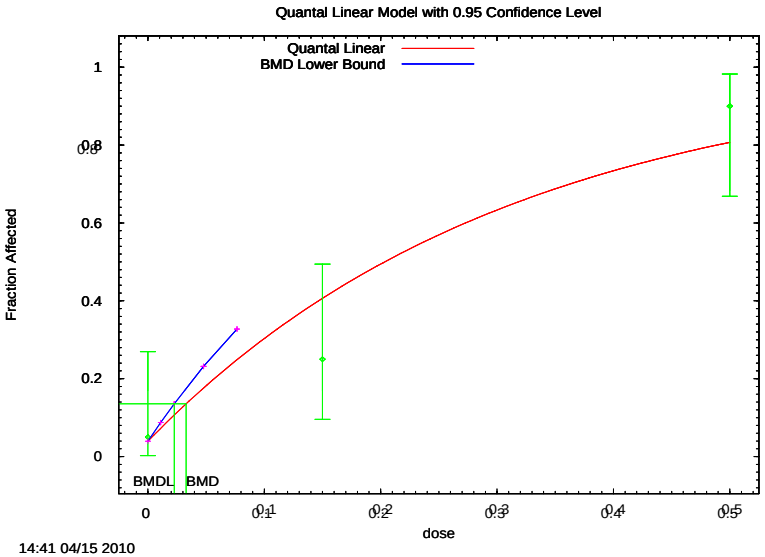


Table C-11. Rice et al. (1988), benz[b,c]aceanthrylene, female mice. Dichotomous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Dichotomous Dose-Response Model (1)	Goodness-of-Fit				POD (% Effect)	Dose (µmol)		Notes
					p-value for Chi-Square Test (2)	AIC (3)	Residuals (4)					
		Animal	Tissue				Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Rice, 1988	BbcAC	female mouse	Unspecified tumor	Gamma	**0.00020**	72	-0.86	**-3.1**	10%	0.10	0.074	
Rice, 1988	BbcAC	female mouse	Unspecified tumor	Logistic	**0.00010**	81	**2.5**	**-2.7**	10%	0.26	0.19	
Rice, 1988	BbcAC	female mouse	Unspecified tumor	LogLogistic	0.55	61	-0.021	-1.0	10%	0.026	0.013	Acceptable Fit:LogLogistic model has the lowest maxmimum scaled residuals, lowest AIC, and highest p-value.
Rice, 1988	BbcAC	female mouse	Unspecified tumor	LogProbit	**0.0011**	68	-0.25	**-3.2**	10%	0.13	0.087	
Rice, 1988	BbcAC	female mouse	Unspecified tumor	Multistage	**0.00020**	72	-0.86	**-3.1**	10%	0.10	0.074	
Rice, 1988	BbcAC	female mouse	Unspecified tumor	Multistage-Cancer	**0.00020**	72	-0.86	**-3.1**	10%	0.10	0.074	
Rice, 1988	BbcAC	female mouse	Unspecified tumor	Probit	**0**	82	**2.5**	**-2.9**	10%	0.30	0.23	
Rice, 1988	BbcAC	female mouse	Unspecified tumor	Weibull	**0.00020**	72	-0.86	**-3.1**	10%	0.10	0.074	
Rice, 1988	BbcAC	female mouse	Unspecified tumor	Quantal-Linear	**0.00020**	72	-0.86	**-3.1**	10%	0.10	0.074	

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

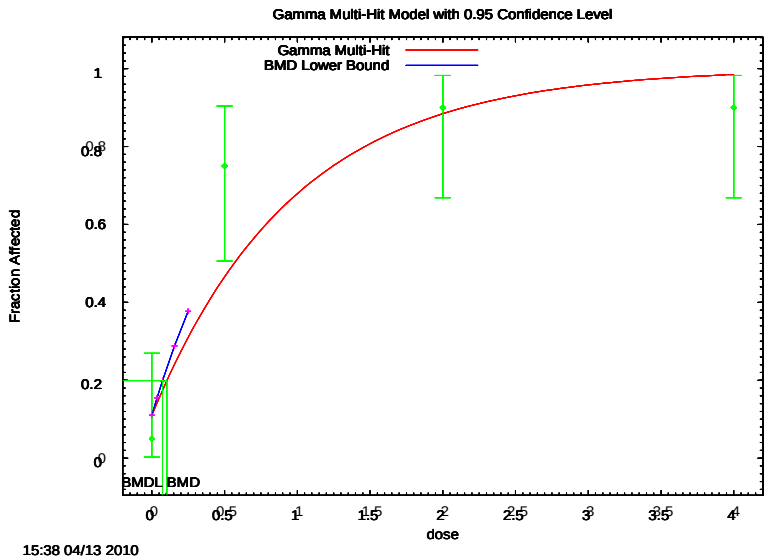
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.

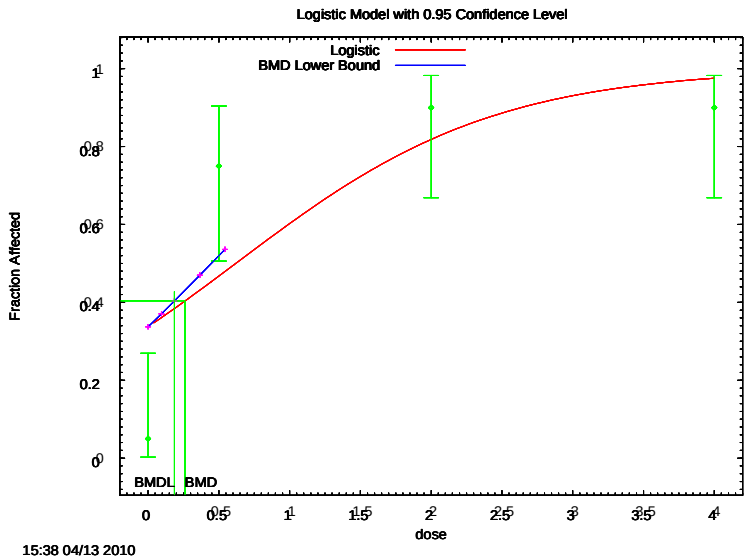
References

Rice, J.E., K. Jordan, P. Little et al. 1988. Comparative tumor-initiating activity of methylene-bridged and bay-region methylated derivatives of benz[a]anthracene and chrysene. Carcinogenesis 9:2275–2278.

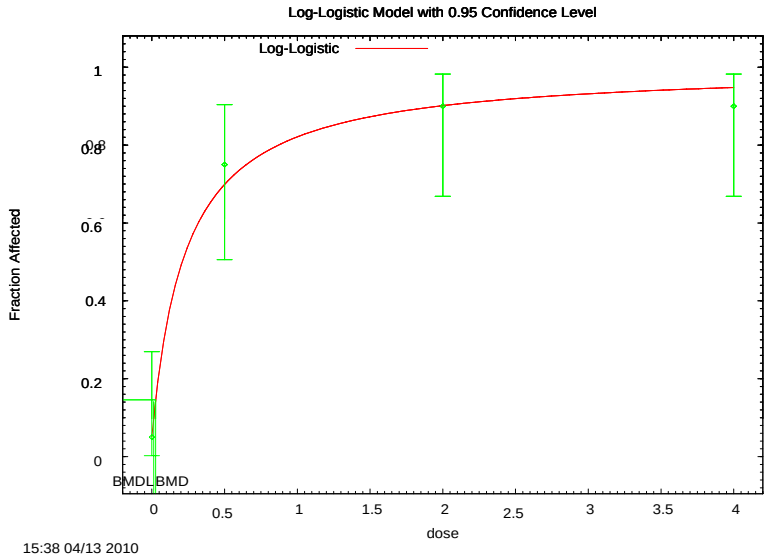
A: Gamma_Rice88_BbcAC_Dich_Gamma



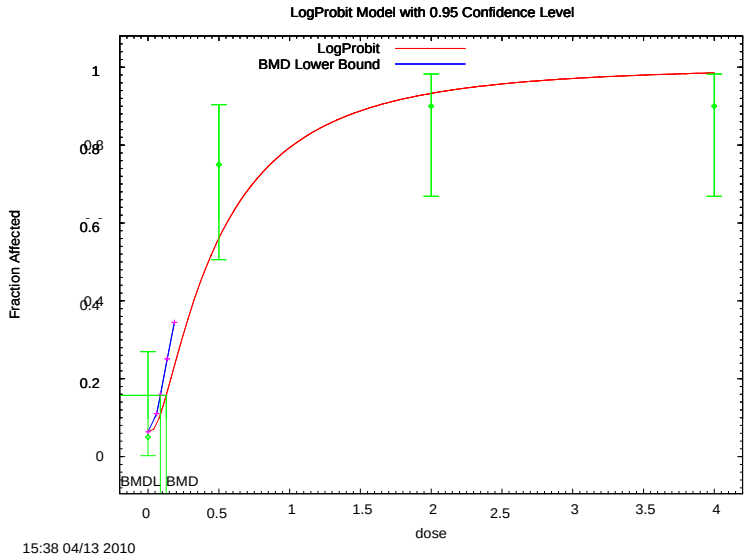
B: Logistic_Rice88_BbcAC_Dich_Logist



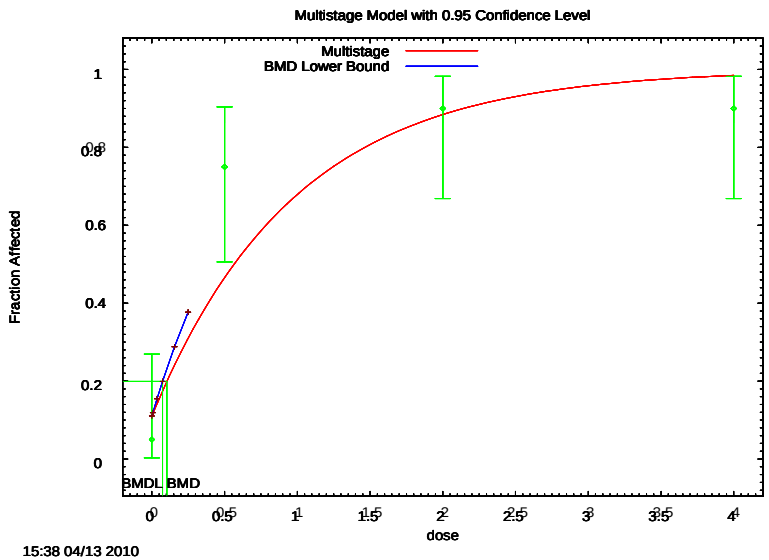
C: LogLogistic_Rice88_BbcAC_Dich_LogLogist



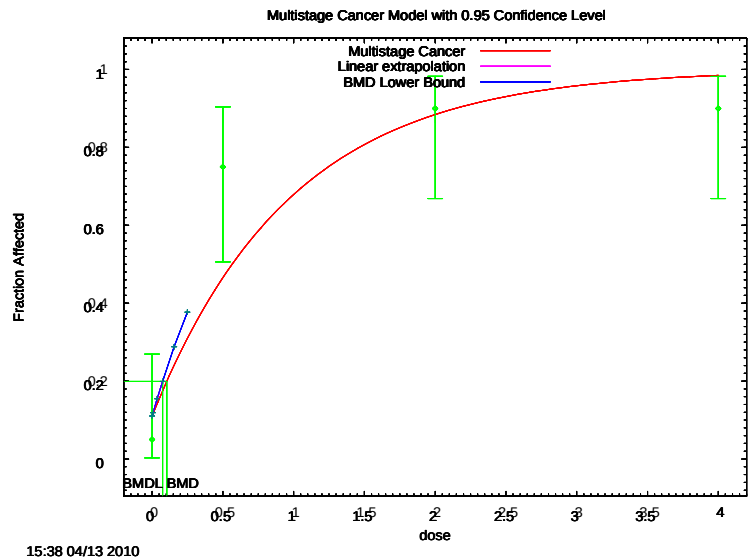
D: LogProbit_Rice88_BbcAC_Dich_LogProb



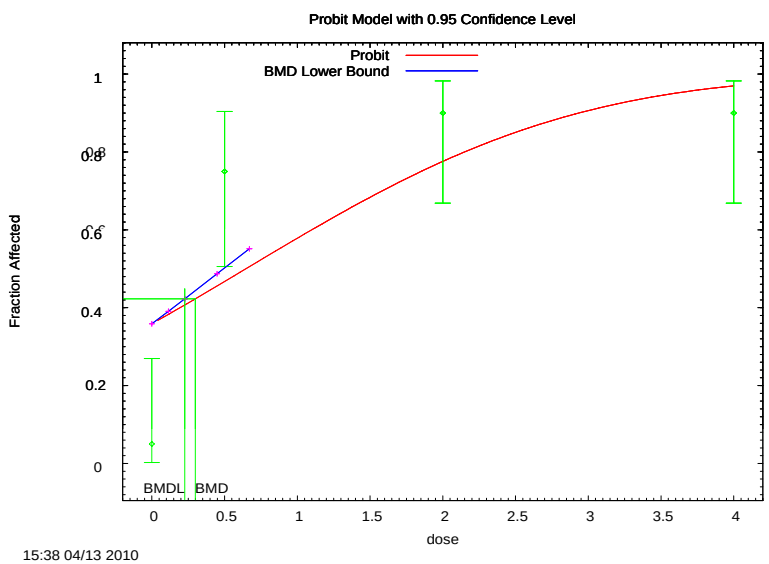
E: Multistage_Rice88_BbcAC_Dich_Multi



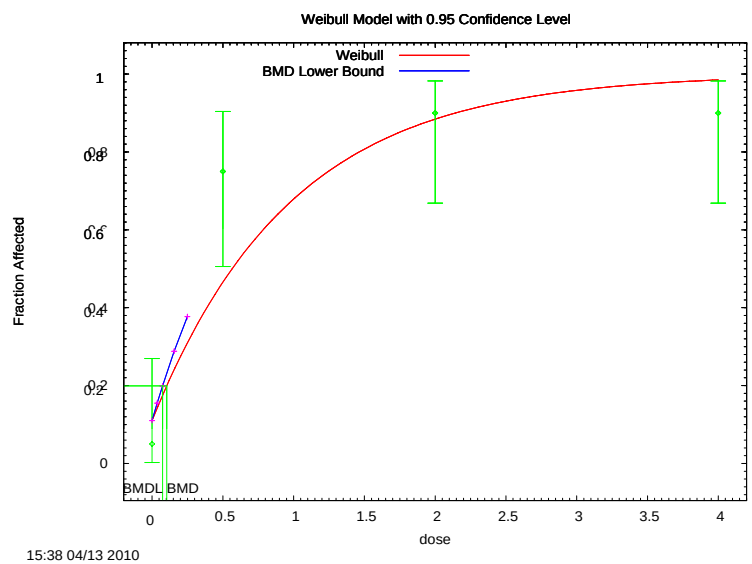
F: Multistage-Cancer_Rice88_BbcAC_Dich_MultiCanc



G: Probit_Rice88_BbcAC_Dich_Probit



H: Weibull_Rice88_BbcAC_Dich_Weibull



I: Quantal-Linear_Rice88_BbcAC_Dich_QntLin

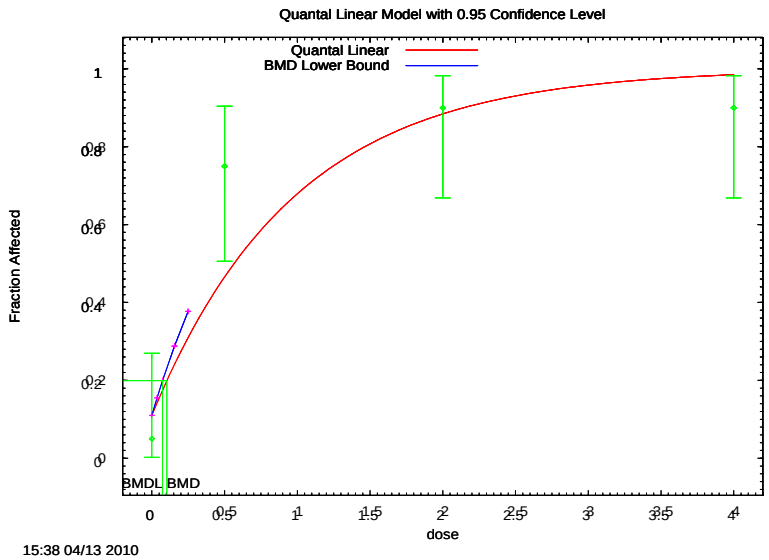


Table C-12. Busby et al. (1984), benzo[a]pyrene, male mouse. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness of Fit							POD Benchmark Response (BMR) (5)	Dose (mg-total)		Notes
					p-value for Maximum Likelihood Tests (2)					Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Busby, 1984	BaP	male mouse	Adenoma + carcinoma	Hill								1 SD			Hill model not applicable
Busby, 1984	BaP	male mouse	Adenoma + carcinoma	Exponential 2	< 0.0001	< 0.0001	< 0.0001	0.0712	361	-0.1	-1.3	1 SD	1.3	1.1	
Busby, 1984	BaP	male mouse	Adenoma + carcinoma	Exponential 3	< 0.0001	< 0.0001	< 0.0001	0.0712	361	-0.1	-1.3	1 SD	1.3	1.1	
Busby, 1984	BaP	male mouse	Adenoma + carcinoma	Exponential 4	< 0.0001	< 0.0001	< 0.0001	NA	360	4.9E-07	6.7E-07	1 SD	0.57	0.30	Acceptable Fit: Exponential 4 model has the lowest residuals and low AIC.
Busby, 1984	BaP	male mouse	Adenoma + carcinoma	Exponential 5								1 SD			Exponential 5 model not applicable
Busby, 1984	BaP	male mouse	Adenoma + carcinoma	Linear	<.0001	<.0001	<.0001	0.21	359	-0.22	1.0	1 SD	1.0	0.8	
Busby, 1984	BaP	male mouse	Adenoma + carcinoma	Polynomial	<.0001	<.0001	<.0001	NA	360	9.8E-11	5.5E-10	1 SD	0.51	0.26	Acceptable Fit: Polynomial model has the lowest residuals and low AIC.
Busby, 1984	BaP	male mouse	Adenoma + carcinoma	Power	<.0001	<.0001	<.0001	0.21	359	-0.22	0.97	1 SD	1.0	0.8	

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

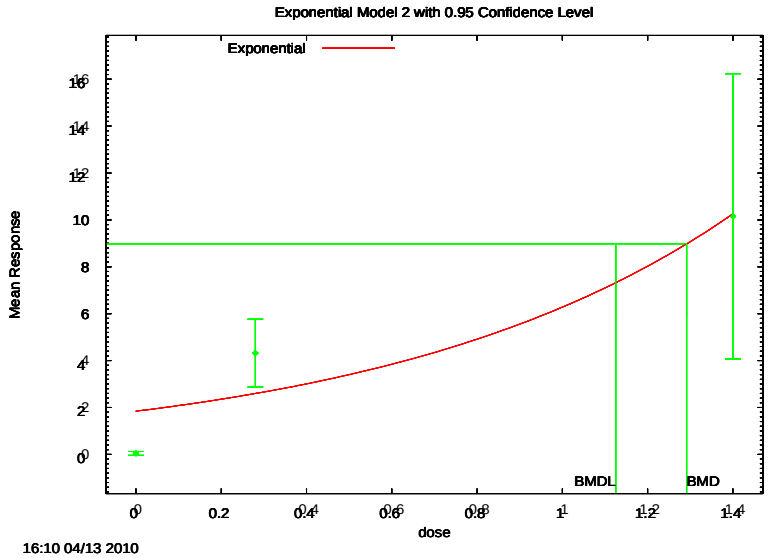
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.
   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.
   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.
   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.
   Test 4. Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.

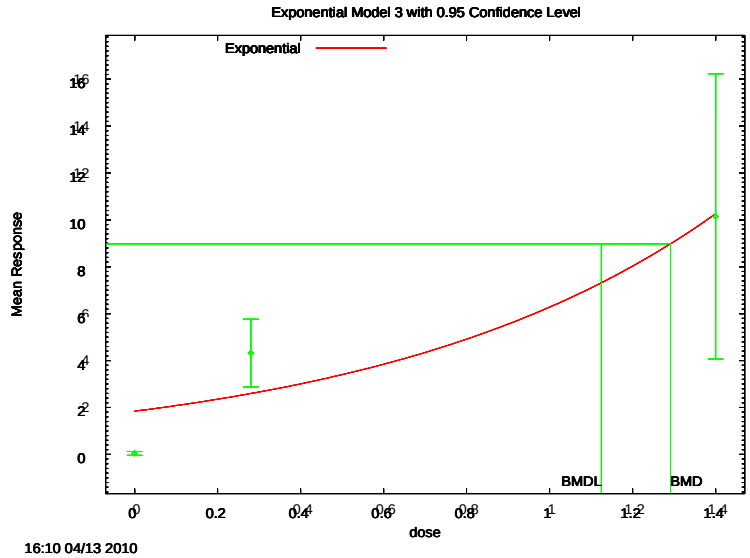
References

Busby, W.F.J., M.E. Goldman, P.M. Newberne et al. 1984. Tumorigenicity of fluoranthene in a newborn mouse lung adenoma bioassay. Carcinogenesis 5:1311–1316.

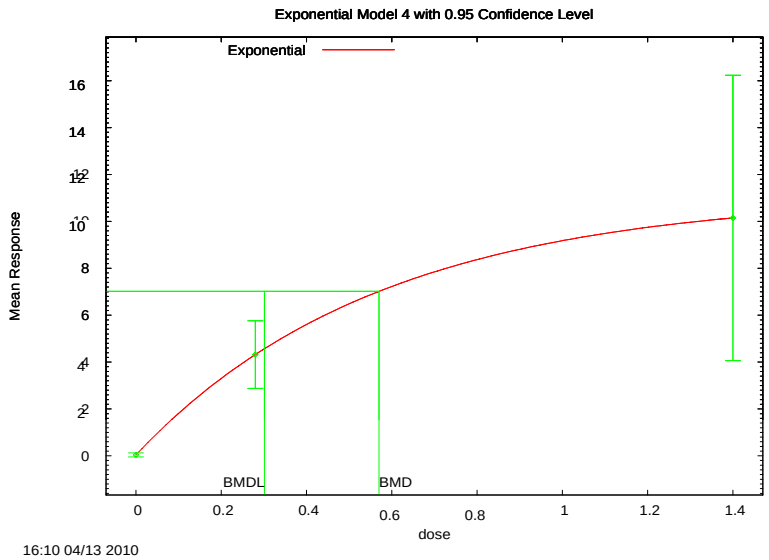
A: Exponential2_Busby84_BaP_M_Cont_Exp



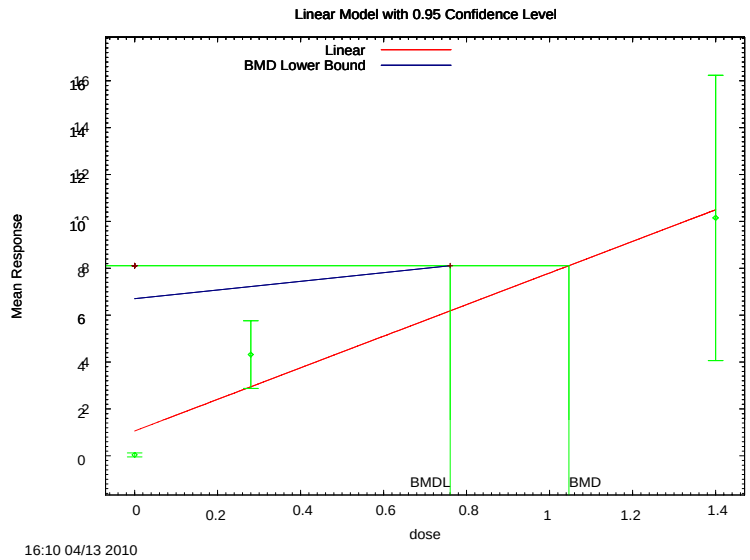
B: Exponential3_Busby84_BaP_M_Cont_Exp



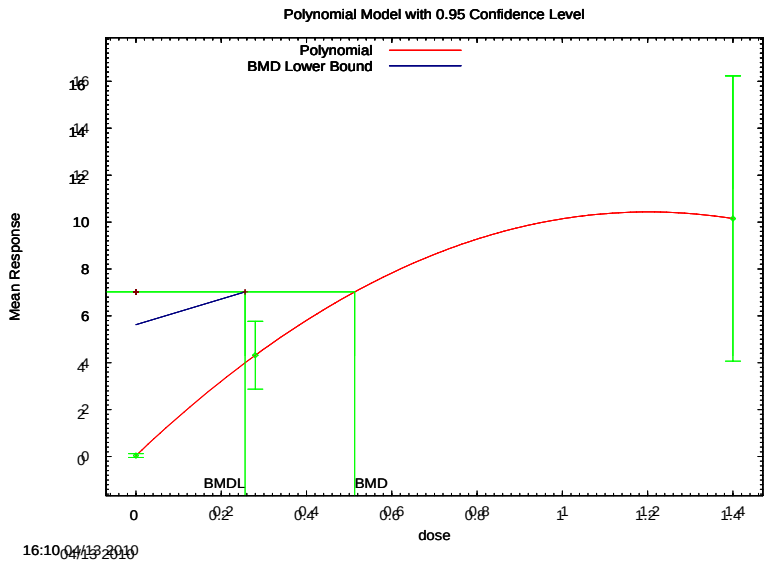
C: Exponential4_Busby84_BaP_M_Cont_Exp



D: Linear_Busby84_BaP_M_Cont_Lin



E: Polynomial_Busby84_BaP_M_Cont_Poly



F: Power_Busby84_BaP_M_Cont_Pow

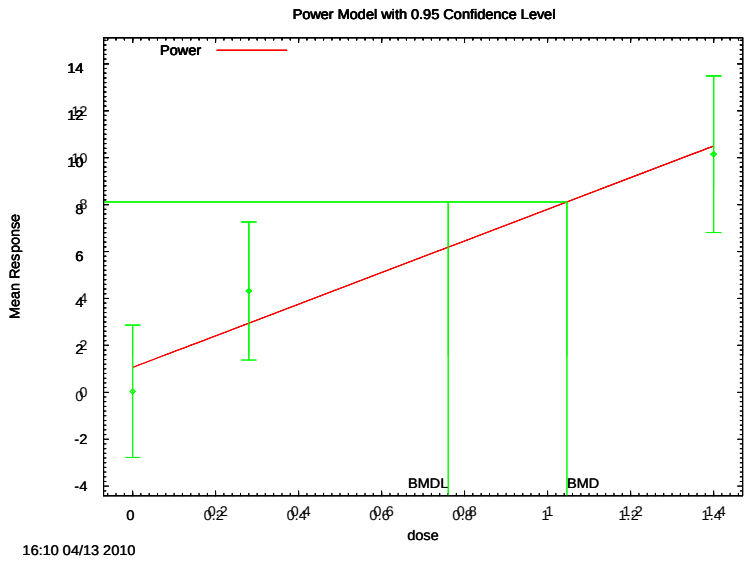


Table C-13. Busby et al. (1984), benzo[a]pyrene, female mouse. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness-of-Fit							POD Benchmark Response (BMR) (5)	Dose (mg-total)		Notes
					p-value for Maximum Likelihood Tests (2)					Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Busby, 1984	BaP	female mouse	Adenoma + carcinoma	Hill								1 SD			Hill model not applicable
Busby, 1984	BaP	female mouse	Adenoma + carcinoma	Exponential 2	< 0.0001	< 0.0001	< 0.0001	0.0002	277	-0.25	**2.5**	1 SD	1.6	1.3	
Busby, 1984	BaP	female mouse	Adenoma + carcinoma	Exponential 3	< 0.0001	< 0.0001	< 0.0001	0.0002	277	-0.25	**2.5**	1 SD	1.6	1.3	
Busby, 1984	BaP	female mouse	Adenoma + carcinoma	Exponential 4	< 0.0001	< 0.0001	< 0.0001	N/A	266	3.4E-06	5.9E-06	1 SD	0.20	0.0010	Acceptable Fit; Exponential 4 model has the lowest residuals and low AIC.
Busby, 1984	BaP	female mouse	Adenoma + carcinoma	Exponential 5								1 SD			Exponential 5 model not applicable
Busby, 1984	BaP	female mouse	Adenoma + carcinoma	Linear	<.0001	<.0001	<.0001	6.2E-04	276	-0.54	**2.6**	1 SD	1.46	0.99	
Busby, 1984	BaP	female mouse	Adenoma + carcinoma	Polynomial	<.0001	<.0001	<.0001	NA	266	4.3E-10	1.6E-09	1 SD	0.24	0.16	Acceptable Fit:Polynomial model has the lowest residuals and low AIC.
Busby, 1984	BaP	female mouse	Adenoma + carcinoma	Power	<.0001	<.0001	<.0001	6.2E-04	276	-0.54	**2.56**	1 SD	1.46	0.99	

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

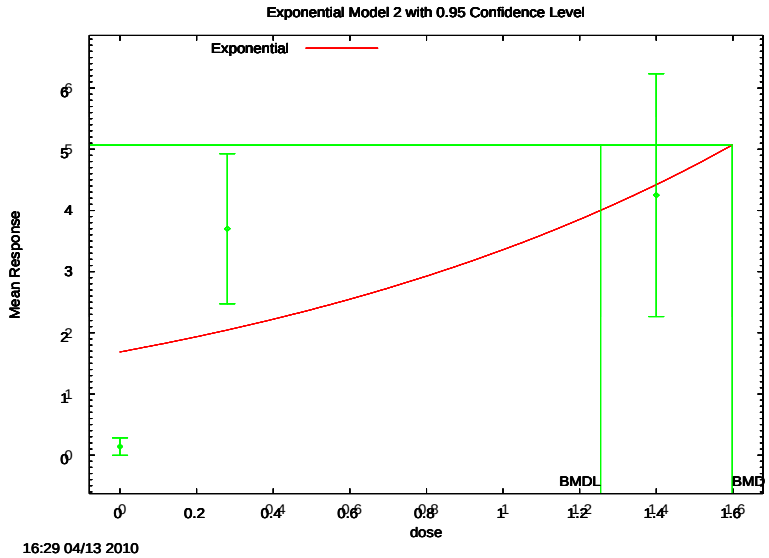
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.
   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.
   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.
   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.
   Test 4. (non-constant variance model). Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.

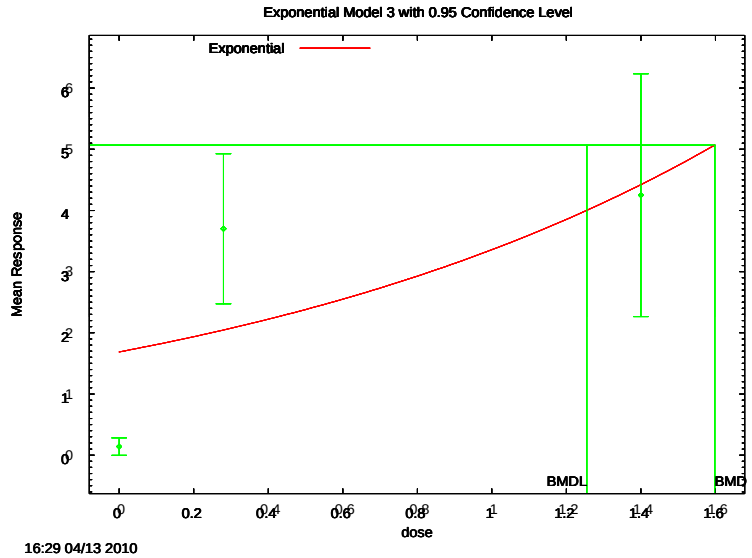
References

Busby, W.F.J., M.E. Goldman, P.M. Newberne et al. 1984. Tumorigenicity of fluoranthene in a newborn mouse lung adenoma bioassay. Carcinogenesis 5:1311–1316.

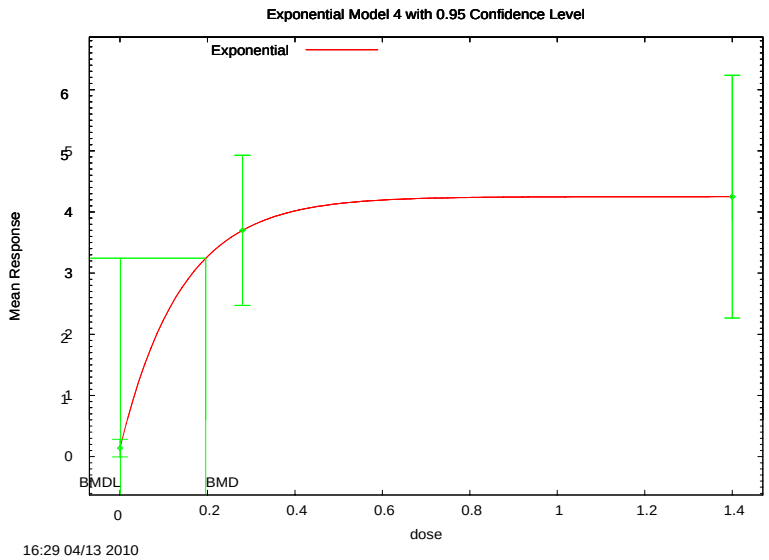
A: Exponential2_Busby84_BaP_F_Cont_Exp



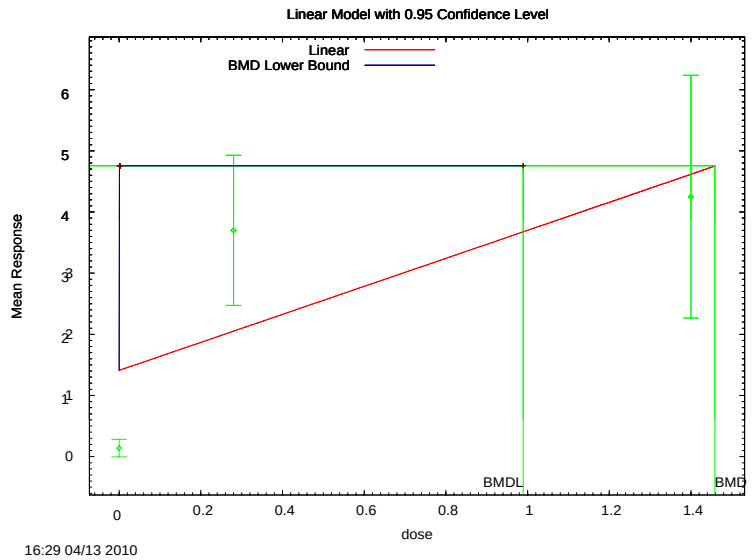
B: Exponential3_Busby84_BaP_F_Cont_Exp



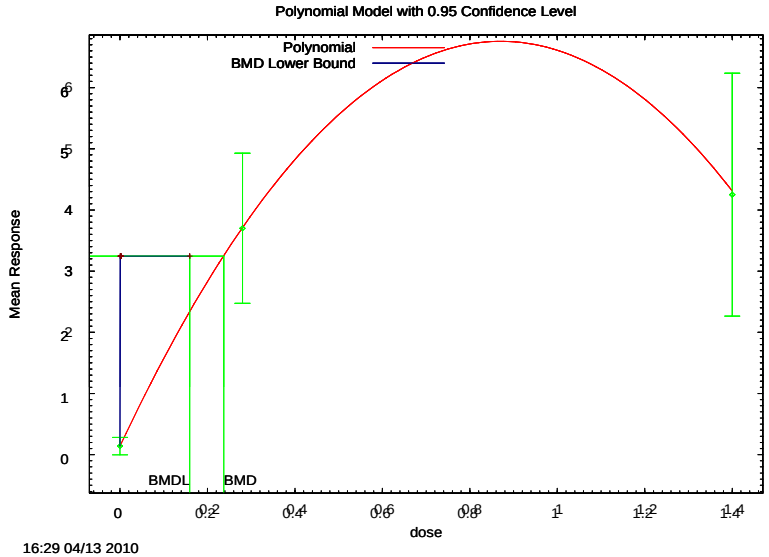
C: Exponential4_Busby84_BaP_F_Cont_Exp



D: Linear_Busby84_BaP_F_Cont_Lin



E: Polynomial_Busby84_BaP_F_Cont_Poly



F: Power_Busby84_BaP_F_Cont_Pow

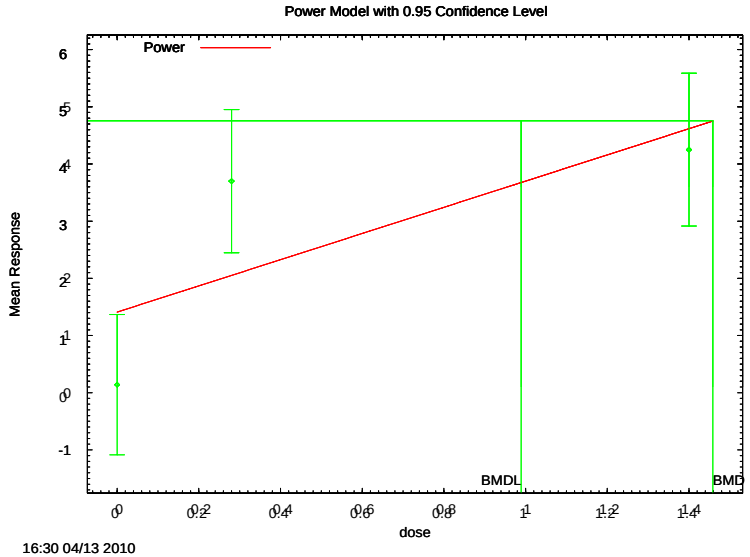


Table C-14. Busby et al. (1984), fluoranthene, male mouse. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness-of-Fit							POD Benchmark Response (BMR) (5)	Dose (mg-total)		Notes
					p-value for Maximum Likelihood Tests (2)					Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Busby, 1984	FA	male mouse	Adenoma + carcinoma	Hill											Hill model not applicable
Busby, 1984	FA	male mouse	Adenoma + carcinoma	Exponential 2	< 0.0001	< 0.0001	< 0.0001	0.55	100	-0.011	-0.49	1 SD	3.1	2.9	Acceptable Fit
Busby, 1984	FA	male mouse	Adenoma + carcinoma	Exponential 3	< 0.0001	< 0.0001	< 0.0001	0.55	100	-0.011	-0.49	1 SD	3.1	2.9	Acceptable Fit
Busby, 1984	FA	male mouse	Adenoma + carcinoma	Exponential 4	< 0.0001	< 0.0001	< 0.0001	N/A	102	2.9E-02	-0.14	1 SD	2.5	1.9	Acceptable Fit
Busby, 1984	FA	male mouse	Adenoma + carcinoma	Exponential 5											Exponential 5 model not applicable
Busby, 1984	FA	male mouse	Adenoma + carcinoma	Linear	<.0001	<.0001	<.0001	0.86	100	0.029	-0.14	1 SD	2.5	1.9	Acceptable Fit
Busby, 1984	FA	male mouse	Adenoma + carcinoma	Polynomial	<.0001	<.0001	<.0001	NA	102	8.0E-11	8.0E-11	1 SD	2.6	1.1	Acceptable Fit
Busby, 1984	FA	male mouse	Adenoma + carcinoma	Power	<.0001	<.0001	<.0001	NA	102	0	0	1 SD	2.6	1.9	Acceptable Fit:Power model has the lowest residuals.

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

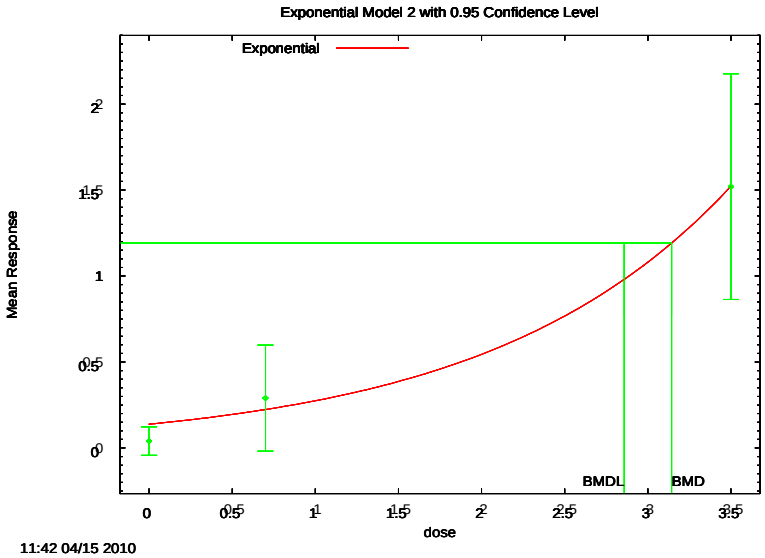
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.
   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.
   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.
   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.
   Test 4. (non-constant variance model) Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.

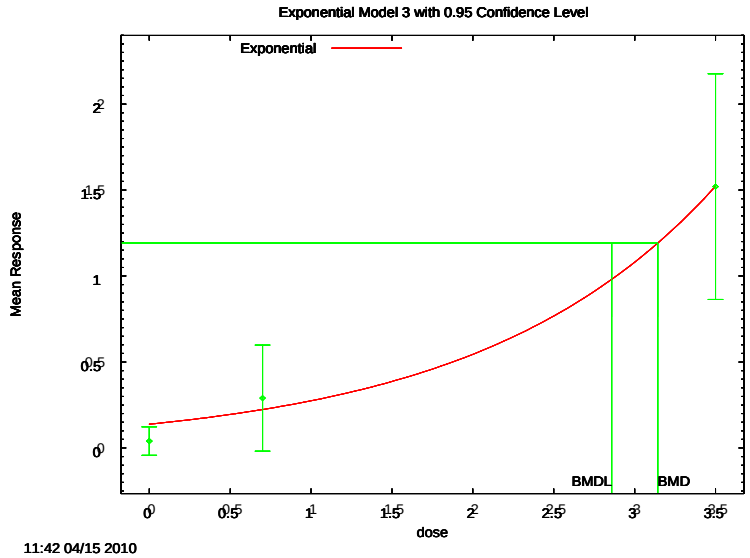
References

Busby, W.F.J., M.E. Goldman, P.M. Newberne et al. 1984. Tumorigenicity of fluoranthene in a newborn mouse lung adenoma bioassay. Carcinogenesis 5:1311–1316.

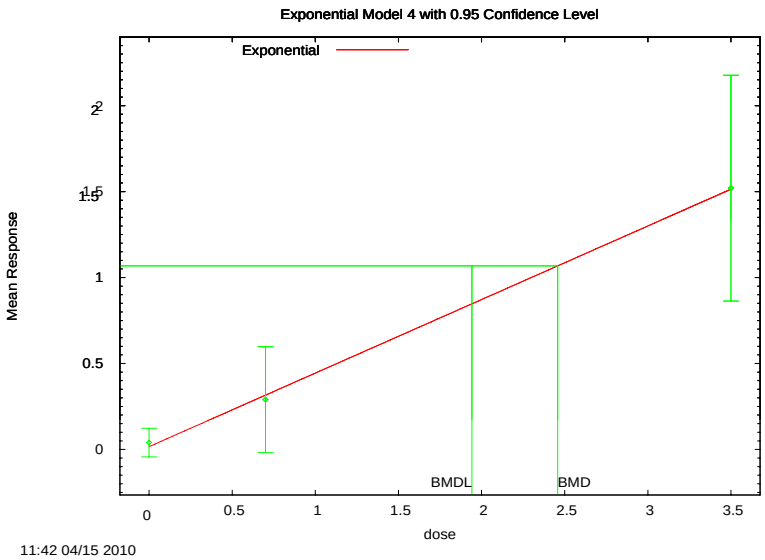
A: Exponential2_Busby84_FA_M_Cont_Exp



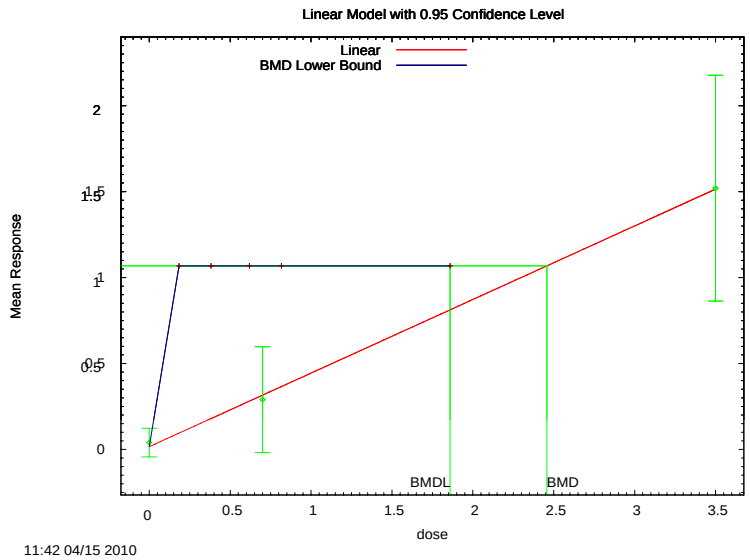
B: Exponential3_Busby84_FA_M_Cont_Exp



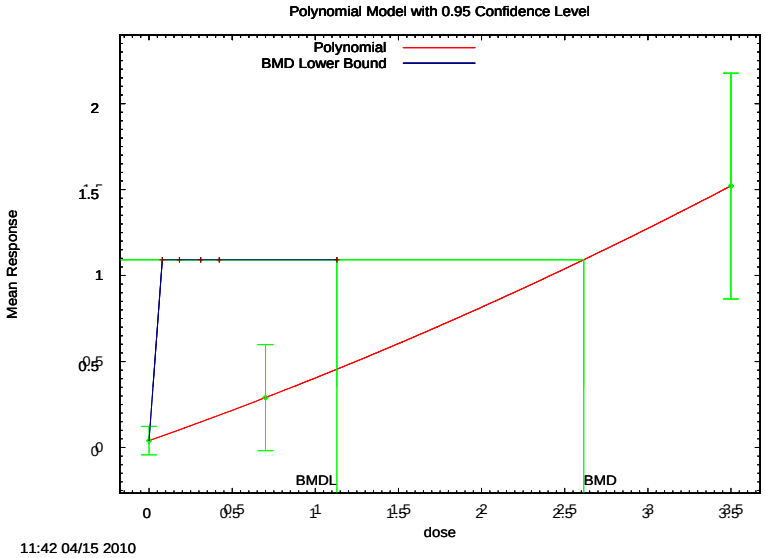
C: Exponential4_Busby84_FA_M_Cont_Exp



D: Linear_Busby84_FA_M_Cont_Lin



E: Polynomial_Busby84_FA_M_Cont_Poly



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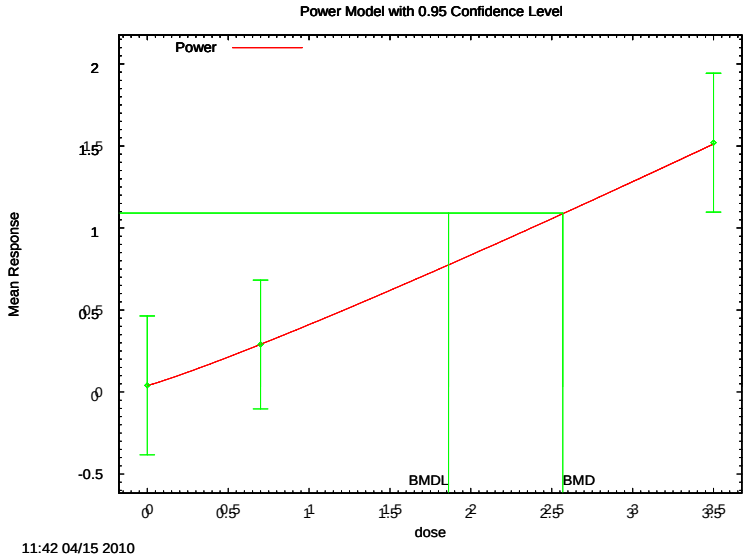

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Table C-15. Busby et al. (1984), fluoranthene, female mouse. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness-of-Fit							POD Benchmark Response (BMR) (5)	Dose (mg-total)		Notes
					p-value for Maximum Likelihood Tests (2)					Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Busby, 1984	FA	female mouse	Adenoma + carcinoma	Hill											Hill model not applicable
Busby, 1984	FA	female mouse	Adenoma + carcinoma	Exponential 2	1.9E-04	3.7E-04	3.7E-04	0.85	-4.8	0.0089	-0.14	1 SD	4.2	3.3	Acceptable Fit
Busby, 1984	FA	female mouse	Adenoma + carcinoma	Exponential 3	1.9E-04	3.7E-04	3.7E-04	N/A	-2.8	9.9E-10	2.8E-09	1 SD	3.9	3.3	Acceptable Fit
Busby, 1984	FA	female mouse	Adenoma + carcinoma	Exponential 4	1.9E-04	3.7E-04	3.7E-04	N/A	-2.6	0.069	-0.35	1 SD	5.0	3.0	Acceptable Fit
Busby, 1984	FA	female mouse	Adenoma + carcinoma	Exponential 5											Exponential 5 model not applicable
Busby, 1984	FA	female mouse	Adenoma + carcinoma	Linear	1.9E-04	3.7E-04	3.7E-04	0.67	-4.6	0.069	-0.35	1 SD	5.0	3.0	Acceptable Fit
Busby, 1984	FA	female mouse	Adenoma + carcinoma	Polynomial	1.9E-04	3.7E-04	3.7E-04	NA	-2.8	5.5E-11	5.5E-11	1 SD	4.2	2.0	Acceptable Fit
Busby, 1984	FA	female mouse	Adenoma + carcinoma	Power	1.9E-04	3.7E-04	3.7E-04	NA	-2.8	0	0	1 SD	4.2	3.0	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

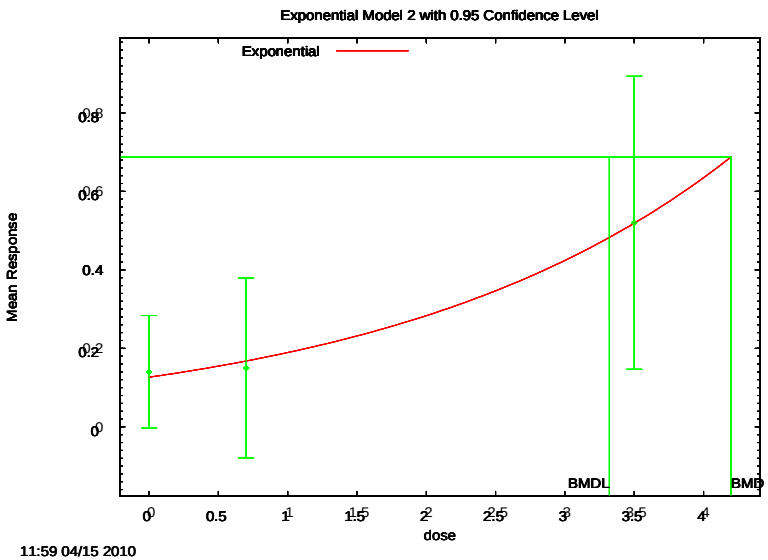
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.
   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.
   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.
   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.
   Test 4. (non-constant variance model). Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.

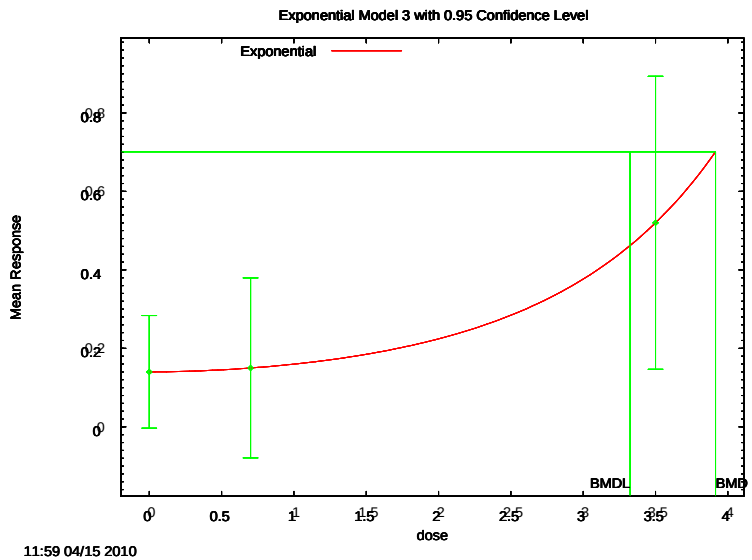
References

Busby, W.F.J., M.E. Goldman, P.M. Newberne et al. 1984. Tumorigenicity of fluoranthene in a newborn mouse lung adenoma bioassay. Carcinogenesis 5:1311–1316.

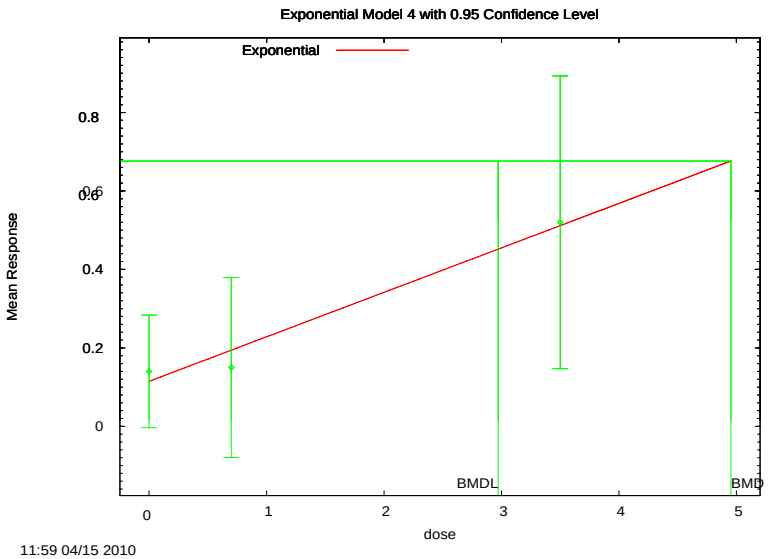
A: Exponential2_Busby84_FA_F_Cont_Exp



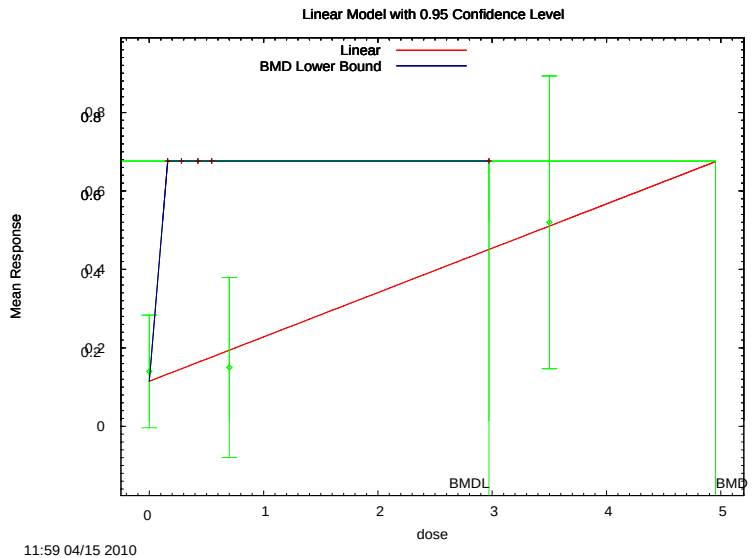
B: Exponential3_Busby84_FA_F_Cont_Exp



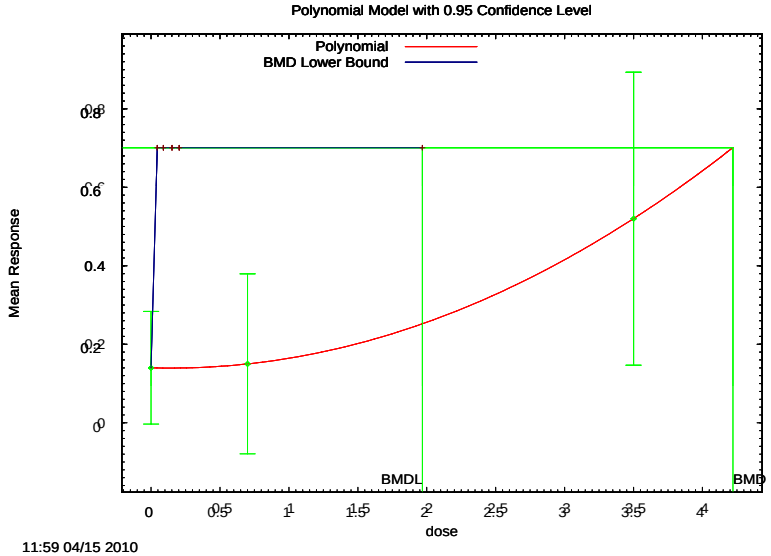
C: Exponential4_Busby84_FA_F_Cont_Exp



D: Linear_Busby84_FA_F_Cont_Lin



E: Polynomial_Busby84_FA_F_Cont_Poly



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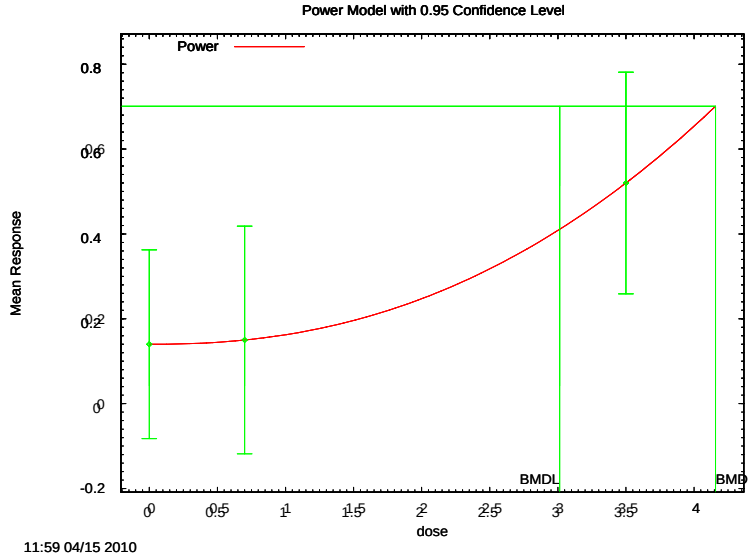

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Table C-16. Nesnow et al. (1998), benzo[a]pyrene. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness-of-Fit							POD Benchmark Response (BMR) (5)	Dose (mg/kg)		Notes
					p-value for Maximum Likelihood Tests (2)					Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Nesnow, 1998	BaP	mouse	Lung tumors	Hill	<.0001	<.0001	<.0001	1.0	497	0.026	0.10	1 SD	57.3	42.6	Best Fit: Hill model has the lowest residuals, the highest p-value in test 4, and the 2nd lowest AIC.
Nesnow, 1998	BaP	mouse	Lung tumors	Exponential 2	< 0.0001	< 0.0001	< 0.0001	**0.0013**	511	**3.0**	**3.0**	1 SD	89.9	82.6	
Nesnow, 1998	BaP	mouse	Lung tumors	Exponential 3	< 0.0001	< 0.0001	< 0.0001	**0.0013**	511	**3.0**	**3.0**	1 SD	89.9	82.6	
Nesnow, 1998	BaP	mouse	Lung tumors	Exponential 4	< 0.0001	< 0.0001	< 0.0001	**< 0.0001**	518	**-4.5**	**23**	1 SD	32.8	28.3	
Nesnow, 1998	BaP	mouse	Lung tumors	Exponential 5	< 0.0001	< 0.0001	< 0.0001	0.87	498	-0.34	-0.34	1 SD	53.6	43.0	Acceptable Fit
Nesnow, 1998	BaP	mouse	Lung tumors	Linear	<.0001	<.0001	<.0001	**0.010**	507	**-2.2**	**-2.2**	1 SD	31.9	28.3	
Nesnow, 1998	BaP	mouse	Lung tumors	Polynomial	<.0001	<.0001	<.0001	0.85	496	-0.54	-0.54	1 SD	50.4	38.6	Acceptable Fit
Nesnow, 1998	BaP	mouse	Lung tumors	Power	<.0001	<.0001	<.0001	0.96	496	-0.34	-0.34	1 SD	53.6	42.0	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion
BMD = benchmark dose
BMDL = 1-sided 95% lower confidence limit for the benchmark dose
POD = point of departure
SD = standard deviation

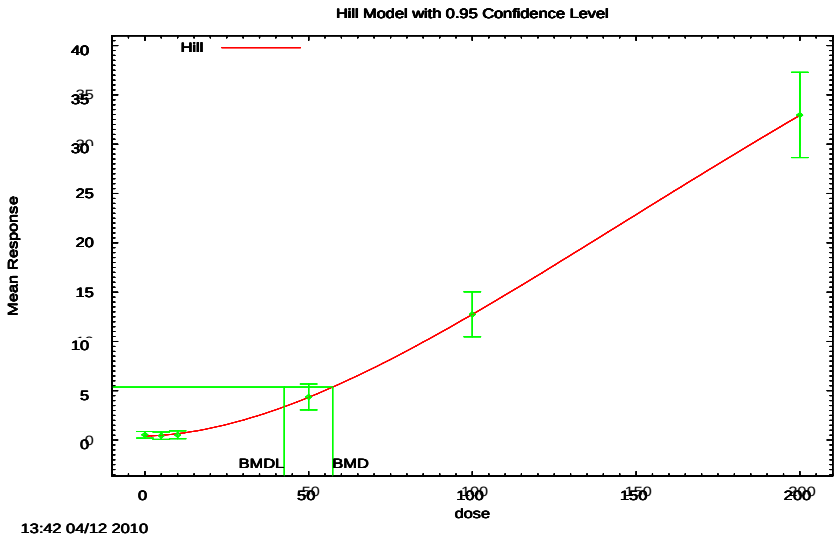
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.
   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.
   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.
   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.
   Test 4. (non-constant variance model) Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.

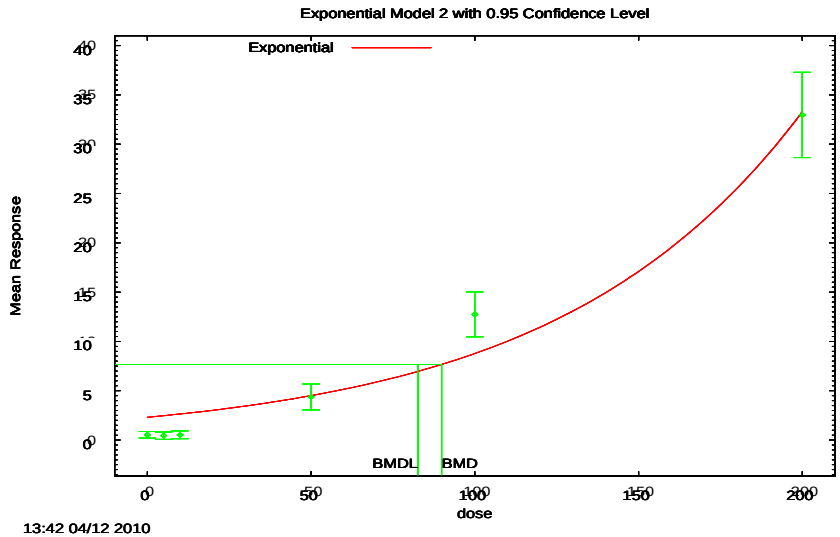
References

Nesnow, S., J.A. Ross, M.J. Mass et al. 1998. Mechanistic relationships between DNA adducts, oncogene mutations, and lung tumorigenesis in strain A mice. Exp Lung Res 24:395-405.

A: Hill_Nesnow98_BaP_Cont_Hill



B: Exponential2_Nesnow98_BaP_Cont_Exp



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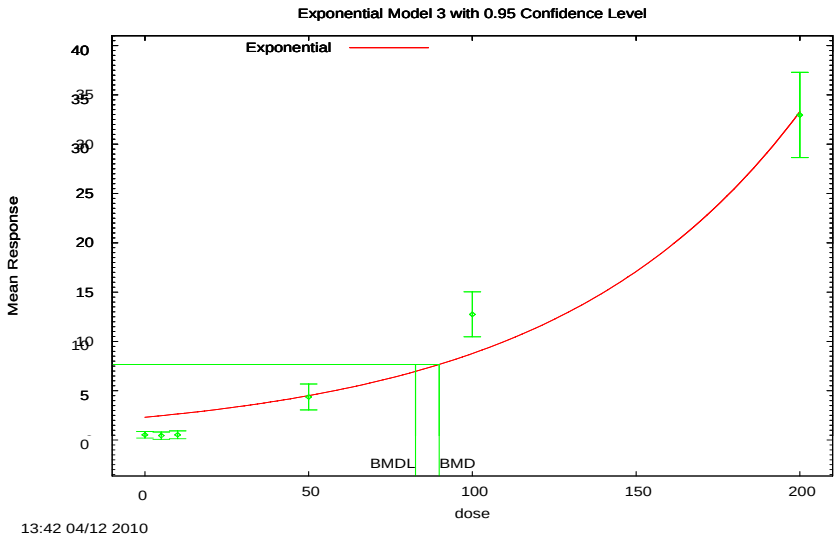
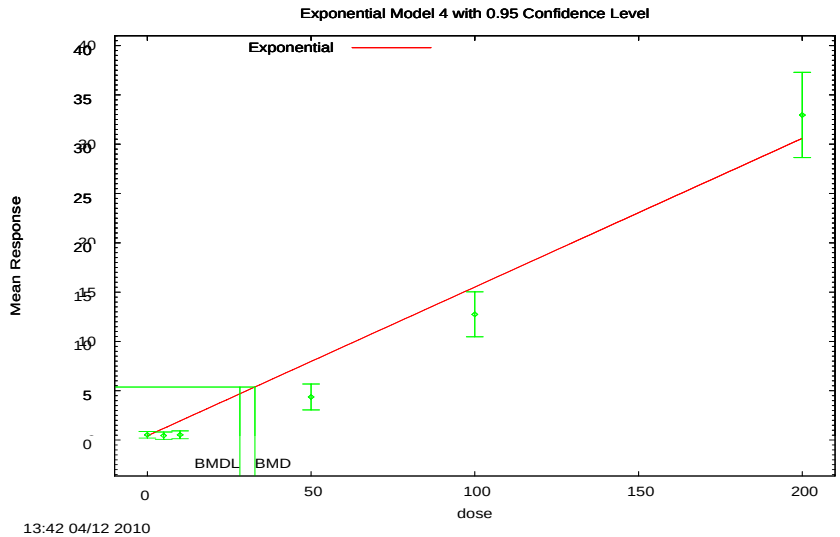
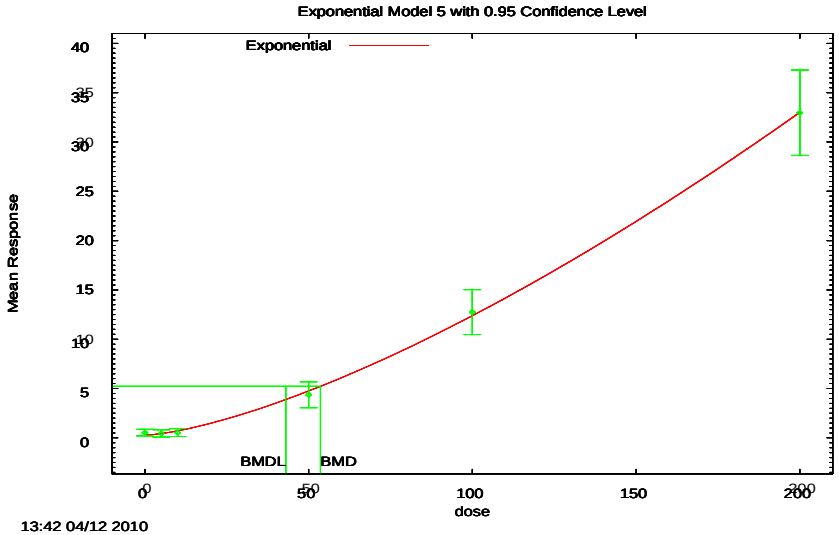


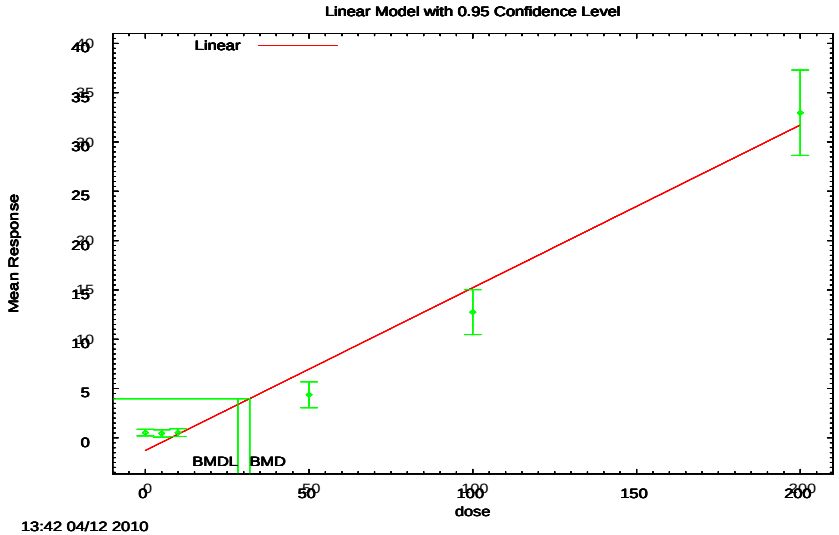
D: Exponential4_Nesnow98_BaP_Cont_Exp



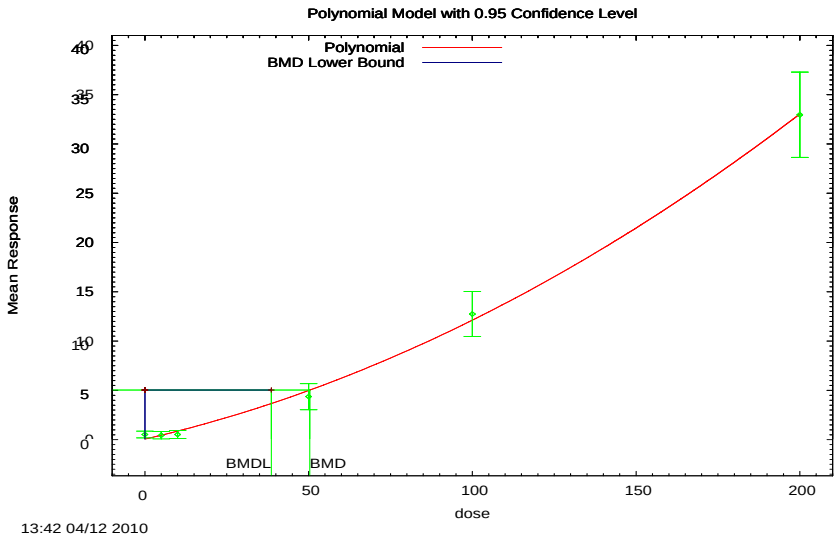
E: Exponential5_Nesnow98_BaP_Cont_Exp



F: Linear_Nesnow98_BaP_Cont_Lin



G: Polynomial_Nesnow98_BaP_Cont_Poly



H: Power_Nesnow98_BaP_Cont_Pow

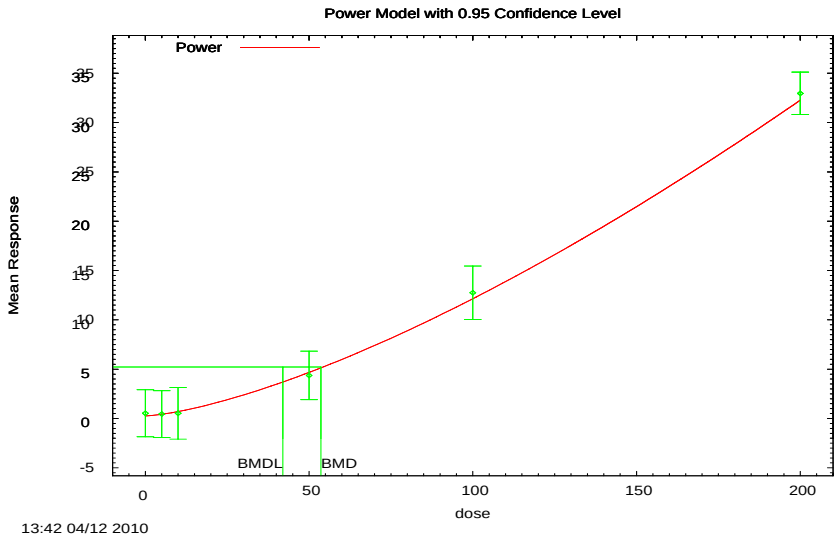


Table C-17. Nesnow et al. (1998), cyclopenta[c,d]pyrene. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness-of-Fit							POD Benchmark Response (BMR) (5)	Dose (mg/kg)		Notes
					p-value for Maximum Likelihood Tests (2)					Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Nesnow, 1998	CPcdP	mouse	Lung tumors	Hill	<.0001	<.0001	<.0001	1.0	624	8.43E-06	0.00042	1 SD	73.9	58.0	Best Fit: Hill model has the lowest residuals, the highest p-value in Test 4, and the lowest AIC.
Nesnow, 1998	CPcdP	mouse	Lung tumors	Exponential 2	< 0.0001	< 0.0001	< 0.0001	**0.0008**	637	**2.8**	**2.8**	1 SD	93.0	84.9	
Nesnow, 1998	CPcdP	mouse	Lung tumors	Exponential 3	< 0.0001	< 0.0001	< 0.0001	**0.0008**	637	**2.8**	**2.8**	1 SD	93.0	84.9	
Nesnow, 1998	CPcdP	mouse	Lung tumors	Exponential 4	< 0.0001	< 0.0001	< 0.0001	**< 0.0001**	680	**-6.9**	**22**	1 SD	32.1	29.8	
Nesnow, 1998	CPcdP	mouse	Lung tumors	Exponential 5	< 0.0001	< 0.0001	< 0.0001	0.12	627	-1.32	-1.32	1 SD	65.2	55.9	Acceptable Fit
Nesnow, 1998	CPcdP	mouse	Lung tumors	Linear	<.0001	<.0001	<.0001	**<.0001**	651	**-3.2**	**-3.2**	1 SD	33.0	28.9	
Nesnow, 1998	CPcdP	mouse	Lung tumors	Polynomial	<.0001	<.0001	<.0001	0.20	626	-1.36	-1.36	1 SD	63.1	48.6	Acceptable Fit
Nesnow, 1998	CPcdP	mouse	Lung tumors	Power	<.0001	<.0001	<.0001	0.31	625	-1.24	-1.24	1 SD	63.6	51.7	Acceptable Fit

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

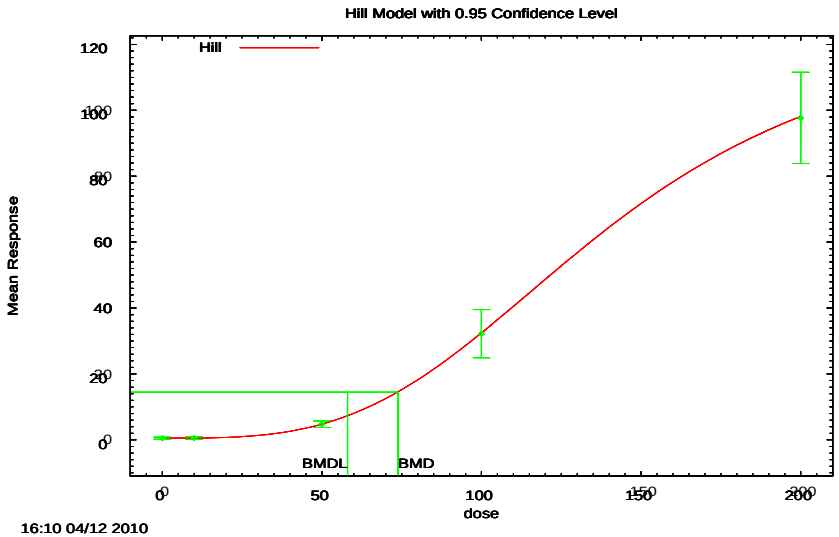
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.
   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.
   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.
   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.
   Test 4. (non-constant variance model). Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.

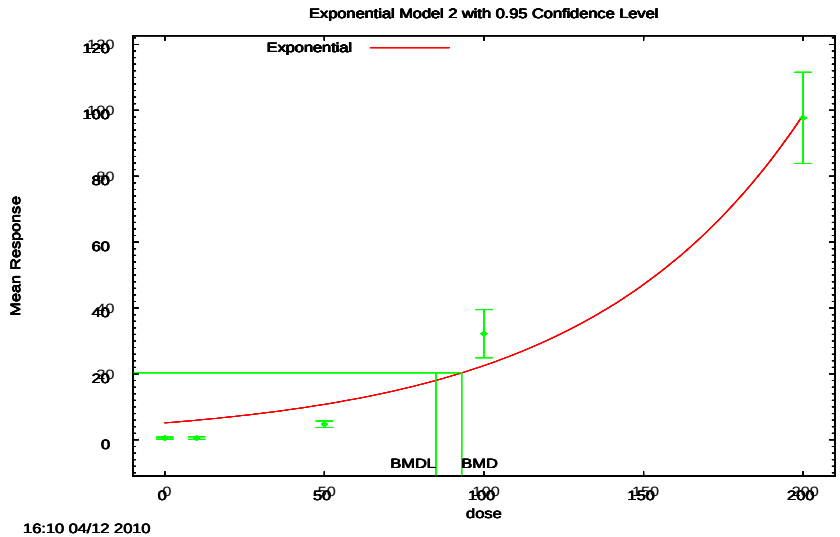
References

Nesnow, S., J.A. Ross, M.J. Mass et al. 1998. Mechanistic relationships between DNA adducts, oncogene mutations, and lung tumorigenesis in strain A mice. Exp Lung Res 24:395-405.

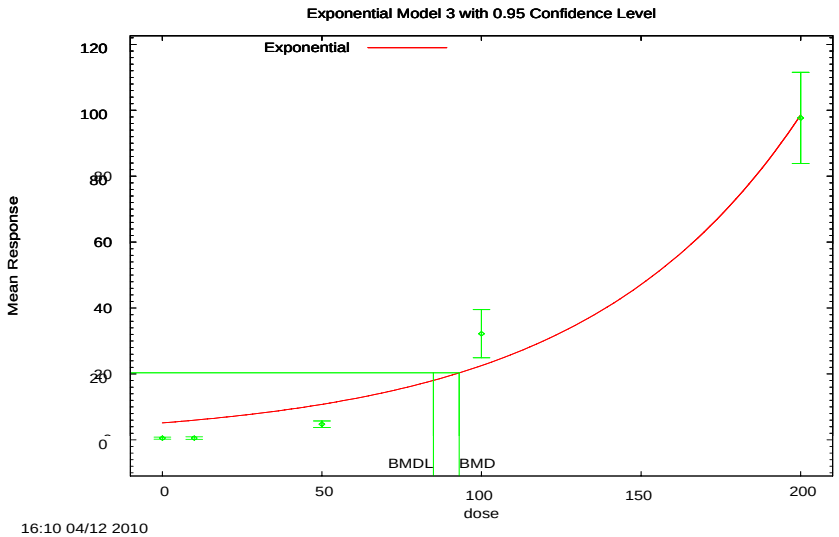
A: Hill_Nesnow98_CPcdP_Cont_Hill



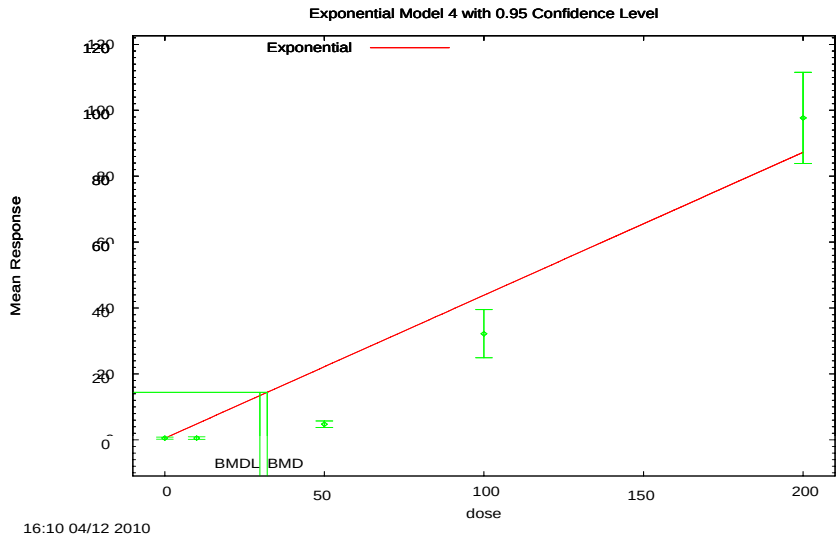
B: Exponential2_Nesnow98_CPcdP_Cont_Exp



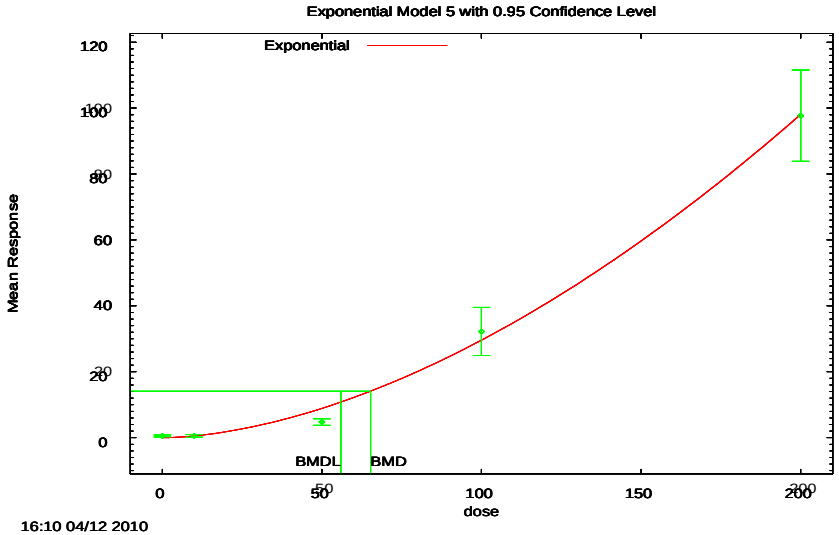
C: Exponential3_Nesnow98_CPcdP_Cont_Exp



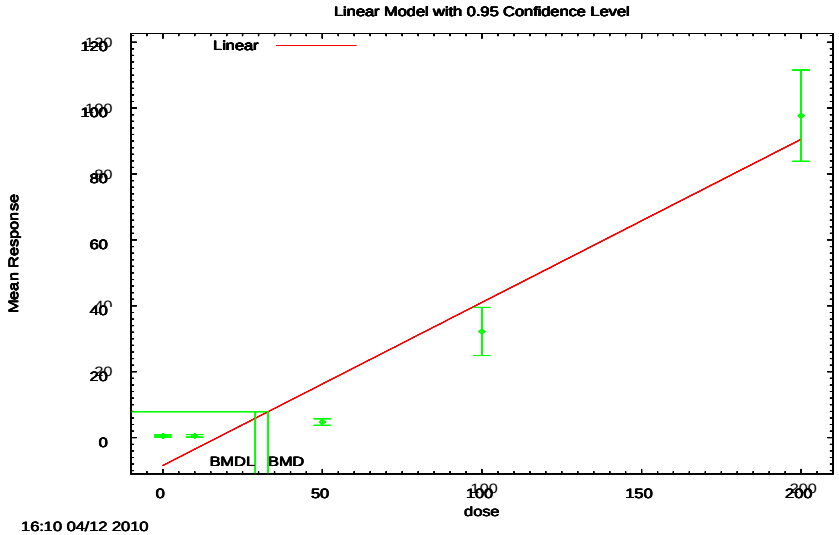
D: Exponential4_Nesnow98_CPcdP_Cont_Exp



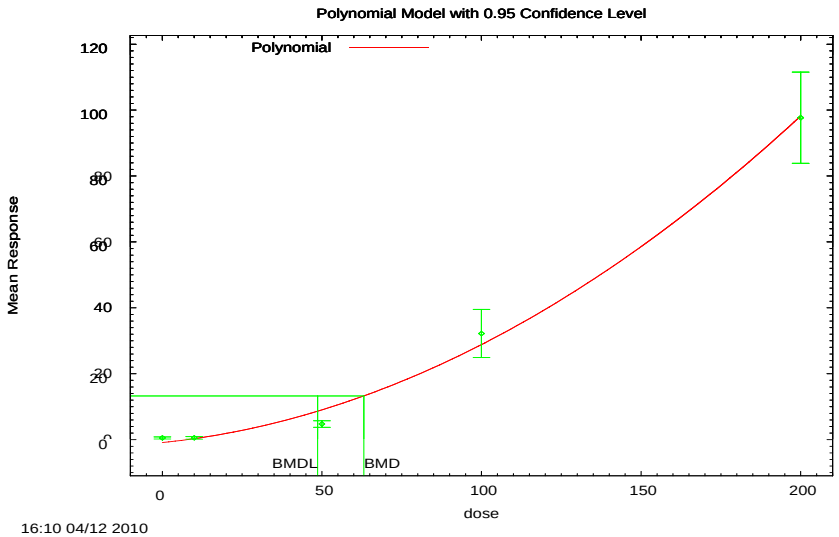
E: Exponential5_Nesnow98_CPcdP_Cont_Exp



F: Linear_Nesnow98_CPcdP_Cont_Lin



G: Polynomial_Nesnow98_CPcdP_Cont_Poly



H: Power_Nesnow98_CPcdP_Cont_Pow

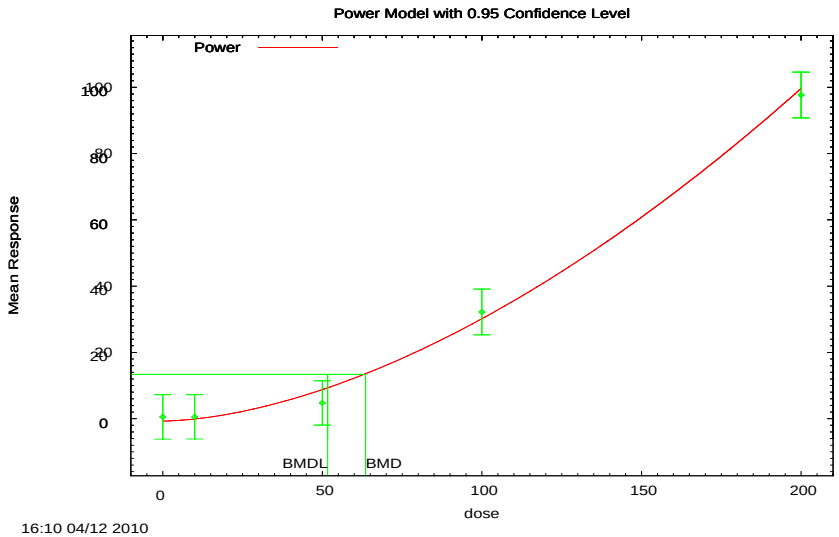


Table C-18. Nesnow et al. (1998), dibenz[a,l]pyrene. Continuous dose-response goodness-of-fit tests and benchmark dose estimates at 10% effect levels applied to study results.

Source	Substance	Effect Endpoint		Continuous Dose-Response Model (1)	Goodness-of-Fit							POD Benchmark Response (BMR) (5)	Dose (mg/kg)		Notes
					p-value for Maximum Likelihood Tests (2)					Residuals (4)					
		Animal	Tissue		Test 1	Test 2	Test 3	Test 4	AIC (3)	Scaled Residual of Interest	Maximum Scaled Residual		BMD	BMDL	
Nesnow, 1998	DBalP	mouse	Lung tumors	Hill	<.0001	<.0001	<.0001	0.2	604	0.75	-0.82	1 SD	1.6	1.2	Acceptable fit.
Nesnow, 1998	DBalP	mouse	Lung tumors	Exponential 2	< 0.0001	< 0.0001	< 0.0001	**0.00020**	619	**2.3**	**-2.8**	1 SD	3.1	2.9	
Nesnow, 1998	DBalP	mouse	Lung tumors	Exponential 3	< 0.0001	< 0.0001	< 0.0001	**0.00020**	619	**2.3**	**-2.8**	1 SD	3.1	2.9	
Nesnow, 1998	DBalP	mouse	Lung tumors	Exponential 4	< 0.0001	< 0.0001	< 0.0001	0.36	603	0.4	0.80	1 SD	1.4	1.3	Acceptable fit.
Nesnow, 1998	DBalP	mouse	Lung tumors	Exponential 5	< 0.0001	< 0.0001	< 0.0001	0.21	604	0.74	-0.82	1 SD	1.6	1.3	Acceptable fit.
Nesnow, 1998	DBalP	mouse	Lung tumors	Linear	<.0001	<.0001	<.0001	0.56	601	0.4	0.81	1 SD	1.4	1.3	Acceptable fit: Linear model has the lowest residuals, the highest p-value in Test 4, and the lowest AIC.
Nesnow, 1998	DBalP	mouse	Lung tumors	Polynomial	<.0001	<.0001	<.0001	0.45	602	0.67	-0.88	1 SD	1.6	1.2	Acceptable fit.
Nesnow, 1998	DBalP	mouse	Lung tumors	Power	<.0001	<.0001	<.0001	0.45	602	0.74	-0.82	1 SD	1.6	1.3	Acceptable fit.

Abbreviations

AIC = Akaike's Information Criterion POD = point of departure

BMD = benchmark dose

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

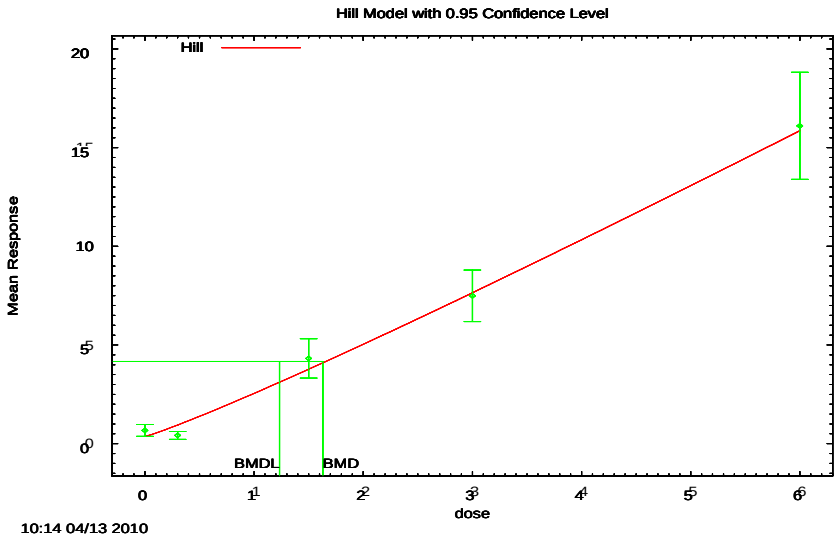
Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response continuous data.
2. There are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.
   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.
   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.
   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately.
   Test 4. (non-constant variance model) Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
3. For each dataset, models with relatively low AIC are indicative of better fits.
4. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Scaled Residual of Interest is a summary output parameter of the BMDS model, while the Maximum Scaled Residual is the maximum of the scaled residuals of each of the individual dose groups. Absolute value less than 2.0 is indicative of a good fit. Values in bold are >2.0.
5. For multidose continuous data, the BMR used in estimating the point of departure was a change of 1 standard deviation (1 SD) from the control mean.

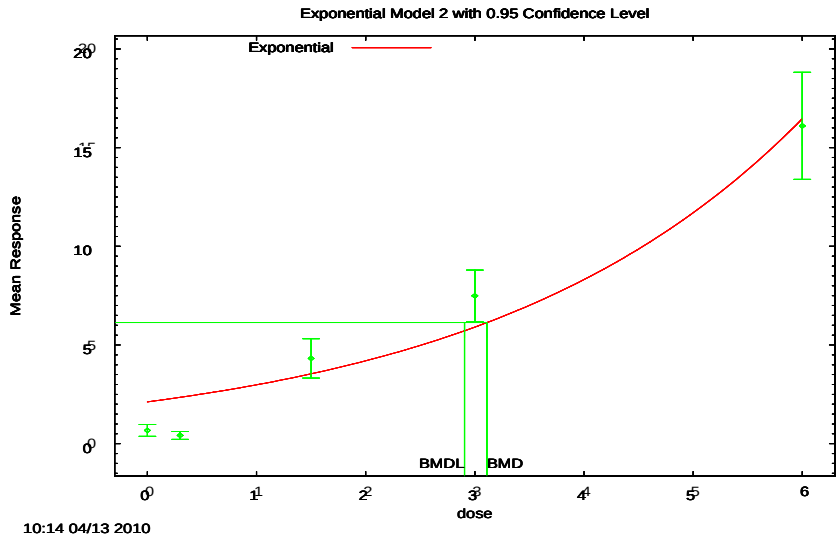
References

Nesnow, S., J.A. Ross, M.J. Mass et al. 1998. Mechanistic relationships between DNA adducts, oncogene mutations, and lung tumorigenesis in strain A mice. Exp Lung Res 24:395-405.

A: Hill_Nesnow98_DBalP_Cont_Hill



B: Exponential2_Nesnow98_DBalP_Cont_Exp



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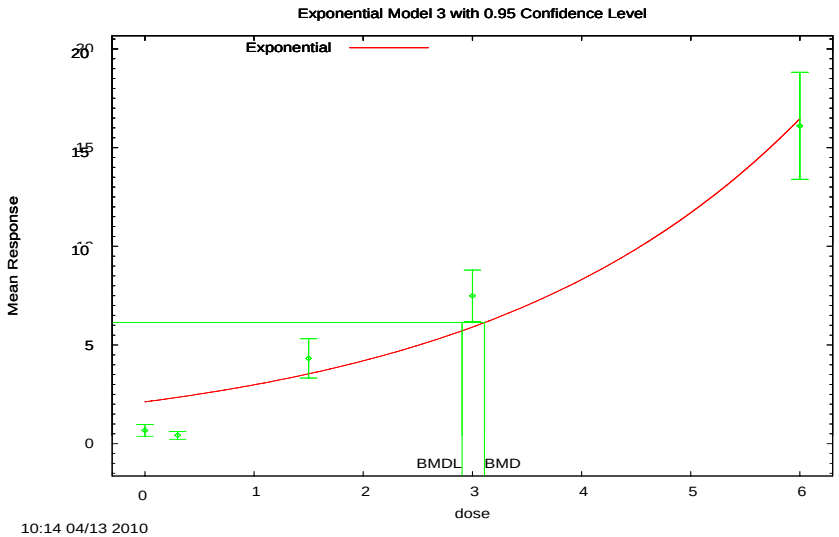

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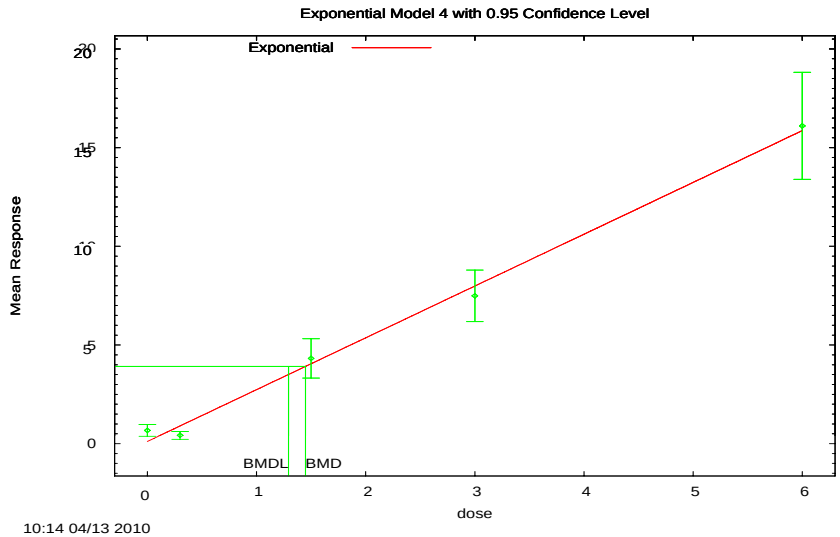
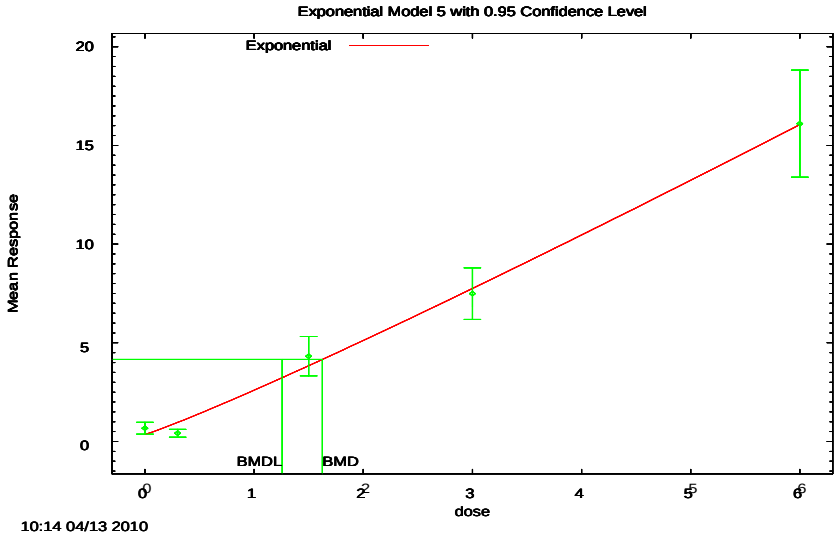
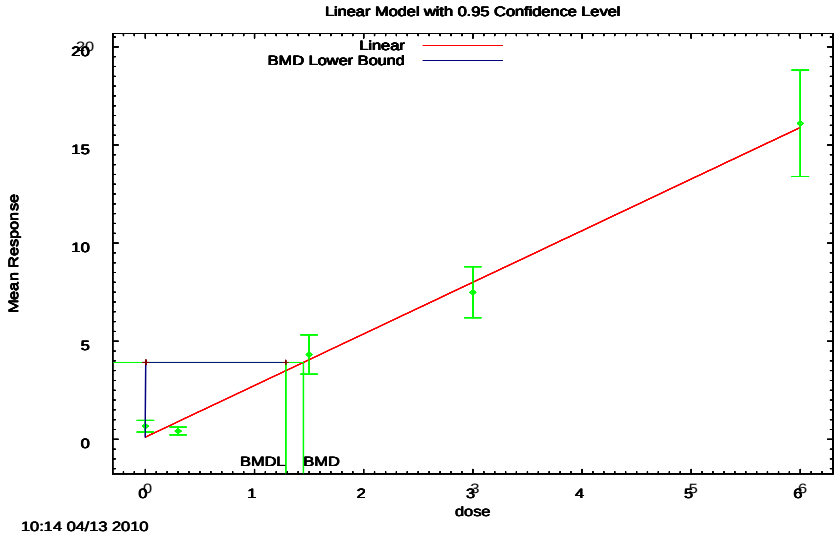


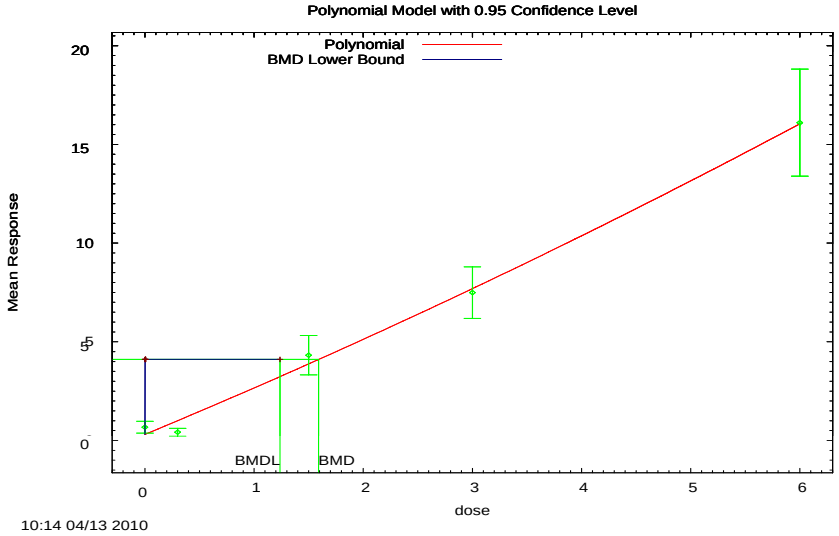
E: Exponential5_Nesnow98_DBalP_Cont_Exp



F: Linear_Nesnow98_DBalP_Cont_Lin



G: Polynomial_Nesnow98_DBalP_Cont_Poly



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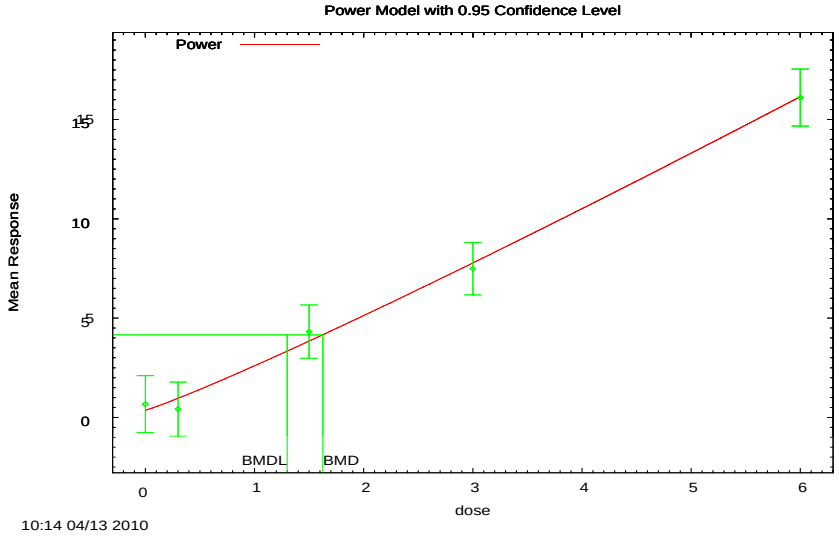

Table D-1. Calculation of Human Equivalent Dose (mg/kg-day) for BMDS Input File

Study Mixture	Dose Group Concentration in Feed[1]		Mass BaP[3]		Mass Coal Tar	Average Feed	Administered Dose			Human Equivalent Dose		
	%	ppm (µg/g)	µg/day		µg/day	grams/day	BW (kg)[4]	µg/kg-day	mg/kg-day	BW (kg)	Scaling Factor[5]	mg/kg-day
Coal Tar Mixture 1 BaP = 0.1837%[2]	0.01	100	0.8	b	435	4.4	0.030	14,516	15	70	7.0	2.1
	0.03	300	2.4	b	1,306	4.4	0.030	43,549	44	70	7.0	6.3
	0.10	1,000	8.0	a	4,355	4.4	0.030	145,164	145	70	7.0	21
	0.30	3,000	23.7	a	12,901	4.3	0.030	430,049	430	70	7.0	62
	0.60	6,000	47.4	b	25,803	4.3	0.030	860,098	860	70	7.0	124
	1.00	10,000	63.0	a	34,295	3.4	0.030	1,143,168	1,143	70	7.0	164
Coal Tar Mixture 2 BaP = 0.2760%[2]	0.03	300	4.0	b	1,449	4.8	0.030	48,309	48.3	70	7.0	7.0
	0.10	1,000	13.2	a	4,783	4.8	0.030	159,420	159	70	7.0	23
	0.30	3,000	36.3	a	13,152	4.4	0.030	438,406	438	70	7.0	63
Benzo(a)pyrene	0.0005	5	20.5	a	NA	4.1	0.030	683	0.68	70	7.0	0.098
	0.00	25	104	a	NA	4.2	0.030	3,467	3.47	70	7.0	0.499
	0.01	100	430	a	NA	4.3	0.030	14,333	14.33	70	7.0	2.062

Notes:

1. % by mass (coal tar in feed)
2. % by mass (benzo(a)pyrene in coal tar)
3. Mass of BaP equals mass daily feed x % by mass coal tar x % by mass BaP. (a) reported in Culp et al. 1998 pages 121-122; (b) estimated as a proportion of concentrations with reported mass BaP.
4. Culp et al. 1998, Figure 1; average body weight ranges from 15 to 40 g during the course of 2 year study, with time-weighted mean of approximately 30 g (or 0.030 kg) Daily food consumption ranges from 2 to 4 g/day.
5. Human equivalent dose (D_2) = Administered dose (D_1) divided by scaling factor. Scaling factor = (BW_human/BW_mouse) $^{1/4}$

$$A_1/BW_1^{3/4} = A_2/BW_2^{3/4}$$

since $1/BW^{3/4} = BW^{1/4}/BW$

$$(A_1/BW_1)\ BW_1^{1/4} = (A_2/BW_2)\ BW_2^{1/4}$$

$$D_1BW_1^{1/4} = D_2BW_2^{1/4}$$

$$D_2 = D_1(BW_1^{1/4}/BW_2^{1/4}) = D_1 / (BW_2/BW_1)^{1/4}$$

Table D-2. Coal Tar Mixture 1 Dose-response Models and Benchmark Dose Estimates at 10% Effect Levels Applied to Study Results

Effect Endpoint			Dose-Response Model (1, 2)	Parameters (2)			Goodness-of-Fit			POD (% Effect)	Dose (mg/kg-day)		Human CSF $(mg/kg\text{-}day)^{-1}$	Notes
Source	Animal	Tissue		β_0	β_1	β_2	p-value (3)	AIC (4)	Maximum Residual (5)		BMD	BMDL		
Culp, 1998 Table III	female B6C3F1 mouse	Liver (hepatocellular adenomas and/or carcinomas)	multistage cancer	0.062	0.00061	0	**0**	196	**4.9**	10%	171.5	78.9	--	BMDL is out of the three times range of dose for some BMR in BMDL curve computation.
Culp, 1998 Table III	female B6C3F1 mouse	Lung (alveolar/bronchiolar adenomas and/or carcinomas)	multistage cancer	0.049	0.0056	0	**0.0016**	308	**3.6**	10%	18.8	15.2	6.58E-03	
Culp, 1998 Table III, without highest 2 doses	female B6C3F1 mouse	Lung (alveolar/bronchiolar adenomas and/or carcinomas)	multistage cancer	0.053	0	0.00020	0.65	164	-1.0	10%	23.2	14.2	7.05E-03	
Culp, 1998 Table III	female B6C3F1 mouse	Forestomach (papillomas and/or carcinomas)	multistage cancer	0.053	0.0021	0	**0.0012**	248	**2.5**	10%	51.0	35.3	2.83E-03	
Culp, 1998 Table III, without highest 2 doses	female B6C3F1 mouse	Forestomach (papillomas and/or carcinomas)	multistage cancer	0.025	0.0052	0	**0.049**	143	**2.2**	10%	20.1	13.0	7.68E-03	
Culp, 1998 Table III	female B6C3F1 mouse	Small intestine (adenocarcinomas)	multistage cancer	0	0	5.53E-05	**0.031**	104	**-3.1**	10%	43.6	38.9	2.57E-03	
Culp, 1998 Table III	female B6C3F1 mouse	Hemangiosarcomas	multistage cancer	0.012	0.0017	0	**0**	190	**-3.6**	10%	60.5	44.0	2.27E-03	
Culp, 1998 Table III	female B6C3F1 mouse	Histiocytic sarcomas	multistage cancer	0.012	5.80E-04	0	**0.0034**	115	**3.2**	10%	181.7	102.7	9.74E-04	BMDL is out of the three times range of dose for some BMR in BMDL curve computation.
Culp, 1998 Table III	female B6C3F1 mouse	Sarcomas	multistage cancer	0.060	0	0	0.13	153	**2.5**	10%	--	--	--	BMD computation failed. BMD is larger than three times maximum input doses. BMDL is out of the three times range of dose for some BMR in BMDL curve computation.

Abbreviations

AIC = Akaike's Information Criterion CSF = cancer slope factor

BMD = benchmark dose POD = point of departure

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Dose response functions for multistage cancer: P[response] = β_0 + (1-β_0)*[1-EXP(-β_1*dose^1-β_2*dose^2)];

   Dose response function for Hill Model: $y + (v * d^n) / (k^n + d^n)$, where v=sign, n=power, and k=slope.
3. For dichotomous data, Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.

   For continuous data, there are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.

   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.

   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.

   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled

   Test 4. (non-constant variance model) Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
4. For each dataset, models with relatively low AIC are indicative of better fits.
5. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Absolute value less than 2.0 is indicative of a good fit.

Values in bold are >2.0

References

Culp, S.J. et al. 1998. Carcinogenesis 19(1):117-124; Table III and Table IV.

Table D-3. Coal Tar Mixture 2 Dose-response Models and Benchmark Dose Estimates at 10% Effect Levels Applied to Study Results

Effect Endpoint			Dose-Response Model (1, 2)	Parameters (2)			Goodness-of-Fit			POD (% Effect)	Dose (mg/kg-day)		Human CSF $(mg/kg\text{-}day)^{-1}$	Notes
Source	Animal	Tissue		β_0	β_1	β_2	p-value (3)	AIC (4)	Maximum Residual (5)		BMD	BMDL		
Culp, 1998 Table III	female B6C3F1 mouse	Liver (hepatocellular adenomas and/or carcinomas)	multistage cancer	0.041	0.003573	0	**0.018**	127	**2.4**	10%	29.5	16.5	6.07E-03	
Culp, 1998 Table III	female B6C3F1 mouse	Lung (alveolar/bronchiolar adenomas and/or carcinomas)	multistage cancer	0.041	0.006972	4.85E-05	0.86	164	-0.13	10%	13.8	8.3	1.20E-02	
Culp, 1998 Table III	female B6C3F1 mouse	Forestomach (papillomas and/or carcinomas)	multistage cancer	0	0.00389	2.04E-05	0.15	100	1.5	10%	24.1	15.3	6.55E-03	
Culp, 1998 Table III	female B6C3F1 mouse	Small intestine (adenocarcinomas)	multistage cancer	0	0	5.8E-06	0.98	12	-0.38	10%	135	70.5	1.42E-03	BMDL is out of the three times range of dose for some BMR in BMDL curve computation.
Culp, 1998 Table III	female B6C3F1 mouse	Hemangiosarcomas	multistage cancer	0.019	3.76E-04	1.00E-04	0.76	115	-0.22	10%	30.6	17.8	5.62E-03	
Culp, 1998 Table III	female B6C3F1 mouse	Histiocytic sarcomas	multistage cancer	0.035	0	5.41E-05	0.48	106	0.93	10%	44.1	22.2	4.51E-03	
Culp, 1998 Table III	female B6C3F1 mouse	Sarcomas	multistage cancer	0.014	0.00174	0	0.29	77	-1.1	10%	60.6	33.0	3.03E-03	BMDL is out of the three times range of dose for some BMR in BMDL curve computation.

Abbreviations

AIC = Akaike's Information Criterion CSF = cancer slope factor

BMD = benchmark dose POD = point of departure

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Dose response functions for multistage cancer: P[response] = β_0 + (1-β_0)*[1-EXP(-β_1*dose^1-β_2*dose^2)];

   Dose response function for Hill Model: $y + (v * d^n) / (k^n + d^n)$, where v=sign, n=power, and k=slope.
3. For dichotomous data, Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.

   For continuous data, there are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.

   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.

   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.

   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled appropriately

   Test 4. (non-constant variance model). Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
4. For each dataset, models with relatively low AIC are indicative of better fits.
5. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Absolute value less than 2.0 is indicative of a good fit.

Values in bold are >2.0

References

Culp, S.J. et al. 1998. Carcinogenesis 19(1):117-124; Table III and Table IV.

Table D-4. Benzo(a)pyrene Dose-response Models and Benchmark Dose Estimates at 10% Effect Levels Applied to Study Results

Effect Endpoint			Dose-Response Model (1, 2)	Parameters (2)			Goodness-of-Fit			POD (% Effect)	Dose (mg/kg-day)		Human CSF $(mg/kg\text{-}day)^{-1}$	Notes
Source	Animal	Tissue		β_0	β_1	β_2	p-value (3)	AIC (4)	Maximum Residual (5)		BMD	BMDL		
Culp, 1998 Table IV	female B6C3F1 mouse	Liver (hepatocellular adenomas)	multistage cancer	0.074	0	0	**0.035**	102	-1.9	10%	--	--	--	BMD computation failed. BMD is larger than three times maximum input doses.
Culp, 1998 Table IV	female B6C3F1 mouse	Lung (alveolar/bronchiolar adenomas and/or carcinomas)	multistage cancer	0.048	0	0	**0.020**	74	1.8	10%	--	--	--	BMD computation failed. BMD is larger than three times maximum input doses.
Culp, 1998 Table IV without 100ppm Dose	female B6C3F1 mouse	Lung (alveolar/bronchiolar adenomas and/or carcinomas)	multistage cancer	0.053	0	0.14	**0.022**	70	-1.7	10%	0.9	0.4	0.23	
Culp, 1998 Table IV	female B6C3F1 mouse	Forestomach (papillomas and/or carcinomas)	multistage cancer	0.014	2.2	0	**0.011**	105	**-2.4**	10%	0.049	0.038	2.61	
Culp, 1998 Table IV without 100ppm Dose	female B6C3F1 mouse	Forestomach (papillomas and/or carcinomas)	multistage cancer	0.019	0	6.0	0.77	84	-0.26	10%	0.133	0.081	1.23	
Culp, 1998 Table IV	female B6C3F1 mouse	Esophagus (papillomas and/or carcinomas)	multistage cancer	0	0	0.21	0.99	81	-0.31	10%	0.72	0.52	0.19	
Culp, 1998 Table IV	female B6C3F1 mouse	Tongue (papillomas and/or carcinomas)	multistage cancer	0	0	0.15	0.99	85	-0.27	10%	0.83	0.55	0.18	
Culp, 1998 Table IV	female B6C3F1 mouse	Larynx (papillomas and/or carcinomas)	multistage cancer	0	0.09	0	0.50	54	1.3	10%	1.2	0.72	0.14	
Culp, 1998 Table IV	female B6C3F1 mouse	Hemangiosarcomas	multistage cancer	0.031	0	0	0.32	55	1.3	10%	--	--	--	BMD computation failed. BMD is larger than three times maximum input doses.
Culp, 1998 Table IV	female B6C3F1 mouse	Histiocytic sarcomas	multistage cancer	0.026	0	0	0.52	48	-1.14	10%	--	--	--	BMD computation failed. BMD is larger than three times maximum input doses.
Culp, 1998 Table IV	female B6C3F1 mouse	Sarcomas	multistage cancer	0.053	0	0	**0.0059**	80	**3.0**	10%	--	--	--	BMD computation failed. BMD is larger than three times maximum input doses.

Abbreviations

AIC = Akaike's Information Criterion CSF = cancer slope factor

BMD = benchmark dose POD = point of departure

BMDL = 1-sided 95% lower confidence limit for the benchmark dose

Notes

1. USEPA's BMDS v.2.1.1 was used to determine dose-response dichotomous data.
2. Dose response functions for multistage cancer: P[response] = β_0 + (1-β_0)*[1-EXP(-β_1*dose^1-β_2*dose^2)];

   Dose response function for Hill Model: $y + (v * d^n) / (k^n + d^n)$, where v=sign, n=power, and k=slope.
3. For dichotomous data, Chi-square test is a hypothesis test in which the null hypothesis is that data fit the dose-response function. Higher p-values indicate better fits.

   For continuous data, there are four Maximum Likelihood tests performed by BMDS that test the null hypothesis that the model fits the data as well as the "true" model.

   Test 1. Tests the hypothesis that response and variance don't differ among dose levels. If this test accepts, there may not be a dose-response. P-values less than 0.1 indicate a model fit.

   Test 2. Tests the hypothesis that variances are homogeneous. If this test accepts, the simpler constant variance model may be appropriate. P-values less than 0.1 indicate a model fit.

   Test 3. (non-constant variance model) Test the hypothesis that the variances are adequately modeled. If this test accepts, it may be appropriate to conclude that the variances have been modeled

   Test 4. (non-constant variance model). Tests the hypothesis that the model for the mean fits the data. If this tests accepts, the user has support for the selected model. P-values greater than 0.1 indicate a model fit.
4. For each dataset, models with relatively low AIC are indicative of better fits.
5. A scaled residual is the difference between the observed and predicted effect (i.e., percent response) divided by the standard deviation. Absolute value less than 2.0 is indicative of a good fit.

Values in bold are >2.0

References

Culp, S.J. et al. 1998. Carcinogenesis 19(1):117-124; Table III and Table IV.

Figure D-1. Coal Tar Mixture 1 BMDS Results for Lung Tumors, Two Highest Doses Removed

Material: **Mixture 1**
Test animal: **Female B6C3F1 Mice**
Effect: **Lung (alveolar/bronchiolar adenomas and/or carcinomas)**

Dose % in feed	Human EqvtDose mg/kg-day	N	Effect
0	0	47	2
0.01	2.1	48	3
0.03	6.3	48	4
0.10	20.9	48	4
0.30	61.9	47	27

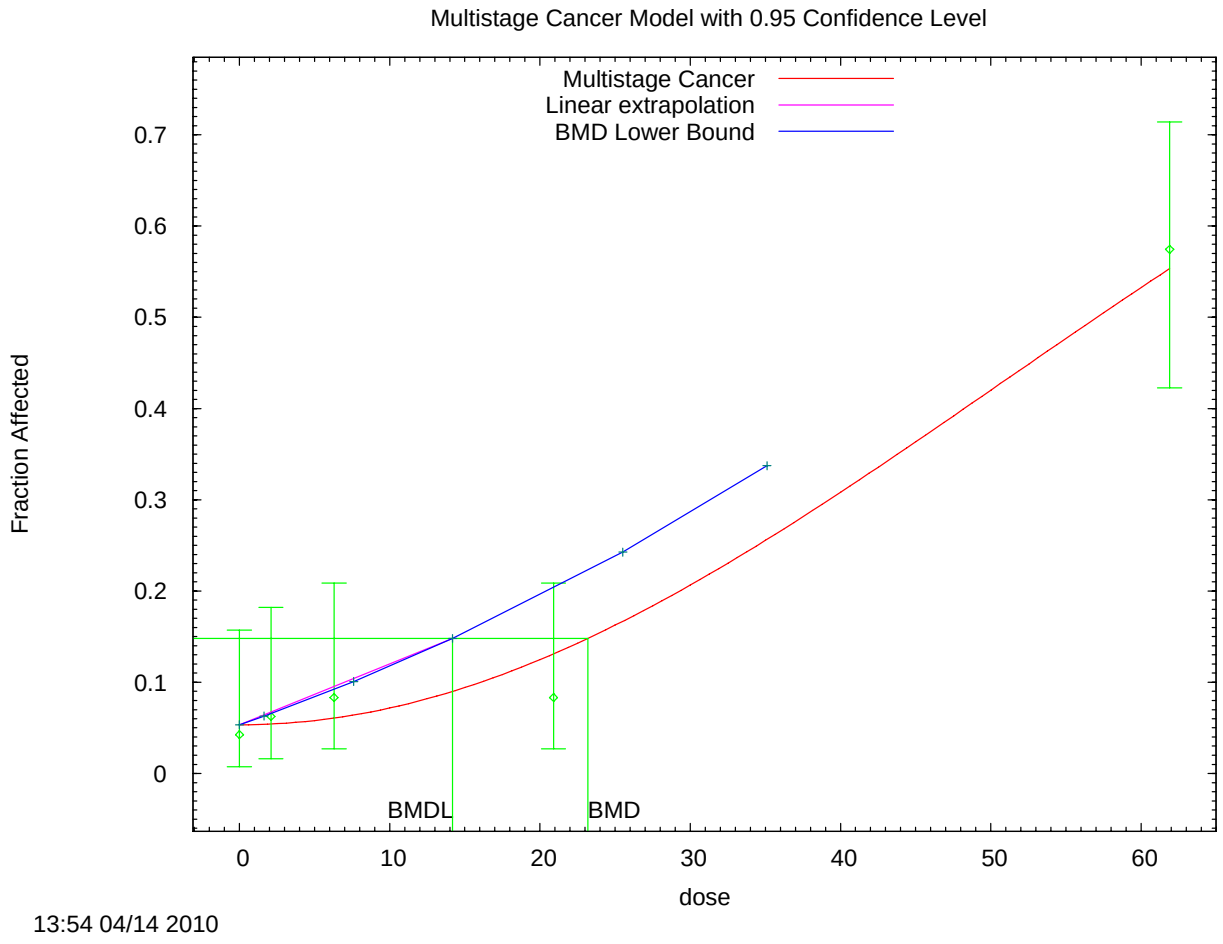

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Figure D-2. Coal Tar Mixture 1 BMDS Results for Forestomach Tumors, Two Highest Doses Removed

Material: **Mixture 1**
Test animal: **Female B6C3F1 Mice**
Effect: **Forestomach (papillomas and/or carcinomas)**

Dose % in feed	Human EqvtDose mg/kg-day	N	Effect
0	0	47	0
0.01	2.1	47	2
0.03	6.3	45	6
0.10	20.9	47	3
0.30	61.9	46	14

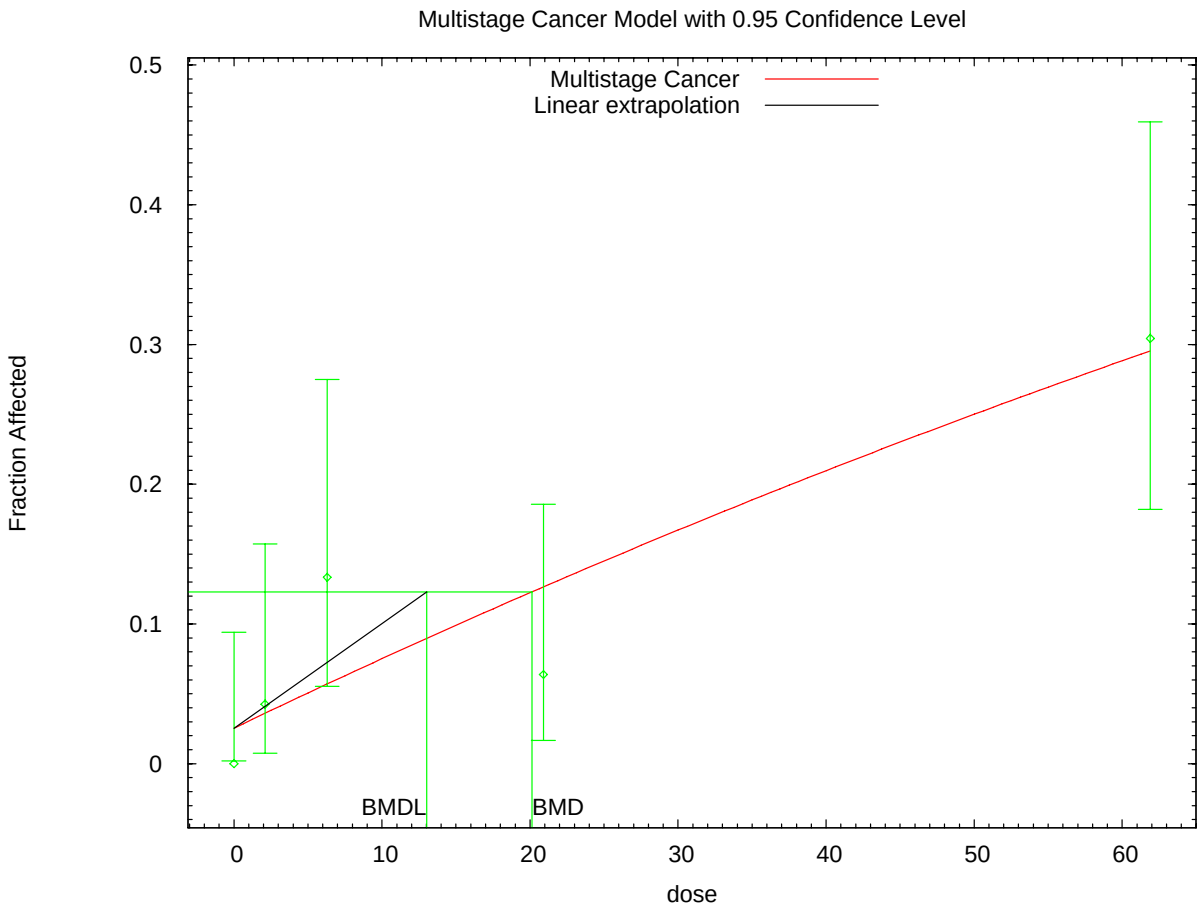

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Figure D-3. Coal Tar Mixture 2 BMDS Results for Lung Tumors

Material: **Mixture 2**
Test animal: **Female B6C3F1 Mice**
Effect: **Lung (alveolar/bronchiolar adenomas and/or carcinomas)**

Dose % in feed	Human EqvtDose mg/kg-day	N	Effect
0	0	47	2
0.03	7.0	48	4
0.10	22.9	48	10
0.30	63.1	47	23

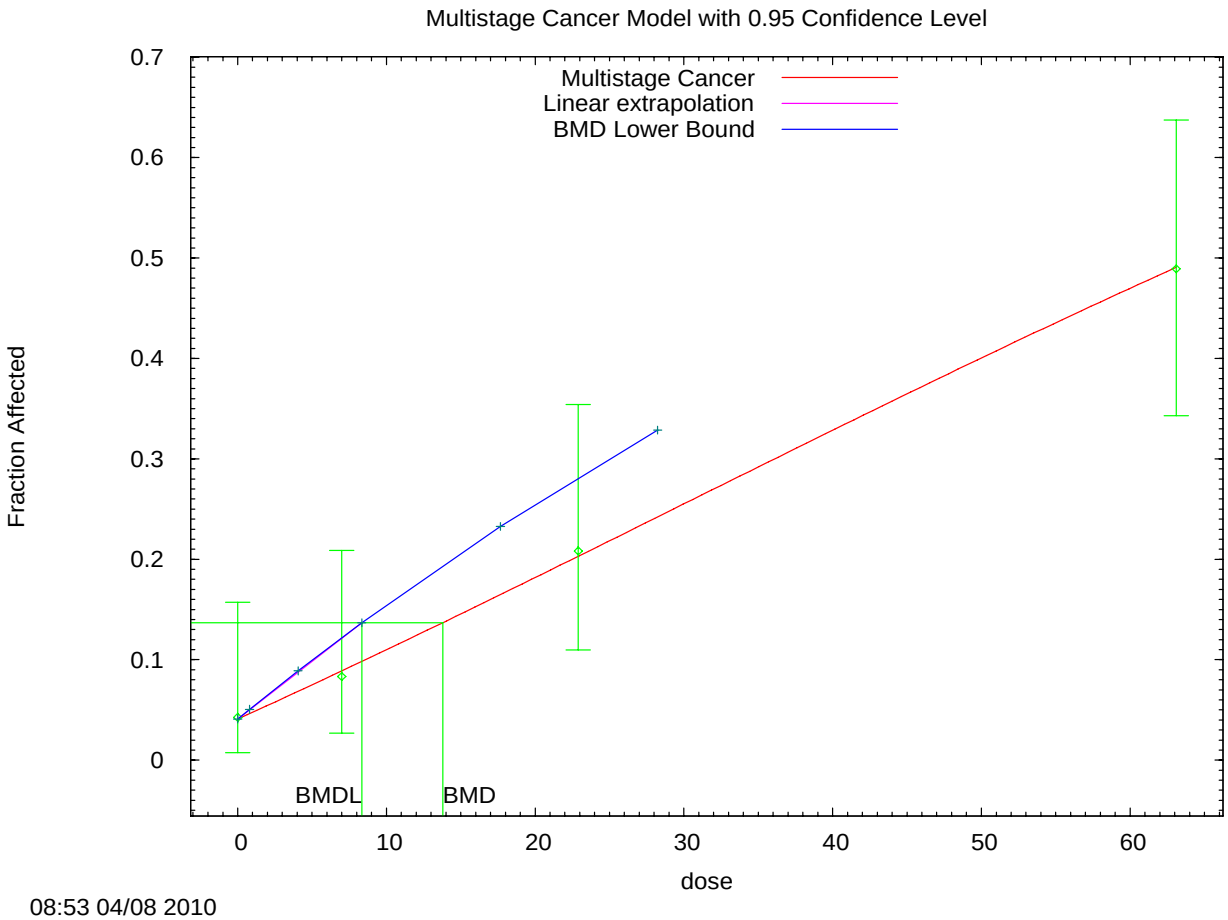

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Figure D-4. Coal Tar Mixture 2 BMDS Results for Forestomach Tumors

Material: **Mixture 2**
Test animal: **Female B6C3F1 Mice**
Effect: **Forestomach (papillomas and/or carcinomas)**

Dose % in feed	Human EqvtDose mg/kg-day	N	Effect
0	0	47	0
0.03	7.0	47	3
0.10	22.9	47	2
0.30	63.1	44	13

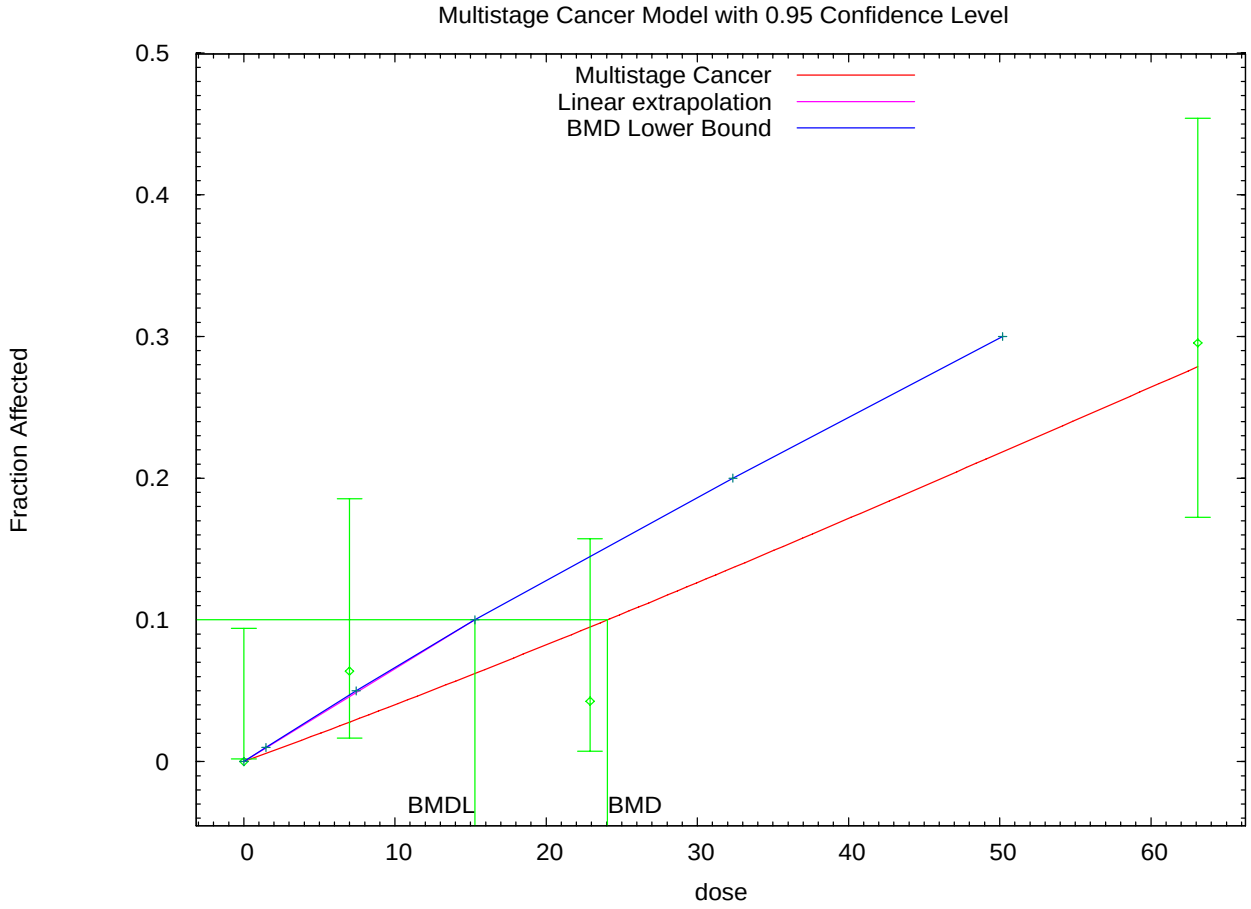

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Figure D-5. Benzo(a)Pyrene BMDS Results for Lung Tumors, 100 ppm Dose Removed

Material: **Benzo[a]pyrene**
Test animal: **Female B6C3F1 Mice**
Effect: **Lung (alveolar/bronchiolar adenomas and/or carcinomas)**

Dose ppm	Human EqvtDose mg/kg-day	Total	Effect
0	0	48	5
5	0.0983	48	0
25	0.4988	45	4

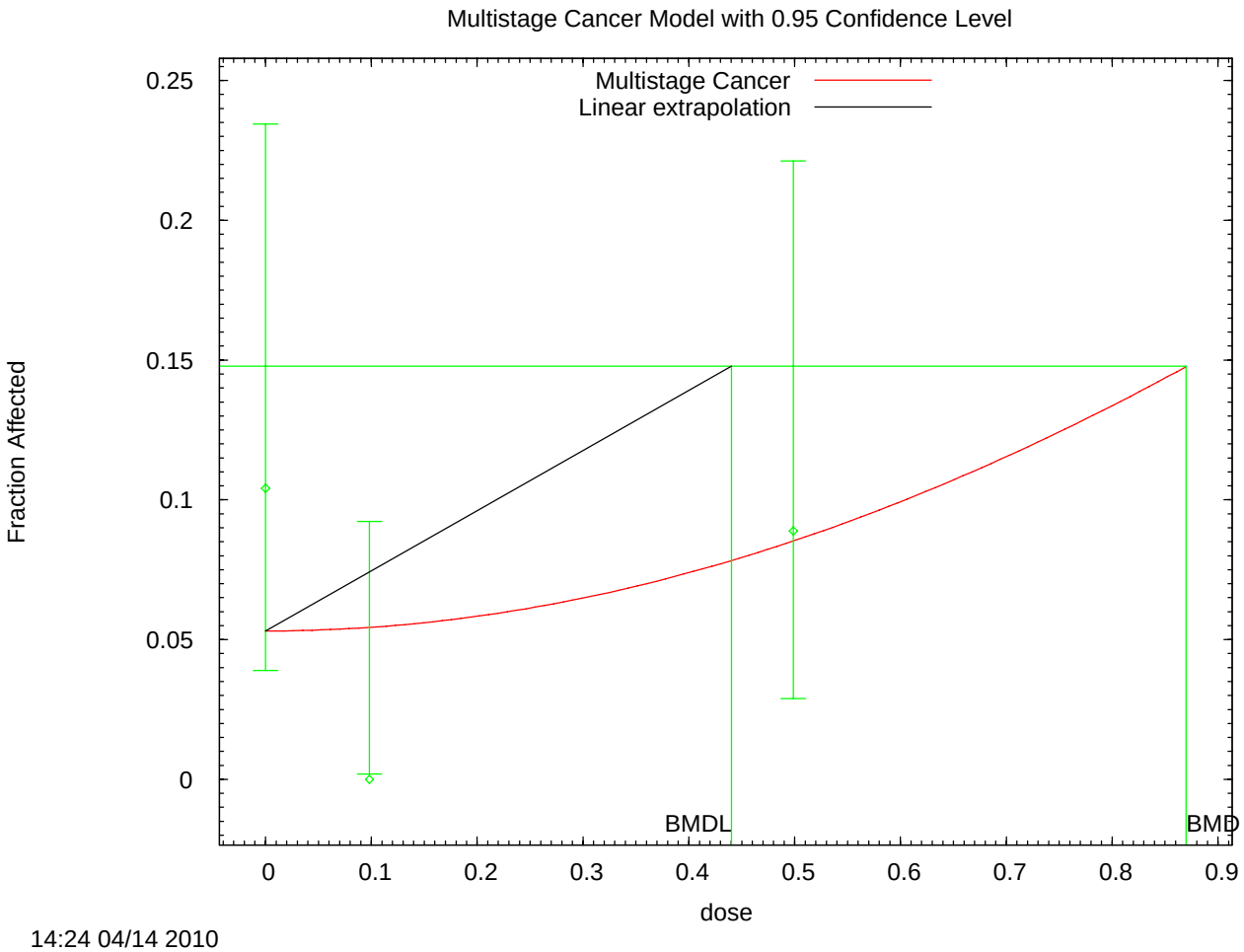

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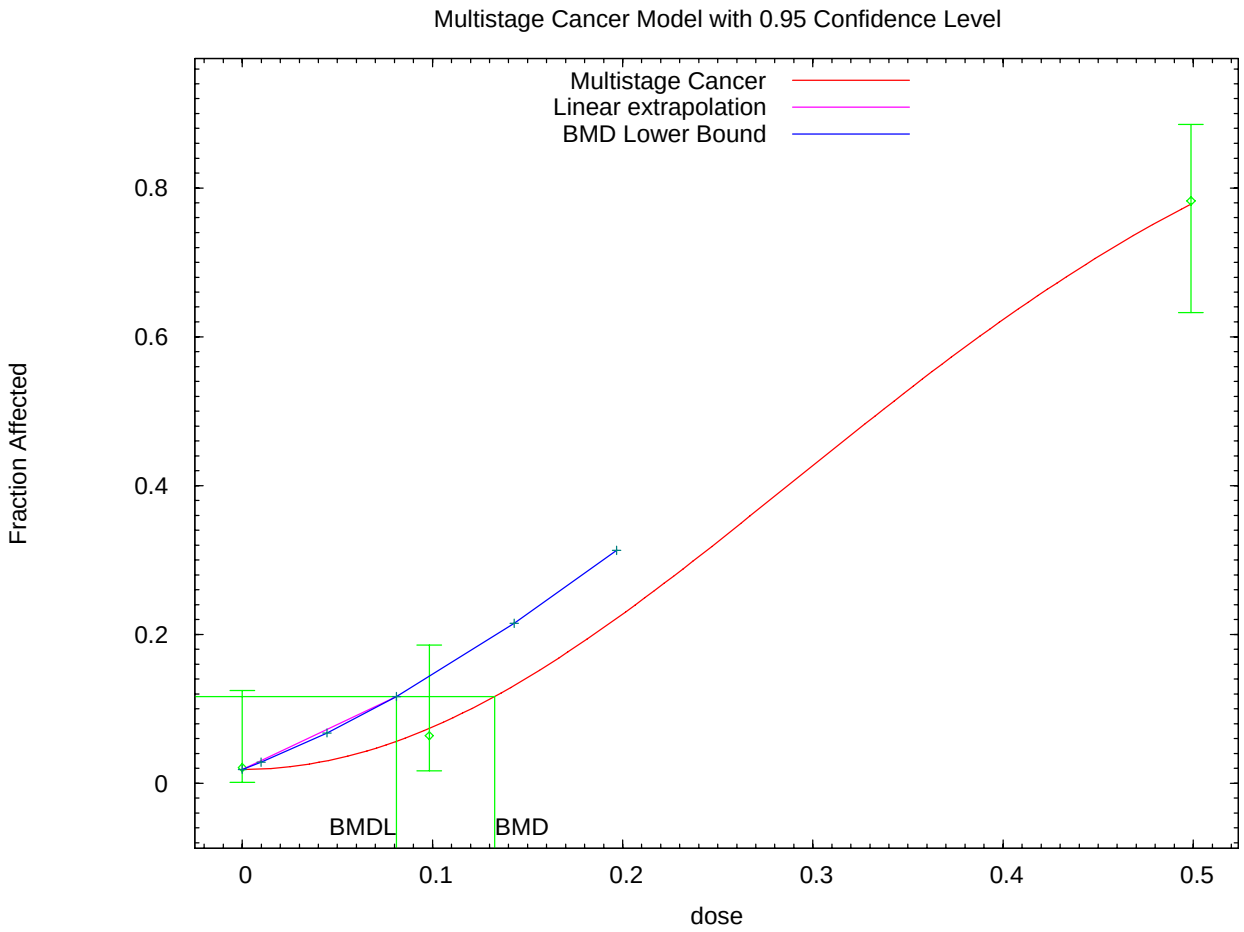
Figure D-6. Benzo(a)Pyrene BMDS Results for Forestomach Tumors, 100 ppm Dose Removed

Material: **Benzo[a]pyrene**
Test animal: **Female B6C3F1 Mice**
Effect: **Forestomach (papillomas and/or carcinomas)**

Dose ppm	Human EqvtDose mg/kg-day	Total	Effect
0	0	48	1
5	0.0983	47	3
25	0.4988	46	36



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