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# DEVELOPMENT OF RELATIVE POTENCY EXTIMATES FOR PAHS AND HYDROCARBON COMBUSTION PRODUCT FRACTIONS COMPARED TO BENZO[A]PYRENE AND THEIR USE IN CARCINOGENIC RISK ASSESSMENTS - (USED AS A REFERENCE IN OU2 RI AND OU5 FS) - CLEMENT INTERNATIONAL, 1990

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# DEVELOPMENT OF RELATIVE POTENCY ESTIMATES FOR PAHs AND HYDROCARBON COMBUSTION PRODUCT FRACTIONS COMPARED TO BENZO[A]PYRENE AND THEIR USE IN CARCINOGENIC RISK ASSESSMENTS

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16. ABSTRACT


As an extension of the work started in a previous contract (EPA 68-02-4403, April 1988), various approaches for estimating the carcinogenic potency of polycyclic aromatic hydrocarbons (PAH) mixtures were investigated. The approach uses the two-stage model described in the previous contract work to convert the tumor incidence data in a variety of bioassays to a low-dose linear slope of the dose-response curve. The potency of mixtures and components of mixtures is expressed as a fraction of the potency of benzo(a)pyrene (B[a]P), which is treated as a reference standard. The basic assumption of the method is that the relative potency of the mixture is the weighted average of the relative potency of the components.

This method is applied to data generated by a single group of investigators on lung cancer in female Osborne-Mendel rats induced by implantation of test material in the lung. Diesel engine exhaust, gasoline engine exhaust, flue gas condensate from coal-fired furnaces and sidestream cigarette smoke were tested, along with their identified chemical components and solvent-extracted components.

Of nine different procedures for evaluating the relative potency of combustion product mixtures from their components, the most accurate one assumes that the relative potency of the solvent-extracted fraction containing greater than three rings is equal to B[a]P. Although this procedure slightly overestimates the potency of the mixture, it is more accurate than the procedures for averaging the relative potencies of the standard set of indicator compounds. The latter procedure, which has been under consideration as the preferred approach, underestimates the potency of the mixtures.


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# Development of Relative Potency Estimates for PAHs and Hydrocarbon Combustion Product Fractions Compared To Benzo[a]pyrene and Their Use in Carcinogenic Risk Assessments

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Summary

Methods are developed for evaluating the carcinogenic potency of materials resulting from the combustion of hydrocarbon sources such as fuel or tobacco, using relative concentrations and carcinogenic potencies of various components of such products. The proposed procedures are based on a two-stage model of carcinogenesis, which is developed and validated with a variety of data in this report. When the two-stage model is assumed, it is natural to define the potency of a carcinogen, in the absence of a promotional effect, as the increased rate of cellular transitions per unit increase in dose delivered at the cellular level. A working hypothesis employed in our analysis is that: 1) various carcinogenic components in the mixture act via a similar carcinogenic mechanism, possibly with different efficiency, and 2) the potency of the combustion product is equal to the weighted average of the potencies of each fraction in the mixture. This hypothesis is referred to as dose-additivity and is recommended in EPA's guidelines for mixtures (EPA 1984). When this hypothesis holds, it is meaningful to express the carcinogenic potency of a combustion product or quantifiable fraction of such a product in terms of carcinogenically equivalent units of a reference agent that acts via the common mechanism. The relative potency of an agent compared to a specified reference agent is defined, in terms of the proposed model, as the dose of reference agent that results in the same increase in the cancer rate as a single unit of the agent of interest. Estimates of relative potencies can be used to estimate a dose of the reference agent carcinogenically equivalent to a unit dose of some other agent of interest; such a reference-equivalent dose can then be used to estimate risk based on procedures appropriate for the reference agent.

The carcinogenicity of hydrocarbon combustion products is widely attributed to the presence of polycyclic aromatic hydrocarbons (PAHs). Procedures are developed here for evaluating the carcinogenic potency of hydrocarbon combustion products, based on relative potencies of the products, quantifiable fractions of the products, or individual PAHs, compared to the reference PAH benzo[a]pyrene (B[a]P). B[a]P is used as a reference standard since it occurs frequently in combustion products, is highly carcinogenic, has been tested in a variety of animal bioassay systems, and used as a positive control for other PAHs and mixtures in numerous experiments.

To increase the precision of the relative potency estimates, our analysis incorporates time-to-tumor information, B[a]P positive controls, and vehicle controls from multiple experiments, and historical controls. Also, the underlying model employed is extended to accommodate possible saturation of mechanisms converting PAHs to their reactive metabolites. Data used to obtain estimates and validate procedures consist of published experimental data on induction of lung epidermoid carcinomas by implantation in the lungs of female Osborne-Mendel rats, collected by a single group of investigators, data on chemical composition of the combustion products obtained

by the same investigators, and published data on background rates of lung carcinomas in female Osborne-Mendel rats. Combustion products considered were diesel engine exhaust, gasoline engine exhaust, flue gas condensate from coal-fired furnaces, and sidestream cigarette smoke. In addition to these data, published data were considered on tumor responses of mice in skin painting experiments. Results obtained from the skin painting experiments were found to be discrepant in some ways from results based on the rat lung implant system; therefore conclusions are based primarily on the latter system, considered to be more directly relevant to human risk from inhalation or ingestion of combustion products.

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Relative potency estimates compared to B[a]P are estimated for various individual PAHs, hydrocarbon combustion products, and fractions of such combustion products. For hydrocarbon combustion products, data were available to test the dose-additivity of fractions of the product, for diesel engine exhaust, gasoline engine exhaust, and flue gas condensate. It was found for these products that the assumption of dose-additivity either accurately predicts the carcinogenicity of a mixture (hydrophobic fraction of diesel engine exhaust and gasoline engine exhaust) or over-predicts carcinogenicity (flue gas condensate), suggesting that the assumption leads to predictions that are accurate or conservative. Estimates based on the dose-additivity assumption are preferred where consistent with the data; this preference maximizes the amount of bioassay information brought to bear in the estimate of a given relative potency. Suggested relative potencies of combustion products, and fractions of these components, compared to B[a]P are summarized, based on final, preferred models.

Nine alternatives are compared, for evaluating the relative potency of a hydrocarbon combustion product relative to B[a]P, based on estimated relative potencies of fractions or component PAHs. These options range from extremely conservative (e.g. considering all solvent extracted components of a combustion product to be as potent as B[a]P) to extremely permissive (considering B[a]P to be the only carcinogenic component). The degree of conservatism of a given procedure is estimated as the ratio of the estimated relative potency of the product computed according to the procedure of interest, to the relative potency estimated directly for the product. Carcinogenicity of the combustion products considered here was found to be attributable primarily to those PAHs with more than three aromatic rings; accordingly, it was found that an accurate procedure, superior in accuracy to the other procedures considered, involves equating the carcinogenicity of a product to the carcinogenicity of the PAHs in the product with more that 3 aromatic rings. However, application of this procedure would require a special animal bioassay to evaluate the carcinogenicity of these highly aromatic PAHs in a given combustion product. Other alternatives are slightly conservative for the combustion products considered here but do not require a special animal bioassay; for example assuming that the carcinogenicity of the combustion product is attributable entirely to the PAHs with more than 3 aromatic rings, and that



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the relative potencies of all such PAHs are equal to the relative potency of B[a]P. Approaches, which involve attributing the carcinogenicity of a combustion product entirely to a handful of detected indicator carcinogenic PAHs, a strategy likely to be considered in regulatory practice, are found to be permissive for the combustion products considered here, whether the potency of relatively carcinogenic PAH (B[a]P) is assumed for each distinct PAH, or specific estimates are used for each PAH.

Certain of the procedures considered involve estimating the relative potency of a mixture of agents using a weighted average of the relative potencies of the individual agents. In these cases the computation of an upper bound for the relative potency of the mixture is problematic. A naive approach to this type of problem, commonly applied in regulatory work, is to estimate a bound for function of multiple model parameters by applying the same function to bounds estimated for the parameters independently; a more exact approach is implemented here based on methods of constrained optimization. The naive approach is compared to the more exact approach for sidestream cigarette smoke, and found to be very conservative.

Relative potency estimates and unit risk estimates for eight PAHs and four hydrocarbon combustion products are displayed below, computed in accordance with procedures and relative potency estimates developed here:

| Agent | Relative potency | Unit risk from inhalation | | Unit risk from ingestion |
|---|---|---|---|---|
| | | 1 $\mu g/m^3$ | 1 mg/kg/day* | 1 mg/kg/day+ |
| B[a]P | 1.0 | $3.89 \times 10^{-5}$ | $1.36 \times 10^{-1}$ | $2.90 \times 10^{-0}$ |
| B[b]F | 0.1228 | $4.78 \times 10^{-6}$ | $1.67 \times 10^{-2}$ | $3.56 \times 10^{-1}$ |
| B[e]F | 0.0070 | $2.72 \times 10^{-7}$ | $9.52 \times 10^{-4}$ | $2.03 \times 10^{-2}$ |
| B[j]F | 0.0523 | $2.03 \times 10^{-6}$ | $7.11 \times 10^{-3}$ | $1.51 \times 10^{-1}$ |
| B[k]F | 0.0532 | $2.07 \times 10^{-6}$ | $7.23 \times 10^{-3}$ | $1.54 \times 10^{-1}$ |
| IND | 0.2780 | $1.08 \times 10^{-5}$ | $3.78 \times 10^{-2}$ | $8.06 \times 10^{-1}$ |
| ANT | 0.3160 | $1.23 \times 10^{-5}$ | $4.29 \times 10^{-2}$ | $9.16 \times 10^{-1}$ |
| B[ghi]P | 0.0212 | $8.25 \times 10^{-7}$ | $2.88 \times 10^{-3}$ | $6.14 \times 10^{-2}$ |
| flue gas from coal fired furnace | 0.0542 | $2.11 \times 10^{-6}$ | $7.37 \times 10^{-3}$ | $1.57 \times 10^{-1}$ |
| diesel engine exhaust | 0.0028 | $1.09 \times 10^{-7}$ | $3.81 \times 10^{-4}$ | $8.12 \times 10^{-3}$ |
| gasoline engine exhaust | 0.0217 | $8.44 \times 10^{-7}$ | $2.95 \times 10^{-3}$ | $6.29 \times 10^{-2}$ |
| sidestream cigarette smoke | 0.0030 | $1.17 \times 10^{-7}$ | $4.08 \times 10^{-4}$ | $8.70 \times 10^{-3}$ |

*Assumes 100% absorption
+Assumes relative potency in lung and digestive tract are equal

It is believed that these estimates will be of considerable use in estimating the cancer risk due to hydrocarbon combustion products and other PAHs mixtures.



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## 1. Introduction

The cancer risk associated with the combustion products of complex hydrocarbons is a well recognized problem. A major portion of such risk is attributable to the carcinogenic PAHs, of which the most intensively studied of the PAHs is Benzo[a]Pyrene (B[a]P).

In previous, companion reports, cancer dose-response models were obtained for ingested and inhaled B[a]P, the most relevant routes of human exposure. Experimental tumorigenicity data is not available for other naturally occurring PAHs administered by these routes. Considerable experimental PAH tumorigenicity data has been generated for other routes of exposure (e.g., lung implants, skin painting). Unfortunately, no known method presently exists for adequately equating the exposure the animals received by these routes to that anticipated for humans. As a result, it is not feasible at this time to obtain human cancer risk estimates for PAHs other than B[a]P directly from the animal tumorigenicity data. It is possible to obtain relative potency estimates of the PAHs compared to B[a]P from the available experimental data, under the assumption that the reactive metabolites of B[a]P and the PAH act via a common general mechanism of action (e.g., point mutation at the same site). Multiplying the PAH exposure level by its estimated relative potency compared to B[a]P gives an estimate of the "B[a]P equivalent exposure level" (i.e., the exposure level of B[a]P that would give the same carcinogenic response as the PAH exposure). These B[a]P equivalent exposure units can then be substituted into a B[a]P ingestion or inhalation dose-response model to indirectly obtain human cancer risk estimates for the PAHs of concern.

In a review of the EPA contract 68-02-4403, several suggestions were made by the review panel for ways of improving the methods previously used to estimate relative potencies of PAHs compared to B[a]P. These suggestions and others supplied by EPA staff are given below in a condensed form.

- Make use of additional available information, such as:
  - survival time of exposure groups in bioassays used,
  - B[a]P positive controls in the same type of experimental systems,
  - historical controls in test species, and
  - vehicle and untreated controls in other studies conducted by the authors of the papers used to make the relative potency estimates.

- Methodological improvements:
  - use all of the data in a bioassay to estimate the model parameters simultaneously,
  - obtain confidence bounds on the relative potency estimates using as much information as possible, and
  - use information on chemical composition of complex PAH mixtures and relative potency estimates of known carcinogenic PAHs to estimate carcinogenic potency of the mixtures.
- Improvements of the mathematical model:
  - incorporate the information on age-specific rate of tumor appearance into the two-stage model and
  - modify the two-stage model to account for the potential saturation of mechanisms that convert the PAHs to their mutagenic metabolites.
- Model verification tasks:
  - test the underlying assumption of dose-additivity and
  - test the proposed method of evaluating the carcinogenicity of a complex mixture of PAHs based on the carcinogenicity of constituents of the mixture.

In this report, these suggestions are incorporated into a method for estimating the carcinogenic potency of individual carcinogenic PAHs, to the fullest extent possible within the resource constraints. Also a method is developed for estimating the carcinogenic potency of a complex mixture of PAHs, which only uses estimates of the amount and relative potency of a few PAHs in the sample. This method is evaluated directly by using known estimates of the carcinogenic potencies of sidestream cigarette smoke, diesel engine exhaust, gasoline engine emissions, and coal-fired residential furnace flue gas condensate.

## 2. Background

One of the main criticisms of the previous attempt to estimate the relative potency of carcinogenic PAHs (EPA contract 68-02-4403) is extensive use of bioassay information of questionable quality. Problems noted in the bioassays previously employed include tumor-independent variation of survival among exposure groups, saturation of the carcinogenic effect of



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dose, outmoded pathological classification, poor experimental design, and inadequate or nonexistent controls.

One method of surmounting these problems is to restrict attention primarily to data generated recently by a single group of investigators, who have used a similar experimental approach and similar pathological classification. One such body of data has been generated by a group of German investigators, who induced lung tumors in rodents using a lung implant system, used the same pathologist and statistician, and reported their results in a similar format, in either the Journal of the National Cancer Institute or Cancer Letters. These data are discussed in greater detail in the following section.

In using this data, it is assumed that relative potencies of a given PAH in lung tissues are similar to relative potencies in target tissues other than the lung. Empirical evidence supporting such a conjecture is limited; however, such a conjecture may be reasonable if the mechanism of metabolism of the PAHs in a target organ is similar to the mechanism of metabolism in the lung.

## 3. Experimental Data Used in Analyses

The experimental data which is to be used in all subsequent analyses is discussed and displayed in this section. Parts of this data will be repeated in subsequent sections in order to clarify precisely what subset of the data has been used for a specific analysis. Three types of data are used in our analyses: 1) bioassay information generated by the German group, 2) historical control lung tumor data for Osborne-Mendal rats, and 3) chemical data on the amounts of selected PAHs in various mixtures.

### 3.1 Bioassay Data

In the previous report (EPA contract 68-02-4403) use was made of a number of test systems for obtaining relative potency estimates. These included intraperitoneal injection in newborn mice, intrapulmonary administration (i.e., lung implants), initiation-promotion on mouse skin, complete carcinogenesis on mouse skin, and subcutaneous injection. In this report we restrict our attention to lung implants and to a much lesser extent complete carcinogenesis on mouse skin. This restriction is due to questionable quality of the data in some test systems, and to resource constraints.

### 3.1.1 Lung Implants

Three studies conducted by Grimmer et al. (1984a, 1987) and Deutsch-Wenzel et al. (1983a), reported in the Journal of the National Cancer Institute, used Osborne-Mendal female rats and a lung implant system to study the carcinogenic effects in lung tissues of gasoline engine exhaust condensate, flue gas condensate from coal-fired residential furnaces, and eight frequently occurring environmental carcinogenic PAHs. Three additional studies reported in Cancer Letters (Deutsch-Wenzel et al. 1983b, Grimmer et al. 1987b, 1988) concerning nitro-PAHs, diesel engine exhaust and sidestream cigarette smoke, respectively, were also based on the lung implant system in female Osborne-Mendal rats. In all of these studies B[a]P was employed as a positive control. However the sidestream cigarette smoke study appears to have used B[a]P controls from the diesel engine exhaust study rather than matched controls. The information obtained from exposed animals in these experiments, relevant to the estimation of the relative potencies for PAHs and fractions of the complex emissions, is displayed in Tables 3.1-3.6.

A short description of the experimental procedures employed to obtain these data follows. Three-month-old inbred Osborne-Mendal female rats were used as test animal in all of the experiments. Various amounts of B[a]P or PAH fractions were dissolved in residue-free acetone and warmed to 50° C, and a 1:1 mixture of beeswax and Trioctanoin was added. The acetone was removed by rotary evaporation under reduced pressure. This material was then warmed to 60° C and introduced by injection into the left lobe of the lung of Nembutal-anesthetized animals following thoracotomy. Following injection of the test material, the thoracotomy aperture was sutured and the skin incision clipped. Following injection, the implant hardened into a pellet from which the test material diffused into the surrounding lung tissue. No further post-operative treatment was needed; operative and post-operative mortality was less than 5%. After surgery, rats were observed until their natural deaths, which occurred up to 32 months following implantation. Moribund animals were killed. When all animals were dead, complete autopsies were performed. The average survival time was obtained for each exposure group, but unfortunately ages at death are not available for individual animals.



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Table 3.1 Rat lung implant carcinogenicity data for pure PAHs

| Agent | Dose mg (x) | Median survival (t) | *Number of animals with epidermoid lung carcinomas /number of animals tested |
|---|---|---|---|
| Benzo[b]Fluoranthene | 0.10 | 110 | 0/35 |
| | 0.30 | 113 | 1/35 |
| | 1.00 | 112 | 9/35 |
| Benzo[e]Pyrene | 0.20 | 117 | 0/35 |
| | 1.00 | 111 | 0/30 |
| | 5.00 | 104 | 1/35 |
| Benzo[j]Fluoranthene | 0.20 | 110 | 1/35 |
| | 1.00 | 117 | 3/35 |
| | 5.00 | 89 | 18/35 |
| Indeno Pyrene | 0.16 | 116 | 3/35 |
| | 0.83 | 109 | 8/35 |
| | 4.15 | 92 | 21/35 |
| Benzo[k]Fluoranthene | 0.16 | 114 | 0/35 |
| | 0.83 | 95 | 3/31 |
| | 4.15 | 98 | 12/27 |
| Anthanthrene | 0.16 | 102 | 1/35 |
| | 0.83 | 88 | 19/35 |
| Benzo[ghi]Fluoranthene | 0.16 | 109 | 0/35 |
| | 0.83 | 114 | 1/35 |
| | 4.15 | 106 | 4/34 |
| Benzo[a]Pyrene | 0.10 | 111 | 4/35 |
| | 0.30 | 77 | 21/35 |
| | 1.00 | 54 | 33/35 |
| Controls | | | |
| Untreated | --- | 100 | 0/35 |
| Vehicle | --- | 100 | 0/35 |

Source: Deutsch-Wenzel et al. (1983a)
*Osborne-Mendal female rats

Table 3.2 Rat lung implant carcinogenicity data for gasoline engine exhaust condensate

| Agent (x.xx) - fraction of total condensate | Dose mg (x) | Median survival (t) | *Number of animals with epidermoid lung carcinomas /number of animals tested |
|---|---|---|---|
| Original condensate | 5.00 | 105 | 3/35 |
| | 10.00 | 99 | 20/35 |
| PAC-free fraction (87.3) | 4.36 | 104 | 3/34 |
| | 8.73 | 108 | 1/34 |
| | 17.45 | 106 | 1/35 |
| PAC with 2 and 3 rings (9.9) | 0.50 | 110 | 0/35 |
| | 0.99 | 106 | 0/35 |
| | 1.98 | 111 | 3/35 |
| PAC with >3 rings (2.8) | 0.14 | 111 | 3/35 |
| | 0.28 | 88 | 15/34 |
| | 0.56 | 80 | 24/35 |
| Benzo[a]Pyrene | 0.03 | 111 | 2/35 |
| | 0.10 | 108 | 5/35 |
| | 0.30 | 94 | 25/35 |
| Controls | | | |
| Untreated | — | 100 | 0/35 |
| Vehicle | — | 108 | 0/34 |

Source: Grimmer et al. (1984a)
*Osborne-Mendal female rats

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Table 3.3 Rat lung implant carcinogenicity data for flue gas condensate from coal-fired residential furnaces

| Agent | Dose mg (x) | Median survival (t) | *Number of animals with epidermoid lung carcinomas /number of animals tested |
|---|---|---|---|
| Flue gas condensate | | | |
| Original condensate | 2.26 | 94 | 15/35 |
| | 4.52 | 89 | 15/35 |
| | 9.04 | 67 | 24/35 |
| PAC-free (fraction I) | 1.00 | 108 | 0/35 |
| | 2.01 | 108 | 0/34 |
| | 4.02 | 111 | 1/27 |
| PACs, 2 and 3 rings (fraction II) | 0.56 | 104 | 1/35 |
| | 1.12 | 106 | 0/35 |
| PAHs and S-PACs, 4-7 rings (fraction III) | 0.35 | 98 | 9/35 |
| | 0.69 | 96 | 14/35 |
| Polar PACs and higher PAHs (fraction IV) | 0.94 | 94 | 18/35 |
| Insoluble carbon | 4.28 | 112 | 0/35 |
| Reconstituted from all fractions (without carbon) | 2.10 | 100 | 8/31 |
| Benzo[a]Pyrene | 0.05 | 100 | 3/35 |
| | 0.10 | 104 | 5/35 |
| | 0.20 | 95 | 15/35 |
| Controls | | | |
| Untreated | --- | 105 | 0/35 |
| Vehicle | --- | 100 | 0/35 |

Source: Grimmer et al. (1987a)
*Osborne-Mendal female rats

Table 3.4 Rat lung implant carcinogenicity data for diesel engine exhaust condensate

| Agent | Dose mg (x) | Median survival (t) | *Number of animals with epidermoid lung carcinomas /number of animals tested |
|---|---|---|---|
| Hydrophilic fraction | 6.70 | 97 | 0/35 |
| Hydrophobic fraction | 20.00 | 99 | 5/35 |
| non-aromatics and PAC 2 and 3 rings | 19.22 | 103 | 0/35 |
| PAH 4-7 rings | 0.21 | 102 | 6/35 |
| Polar PAC | 0.29 | 97 | 0/35 |
| Nitro-PAH | 0.19 | 106 | 1/35 |
| Reconstituted hydrophobics | 19.91 | 93 | 7/35 |
| Benzo[a]Pyrene | 0.03 | 97 | 3/35 |
| | 0.10 | 98 | 11/35 |
| | 0.30 | 69 | 27/35 |
| Control | | | |
| Untreated | --- | 110 | 0/35 |
| Vehicle | --- | 103 | 0/35 |

Source: Grimmer et al. (1987b)
*Osborne-Mendal female rats



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Table 3.5 Rat lung implant carcinogenicity data for cigarette sidestream smoke constituents

| Agent | Dose mg (x) | Median survival (t) | *Number of animals with epidermoid lung carcinomas /number of animals tested |
|---|---|---|---|
| PAH-free material and PAH 2 and 3 rings | 16.00 | 102 | 1/35 |
| PAH 4 and more rings | 1.06 | 105 | 5/35 |
| Semivolatiles (gaseous phase) | 11.80 | 104 | 0/35 |
| Benzo[a]Pyrene | 0.03 | 93 | 3/35 |
| | 0.10 | 98 | 11/35 |
| | 0.30 | 75 | 27/35 |
| Controls | | | |
| Untreated | --- | 102 | 0/35 |
| Vehicle | --- | 105 | 0/35 |

Source: Grimmer et al. (1988)
*Osborne-Mendal female rats

Table 3.6 Rat lung implant carcinogenicity data for nitro-PAHs

| Agent | Dose mg (x) | Median survival (t) | *Number of animals with epidermoid lung carcinomas /number of animals tested |
|---|---|---|---|
| Benz[c]Acridine | 0.1 | 114 | 0/35 |
| | 0.3 | 112 | 0/35 |
| | 1.0 | 116 | 0/35 |
| Dibenz[a,j]Acridine | 0.1 | 102 | 0/35 |
| | 0.3 | 111 | 0/35 |
| | 1.0 | 104 | 0/35 |
| Dibenz[a,h]Acridine | 0.1 | 113 | 0/35 |
| | 0.3 | 104 | 3/35 |
| | 1.0 | 99 | 9/35 |
| Acridine | 0.2 | 109 | 0/35 |
| | 1.0 | 106 | 0/35 |
| | 5.0 | 109 | 0/35 |
| Benz[a]Acridine | 0.2 | 106 | 0/35 |
| | 1.0 | 111 | 0/35 |
| | 5.0 | 109 | 0/35 |
| Benzo[a]Pyrene | 0.1 | 103 | 5/35 |
| | 0.3 | 73 | 24/35 |
| | 1.0 | 64 | 27/35 |
| Controls | | | |
| Untreated | --- | 110 | 0/35 |
| Vehicle | --- | 103 | 0/35 |

Source: Deutsch-Wenzel et al. (1983b)
*Osborne-Mendal female rats

### 3.1.2 Skin Painting

Considerable additional work on complex mixture of PAH exposure has also been carried out by the same group of German investigators using the mouse skin bioassay system (Habs et al. 1980 and Grimmer et al. 1982, 1983, 1984c, 1985). The information from these experiments may be used in the future to develop estimates of the relative potencies of complex mixture fractions, which would then be compared to the estimates based on lung implant data. Because of resource constraints such an analysis has not been performed. However, skin painting data for pure PAHs, found in Habs et al. (1980) and displayed in Table 3.7 is used here to estimate relative potencies of several PAHs, and these estimates are compared to results based on lung implant data.

In the Habs et al. (1980) study, the carcinogenicity of a number of pure PAHs was investigated using 10 week old female NMRI mice. Solutions of the PAHs in acetone were applied topically to clipped skin twice a week, except that coronene was applied four times a week in order to induce an adequate tumor response. Animals were followed until their deaths or until an advanced stage of clearly infiltrative tumor growth could be detected macroscopically, at which point they were killed and autopsied. Note that statistics were given on "local tumors". It is difficult to determine from the description what types of tumors are included. For purposes of this analysis, all tumors are assumed to be carcinomas; however, the authors also report that other types of tumors were induced by certain compounds, e.g., lymphosarcoma and papilloma in response to treatment with coronene.

## 3.2 Historical Controls

No tumors were reported in any of the control groups. Therefore, historical control data was used to obtain a non-zero estimate of the background lung cancer rate. This was done in order to obtain a biological meaningful model, and to avoid potential computational problems. Historical control data for Osborne-Mendal rats, compiled by Goodman et al. (1980), are displayed in Table 3.8. We note that the rates are not statistically different between male and female rats for any type of lung tumor. Therefore, we pool the information for males and females, for epidermoid carcinomas (i.e., the specific tumor type elicited in the bioassays) for potential use in our subsequent analyses.

Table 3.7 Mouse skin painting carcinogenicity data for pure PAHs

| Agent | Dose | | *Number of animals with tumors/number of animals tested |
|---|---|---|---|
| | pg/animal | doses/week | |
| Solvent (acetone) | --- | --- | 0/35 |
| DMSO | --- | --- | 0/36 |
| Benzo[a]Pyrene | 1.7 | 2 | 8/34 |
| | 2.8 | 2 | 24/35 |
| | 4.6 | 2 | 22/36 |
| Benzo[b]Fluoranthene | 3.4 | 2 | 2/38 |
| | 5.6 | 2 | 5/34 |
| | 9.2 | 2 | 20/37 |
| Benzo[j]Fluoranthene | 3.4 | 2 | 1/38 |
| | 5.6 | 2 | 1/35 |
| | 9.2 | 2 | 2/38 |
| Benzo[k]Fluoranthene | 3.4 | 2 | 1/39 |
| | 5.6 | 2 | 0/38 |
| | 9.2 | 2 | 0/38 |
| Indeno[1,2,3-cd]Pyrene | 3.4 | 2 | 1/36 |
| | 5.6 | 2 | 0/35 |
| | 9.2 | 2 | 0/37 |
| Cyclopentadieno[cd]Pyrene | 1.7 | 2 | 0/34 |
| | 6.8 | 2 | 0/35 |
| | 27.2 | 2 | 3/38 |
| Coronene | 5.0 | 4 | 1/39 |
| | 15.0 | 4 | 2/40 |

Source: Habs et al. (1980)
*NMRI female mice

Table 3.8 Historical lung tumor control data for Osborne-Mendal rats

| Lung tumors | Male | Female | Combined |
|---|---|---|---|
| Epidermoid carcinomas | 1/975 | 0/970 | 1/1945 |
| Alveolar/bronchiolar adenoma | 4/975 | 2/970 | 6/1945 |
| Alveolar/bronchiolar carcinoma | 3/975 | 3/970 | 6/1945 |

Source: Goodman et al. (1980)

## 3.3 Chemical Composition of Selected PAHs in Emission Samples

An important advantage that is gained by using the lung implant information obtained by the German group is that extensive chemical analysis has been conducted on the emission samples placed in the implants. This allows evaluation of procedures for estimating the potency of a sample from the amount and relative potencies of some of its constituents. For each of the four complex mixtures the amounts of eleven PAHs are displayed in Table 3.9. Eight of these PAHs have lung implant bioassay data available for them. In addition, the amounts of the other three PAHs anthracene, fluoranthene, and pyrene are also given. These three PAHs have been heavily tested in bioassay systems, and all are classified by the International Agency for the Research of Cancer (IARC) as "adequately tested with no evidence of carcinogenicity" (IARC 1983). Measurements were not obtained for some of the eleven PAHs in some of the studies, which is indicated by a --- in Table 3.9.

# 4. Mathematical Dose-Response Model Development

In this section the previous model used for obtaining relative potency estimates is reviewed and a new general dose-response model is developed. The new model has been made flexible enough to accommodate a wide variety of different types of data, including survival information, tumor responses to simultaneous exposure to multiple agents, and simultaneous use of information from different types of bioassay systems. The new model can also account for tumors generated by an experimentally-induced irritation effect (i.e., an increment in tumor response associated with the experimental procedure and independent of the PAH dose), and saturation of the mechanisms which converts PAHs to their mutagenic metabolites.

For ease of expression, all of the models will be represented in this report by their cumulative hazard function $H(x,t)$, where $x$ is the exposure level and $t$ is the age of observation. The relationship between probability of response (e.g., detection of cancer) and cumulative hazard is

$$P(x,t) = 1\text{-}\exp\text{-}H(x,t).$$

The unknown parameters in all the proposed models are estimated by the method of maximum likelihood. It is assumed that the number of animals with carcinoma at a given exposure level is a binomial random variable independent of the number of animals responding at other exposure levels. It follows that the likelihood of any set of data is the product of the likelihoods



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Table 3.9 Amounts of selected PAHs in hydrocarbon combustion product emission samples

| Type of PAH | Gasoline engine exhaust condensate[1] (μg/ℓ) | Diesel engine exhaust[2] (μg/g) | Coal-fired residential furnace flue gas condensate[3] (mg/kg) | Sidestream cigarette smoke[4] (μg/cig) (sum particle filter & porapak) |
|---|---|---|---|---|
| Anthracene | 642 | --- | 2.113 | 710.0 |
| Fluoranthene | 1662 | 725 | 28.357 | 685.9 |
| Pyrene | --- | 756 | 26.589 | 476.3 |
| Benzo[b]Fluoranthene | 19 | 57 | 6.102 | 65.8 |
| Benzo[k]Fluoranthene | 7 | 46 | --- | 65.8 |
| Benzo[j]Fluoranthene | 11 | 116 | 6.782 | 65.8 |
| Benzo[e]Pyrene | 37 | 95 | 3.994 | 75.7 |
| Benzo[a]Pyrene | 50 | 92 | 4.303 | 103.5 |
| Indeno Pyrene | 32 | 85 | 4.600 | 51.4 |
| Benzo[ghi]Perylene | 115 | 115 | 3.855 | 41.6 |
| Anthanthrene | 26 | --- | 0.665 | --- |
| Total | 2601 | 2087 | 87.360 | 2341.8 |

[1]Source: Grimmer et al. (1977)
[2]Source: Grimmer et al. (1987a)
[3]Source: Grimmer et al. (1985)
[4]Source: Grimmer et al. (1987b)

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corresponding to different exposure levels. The general approach discussed by Gart et al. (1986, pg. 125, Section iii) is used to obtain maximum likelihood estimates for the unknown parameters in the models. The estimation procedures were implemented using the GMF computer program developed for EPA.

## 4.1 Previously Employed Model

In the previous "Interim Final Report" for EPA contract 68-02-4403, the rationale for a simple two-stage model was developed and the model was used to estimate relative potencies of selected PAHs compared to B[a]P, based on the assumptions that:

- tumor-independent survival is not exposure-dependent,
- the critical carcinogenic PAH metabolites affect the cell transition rates but not the preneoplastic clone growth rate,
- the concentration of PAH reactive metabolites in target tissues is proportional to the exposure dose,
- the relative change in the transition rate per unit of exposure (i.e., the incremental change per unit exposure, divided by the background rate) is equal for the two transitions,
- the reactive metabolites of different PAHs cause the same mutations, via the same general mechanism of action (e.g., point mutation), and
- the combined carcinogenic effect of multiple PAHs is dose additive.

Under these assumptions the cumulative hazard rate at the end of typical lifespan, given constant exposure to the jth agent at level $x_j$, has the simple form

$$H(x_j) = M \cdot (1+R_j x_j)^2 \qquad j=1,2,\ldots,m.$$

In this model M is approximately the background cancer rate, $R_j$ is the relative cell transition rate for the jth PAH, and m is the number of PAHs under consideration. To define $R_j$ precisely, let $T(x_j)$ be the transition rate at an exposure to the jth type of PAH, of magnitude $x_j$; then

$$R_j = \frac{T(x_j) - T(0)}{x_j \cdot T(0)}$$



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The relative potency of the jth PAH compared to B[a]P is here defined as

$$\rho_j = \frac{R_j}{R_1}$$

where $R_1$ is the relative transition rate per unit of exposure to B[a]P. In the previous use of the model the parameters $R_j$ were estimated independently using just control data and the jth PAH exposure group. The procedure employed to estimate the potencies was not statistically optimal. These non-optimal estimates were then used to obtain relative potency estimates by dividing the estimated relative transition rate for an agent by the estimated relative transition rate for B[a]P.

## 4.2 Model Extensions

In this report, several of the basic assumptions of the previous model are generalized and the model is extended in several other important ways. The model that results can accommodate a wider range of experimental data than the previous model, and allows more precise parameter estimation by using data from multiple agents and bioassay systems simultaneously. The new model allows for differences in survival time between the exposure groups by employing the age-dependent form of the two-stage model. In addition we relax the assumption of equal relative rates for the two assumed transitions.

Under these modified assumptions the cumulative hazard for exposure to B[a]P at level x for duration t has the form

$$H(x,t) = \frac{A}{G^2}(1+S_0x)(1+S_1x)[\exp(Gt)-Gt-1]$$

where the parameters are defined below.

| | |
|---|---|
| A | is the product of background transition rates |
| G | is the growth rate of the preneoplastic clone |
| $S_0$ | is the relative transition rate from stem to preneoplastic cell |
| $S_1$ | is the relative transition rate from preneoplastic to neoplastic cell |

## 4.3 Adjustments for Saturation of PAH Metabolism

In cancer bioassay experiments it is often observed that the tumor response rate flattens

or even decreases at the highest exposure levels. This phenomena has been attributed to such factors as:

1. exposure-dependent non-tumor mortality
2. toxic effects on pre- and neoplastic cells
3. saturation of absorbed dose
4. saturation of mechanism that converts an agent to its reactive metabolite
5. natural consequence of a birth-and-death model of growth of a preneoplastic clone (see Moolgavkar et al. 1990)
6. population heterogeneity, where most of the individuals in a sub-population are very sensitive and have responded at the higher dose levels

If any of these effects occur, then neither the two-stage model used here nor the multistage model will adequately describe the dose-response relationship without modification.

For the PAHs the alteration in the anticipated shape of the dose-response curve at higher doses is most likely due to some type of saturation of the metabolic processes that create the active carcinogen. If more information were available about the exact nature of the saturation effect, then the true functional relationship between the concentration of delivered active metabolite and the administered dose could be used directly in the dose-response model. In the absence of such information the simplest type of saturation mechanism will be assumed.

Following Rai and Van Ryzin (1983; see Gart et al. 1986) we make the simple assumption that the agent is metabolized by a saturable mechanism with rate described by the Michaelis-Menton equation

$$\frac{dD}{dt} = \frac{V_{max} \cdot x}{(k_m + x)}$$

where dD/dt is the rate of production of metabolite (D) from parent (x), $V_{max}$ is the maximum rate of production of metabolite, and $k_m$ is the value of x at which the rate is $V_{max}/2$. The true "effective dose" is regarded as the steady-state concentration of the metabolite D in target tissues, here denoted $D^*$, which can be derived in closed form with certain additional simplifying assumptions. More realistic (hence more complicated) pharmacokinetic assumptions may require additional model development, extensive data on values of pharmacokinetic parameters (including possibly direct experimental measurements), and solution of differential equations by numerical

procedures.

We assume that the rate of $T(x_i)$ of the transitions induced by an administered dose $x_i$ for the ith PAH is linear in the effective dose, i.e.,

$$T(x_i) = \alpha + \beta_i \cdot D^* = \alpha + \beta_i \cdot \left[\frac{B_i x_i}{1 + k_i x_i}\right]$$

where $\alpha$ is the background transition rate, $\beta_i$ is the increase in transition rate per unit increase in *effective dose*, and $B_i$ and $k_i$ are functions of $V_{max}$ and $k_m$ as described by Gart et al. (1986), with subscript i indicating possible dependence on the agent of interest. As $x_i$ is made very small, this expression becomes approximately

$$T(x_i) = \alpha + (\beta_i B_i) \cdot x_i,$$

from which it is clear that the product $\beta_i B_i$ is appropriately described as the low-dose potency of the *administered dose* of the agent (i.e., the increase in transition per unit increase in administered dose. A final, related definition, is the relative potency of the ith PAH compared to the first PAH (here B[a]P)

$$Q_i = \frac{\beta_i B_i}{\beta_1 B_1}.$$

4.4 Exposure to Multiple Agents

Under the assumption of dose additivity the total number of B[a]P equivalent exposure units, when exposure is to m agents, has the generalized form

$$y = \sum_{j=1}^{m} y_j = \sum_{j=1}^{m} \frac{Q_j x_j}{1 + k_j x_j}$$

where the subscript j refers to the jth agent. The cumulative hazard for such a joint exposure has the obvious form

$$H(y, t) = \frac{A}{G^2}(1 + S_0 y)(1 + S_1 y)[\exp(Gt) - Gt - 1]$$

with y defined as above.

## 4.5 Irritation Effects

There is evidence for some of the complex combustion product emissions, of what may be termed an irritation effect in the induction of epidermoid carcinomas. Such an effect is hypothesized to be caused by the interaction of such factors as

- mechanical injury associated with the surgical procedure,
- immune response to the implant, and
- the irritation of leaching materials from the implant

Assuming that this effect occurs independently of other carcinogenic effects and induces both stage transition rates (i.e., the simplest method of incorporating an irritation effect), the cumulative hazard function has the form

$$H(y,t) = \phi + \left\{ \frac{A}{G^2}(1+S_0 y)(1+S_1 y) \right\} [\exp(Gt) - Gt - 1]$$

where $\phi$ denotes the magnitude of the irritation effect, which is to be estimated from the data.

In the following sections specific forms of this general model that depend upon the type and extent of data available will be employed in a variety of analyses.

## 5. Selection of Positive and Negative Control Data

To increase the precision of relative potency estimates for pure PAHs and fractions of hydrocarbon combustion products, we want to use as much negative and positive (i.e., B[a]P) control data as is possible. To justify combining control data between studies it is necessary to establish that cancer response rates are not statistically different between studies. The positive and negative control data from the six lung implant studies is taken from Tables 3.1-3.6 and combined to form Table 5.1.

The six-experiment negative control data is clearly consistent between studies since no tumors were observed in the total 419 control animals. The 0/419 for the combined negative controls is also consistent with the historical control rate of 1/1945. Therefore, it appears that all of the negative control data (i.e., six-experiment and historical) can be used in all subsequent analyses.

Since survival times and exposure levels differ between experiments for the B[a]P positive controls, it is not possible to compare the rates directly. Several other potential problems also exist in the analysis of the consistency of the response data. First, survival times for the coal flue

Table 5.1 Negative controls and positive B[a]P control data for lung implant experiments

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| Journal in which study published | Agents used in study | | Exposure (x) | Median survival (t) | # with epidermoid carcinoma/# on test |
|---|---|---|---|---|---|
| JNCI | Pure PAHs | Controls | | | |
| | | Vehicle | — | 104 | 0/35 |
| | | Matched | — | 118 | 0/35 |
| | | B[a]P | 0.10 | 111 | 4/35 |
| | | | 0.30 | 77 | 21/35 |
| | | | 1.00 | 54 | 33/35 |
| JNCI | Gasoline Engine Exhaust | Controls | | | |
| | | Vehicle | — | 108 | 0/34 |
| | | Matched | — | 100 | 0/35 |
| | | B[a]P | 0.03 | 111 | 2/35 |
| | | | 0.10 | 108 | 5/35 |
| | | | ≥0.30 | 94 | 25/35 |
| JNCI | Coal Flue Gas Condensate | Controls | | | |
| | | Vehicle | — | 100 | 0/35 |
| | | Matched | — | 105 | 0/35 |
| | | B[a]P | 0.05 | 100 | 3/35 |
| | | | 0.10 | 104 | 5/35 |
| | | | 0.20 | 95 | 15/35 |
| Cancer Letters | Diesel Engine Exhaust | Controls | | | |
| | | Vehicle | — | 103 | 0/35 |
| | | Matched | — | 110 | 0/35 |
| | | B[a]P | 0.03 | 97 | 3/35 |
| | | | 0.10 | 98 | 11/35 |
| | | | 0.30 | 69 | 27/35 |
| Cancer Letters | Cigarette Sidestream Smoke | Controls | | | |
| | | Vehicle | — | 102 | 0/35 |
| | | Matched | — | 105 | 0/35 |
| | | B[a]P | 0.03 | 93 | 3/35 |
| | | | 0.10 | 98 | 11/35 |
| | | | 0.30 | 75 | 27/35 |
| Cancer Letters | Nitro-PAHs | Controls | | | |
| | | Vehicle | — | 103 | 0/35 |
| | | Matched | — | 110 | 0/35 |
| | | B[a]P | 0.10 | 103 | 5/35 |
| | | | 0.30 | 73 | 24/35 |
| | | | 1.00 | 64 | 27/35 |

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gas and nitro-PAH studies are expressed as mean survival, while for the other four studies the median survival time is given. Second, either a very rare event has occurred (i.e., the exact cancer response rates were obtained for two studies) or the B[a]P positive controls used in the cigarette sidestream smoke study (Grimmer et al. 1988) were actually taken from the diesel engine exhaust study (Grimmer et al. 1987b). Thus, whether to include the tumor rates for these two studies once or twice in the analysis is not clear.

A method for testing the consistency of the response is to fit a single dose-response model to the entire data set. The model chosen has the following simple form for the cumulative hazard

$$H(x,t) = A(1+S_0x)(1+S_1x)t^2$$

which results from our general model with the simplifying assumptions:

1. The product of the background transition A is the same across all six lung implant experiments.

2. The postulated preneoplastic clone of the epidermoid carcinoma appears to be virtually dormant after its initial early appearance. Therefore, we assume that

$$G \to 0 \quad so \quad that \frac{[\exp(Gt) - Gt - 1]}{G^2} \to \frac{t^2}{2}$$

3. No irritation effect is present for B[a]P (i.e., $\phi=0$).

4. Metabolic saturation is not present for B[a]P (i.e., $k \to 0$)

It was found that

- the model did not adequately describe the entire six-experiment data set,
- permitting positive estimates for $\phi$ and/or k did not materially improve the fit,
- the estimates of $S_0$ and $S_1$ are equal, and
- the three JNCI studies (i.e., pure PAHs, gasoline engine exhaust, and coal flue gas condensate) can be fitted to the same model.

Therefore, it was decided to use the three-experiment JNCI controls for any experiment analyzed within this group and matched B[a]P controls for the other three experiments.



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Also it was found that convergence of the numerical estimation algorithm was achieved much more easily if it was assumed that $S_0=S_1=S$ from the start, an assumption which has some biological basis. To check the effect of some of these decisions on the relative potency estimates obtained for a complex mixture, the sensitivity experiment described in the next section was conducted.

## 6. Evaluation of the Sensitivity of Relative Potency Estimates to Selection of Control Data and to the Assumption of Equal Cell Transition Rates

Due to its monotonic dose-response and typical results, the gasoline engine exhaust lung implant study (Grimmer et al. 1984a) was chosen to conduct a sensitivity analysis. To investigate the effects of alternative definitions of tumors among control animals and alternative transition rate assumptions, the following analysis was performed on the data displayed in Table 6.1 (selected from Tables 3.1-3.3, 3.7).

The relative potencies of the entire gasoline engine exhaust condensate (i.e., original) and the PAC > 3 ring portion of the condensate, were calculated under all eight possible combinations of the following alternatives conditions:

- positive controls:
  - (1) matched controls from the B[a]P gasoline engine experiment, or
  - (2) B[a]P controls from three experiments: gasoline engine exhaust, coal fired flue gas from residential furnaces, and pure PAH experiments
- historical controls:
  - (1) lung epidermoid carcinomas in male and female Osborne-Mendal rats, or
  - (2) lung carcinomas in female Osborne-Mendal rats
- cell transition rate assumption:
  - (1) relative transition rates assumed equal (i.e., $S_0=S_1$), or
  - (2) relative transition rates not constrained (i.e., $S_0$ not necessarily equal to $S_1$)

The model used was the same as for B[a]P under the assumption that $k=0$ and $\phi=0$ for all agents. Both the original condensate and the PAC with > 3 ring fraction were treated like B[a]P for purposes of this analysis. In a more complete analysis of gasoline engine exhaust

Table 6.1 Data used to evaluate the stability of relative potency estimates using different assumption and control data

| Agent | Dose (mg) | Median survival | # animals on test (Osborne-Mendal female rats) | Observed # epidermoid carcinomas |
|---|---|---|---|---|
| Historical Controls | 0.00 | 104 | 1945* | 1 |
| Gasoline Engine Exhaust Condensate Study | | | | |
| Original condensate | 5.00 | 105 | 35 | 3 |
| | 10.00 | 99 | 35 | 20 |
| PAC with >3 rings | 0.14 | 111 | 35 | 3 |
| | 0.28 | 88 | 34 | 15 |
| | 0.56 | 80 | 35 | 24 |
| Untreated controls | 0.00 | 104 | 35 | 0 |
| Vehicle controls | 0.00 | 118 | 35 | 0 |
| B[a]P | 0.10 | 111 | 35 | 4 |
| | 0.30 | 77 | 35 | 21 |
| | 1.00 | 54 | 35 | 33 |
| Coal-Fired Residential Furnace Flue Gas Condensate Study | | | | |
| Untreated controls | 0.00 | 108 | 34 | 0 |
| Vehicle controls | 0.00 | 100 | 35 | 0 |
| B[a]P | 0.03 | 111 | 35 | 2 |
| | 0.10 | 108 | 35 | 5 |
| | 0.30 | 94 | 35 | 25 |
| Pure PAHs | | | | |
| Untreated controls | 0.00 | 100 | 35 | 0 |
| Vehicle controls | 0.00 | 100 | 35 | 0 |
| B[a]P | 0.05 | 100 | 35 | 3 |
| | 0.10 | 104 | 35 | 5 |
| | 0.20 | 95 | 35 | 15 |

*males and females combined

fractions in a following section, use will be made of the entire data obtained in the Grimmer et al. (1984a) study.

The relative potency estimates and some of their 95% upper and lower confidence bounds under the eight alternatives are displayed in Table 6.2. We note that none of the alternative assumptions have a very pronounced effect on the relative potency estimates. For the entire condensate samples, the ratio for the highest to lowest estimate among the eight conditions is only 1.053, while it is an even smaller ratio, 1.049, for the PAC > 3 ring portion samples. The biological variability, although small as measured by the confidence intervals, appears to contribute much more significantly to total uncertainty regarding the relative potency estimates than the alternative assumptions. Thus, our previous use of the 3-experiment positive B[a]P controls, historical controls for both sexes combined, and assumption of equal relative transition rates appear reasonable and will be continued in subsequent analyses.

## 7. Relative Potency Estimates for Single PAHs and Nitro-PAHs

In this section a dose-response model for PAHs and nitro-PAHs is developed that uses all of the data in an experiment in the estimation of relative potencies. Confidence bounds are obtained for the estimates and comparisons made with previous estimates.

### 7.1 Model for Pure PAHs

It is obvious that the most general model for the accumulated pure PAH data in the lung implant system, shown in Table 7.1 (obtained from information found in Tables 3.1-3.3, and 3.7), would contain a large number of unknown parameters. This is illustrated in Display 1 where we note that 31 parameters would have to be estimated using only 36 data points, in order to use the full model.

Display 1

General model: Total possible parameters for data reported in Table 7.1

| | | |
|---|---|---|
| A | separate for each experiment | 3 values |
| G | separate for each experiment | 3 values |
| $\phi_j$, $k_j$ | for each of eight agents | 2 × 8 = 16 values |
| $Q_j$ | for seven agents $Q_1=1$ for B[a]P | 7 values |
| $S_0$, $S_1$ | constant across all agents and experiments | 2 values |
| | 36 Separate Experimental Groups | 31 Parameters |

Table 6.2 Summary of relative potencies of fractions of gasoline engine exhaust condensate under various assumptions

| Model form | Value* | Alternative background rate assumptions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Lung epidermoid carcinoma in Osborne-Mendal rats (1/1945) | | | | | | Lung carcinoma in Osborne-Mendal female rats (3/970) | |
| | | 3-experiment positive controls | | | Matched-experiment positive controls | | | 3-experiment positive controls | Matched experiment positive controls |
| | | 95% lower bound | M.L.E. | 95% upper bound | 95% lower bound | M.L.E. | 95% upper bound | M.L.E. | M.L.E. |
| Equal relative transition rate model $S_0=S_1=S$ | $\rho_1$ | 0.017821 | 0.021997 | 0.02673 | 0.01647 | 0.021002 | 0.026628 | 0.021920 | 0.020968 |
| | $\rho_2$ | 0.567440 | 0.670413 | 0.78739 | 0.50181 | 0.639888 | 0.789670 | 0.673781 | 0.644825 |
| | lnL | | -240.18961 | | | -145.36945 | | -252.29642 | -157.4805 |
| Non-constrained relative transition rate model $S_0 \neq S_1$ | $\rho_1$ | | 0.021915 | | | | | | |
| | $\rho_2$ | | 0.672456 | | | (same as above) | | (same as above) | (same as above) |
| | lnL | | -240.07874 | | | | | | |

* $\rho_1$ = relative potency of gasoline engine exhaust entire original condensate compared to B[a]P.
$\rho_2$ = relative potency of PAC with > 3 rings of gasoline engine exhaust condensate compared to B[a]P.
lnL = log-likelihood of the M.L.E. fit to the data.

Table 7.1 Data used to evaluate the stability of relative potency estimates for pure PAHs

| Agent | Dose (mg) | Median survival | # animals on test (Osborne-Mendal female rats) | Observed # epidermoid carcinomas |
|---|---|---|---|---|
| Historical Controls | 0.00 | 104 | 1945* | 1 |
| Gasoline Engine Exhaust Condensate Study | | | | |
| Untreated controls | 0.00 | 104 | 35 | 0 |
| Vehicle controls | 0.00 | 118 | 35 | 0 |
| B[a]P | 0.10 | 111 | 35 | 4 |
| | 0.30 | 77 | 35 | 21 |
| | 1.00 | 54 | 35 | 33 |
| Coal-Fired Residential Furnace Flue Gas Condensate Study | | | | |
| Untreated controls | 0.00 | 108 | 34 | 0 |
| Vehicle controls | 0.00 | 100 | 35 | 0 |
| B[a]P | 0.03 | 111 | 35 | 2 |
| | 0.10 | 108 | 35 | 5 |
| | 0.30 | 94 | 35 | 25 |
| Pure PAHs | | | | |
| Untreated controls | 0.00 | 100 | 35 | 0 |
| Vehicle controls | 0.00 | 100 | 35 | 0 |
| B[a]P | 0.05 | 100 | 35 | 3 |
| | 0.10 | 104 | 35 | 5 |
| | 0.20 | 95 | 35 | 15 |

Table 7.1 continued

| Agent | Dose (mg) | Median survival | # animals on test (Osborne-Mendal female rats) | Observed # epidermoid carcinomas |
|---|---|---|---|---|
| Benzo[b]Fluoranthene | 0.10 | 110 | 35 | 0 |
| | 0.30 | 113 | 35 | 1 |
| | 1.00 | 112 | 35 | 9 |
| Benzo[e]Pyrene | 0.20 | 117 | 35 | 0 |
| | 1.00 | 111 | 30 | 0 |
| | 5.00 | 104 | 35 | 1 |
| Benzo[j]Fluoranthene | 0.20 | 110 | 35 | 1 |
| | 1.00 | 117 | 35 | 3 |
| | 5.00 | 89 | 35 | 18 |
| Benzo[k]Fluoranthene | 0.16 | 114 | 35 | 0 |
| | 0.83 | 95 | 31 | 3 |
| | 4.15 | 98 | 27 | 12 |
| Indeno Pyrene | 0.16 | 116 | 35 | 3 |
| | 0.83 | 109 | 35 | 8 |
| | 4.15 | 92 | 35 | 21 |
| Anthanthrene | 0.16 | 102 | 35 | 1 |
| | 0.83 | 88 | 35 | 19 |
| Benzo[ghi]Perylene | 0.16 | 109 | 35 | 0 |
| | 0.83 | 114 | 35 | 1 |
| | 4.15 | 106 | 34 | 4 |

*males and females combined



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It is well known that such over-parameterized models tend to be difficult to fit and to result in relatively imprecise predictions. A more prudent approach is to start with a simpler model and only add additional parameters if they significantly improve the model fit. The following simplifying assumptions provide a reasonable starting point for a manageable model:

1. The product of the background transition A is the same across all three lung implant experiments reported in the JNCI.

2. The postulated clone of epidermoid carcinoma precursor cells appears to be virtually dormant after its initial early appearance. Therefore, we assume that

$$G \to 0 \quad so \ that \quad \frac{[\exp(Gt) - Gt - 1]}{G^2} \to \frac{t^2}{2}$$

3. The relative transition rates between stages are equal, i.e., $S_0 = S_1 = S$.

4. No irritation effect is present for the pure PAHs, i.e., $\phi = 0$ for all agents.

5. Metabolic saturation is not present for any of the pure PAHs, i.e., $k \to 0$ for all agents.

6. Exposure to different PAHs is dose-additive.

The cumulative hazard function under this set of simplifying assumptions has the form

$$H(y, t) = A(1 + Sy)^2 t^2$$

where

$$y = \sum_{j=1}^{m} Q_j x_j$$

is the B[a]P equivalent exposure for up to m agents.

This model is fitted to the entire data in Table 7.1 and the resulting low-dose relative potency estimates (i.e., $Q_j$'s) are displayed in Table 7.2 ("Case 1"). We note that the $\chi^2$ goodness-of-fit test results in a p-value of 0.0034, which suggests that the model does not adequately describe the observed tumor data. However, a closer inspection of the discrepancies between observed and the predicted values suggest that the significance of the $\chi^2$ test is largely due to the single agent Indeno Pyrene (IND). This is verified by removing the IND information from the

Table 7.2 Low-dose relative potency estimate $Q_j$ and goodness-of-fit values for four different approaches

| Agent (pure PAH) | Case 1 all $k_j \to 0$ | Case 2 (IND omitted) all $k_j \to 0$ | Case 3 separate $Q_j$ for each exposure level IND all $k_j \to 0$ | Case 4 $k_j > 0$ for IND all other $k_j \to 0$ |
|---|---|---|---|---|
| B[a]F | 0.12106 | 0.12299 | 0.12299 | 0.12277 |
| B[e]P | 0.00654 | 0.00710 | 0.00710 | 0.00704 |
| B[j]F | 0.05214 | 0.05234 | 0.05234 | 0.05232 |
| B[k]F | 0.05298 | 0.05325 | 0.05325 | 0.05322 |
| IND | 0.07725 | --- | low dose 0.39291 | 0.27800 |
| | --- | --- | middle dose 0.14446 | --- |
| | --- | --- | high dose 0.06652 | --- |
| ANT | 0.31626 | 0.31594 | 0.31594 | 0.31598 |
| B[ghi]P | 0.02065 | 0.02132 | 0.02132 | 0.02124 |
| Number of Parameters Estimated | 9 | 8 | 11 | 10 |
| $\chi^2$ | 51.09 (33.79)* | 19.03 | 19.03 | 21.40 |
| d.f. | 27 | 25 | 25 | 26 |
| p | 0.0034 | 0.796 | 0.796 | 0.721 |
| lnL | -354.4303 | -292.9153 | -345.5225 | -346.6435 |

*amount of $\chi^2$ due to IND



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data set and refitting the model. The results are shown as Case 2 in Table 7.2. We note that the relative potency estimate for the remaining agents are little affected by removal of the data for IND, but the goodness-of-fit is excellent with a p-value of 0.796.

Alternatively, we could obtain a relative potency estimate for IND at each of the three different exposure levels. This results in the values shown as Case 3 in Table 7.2. The estimates of the relative potency for the agents other than IND are essentially the same as for Case 2, as is the result of the $\chi^2$ test, since using three parameters to estimate the effect for IND at three levels results in an exact fit of the IND data. We note that the dose-specific relative potency estimates for IND decline as exposure increases, which suggests that saturation of IND metabolism is occurring at higher doses. To account for this possibility it is assumed that $k>0$ for IND and the model is refitted and the results shown as Case 4. We propose that the parameter estimates for Case 4 should be regarded as the "best" estimates which can be derived solely from the data in Table 7.1. Again, the estimates for agents other than IND shown in Case 4 in Table 7.2 are little changed from the estimates of Case 1 in Table 7.2. However, the value for IND is increased by a factor of approximately 3.6 because of the greater relative potency of IND at the lower exposure levels, where reaction kinetics are essentially 1st-order. Predicted and observed tumor rates are compared in Table 7.3 for the four cases, where Case 2 is the same as Case 3, except that the data for IND is removed.

Upper and lower 95% confidence bounds for the potency of each agents are calculated (Table 7.4) by taking the largest and smallest potency value of the individual agent that results in a log-likelihood, maximized over parameters other than the potency of interested that is precisely 1.35 (i.e., $\chi^2_{1,0.05}/2$) smaller than the log-likelihood maximized over all parameters. These results indicate that except for B[e]P and IND the estimates are very precise. Even for these two agents the upper bounds are only about twice the maximum likelihood estimates. However, the lower bound for B[e]P is very low because the increase in the tumor rates in the B[e]P-exposed group compared to the rates among controls is very small.

It is of some interest to compare the potency obtained by this analysis with results of the relatively crude approach used in the previously report, which relied to some extent upon the intuition of the primary investigator. Such a comparison is shown in Table 7.5. We note the excellent agreement between the old and new methods, which is almost embarrassingly close considering the orders of magnitude greater computational effort required to obtain the new estimates. In addition, the relative potency estimates and their confidence bounds derived from

Table 7.3 Goodness-of-fit of models to pure PAH data under different assumptions

| Group | Dose (mg) | Observed # carcinomas | Estimates of expected # or epidermoid carcinomas using four different approaches (Case #'s as defined in Table 2) | | |
|---|---|---|---|---|---|
| | | | Case 1 | Case 3 | Case 4 |
| Historical control | 0.00 | 1 | 3.113 | 1.575 | 1.724 |
| **Gasoline Engine Exhaust Condensate** | | | | | |
| Untreated controls | 0.00 | 0 | 0.056 | 0.028 | 0.031 |
| Vehicle controls | 0.00 | 0 | 0.072 | 0.036 | 0.040 |
| B[a]P controls | 0.10 | 4 | 6.259 | 6.102 | 6.120 |
| | 0.30 | 21 | 18.423 | 18.546 | 18.531 |
| | 1.00 | 33 | 34.284 | 34.350 | 34.342 |
| **Pure PAHs** | | | | | |
| Untreated controls | 0.00 | 0 | 0.052 | 0.026 | 0.029 |
| | 0.00 | 0 | 0.052 | 0.026 | 0.029 |
| B[a]P | 0.05 | 3 | 1.642 | 1.522 | 1.535 |
| | 0.10 | 5 | 5.559 | 5.418 | 5.434 |
| | 0.20 | 15 | 14.254 | 14.256 | 14.255 |
| Benzo[b]Fluoranthene | 0.10 | 0 | 0.285 | 0.222 | 0.229 |
| | 0.30 | 1 | 1.264 | 1.164 | 1.175 |
| | 1.00 | 9 | 8.659 | 8.744 | 8.734 |

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Table 7.3 continued

| Group | Dose (mg) | Observed # carcinomas | Estimates of expected # or epidermoid carcinomas using four different approaches (Case #'s as defined in Table 2) | | |
|---|---|---|---|---|---|
| | | | Case 1 | Case 3 | Case 4 |
| Benzo[e]Pyrene | 0.20 | 0 | 0.089 | 0.051 | 0.055 |
| | 1.00 | 0 | 0.142 | 0.150 | 0.109 |
| | 5.00 | 1 | 0.916 | 0.929 | 0.927 |
| Benzo[j]Fluoranthene | 0.20 | 1 | 0.245 | 0.183 | 0.189 |
| | 1.00 | 3 | 2.382 | 2.233 | 2.249 |
| | 5.00 | 18 | 18.660 | 18.840 | 18.818 |
| Benzo[k]Fluoranthene | 0.16 | 0 | 0.217 | 0.156 | 0.162 |
| | 0.83 | 3 | 1.071 | 0.989 | 0.998 |
| | 4.15 | 12 | 13.136 | 13.252 | 13.238 |
| Indeno Pyrene | 0.16 | 3 | 0.324 | 3.0 | 1.384 |
| | 0.83 | 8 | 2.908 | 8.0 | 10.177 |
| | 4.15 | 21 | 24.593 | 21.0 | 20.270 |
| Anthanthrene | 0.16 | 1 | 1.739 | 1.612 | 1.626 |
| | 0.83 | 19 | 18.542 | 18.607 | 18.599 |
| Benzo[ghi]Perylene | 0.16 | 0 | 0.106 | 0.066 | 0.070 |
| | 0.83 | 1 | 0.456 | 0.385 | 0.393 |
| | 4.15 | 4 | 4.329 | 4.419 | 4.408 |

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Table 7.4 Relative potency estimates obtained from the combined analysis of three lung implant studies and historical controls

| Compound | 95% lower bound | Maximum likelihood estimate | 95% upper bound |
|---|---|---|---|
| Benzo[b]Fluoranthene (B[b]F) | 0.0886 | 0.1228 | 0.1616 |
| Benzo[e]Pyrene (B[e]P) | 0.0011 | 0.0070 | 0.0151 |
| Benzo[j]Fluoranthene (B[j]F) | 0.0420 | 0.0523 | 0.0641 |
| Benzo[k]Fluoranthene (B[k]F) | 0.0409 | 0.0532 | 0.0672 |
| Indeno(1,2,3-cd)Pyrene (IND) | 0.1609 | 0.2780 | 0.5132 |
| Anthanthrene (ANT) | 0.2521 | 0.3160 | 0.3887 |
| Benzo[ghi]Perylene (B[ghi]P) | 0.0128 | 0.0212 | 0.0310 |

Table 7.5 Comparison of PAH relative potency estimates obtained in different studies

| Agent | Study* | 95% lower bound | Maximum likelihood estimate | 95% upper bound |
|---|---|---|---|---|
| B[b]F | DW-83 | 0.04 | 0.11 | 0.74 |
| | R | 0.089 | 0.123 | 0.162 |
| | CEOH | | 0.036 | |
| | P-EPA | | 0.140 | |
| B[e]P | DW-83 | 0.000 | 0.003 | 0.020 |
| | R | 0.001 | 0.007 | 0.015 |
| | P-EPA | | 0.004 | |
| B[j]F | DW-83 | 0.005 | 0.03 | 0.24 |
| | R | 0.042 | 0.052 | 0.064 |
| | CEOH | | 0.076 | |
| | P-EPA | | 0.053 | |
| B[k]F | DW-83 | 0.005 | 0.03 | 0.24 |
| | R | 0.041 | 0.053 | 0.067 |
| | P-EPA | | 0.066 | |
| IND | DW-83 | 0.01 | 0.08 | 0.57 |
| | R | 0.161 | 0.278 | 0.513 |
| | CEOH | | 0.006 | |
| | P-EPA | | 0.232 | |
| ANT | DW-83 | 0.02 | 0.19 | 1.32 |
| | R | 0.252 | 0.316 | 0.389 |
| | P-EPA | | 0.320 | |
| B[ghi]P | DW-83 | 0.00 | 0.01 | 0.06 |
| | R | 0.013 | 0.021 | 0.031 |
| | P-EPA | | 0.022 | |

*DW-83 - Deutsch-Wenzel (1983a); R - Present report; CEOH - Rugen et al. (1989); P-EPA - Previous EPA report contract # 68-02-4403.

the probit model by Deutsch-Wenzel et al. (1983a) and a linear model used by Rugen et al. (1989) which employed old mouse skin painting experiments conducted by Wynder and Hoffman (1959, 1961) are also given for comparison. We note that for the Deutsch-Wenzel et al. (1983a) approach the maximum likelihood estimates are similar but the confidence bounds are much smaller for the new estimates, which use all the PAH data simultaneously, and make use of additional positive B[a]P controls. The Rugen et al. (1989) estimate are based upon both questionable data selection and analytical approach and tend to be considerably lower, except for B[j]F.

## 7.2 Model and Relative Potency Estimates for Nitro-PAH

Parameters estimated using the data for nitro-PAHs (Table 3.6) are displayed in Table 7.6, along with results of a chi-square test and the maximized value of the log-likelihood. These estimates are based on the model derived in Section 7.1 for pure PAHs, with saturation constants ($k_j$) and irritation effects ($\phi_j$) assumed to equal zero for each nitro-PAH tested. In addition to the data displayed in Table 3.6, the historical control group was incorporated into the analysis. For the data from the nitro-PAH experiment, positive responses were obtained only using dibenz[a,j]acridine (DBa,jAC). For this PAH a positive dose-response relationship is observed in the experimental data. Consequently, the estimated relative potency is positive for DBa,jAC and zero for all other nitro-PAHs.

The model fits the total data set very poorly ($p = 0.0001$), but fits very well when the high-dose B[a]P control group (receiving 1 mg) is removed from the analysis ($p \approx 1$). This is consistent with the observation that, with all data included in the analysis, the three B[a]P groups contribute a value of 46.1 to the total chi-square of 48.4. Parameters estimated with and without the high-B[a]P group included are displayed in Table 7.6. The effect of removing the high-B[a]P group on the values of the estimates is to approximately double the estimate of S (the potency of B[a]P) and approximately halve the relative potency estimate for DBa,jAC.

We are not confident of the explanation for the poor fit of the model associated with the results for the B[a]P group. Possibly the approach used here for incorporating survival effects, i.e. assuming that the age of death of each animal in a group is equal to the mean age of death for the group, is simply too crude when average animal survival time is markedly reduced by exposure. Fitting a saturation constant k for B[a]P (see Section 7.1) results in a model that fits adequately ($p = 0.93$), but not nearly as well as the model with the high-B[a]P group removed:

Table 7.6 Parameter estimates and model fit evaluation for nitro-PAHs with and without 1 mg B[a]P group included

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| Estimates of model parameters A, S, and k | | | |
|---|---|---|---|
| | Using all data | Excluding 1 mg B[a]P group | Using all data k≥0 for B[a]P |
| A | $5.77 \times 10^{-8}$ | $3.61 \times 10^{-8}$ | $3.61 \times 10^{-8}$ |
| S | 108.6 | 239.3 | 328.8 |
| k | 0 (fixed) | 0 (fixed) | 2.088 |
| Relative Potency Estimates (Upper bounds) | | | |
| BcAC | 0 | 0 (0.031293) | 0 |
| DBa,jAC | 0 | 0 (0.034952) | 0 |
| DBa,hAC | 0.231339 | 0.134471 (0.179227) | 0.097787 |
| AC | 0 | 0 (0.006965) | 0 |
| BaAC | 0 | 0 (0.007217) | 0 |
| Model Fit Summary | | | |
| $\chi^2$ | 48.36 | 3.565 | 9.270 |
| Degrees of Freedom | 18 | 17 | 17 |
| p-value | 0.000133 | 0.999765 | 0.931417 |
| Log-likelihood | -111.45 | -94.38* | -97.37 |

*In order for this value to be comparable with the log-likelihoods for the other models, a value of -18.81 has been added. This is the maximum log-likelihood attainable for the group removed, given the response rate in that group of 27/35 animals.



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the maximized log-likelihood with the high-B[a]P group removed and $k = 0$, is substantially larger than the value with the high-B[a]P group included and $k \geq 0$, after correcting for the effect of using a smaller data set. For the model with saturable B[a]P metabolism, the estimated relative potency of DBa,jAC was 0.098, which is similar to the estimate with non-saturable metabolism and the high-B[a]P group removed (0.13).

Based on the these results, we consider the best estimate of the relative potency of DBa,jAC to be 0.1345, which is the result based on the model with $k = 0$, with the high-B[a]P group removed. For the other nitro-PAHs considered here, we consider the best estimates of relative potencies to be zero, which is the maximum-likelihood estimate for each of these agents, based on the data considered here. Corresponding to the preferred set of relative potency estimates, 95% upper bounds have been computed and are displayed in Table 7.6. It will often happen, for an agent with a very small positive relative potency, that the estimated relative potency will be zero. For risk assessment contexts involving complex mixtures of agents, we believe that, considering the magnitudes of various uncertainties, treating the least-carcinogenic agents as non-carcinogenic is ordinarily a sufficiently precise approach. This conclusion is based partly on our favorable experience in predicting the carcinogenic potency of a complex mixture from the potencies of its components, as described in Section 9.

## 8. The Use of Different Types of Experiments to Make Joint Relative Potency Estimates

Sections 5-7 demonstrate our approach toward the derivation of comparative potency estimates from a single experimental system, the induction of tumors by implantation of carcinogenesic material in the rat lung. In this section, we demonstrate how a similar approach may be used to efficiently integrate information from two different experimental systems (i.e., the rat lung implant system and a skin painting system in mice) to obtain combined relative potency estimates.

To use data from different sex-species-strain combinations simultaneously to obtain relative potency estimates, we assume that the parameters A and G in our general model depend upon the tumor type and the specific bioassay animal used (i.e., background tumor rates differ among sexes, species, and strains). The relative potency parameters $Q_j$, $j=1,2,..,m$, should be regarded as constant (i.e., across sex-species, strains, and routes of exposure). However, if the metabolic pathway is different in an organ (e.g., mammary tumors), this information should be taken into consideration. The parameters, $k_j$ (saturation) and $\phi_j$ (irritation), may in some cases be

independent of sex and strain or even species but would always be specific to organ and exposure route.

To illustrate the use of two different experiments employed jointly to estimate relative potency, we will combine the information found in the Deutsch-Wenzel et al. (1983a) and Habs et al. (1980) studies. There are a number of problems associated with using the Habs et al. (1980) data to estimate relative potencies. The more serious are:

- the type of tumors were assumed to be carcinomas of uniform origin; in some cases, this may not be true,
- average survival was not given even though dose-dependent survival is apparent,
- experimental conditions for coronene (COR) were different than those for the positive control B[a]P,
- the limited tumor responses observed with exposures to B[k]F and IND were not exposure-dependent and, along with the B[j]F and COR response, suggest a non-exposure-dependent irritation factor,
- the highest dose of B[a]P resulted in a depression of the tumor response, and
- no tumors were observed in the controls.

In spite of these potentially serious limitations, a rigorous analysis was performed on the data after the following adjustments were made:

- COR was omitted from the analysis since its protocol was inconsistent with the positive control B[a]P,
- B[k]F and IND were omitted from the analysis due to absence of a positive dose-response (i.e., IND is assumed to have zero potency in mouse skin systems),
- the high dose of B[a]P was not used because of differential survival,
- the survival rates of the rest of the exposure groups were assumed to be equal, and
- the acetone and DMSO vehicle control groups were combined and the zero observed response rates in the controls were replaced with an ad-hoc assumption of 0.25 tumor in this combined group, to insure a linear term for the dose-response function. It can be shown that the results are relatively insensitive to the value assumed, with virtually identical results obtained if as an alternative 0.125 or 0.5 is the assumed number of tumors in the control group.

The same dose-response model was used as in previous analyses but with the simplifying assumptions that $k_j \rightarrow 0$ and $\phi_j = 0$ for all compounds in each test system. This results in a

cumulative hazard of the form

$$H(y,t) = \frac{\lambda}{G^2}(1+Sy)^2[\exp(Gt)-Gt-1],$$

$$y = \sum_{j=1}^{m} \rho_j x_j$$

where $\rho_j$ and $x_j$ are the relative potency compared to B[a]P and exposure level for the jth PAH, respectively (i.e., $\rho_j = Q_j$ since $k_j = 0$).

A slightly condensed form of the basic model was used for the Habs et al. (1980) data set, for which survival data was not available. For the Habs data it is assumed that tumor independent mortality is not exposure-dependent. Under this assumption the cumulative hazard can be approximated with the collapsed form

$$H(y) = M_s(1+S_s y)^2$$

where

$$M_s = \frac{\lambda_s}{G_s^2}[\exp(G_s t^*) - G_s t^* - 1]$$

is a composite parameter that is approximately equal to the background tumor rate at the end of the average duration denoted as $t^*$. The subscript s denotes that the parameters are for the female NMRI mouse skin. As before, the cumulative hazard for lung carcinomas in female Osborne-Mendal rats is expressed as

$$H(y,t) = A_\ell(1+S_\ell y)^2 \frac{t^2}{2}$$

where the subscript $\ell$ indicates that the parameter refers to lung tissue. A common feature of the two models is the relative potency parameters $\rho_1, \rho_2, \ldots, \rho_m$, which are assumed to be constant across test systems. In the present analysis only one PAH is given in each exposure group so that for the jth PAH the total B[a]P equivalent exposure is simply $y = \rho_j x_j$. The IND data was omitted from this analysis so that the simpler model form could be used. It can be shown that the inclusion of IND exposures does not alter any of the parameter estimates if the appropriate adjustments are made to account for metabolic saturation.

The two simple models were fitted simultaneously to the combined data sets shown in Table 8.1. The potencies for the two agents evaluated in both experiments, B[b]F and B[j]F, must be similar for the two routes of exposure (i.e., skin painting and lung implant) in order for a valid fit to be obtained. A summary of the results of fitting the model is shown in Table 8.2. We note that in spite of the use of eight different agents, different species and organs affected, and marked differences in survival between the groups, only eleven parameters needed to be estimated to obtain a good fit to the data ($\chi^2_{24}$=23.56 and p=0.487). We also note the rather narrow confidence bounds on the estimates. To evaluate the effect of using only a single type of bioassay, the two types of experiments were used independently to obtain relative potency estimates. Results for the agents common to both studies (i.e., B[j]F and B[b]F) are compared for the individual and combined studies in Table 8.3.

This analysis shows that for at least two agent, B[j]F and B[b]F, comparable results can be obtained in skin painting and lung implant bioassay systems. On the other hand, if IND and B[k]F were as potent in the skin painting system as in the lung implant system, we would have expected to see a large number of skin tumors associated with the levels of exposures administered. In reality no response was observed for IND or B[k]F even at the highest skin exposures, indicating the possibility of a tissue-specific PAH metabolic interaction. This suggests that route of exposure should be considered critically in evaluating the relative potency estimates.

## 9. Estimation of Relative Potencies of Hydrocarbon Combustion Products and Their Fractions

In this section estimates are obtained for relative potencies compared to B[a]P of four hydrocarbon combustion products and their fractions. These include gasoline engine exhaust condensate, diesel engine exhaust, coal-fired residential furnace flue gas condensate, and sidestream cigarette smoke. These estimates are, in addition to their intrinsic interest, useful in other ways. In particular, the estimates provide methods for (1) testing the assumption of dose-additivity, and (2) evaluating methods for estimating the relative potency of a complex combustion product mixture from the relative amounts of selected PAHs components.

In this section the relative potency of each combustion product and their fractions are obtained. The assumption of dose-additivity is also tested. In the following section nine potential methods are evaluated for estimating the relative potency of a complex mixture using chemical and biological data.

Table 8.1 Data from Deutsch-Wenzel et al. (1983) and Habs et al. (1980) used to estimate potency of pure PAHs

| Material | Dose (mg)[a] | Dose (pg/week)[b] | Median survival t | # animals with carcinomas/# animals |
|---|---|---|---|---|
| Benzo[a]Pyrene | 0.10 | | 111 | 4/35 |
| | 0.30 | | 77 | 21/35 |
| | 1.00 | | 54 | 33/35 |
| | | **1.70** | | **8/34** |
| | | **2.80** | | **24/35** |
| Benzo[b]Fluoranthene | 0.10 | | 110 | 0/35 |
| | 0.30 | | 113 | 1/35 |
| | 1.00 | | 112 | 9/35 |
| | | **6.80** | | **2/38** |
| | | **11.20** | | **5/34** |
| | | **18.40** | | **20/37** |
| Benzo[e]Pyrene | 0.20 | | 117 | 1/35 |
| | 1.00 | | 111 | 0/30 |
| | 5.00 | | 104 | 1/35 |
| Benzo[j]Fluoranthene | 0.20 | | 110 | 1/35 |
| | 1.00 | | 117 | 3/35 |
| | 5.00 | | 89 | 18/35 |
| | | **6.80** | | **1/38** |
| | | **11.20** | | **1/35** |
| | | **18.40** | | **2/38** |
| Benzo[k]Fluoranthene | 0.16 | | 114 | 0/35 |
| | 0.83 | | 95 | 3/31 |
| | 4.15 | | 98 | 12/27 |



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Table 8.1 continued

| Material | Dose (mg)[a] | Dose (pg/week)[b] | Median survival t | # animals with carcinomas/# animals |
|---|---|---|---|---|
| Anthanthrene | 0.16 | | 102 | 1/35 |
| | 0.83 | | 88 | 19/35 |
| Benzo[ghi]Perylene | 0.16 | | 109 | 0/35 |
| | 0.83 | | 114 | 1/35 |
| | 4.15 | | 106 | 4/34 |
| Cyclopentadieno[cd]Pyrene | | **3.40** | --- | **0/34** |
| | | **17.60** | --- | **0/38** |
| | | **54.40** | --- | **3/35** |
| Controls | | | | |
| Untreated[a] | --- | | 100 | 0/35 |
| Vehicle[a] | --- | | 100 | 0/35 |
| Solvent acetone[b] | --- | | | 0/35 |
| DMSO[b] | --- | | | 0/35 |
| Historical[c] | --- | | 104 | 1/1945 |

[a]Source: Deutsch-Wenzel et al. (1983)
[b]Source: Habs et al. (1980) in bold
[c]Source: Goodman et al. (1980)

Table 8.2 Relative potency estimates from simultaneous analysis of two experiments

| Agent | 95% lower bound | Maximum likelihood estimate | 95% upper bound | Studies combined | |
|---|---|---|---|---|---|
| | | | | Deutsch-Wenzel | Habs |
| Benzo[b]Fluoranthene | 0.1518 | 0.1840 | 0.2199 | x | x |
| Benzo[j]Fluoranthene | 0.0475 | 0.0602 | 0.0747 | x | x |
| Cyclopentadieno[cd]Pyrene | 0.0098 | 0.0228 | 0.0375 | | x |
| Benzo[e]Pyrene | 0.0011 | 0.0071 | 0.0184 | x | |
| Benzo[k]Fluoranthene | 0.0521 | 0.0609 | 0.0702 | x | |
| Anthanthrene | 0.2846 | 0.3649 | 0.4602 | x | |
| Benzo[ghi]Pyrene | 0.0132 | 0.0232 | 0.0352 | x | |

Two experiments: Deutsch-Wenzel: Lung Implant - female rat
Habs: Skin Painting - female mice

Eight different agents used (7 listed above plus Benzo[e]Pyrene)

Seven PAHs: 4 Deutsch-Wenzel unique
1 Habs unique
2 common

Eleven parameters estimated, historical controls also used, 35 total dose groups employed in analysis
$\chi^2 = 23.56$ d.f. = 24 p = 0.487



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Table 8.3 Comparison of relative potency estimates using different assay systems

| Compound | Assay system | Relative potency estimates | | |
|---|---|---|---|---|
| | | 95% lower bound | Maximum likelihood estimate | 95% upper bound |
| Benzo[j]Fluoranthene | Lung implant[a] (rat) | 0.041 | 0.053 | 0.068 |
| | Skin painting[b] (mice) | 0.050 | 0.082 | 0.122 |
| | Joint | 0.048 | 0.060 | 0.075 |
| Benzo[b]Fluoranthene | Lung implant (rat) | 0.075 | 0.115 | 0.162 |
| | Skin painting (mice) | 0.194 | 0.242 | 0.302 |
| | Joint | 0.152 | 0.184 | 0.220 |

[a]Deutsch-Wenzel et al. (1983)
[b]Habs et al. (1980)



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## 9.1 Diesel Engine Exhaust

In the diesel engine exhaust study the hydrophobic fraction of the emissions were:

- tested directly in the lung implant bioassay system;
- separated into the four mutually exclusive fractions (non-aromatics + PAC 2 and 3 rings, PAH 4-7 rings, polar PAC, nitro-PAH); and
  - each of the four fractions tested separately,
  - the four fractions were reconstituted and tested jointly.

Under the assumptions that doses are additive and that the emissions separation system does not change the potencies of the resulting fractions, it is possible to predict the response rates due to the total hydrophobic fraction and the total reconstituted hydrophobic fraction from the potency estimates of the individual fractions. To do so, estimates are required for emission fraction potencies relative to B[a]P potency. Under the dose-additivity model, with equal relative transition rates, the cumulative hazard for a joint exposure to m agents has the form:

$$H(y,t) = \phi + A(1+Sy)^2 t^2$$

with

$$y = \sum_{j=1}^{m} \rho_j x_j$$

where $\rho_j$ is the relative potency of the jth fraction with respect to B[a]P, $x_j$ is the exposure level for the jth fraction, and y is the total B[a]P equivalent exposure for a mixture made up of m emission fractions (the relative potency of B[a]P itself is one by definition). The terms $\phi$, A, S, and t are as previously defined. Using the data in Table 9.1, the maximum likelihood estimates for the unknown parameters are obtained under various assumptions and displayed in Table 9.2.

Three different approaches for estimating model parameters are shown in Table 9.2, denoted Case 1 to Case 3. These approaches result in mutually consistent results. In the first case the tumor rates observed in the groups exposed to the hydrophobic fraction and the reconstituted hydrophobic fraction were not used to estimate model parameters. We attempt to predict the tumor rates in these groups from the model obtained, by assuming that:

- the part of the hydrophobic fraction that was lost in the separation is non-carcinogenic and
- the fractions of the complex emission mixture are dose-additive.

Table 9.1 Data used in relative potency estimation for diesel engine exhaust condensate

| Group | Dose (mg) | Median survival | # animals on test (Osborne-Mendal female rats) | # epidermoid carcinomas | |
|---|---|---|---|---|---|
| | | | | Observed | Case 2 Predicted assuming dose-additivity |
| Hydrophilic fraction | 6.70 | 97 | 35 | 0 | 0.02 |
| Hydrophobic fraction | 20.00 | 99 | 35 | 5 | 7.07 |
| non-aromatics + PAC 2 & 3 rings | 19.22 | 103 | 35 | 0 | 0.02 |
| PAH 4-7 rings | 0.21 | 102 | 35 | 6 | 4.86 |
| polar PAC | 0.29 | 97 | 35 | 0 | 0.02 |
| nitro-PAH | 0.19 | 106 | 35 | 1 | 0.60 |
| Reconstituted hydrophobics | 19.91 | 93 | 35 | 7 | 6.32 |
| Control: | | | | | |
| Historical | --- | 104 | 1945 | 1 | 1.15 |
| Untreated | --- | 110 | 35 | 0 | 0.02 |
| Beeswax/trioctanoin | --- | 103 | 35 | 0 | 0.02 |
| Benzo[a]Pyrene | 0.3 | 69 | 35 | 27 | 27.82 |
| | 0.1 | 96 | 35 | 11 | 10.91 |
| | 0.03 | 97 | 35 | 3 | 1.34 |

$\chi^2_9 = 3.818$
$p = 0.923$

Source: Grimmer et al. (1987b)



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Table 9.2 Estimates of relative potency of total and fractions of diesel engine exhaust and other model parameters

| Parameter symbol | Physical meaning | Numerical estimates | | |
|---|---|---|---|---|
| | | Case 1 Only fractions used | Case 2 Total and reconstituted emissions also used | Case 3 All estimated independently from data |
| $\phi$ | irritation effect | 0.0 | 0.0 | 0.0 |
| A | product of background transition rates | $5.1372 \times 10^{-8}$ | $5.4884 \times 10^{-8}$ | $5.1372 \times 10^{-8}$ |
| S | increase in relative transition rate per unit exposure to B[a]P | 265.01 | 256.17 | 265.01 |
| | relative potency estimates | | | |
| $\rho$ | entire emission (no bioassay data) | 0.0034* | 0.0028* | --- |
| $\rho_1$ | hydrophilic fraction | 0.0 | 0.0 | 0.0 |
| $\rho_2$ | hydrophobic fraction | 0.0045* | 0.0038* (0.0029, 0.0049)+ | 0.0031 |
| $\rho_2$ | reconstituted fraction | 0.0045* | 0.0038* | 0.0041 |
| $\rho_{21}$ | non-aromatics + PAC 2 & 3 rings | 0.0 | 0.0 | 0.0 |
| $\rho_{22}$ | PAH 4-7 rings | 0.31908 | 0.28214 | 0.31908 |
| $\rho_{23}$ | polar PAC | 0.0 | 0.0 | 0.0 |
| $\rho_{24}$ | nitro-PAH | 0.12088 | 0.08833 | 0.12088 |

*method used to obtain estimates shown in Table 9.3.
+90% confidence bounds (i.e., 5% lower, 95% upper).

Substituting the parameter estimates obtained for A and S shown as Case 1 in Table 9.2 into the cumulative hazard function yields the result:

$$H(y,t) = 5.1372 \times 10^{-8} \cdot (1 + 265.01y)^2 t^2$$

where y is the B[a]P equivalent exposure units and t is the median survival time. Based on the described assumptions, the exposure units shown in Table 9.1, and the estimated relative potencies displayed as Case 1 in Table 9.2, the number of B[a]P equivalent exposure units in either the total or reconstituted hydrophobic fraction is estimated to be

$$y = \rho_{22} \cdot x_{22} + \rho_{24} \cdot x_{24} = 0.21 \cdot 0.31908 + 0.19 \cdot 0.12088 = 0.09$$

where the subscripts refer to the notation in the first column of Table 9.2. Substituting the B[a]P equivalent exposure unit estimate and the median survival into the cumulative hazard function and making the indicated transformations results in the predicted number of carcinomas displayed in Table 9.3. The $\chi^2$ goodness-of-fit test comparing predicted and observed tumor counts indicates that their is no evidence to reject the hypothesis of dose-additivity (i.e., $\chi^2_2 = 3.375$, $p = 0.18$). The discrepancies observed in Table 9.3 suggest that, to the degree that the data does not fit the model, it may overpredict risk. We need to emphasize that the expected number of tumors in the two exposure groups, displayed in Table 9.3 were estimated without using the actual observed number of tumors in any manner.

It is possible to estimate the relative potency of the total emissions (i.e., hydrophobic and hydrophilic fractions), combined under the assumption of dose-additivity, using all the data available, both for mixtures and individual PAHs. To do so we express the B[a]P equivalent exposure units for the two fractions as sums of the fractional concentrations of component PAHs, weighted by corresponding unknown relative potency values, to be estimated from the data. Taking this approach we obtain the parameter estimates denoted as Case 2 in Table 9.2. The relative potency estimates for the complete mixture and hydrophobic fraction are obtained from linear combinations of the fractional concentrations as indicated in Table 9.4. The potency estimate of the complete mixture ($\rho$=0.0028) calculated in this manner will be used in subsequent analyses.

Table 9.3 Estimated number of tumors under the assumption of dose-additivity

| Exposure group | n # animals on test | x B[a]P equivalent exposure units | t median survival | H(x,t) cumulative hazard | # animals with carcinomas | |
|---|---|---|---|---|---|---|
| | | | | | Observed | Predicted* (case 1) |
| Total hydrophobics | 35 | 0.09 | 99 | 0.3109 | 5 | 9.35 |
| Reconstituted hydrophobics | 35 | 0.09 | 93 | 0.2744 | 7 | 8.40 |

case 1:
$\chi^2 = 3.375$
$p = 0.18$

*predicted = [1-exp-H(x,t)] • n



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Table 9.4 Calculation of relative potency of total and major fractions of diesel engine exhaust from concentrations and relative potencies of component PAHs

| Parameter symbol | Equation | Numerical result | Table 8.2 case # |
|---|---|---|---|
| $\rho_2$ | $\frac{\rho_{21} \cdot x_{21} + \rho_{22} \cdot x_{22} + \rho_{23} \cdot x_{23} + \rho_{24} \cdot x_{24}}{x_2}$ | $\frac{0.31098x0.21+0.12088x0.19}{20} = 0.0045$ | 1 |
| | | $\frac{0.28214x0.21+0.08833x0.19}{20} = 0.0038$ | 2 |
| $\rho$ | $\frac{\rho_1 \cdot x_1 + \rho_2 x_2}{x}$ | $\frac{0.0045x20}{26.7} = 0.0034$ | 1 |
| | | $\frac{0.0038x20}{26.7} = 0.0028$ | 2 |



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To obtain a feeling for the uncertainty in the parameters resulting from variation in tumor counts a 90% confidence interval for the relative potency of the hydrophobic fraction is computed. This is achieved by finding the extreme values of relative potencies subject to the constraint that the deviation of the fit of the model from the fit of the best-fitting model, as measured by the log-likelihood criterion is equal to $1.35 = \chi^2_{1,0.95}/2$. Because the relative potency of the fraction is a linear combination of the actual model parameters, i.e., relative potencies of individual PAHs weighted by corresponding concentrations, Lagrange methods of constrained optimization were used to compute the bounds. The limits computed were 0.0029 and 0.0049, which are quite narrow for values based on biological responses. How well the predictions of the model developed in Case 2 conform to the actual values is illustrated in the last column of Table 9.1. We note the excellent fit as indicated by the standard $\chi^2$ goodness-of-fit test ($\chi^2_9$=3.818, p=0.923).

An alternative, subjective method for assessing the biological variability of the potency estimates is to estimate a relative potency for each fraction or combination of fractions directly from the bioassay data without any assumptions of dose-additivity, and compare the values obtained to the estimates obtained in Cases 1 and 2. If the model is reasonably accurate, then the discrepancies among estimates from the different cases results primarily from variation among groups of animals exposed at the same magnitude. Results of this approach, denoted as Case 3, are displayed in Table 9.2. We note that the estimates for Cases 2 and 3 are reasonably close. However, in order for the potency of the hydrophobic reconstituted fraction potency to be larger than that of the original fraction, as is suggested by the results for Case 3, it is necessary to assume that the small fraction lost in the reconstituted sample would have had an inhibitory effect on cancer potency. A more plausible explanation, that is consistent with the observed data, is that the differences are due to random biological variation in the bioassay results.

## 9.2 Gasoline Engine Exhaust Condensate

It is also possible to estimate relative potencies and test the assumption of dose-additivity in the gasoline engine exhaust condensate experiment by the same methods applied to diesel engine exhaust. Estimates of the relative potencies for different fractions of gasoline engine exhaust condensate, obtained by the three methods discussed in the previous section, are derived from the data shown in Table 9.5 and displayed in Table 9.6.



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Table 9.5 Data used in relative potency estimation for gasoline engine exhaust condensate

| Group | Dose (mg) | Median survival | # animals on test (Osborne-Mendal female rats) | # carcinomas | |
|---|---|---|---|---|---|
| | | | | Observed | Predicted assuming dose-additivity |
| Original condensate | 5.0 | 105 | 35 | 3 | 6.29 |
| | 10.0 | 99 | 35 | 20 | 17.08 |
| PAC-free fraction (87.3) | 4.36 | 104 | 34 | 3 | 1.59 |
| | 8.73 | 106 | 34 | 1 | 1.71 |
| | 17.45 | 106 | 35 | 1 | 1.70 |
| PAC with 2 & 3 rings (9.9) | 0.50 | 110 | 35 | 0 | 0.24 |
| | 0.99 | 106 | 35 | 0 | 0.64 |
| | 1.98 | 111 | 35 | 3 | 2.31 |
| PAC with > 3 rings (2.8) | 0.14 | 111 | 35 | 3 | 5.44 |
| | 0.28 | 88 | 34 | 15 | 11.23 |
| | 0.56 | 80 | 35 | 24 | 25.33 |
| Controls*: | | | | | |
| Historical | --- | 104 | 1945 | 1 | 0.94 |
| Untreated | --- | 100 | 35 | 0 | 0.02 |
| Beeswax/trioctanoin | --- | 108 | 34 | 0 | 0.02 |
| Benzo[a]Pyrene | 0.03 | 111 | 35 | 2 | 0.75 |
| | 0.10 | 106 | 35 | 5 | 5.72 |
| | 0.03 | 94 | 35 | 25 | 23.71 |

$\chi^2 = 18.58$
d.f. = 22; p = 0.67

Source: Grimmer et al. (1984)
*Other B[a]P and control data found in Grimmer JNCI papers also employed in model fits but not displayed.

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Table 9.6 Estimates of relative potency of total and fractions of gasoline engine exhaust condensate

| Parameter symbol | Physical meaning | Numerical estimates | | |
|---|---|---|---|---|
| | | Case 1 Only fractions used | Case 2 Fractions and original condensate used | Case 3 All estimated independently from data |
| $\phi$ | irritation effect | $4.37747 \times 10^{-6}$ | $4.38150 \times 10^{-6}$ | $4.38141 \times 10^{-6}$ |
| A | product of background transition rates | $4.85668 \times 10^{-8}$ | $4.45371 \times 10^{-8}$ | $4.46272 \times 10^{-8}$ |
| S | relative transition rate of B[a]P per unit of exposure | 167.75 | 175.40 | 175.22 |
| $\rho$ | original total condensate | 0.02162* | 0.02171* | 0.0220 |
| $\rho_1$ | PAC-free fraction | 0.0 | 0.0 | 0.0 |
| $\rho_2$ | PAC with 2 & 3 rings | 0.02876 | 0.02924 | 0.02887 |
| $\rho_3$ | PAC with > 3 rings | 0.67040 | 0.67382 | 0.67027 |

*calculated in the following manner:

Case 1

$$\rho = \frac{9.9 \times 0.02876 + 2.8 \times 0.67040}{100} = 0.02162$$

Case 2

$$\rho = \frac{9.9 \times 0.02179 + 2.8 \times 0.67382}{100} = 0.02171$$

The estimated relative potency of the entire condensate ($\rho$=0.02171) from Table 9.6, Case 2, will be used in all subsequent analyses. A test of dose-additivity is obtained from Case 1 in Table 9.6 (i.e., results, which do not use the original condensate total in the parameter estimation). Proceeding as in the previous section, an estimate of the B[a]P equivalent exposure units is:

$$y = 0.50 \bullet 0.02876 + 0.14 \bullet 0.67040 = 0.10824$$

for the 5 mg exposure group and 2 x 0.10824 = 0.21648 for the 10 mg exposure group. Using these values and the parameter estimates shown as Case 1 in Table 9.6 results in the predicted cases displayed in Table 9.7. We note that the discrepancies between the observed and predicted numbers of carcinomas, assuming dose-additivity, are within the limits of normal biological variability, as indicated by the standard $\chi^2$ goodness-of-fit test (i.e., $\chi^2_2 = 3.115$, $p = 0.212$). Again, it needs to be emphasized that the data for groups exposed to the original condensate were not used in any manner to obtain the parameter estimates, and as a result provide a completely independent test of the additivity assumption.

## 9.3 Coal-Fired Residential Furnace Flue Gas Condensate

The data used to obtain the relative potency estimates for coal-fired gas condensate is displayed in Table 9.8. The same model as was employed with diesel engine exhaust is also used. In this case all of the data was used to estimate the potency parameters independently without the dose-additivity assumption. The goodness-of-fit of the model and the relative potency estimates are also displayed in Table 9.8. We note that the model is consistent with the observed data at the $p>0.1$ level. However, almost half (i.e., 13.87 out of 29.39) of the value of the $\chi^2$ statistic is contained in the low exposure total condensate group.

A test of the dose-additivity assumption can be derived in the following manner. The parameter estimates $A=7.382\times10^{-8}$ and $S=135.1$ obtained in the fit define the cumulative hazard

$$H(y,t) = 7.382\times10^{-8} \bullet (1+135.1y)^2 t^2$$

where y is the B[a]P equivalent number of exposure units. Under the dose-additivity assumption, the value for y for the original condensate and the reconstituted fraction is obtained from the

Table 9.7 Test of dose-additivity for gasoline engine exhaust fractions

| Exposure of total condensate | # animals on test | # carcinomas | |
|---|---|---|---|
| | | Observed | Predicted assuming dose-additivity |
| 5 mg | 35 | 3 | 6.23 |
| 10 mg | 35 | 20 | 16.93 |

$\chi^2 = 3.115$
$p = 0.212$



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Table 9.8 Rat lung implant carcinogenicity data for flue gas condensate from coal-fired residential furnaces

| Agent | Relative potency | Dose mg (x) | Median survival (t) | *Number of animals with epidermoid lung carcinomas /number of animals tested | |
|---|---|---|---|---|---|
| | | | | Observed | Predicted |
| Flue gas condensate | | | | | |
| Original condensate | 0.05417 | 2.26 | 94 | 15/35 | 6.36 |
| | | 4.52 | 89 | 15/35 | 17.25 |
| | | 9.04 | 67 | 24/35 | 27.15 |
| PAC-free (fraction I) | 0.00790 | 1.00 | 108 | 0/35 | 0.13 |
| | | 2.01 | 108 | 0/34 | 0.29 |
| | | 4.02 | 111 | 1/27 | 0.68 |
| PACs, 2 and 3 rings (fraction II) | 0.02443 | 0.56+ | 104 | 1/35 | 0.23 |
| | | 1.12 | 106 | 0/35 | 0.63 |
| PAHs and S-PACs, 4-7 rings (fraction III) | 0.37934 | 0.35 | 98 | 9/35 | 7.86 |
| | | 0.69 | 96 | 14/35 | 14.83 |
| Polar PACs and higher PAHs (fraction IV) | 0.25413 | 0.94@ | 94 | 18/35 | 18.00 |
| Insoluble carbon | 0.0 | 4.28 | 112 | 0/35 | 0.03 |
| Reconstituted from all fractions (without carbon) | 0.06735 | 2.10 | 100 | 8/31 | 8.00 |
| Benzo[a]Pyrene | 1.0 | 0.05 | 100 | 3/35 | 1.52 |
| | | 0.10 | 104 | 5/35 | 5.42 |
| | | 0.20 | 95 | 15/35 | 14.26 |
| Controls | | | | | |
| Historical | | — | 104 | 1 | 1.55 |
| Untreated | | — | 105 | 0/35 | 0.03 |
| Vehicle | | — | 100 | 0/35 | 0.03 |

Source: Grimmer et al. (1987a)
*Osborne-Mendal female rats
+twice lowest dose amount in original condensate (i.e., same as middle dose)
@four times lowest dose amount in original condensate (i.e., same as high dose)

$\chi^2=29.39$
d.f.=21
p=0.105

relative potency estimates and exposure levels of the individual fractions, shown in Table 9.8, in the following manner

$$y = 1x0.00790+(0.56/2)x0.02443+0.35x0.37934+(0.94/4)x0.25413 = 0.233.$$

The resulting B[a]P equivalent exposures are shown in Table 9.9. In conjunction with the survival times these exposures are used to estimate the cumulative hazard. The corresponding expected numbers of tumors are compared to the observed number in Table 9.9. We note that the true rates are significantly overpredicted using the dose-additive model and therefore we infer that assuming such a model would be conservative for predicting risks from exposure to coal flue gas emissions.

## 9.4 Estimation of Relative Potency of Sidestream Cigarette Smoke Constituents

The data that is used to estimate the relative potency of sidestream cigarette constituents is displayed in Table 3.5 with the addition of historical controls. In this analysis only the experiment matched B[a]P positive controls are employed rather than the "three-experiment" B[a]P positive controls. The rationale for this choice is the observation that the relative potencies for the B[a]P groups are statistically significantly different.

The standard model with $\phi=0$ and $k=0$ for all constituents was used in the analysis. Due to the limited available data no other model is possible. The relative potency estimates for the fractions of sidestream smoke are displayed in Table 9.10.

Unfortunately, the carcinogenicity of the entire emission mixture was not directly evaluated in this experiment. However, under the assumption of dose-additivity an estimate of the relative potency of the entire sample is simply the concentration-weighted average of the relative potencies. Using this approach we obtain the estimate

$$\rho = \frac{x_1\rho_1+x_2\rho_2+x_3\rho_3}{x_1+x_2+x_3} = \frac{16x\cdot 0.00158+1.06x\cdot 0.05833}{16+1.06+11.8} = 0.00302$$

which shall be used in our subsequent analyses.

To illustrate several points the upper bound relative potency estimates for the individual fractions and total emission are obtained in two different ways. An upper bound is obtained directly for the relative potency $\rho$ as defined above. This is denoted as the 95% joint upper

Table 9.9 Predicted number of tumors in rats exposed to the complete and reconstituted coal flue gas condensate under the assumption of dose-additivity

| Agent | Dose mg (x) | B[a]P equivalent mg (y) | Survival time t | Number exposed | Observed tumors | Predicted under assumption of dose-additivity |
|---|---|---|---|---|---|---|
| Total (original) condensate | 2.26 | 0.233 | 94 | 35 | 15 | 16.78 |
| | 4.52 | 0.466 | 89 | 35 | 15 | 31.80 |
| | 9.04 | 0.932 | 67 | 35 | 24 | 34.83 |
| Reconstituted | 2.10 | 0.233 | 100 | 31 | 8 | 16.78 |

Table 9.10 Relative potency estimate of sidestream cigarette smoke constituents

| Material | j | Dose ($x_j$) | Fraction of sample ($f_j$) | Maximum Likelihood Relative Potency Estimates ($\rho_j$) | 95% Joint Upper Bounds ($\bar{\rho}_j^*$) | 95% Upper Bound Individual Relative Potency ($\rho_j^*$) |
|---|---|---|---|---|---|---|
| PAH-free | 1 | 16.00 | 0.55440 | 0.00158 | 0.00250 | 0.00332 |
| PAH 2,3 rings | | | | | | |
| PAH 4 and more rings | 2 | 1.06 | 0.03673 | 0.02533 | 0.07289 | 0.08523 |
| Semivolatiles (gaseous phase) | 3 | 11.80 | 0.40887 | 0.00000 | 0.00145 | 0.00252 |
| Weighted total | | 28.86 | 1.00000 | 0.00302 | 0.00466 | 0.00600 |



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bound. The contribution to this upper bound of the individual upper bound fraction are also displayed in Table 9.10. The 95% upper bound obtained in this manner is less that 50% greater than the maximum likelihood estimate indicating greater precision than might have been anticipated considering the rather limited data. An alternative much simpler computation approach is to obtain the 95% upper bound on the relative potencies of each fraction separately and then combine the results. This is the same type of approach that is often done for multiple agent joint exposures. As shown in Table 9.10, under the title 95% upper bound of individual relative potency, estimates obtained in this manner give an overly conservative upper bound for the total. This upper bound is almost twice the maximum likelihood estimate.

## 9.5 Summary of Relative Potency Estimates

For the convenience in future references we summarize the information on the relative potency estimates of hydrocarbon combustion product emissions and their fractions in Table 9.11. Also shown in the table are the 95% upper bound estimates for the relative potencies. This information will be used in various ways in the next section.

# 10. Evaluation of Potential Methods for Estimating Cancer Potency of Complex Hydrocarbon Combustion Products from their Chemical Composition

A number of approaches can be used to estimate the relative potency of a complex hydrocarbon combustion products from some information about its chemical composition. The extremes in this regard range from assuming that the entire emission is as potent as B[a]P, (the highly conservative approach traditionally used by EPA) to assuming that all of the carcinogenicity is due to B[a]P (highly permissive; many other known and unknown carcinogens can be found in the emissions). Between these extremes are a number of other possibilities that can be based on the relative potency estimates and tested using the hydrocarbon combustion product bioassay information. The options for estimating total carcinogenicity of a mixture that will be evaluated in this report in order of expected conservatism are:

Table 9.11 Summary of relative potency estimates for combustion products of hydrocarbons

| Hydrocarbon combustion product | Definition of emission | Relative potency | |
|---|---|---|---|
| | | Maximum likelihood estimate* | 95% upper bound |
| Sidestream Cigarette Smoke | PAH-free material and PAH 2,3 rings | 0.00158 | 0.00332 |
| | PAH ≥ 4 rings | 0.05833 | 0.08523 |
| | Semivolatiles (gaseous phase) | 0.0 | 0.00252 |
| | Total | (0.00302) | |
| Diesel Engine Exhaust | Fraction I: Hydrophilic fraction | 0.0 | 0.00455 |
| | Fraction II: Hydrophobic fraction | 0.00311 (0.0038) | 0.00454 |
| | Fraction IIa: PAC 2 + 3 rings | 0.0 | 0.00149 |
| | Fraction IIb: PAH 4-7 rings | 0.31908 (0.28214) | 0.45338 |
| | Fraction IIc: Polar PAC | 0.0 | 0.10521 |
| | Fraction IId: Nitro-PAH | 0.12088 (0.08833) | 0.25381 |
| | Reconstituted hydrophobics: . Fractions IIa, b, c, d | 0.00406 (0.0038) | 0.00564 |
| Gasoline Engine Exhaust | Original condensate | 0.02200 (0.02171) | 0.2673 |
| | Fraction: PAC 2-3 rings | 0.02887 (0.02924) | 0.04569 |
| | Fraction: PAC > 3 | 0.67027 (0.67382) | 0.78708 |
| Coal Flue Gas Condensate | Original condensate | 0.05417 (0.09660) | 0.06295 |
| | Fraction I: PAC-free | 0.00790 | 0.01715 |
| | Fraction II: PAC's 2-3 rings | 0.02443 | 0.05770 |
| | Fraction III: PAHs & S-PACs 4-7 rings | 0.37934 | 0.46184 |
| | Fraction IV: Polar PACs & higher PAHs | 0.25413 | 0.31498 |
| | Reconstituted without carbon | 0.06735 | 0.09046 |

values in parentheses are estimated under the assumption of fractional dose-additivity.

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| Option | Explanation |
|---|---|
| 1 | The entire emission sample is as potent as B[a]P. |
| 2 | The entire emission sample is as potent as the PAHs with more than 3 rings. |
| 3 | The entire emission sample is as potent as the sub-sample made up of the eight PAHs for which we have derived relative potency estimates. |
| 4 | The emission sample is as potent as the sub-sample with the eight PAHs plus the three PAHs in the IARC category "adequately tested with no evidence of carcinogenicity". |
| 5 | The entire sample potency is due to the PAHs with more than 3 rings, which are all assumed to be as potent as B[a]P. |
| 6 | The entire sample potency is due to the PAHs with more than 3 rings. |
| 7 | The entire sample potency is due to the eight carcinogenic PAHs which are all assumed to be as potent as B[a]P. |
| 8 | The entire sample potency is due to the eight carcinogenic PAHs. |
| 9 | The entire sample potency is due to B[a]P. |

Of these options only numbers (2) and (6) require the use of bioassay information for the mixture of concern to obtain potency estimates. The other seven options make use of only the relative amount of PAHs in a sample and our previously derived relative potency estimates for the eight carcinogenic PAHs.

To evaluate the different options, we compare them to the results obtained from the direct bioassay information. Three methods are used to obtain relative potency estimates for the entire samples using the bioassay information. They are:

Method 1: This approach used all of the available bioassay data but did not assume dose-additivity. As a result, nearly all of the information about the relative potency is based on exposure to the total condensate.

Method 2: All of the available bioassay information is used and a functional dependency between the exposure groups established by assuming dose-additivity. If the resulting model adequately fits the data this should be the most accurate method of estimating relative potency.

Method 3: The same data was used as for Method 1, but the relative potency estimates for the individual fractions were combined under the assumption of dose-additivity. This method would be appropriate if at high exposures due to toxicity, saturation of target dose, or other factors, dose-additivity was not operative. In this case the relative potency would be relevant for low but not high exposure situations.

Estimates obtained by these three methods are displayed in Table 10.1. In that table, situations where data is not available to make an estimate are indicated by a —. Also, where a method has been shown to be inconsistent with the data, this is indicated by a *. We note that very similar results are obtained by each approach, however Method 2 is to be preferred if dose-additivity is not rejected. The preferred estimates are shown in Table 10.1 in bold and given as the best direct bioassay estimate in Table 10.2.

The manner in which estimates were obtained is explained briefly in Display 10.1 and the numerical results given in Table 10.2 and in Figure 1. More details of how Options 3 and 4 were employed is given in Tables 10.3-10.6 and Option 6 in Table 10.7.

Display 10.1

Description of Simple Methods Used to Estimate Relative Potencies of Complex Combustion Product Emissions

| Option # | Method used to calculate relative potency |
|---|---|
| 1 | assumed equal to one |
| 2 | estimated from bioassay data of PAHs with more than 3 rings |
| 3 | weighted average of relative potency of eight carcinogenic PAHs |
| 4 | weighted average of relative potency of eight carcinogenic and three non-carcinogenic PAHs |
| 5 | fraction of PAHs with more than 3 rings in entire sample |
| 6 | relative potency of PAHs with more than 3 rings times fraction of PAHs with more than 3 rings in entire sample |
| 7 | fraction of eight carcinogenic PAHs in entire sample |
| 8 | weighted relative potency eight carcinogenic PAHs times fraction of entire sample made up of the eight |
| 9 | fraction of B[a]P in entire sample |



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Table 10.1 Relative potency estimates for entire sample obtained by three methods

| Source of emissions | Based on total condensate exposures (Method 1) | Based on all data and dose-additivty (Method 2) | Based on dose-additivity of fractional parts (Method 3) |
|---|---|---|---|
| Flue gas from coal-fired residential furnaces | 0.0542 | * | 0.0966 |
| Diesel engine exhaust | | | |
| Total | --- | 0.0028 | 0.0034 |
| Hydrophobic fraction | 0.0031 | 0.0038 | 0.0045 |
| Gasoline engine exhaust | 0.0220 | 0.0217 | 0.0216 |
| Sidestream cigarette smoke | --- | --- | 0.0030 |

* model does not fit data, suppresion of additivity effect present
--- bioassay information required to make estimate is not available

Table 10.2 Relative potency estimates of hydrocarbon combustion product determined by eight methods

| Option # | Method of estimation | Coal flue gas | Diesel engine exhaust | Gasoline engine exhaust | Sidestream cigarette smoke |
|---|---|---|---|---|---|
| + | Best direct bioassay estimate | (1.0)* 0.0542 | (1.0) 0.0028 | (1.0) 0.0217 | (1.0) 0.0030 |
| 1 | Total sample as potent as B[a]P | (18.5) 1.0 | (357.14) 1.0 | (46.1) 1.0 | (331.1) 1.0 |
| 2+ | Total sample as potent as >3 ring PAHs | (7.00) 0.3793 | (100.75) 0.2821 | (31.04) 0.6738 | (19.30) 0.0583 |
| 3 | Total sample as potent as weighted average eight carcinogens | (4.27) 0.2313 | (79.14) 0.2216 | (6.73) 0.1461 | (94.63) 0.2858 |
| 4 | Total sample as potent as weighted sample average eight carcinogens, three non-carcinogens | (1.34) 0.0724 | (22.98) 0.06435 | (1.50) 0.03246 | (18.90) 0.05707 |
| 5 | Total carcinogenicity due to >3 ring PAH which are assumed to be as equally potent as B[a]P | (2.86) 0.1549 | (2.81) 0.0079 | (1.29) 0.028 | (12.24) 0.0367 |
| 6+ | Total carcinogenicity due to >3 ring PAH | (0.64) 0.03475 | (1.06) 0.00296 | (0.90) 0.01948 | (0.71) 0.00214 |
| 7 | Total carcinogenicity due to eight carcinogens assumed as potent as B[a]P | (0.092) $4.78 \times 10^{-3}$ | (2.85) $7.98 \times 10^{-3}$ | (0.131) $2.85 \times 10^{-3}$ | (0.005) $1.58 \times 10^{-5}$ |
| 8 | Total carcinogenicity due to eight carcinogens | (0.020) $1.11 \times 10^{-3}$ | (0.650) $1.82 \times 10^{-3}$ | (0.032) $7.02 \times 10^{-4}$ | (0.0015) $4.50 \times 10^{-6}$ |
| 9 | Total carcinogenicity due to B[a]P | (0.013) $6.80 \times 10^{-4}$ | (0.446) $1.25 \times 10^{-3}$ | (0.022) $4.80 \times 10^{-4}$ | (0.0012) $3.47 \times 10^{-6}$ |

*number in parentheses is the ratio of the relative potency estimates derived for each option to the direct bioassay estimate.
+alternatives require an animal bioassay for the emission of concern, or for some fraction of emission

Figure 1. Comparison of methods for estimating the potency of a mixture for four hydrocarbon combustion products

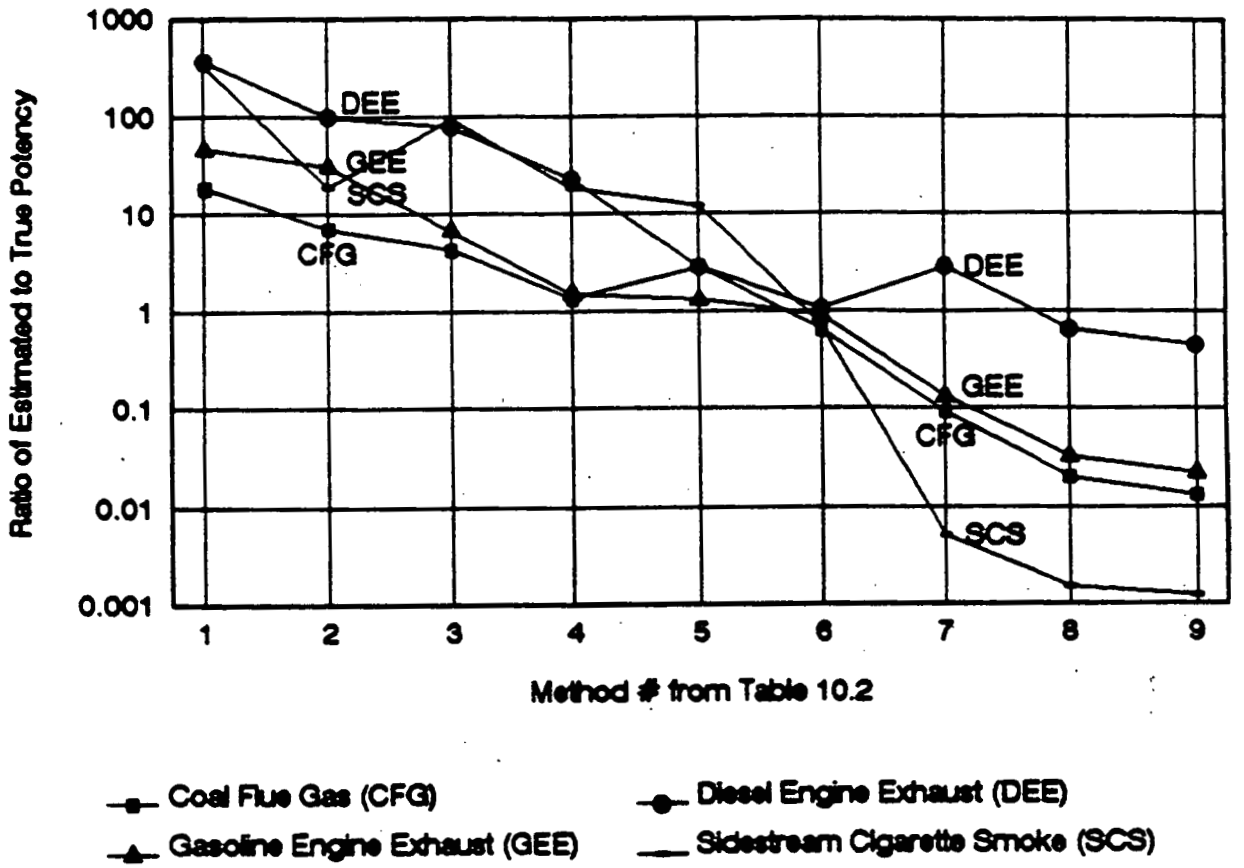

Table 10.3 Calculatuion of critical sub-sample relative potency for coal-fired residential furnace flue gas condensate

| Agent | Relative potency best bioassay estimate | Amount sample (mg/kg) | Relative amount of agent in sample | | Relative potency • relative amount | |
|---|---|---|---|---|---|---|
| | | | All agents | Only carcinogens | All agents | Only carcinogens |
| Coal Flue Gas Condensate | $\rho_T$=0.0542 | | | | | |
| Anthracene | 0.0 | 2.113 | 0.024190 | --- | --- | --- |
| Fluoranthene | 0.0 | 28.357 | 0.324637 | --- | --- | --- |
| Pyrene | 0.0 | 26.589 | 0.304396 | --- | --- | --- |
| Benzo[b]Fluoranthene | 0.12277 | 6.102 | 0.069857 | 0.201379 | 0.00086 | 0.02472 |
| Benzo[k]Fluoranthene | 0.05322 | --- | --- | --- | --- | --- |
| Benzo[j]Fluoranthene | 0.05232 | 6.782 | 0.077642 | 0.223820 | 0.00406 | 0.01171 |
| Benzo[e]Pyrene | 0.00704 | 3.994 | 0.045724 | 0.131811 | 0.00032 | 0.00093 |
| Benzo[a]Pyrene | 1.00000 | 4.303 | 0.049262 | 0.142009 | 0.04926 | 0.14201 |
| Indeno Pyrene | 0.27800 | 4.600 | 0.052662 | 0.151810 | 0.01464 | 0.04220 |
| Benzo[ghi]Perylene | 0.02124 | 3.855 | 0.044133 | 0.127224 | 0.00094 | 0.00270 |
| Anthanthrene | 0.31598 | 0.665 | 0.007499 | 0.021946 | 0.00237 | 0.00693 |
| | Total | 87.360 | 1.000000 | 1.000000 | $\rho_S$=0.0724 | $\rho_S$=0.2312 |

Table 10.4 Calculation of critical sub-sample relative potency for gasoline engine exhaust condensate

| Agent | Relative potency | Amount sample (μg/ℓ) | Relative amount of agent in sample | | Relative potency • relative amount | |
|---|---|---|---|---|---|---|
| | | | All agents | Only carcinogens | All agents | Only carcinogens |
| Gasoline Engine Condensate | $\rho_T$=0.0217 | | | | | |
| Anthracene | 0.0 | 642 | 0.284953 | --- | --- | --- |
| Fluoranthene | 0.0 | 1662 | 0.199290 | --- | --- | --- |
| Pyrene | 0.0 | --- | 0.383933 | --- | --- | --- |
| Benzo[b]Fluoranthene | 0.12277 | 19 | 0.008433 | 0.063973 | 0.00104 | 0.00785 |
| Benzo[k]Fluoranthene | 0.05322 | 7 | 0.003107 | 0.023570 | 0.00017 | 0.00125 |
| Benzo[j]Fluoranthene | 0.05232 | 11 | 0.004882 | 0.037037 | 0.00026 | 0.00194 |
| Benzo[e]Pyrene | 0.00704 | 37 | 0.016423 | 0.124580 | 0.00012 | 0.00088 |
| Benzo[a]Pyrene | 1.00000 | 50 | 0.022193 | 0.168350 | 0.02219 | 0.16835 |
| Indeno Pyrene | 0.27800 | 32 | 0.014203 | 0.107744 | 0.00395 | 0.02995 |
| Benzo[ghi]Perylene | 0.02124 | 115 | 0.051043 | 0.387205 | 0.00108 | 0.00822 |
| Anthanthrene | 0.31598 | 26 | 0.011540 | 0.087542 | 0.00365 | 0.02766 |
| | Total | 2601 | 1.000000 | 1.000000 | $\rho_S$=0.03246 | $\rho_S$=0.2461 |

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Table 10.5 Calculation of critical sub-sample relative potency for diesel engine exhaust

| Agent | Relative potency | Amount sample (μg/g) | Relative amount of agent in sample | | Relative potency • relative amount | |
|---|---|---|---|---|---|---|
| | | | All agents | Only carcinogens | All agents | Only carcinogens |
| Diesel Engine Exhaust | $\rho_T$=0.0028 | | | | | |
| Anthracene | 0.0 | --- | --- | --- | --- | --- |
| Fluoranthene | 0.0 | 725 | 0.34739 | --- | --- | --- |
| Pyrene | 0.0 | 756 | 0.36224 | --- | --- | --- |
| Benzo[b]Fluoranthene | 0.12277 | 57 | 0.02731 | 0.094059 | 0.00335 | 0.01155 |
| Benzo[k]Fluoranthene | 0.05322 | 46 | 0.05558 | 0.075908 | 0.00296 | 0.00404 |
| Benzo[j]Fluoranthene | 0.05232 | 116 | 0.02204 | 0.191419 | 0.00115 | 0.01002 |
| Benzo[e]Pyrene | 0.00704 | 95 | 0.04552 | 0.156766 | 0.00032 | 0.00110 |
| Benzo[a]Pyrene | 1.00000 | 92 | 0.04408 | 0.151816 | 0.04408 | 0.15182 |
| Indeno Pyrene | 0.27800 | 85 | 0.04073 | 0.140264 | 0.01132 | 0.03900 |
| Benzo[ghi]Perylene | 0.02124 | 115 | 0.05510 | 0.189769 | 0.00117 | 0.00403 |
| Anthanthrene | 0.31598 | --- | --- | --- | --- | --- |
| | Total | 2087 | 1.000000 | 1.000000 | $\rho_S$=0.06435 | $\rho_S$=0.22156 |

Table 10.6 Calculation of critical sub-sample relative potency for sidestream cigarette smoke

| Agent | Relative potency | Amount sample (μg/cig) | Relative amount of agent in sample | | Relative potency • relative amount | |
|---|---|---|---|---|---|---|
| | | | All agents | Only carcinogens | All agents | Only carcinogens |
| Sidestream Cigarette Smoke | $\rho_T$=0.0030 | | | | | |
| Anthracene | 0.0 | 710.0 | 0.30329 | --- | --- | --- |
| Fluoranthene | 0.0 | 685.9 | 0.29304 | --- | --- | --- |
| Pyrene | 0.0 | 476.3 | 0.20333 | --- | --- | --- |
| Benzo[b]Fluoranthene | 0.12277 | 65.8 | 0.02802 | 0.140120 | 0.00344 | 0.01720 |
| Benzo[k]Fluoranthene | 0.05322 | 65.8 | 0.02806 | 0.140120 | 0.00149 | 0.00746 |
| Benzo[j]Fluoranthene | 0.05232 | 65.8 | 0.02807 | 0.140120 | 0.00147 | 0.00733 |
| Benzo[e]Pyrene | 0.00704 | 75.7 | 0.03246 | 0.161201 | 0.00023 | 0.00113 |
| Benzo[a]Pyrene | 1.00000 | 103.5 | 0.04400 | 0.220400 | 0.04400 | 0.22040 |
| Indeno Pyrene | 0.27800 | 51.4 | 0.02179 | 0.109455 | 0.00606 | 0.03043 |
| Benzo[ghi]Perylene | 0.02124 | 41.6 | 0.01794 | 0.088586 | 0.00038 | 0.00188 |
| Anthanthrene | 0.31598 | --- | --- | --- | --- | --- |
| | Total | 2341.8 | 1.000000 | 1.000000 | $\rho_S$=0.05707 | $\rho_S$=0.28583 |

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Table 10.7 Relative contribution of higher ring PAHs to the carcinogenicity of complex mixtures

| Source of complex mixture | Constituent | Relative potency compared to B[a]P | Estimated fraction of total carcinogenic potency of complex mixture due to constituent assuming dose-additivity* |
|---|---|---|---|
| Flue Gas Coal-Fired Residential Furnaces | PAHs & S-PACs 4-7 rings | 0.3793 | 0.6407 |
| Diesel Engine Exhaust | PAH 4-7 rings | 0.2821 | ~ 1.0 |
| Gasoline Engine Exhaust | PAC ≥ 4 rings | 0.6738 | 0.8690 |
| Sidestream Cigarette Smoke | PAH ≥ 4 rings | 0.0583 | 0.7094 |

*ratio of constituent to total B[a]P equivalent exposure units (e.g., gasoline engine exhaust ~ 0.14 x 0.6738 + 5.0 x 0.02171 = 0.8690).

We note that as anticipated, assuming the entire emission is as potent as B[a]P considerably overestimates the true potency in each case. The most accurate approach by far, of the nine investigated, is to assume that the carcinogenicity in the entire sample is due to the PAH fraction consisting of compounds with more than 3 rings (the exact type of fraction is specified more accurately in Table 10.7). Such an approach would only slightly underestimate the true relative potency. Unfortunately, in most situations the relative potencies for the required PAHs with more than 3 rings would not be known. If only the total amount of PAHs with more than 3 rings were available Option 5 could be employed. Lacking a way of fractioning a sample so that the amount of PAHs with more than 3 rings could be estimated, Option 4 could be employed. In that case, chemical analysis of the amount of eleven PAHs present in the sample is all that would be required.

It is also clear that, except in the case of diesel engine exhaust, B[a]P accounts for only a small portion of the total carcinogenicity of the sample. Even the total of the eight known carcinogenic PAHs (i.e., option 7) contributes only a minor portion of the total carcinogenic effect. As a result, it would not appear to be very prudent to use such indicator type approaches to estimate cancer risk due to complex mixtures.

## 11. Discussion

Virtually the entire human population is exposed to some extent to complex mixtures of hydrocarbon combustion byproducts. Exposure to humans can result from a variety of different industrial and nonindustrial sources. Industrial sources include cokeing operations from steel mills and aluminum reduction plants; generation of power by combustion of coal, oil, gas, or garbage; synthetic fuel production; and dump sites of defunct coal-to-gas or oil-to-gas conversion plants. Contexts where nonindustrial exposures occur include barbecuing meat or consumption of barbecued meat, exposure to exhaust from diesel and gasoline engines; exposure to emissions from home oil, gas, or coal furnaces, fireplaces, and wood-burning stoves; and exposure to environmental tobacco smoke.

The carcinogenicity of hydrocarbon combustion products is believed to be attributable largely to the presence of polycyclic aromatic hydrocarbons (PAHs), and the carcinogenicity of the PAHs is in turn largely attributable to those PAHs with chemical structures including more that three aromatic rings. This suggests that approximate approaches for evaluating the carcinogenicity of hydrocarbon combustion products may be based on the carcinogenic potencies of PAHs that occur in these products.

A number of different approaches involving assumptions about PAH carcinogenicity can be used to estimate the cancer risk associated with exposure to hydrocarbon combustion products. These include 1) a *direct approach* of basing the dose-response model for an emission source on epidemiological data directly relating human cancer incidence to the emission of interest [e.g. coke oven emissions (EPA 1984); sidestream cigarette smoke (EPA, 1990)], 2) A generally conservative *regulatory approach* of assuming that all emissions are as potent as B[a]P, or else that all PAHs are as potent as B[a]P and that the carcinogenicity of the sample is attributable entirely to PAHs, and 3) a *relative potency* approach which involves obtaining a relative carcinogenic potency for a hydrocarbon combustion product by comparing the agent to a standard or reference agent, usually in an animal bioassay: this relative potency is then multiplied by the exposure level for the emission of concern to obtain an exposure measurement in units of carcinogenically equivalent exposure units of the reference agent. Such a reference-equivalent dose can be used to estimate risk based on a dose-response model for the reference agent (e.g. Albert et al., 1983). Each of these approaches has strengths and weaknesses. The methods and potency estimates obtained in this report may be used for evaluating and improving the quality of risk estimates based on any of these approaches.

Superficially it may appear that the direct approach should be preferred whenever appropriate data are available. The obvious reason is that both the disease endpoint (human cancer incidence) and the exposure variable (human exposure) are directly related to the cancer risk of concern. However the validity of such an approach may be reduced by such factors as:

1. Exposure histories in epidemiological cohorts may be very complex and difficult to quantify accurately, in regards to both level and timing;

2. Unit risk estimates may be extremely sensitive to the accuracy of information on prevalence of cigarette smoking in the cohort;

3. Problems in extrapolating results from an epidemiological cohort to other populations may result from differences in the composition of the emissions, differences in the demographic composition of the populations, and differences in exposure route.

Many examples may be given of the third type of problem (non-comparability of the context of an epidemiological study with a risk-assessment context), relevant to risk assessment for hydrocarbon combustion products. Emissions may differ qualitatively between an industrial worksplace where occupational exposures occur, and the community surrounding an industrial



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source, because of use of pollution control equipment (e.g. scrubbers) and photodegredation of the emissions. Use of epidemiological data on effects of smoking to directly assess risks from environmental tobacco smoke (primarily sidestream smoke) is complicated by many factors. Fresh sidestream and mainstream cigarette smoke differ in various ways (NRC 1986 especially Tables 2-1 and 2-2) and smokers may differ physiologically from nonsmokers because of chronic effects of smoking. Epidemiological data on the carcinogenic effects of coke oven emissions are based on healthy, black males engaged in strenuous labor (EPA, 1984), who may be a poor surrogate for the general population without appropriate adjustments.

The regulatory approach has often been criticized as being overly conservative. Such critism is most likely valid when all of the PAHs (e.g., benzene soluble organics) or the total emissions is considered to be as potent as B[a]P (see Table 10.1). However, the evidence from the lung implant studies strongly suggests that the use of PAH "indicator" carcinogens, all considered as potent as B[a]P, could seriously underestimate the true risk (see Table 10.2, option #8). This is probably because many of the greater than 3 ring PAHs that are unknown or are unstudied in carcingenic bioassays are in fact carcinogenic. This reasonable conjecture calls into question the common practice of basing risk estimates from complex mixtures on the potencies of indicator carcinogens.

For airborne hydrocarbon combustion products and perhaps PAHs in the groundwater, a more fruitful approach would appear to be estimating the concentration of greater than 3 ring PAHs in the air or water and assuming that all of them to be as potent as B[a]P (see Table 10.2, option #5). This has the advantage of being conservative but apparently not unduly so, and much less expensive than obtaining measurements for multiple indicator chemicals. Of course if bioassay estimates of the total solvent extractable emissions is available for the greater than 3 ring PAHs, such estimates usually would be prefered. However, this assumes that the samples bioassayed were representative of the entire emissions over a reasonable time frame from the source of concern.

The relative potency approach of Albert et al. (1983) depends upon having a relative potency estimate of an emission source compared to a suitable standard (e.g., perhaps coke oven emissions) and a dose-response model for the standard. Problems with this approach are:

1. Epidemiological data for the suggested standards, coke oven emissions, cigarette smoke condensate, and roofing tar fumes, are out of date, because of new data and methodological approaches for obtaining potency estimates.

2. Relative potency estimates would have to be based on a double transformation (e.g., potency of coke oven emissions compared to B[a]P then in a separate system potency of souce of concern compared to B[a]P.

3. Estimates of relative potencies are in mouse skin rather than lung tissue, the actual site of concern for inhaled emissions.

4. The models and methods previously used to obtain the relative potency estimates using the mouse skin tumor responses are of questionable validity.

It is clear that each of the three approaches has potential major problems associated with it. Thus, careful consideration of the shortcomings of the approach chosen for a risk analysis in a given circumstance needs to be conducted before any analysis is based on the approach.

In some circumstances the unit risk for a single carcinogenic PAH may be required. These are estimated by multiplying the unit risk for inhalation and ingestion for B[a]P obtained in the companion documents times the maximum likelihood estimate of the relative potency for the individual PAHs compared to B[a]P, displayed in Table 7.4. This results in the unit risk estimates for the individual PAHs shown in Table 11.1. Also displayed in Table 11.1 are unit risks for emission sources, with relative potency estimates taken from Tabel 10.1.

## 12. Further Research Needs (In preparation)

Table 11.1 Unit risk estimates for selected PAHs and hydrocarbon combustion product mixtures

| Agent | Relative potency | Unit risk from inhalation | | Unit risk from ingestion |
|---|---|---|---|---|
| | | 1 $\mu g/m^3$ | 1 mg/kg/day* | 1 mg/kg/day+ |
| B[a]P | 1.0 | $3.89 \times 10^{-5}$ | $1.36 \times 10^{-1}$ | $2.90 \times 10^{-0}$ |
| B[b]F | 0.1228 | $4.78 \times 10^{-6}$ | $1.67 \times 10^{-2}$ | $3.56 \times 10^{-1}$ |
| B[e]F | 0.0070 | $2.72 \times 10^{-7}$ | $9.52 \times 10^{-4}$ | $2.03 \times 10^{-2}$ |
| B[j]F | 0.0523 | $2.03 \times 10^{-6}$ | $7.11 \times 10^{-3}$ | $1.51 \times 10^{-1}$ |
| B[k]F | 0.0532 | $2.07 \times 10^{-6}$ | $7.23 \times 10^{-3}$ | $1.54 \times 10^{-1}$ |
| IND | 0.2780 | $1.08 \times 10^{-5}$ | $3.78 \times 10^{-2}$ | $8.06 \times 10^{-1}$ |
| ANT | 0.3160 | $1.23 \times 10^{-5}$ | $4.29 \times 10^{-2}$ | $9.16 \times 10^{-1}$ |
| B[ghi]P | 0.0212 | $8.25 \times 10^{-7}$ | $2.88 \times 10^{-3}$ | $6.14 \times 10^{-2}$ |
| flue gas from coal fired furnace | 0.0542 | $2.11 \times 10^{-6}$ | $7.37 \times 10^{-3}$ | $1.57 \times 10^{-1}$ |
| diesel engine exhaust | 0.0028 | $1.09 \times 10^{-7}$ | $3.81 \times 10^{-4}$ | $8.12 \times 10^{-3}$ |
| gasoline engine exhaust | 0.0217 | $8.44 \times 10^{-7}$ | $2.95 \times 10^{-3}$ | $6.29 \times 10^{-2}$ |
| sidestream cigarette smoke | 0.0030 | $1.17 \times 10^{-7}$ | $4.08 \times 10^{-4}$ | $8.70 \times 10^{-3}$ |

*Assumes 100% absorption
+Assumes relative potency in lung and digestive tract are equal

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