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Authors

Bois, F.Y.
Spear, R.C.

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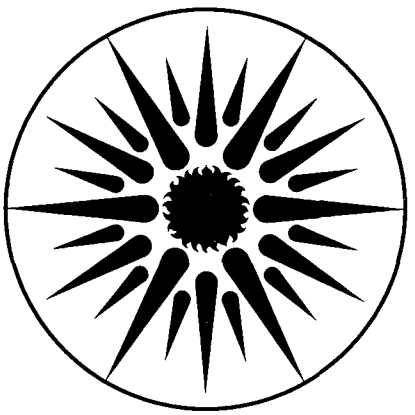

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ANALYSIS OF PARAMETRIC UNCERTAINTY IN PHYSIOLOGICAL PHARMACOKINETIC AND MULTISTAGE CANCER MODELS

by

Frédéric Y. Bois and Robert C. Spear*

Indoor Environment Program
Applied Science Division
Lawrence Berkeley Laboratory
University of California
Berkeley, California 94720

*Biomedical and Environmental Health Sciences
School of Public Health
University of California
Berkeley, California 94720

March 1990

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Frédéric Y. Bois and Robert C. Spear.

Biomedical and Environmental Health Sciences, School of Public Health and Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720.

SUMMARY

A global optimization, using Monte Carlo simulations, was performed on the 29 parameters of a coupled physiological pharmacokinetic and multistage cancer model. The parameters were weighted according to their relative likelihood, and used to obtain the distribution of cancer incidence at low exposure levels. As an example, the case of benzene carcinogenicity is investigated. Using this new approach it appears that much more confidence can be placed on extrapolations made with a pharmacokinetic model, than with the multistage carcinogenesis component. The distributions of estimated low-exposure risks are bimodal, and extend over several orders of magnitude. These results demonstrate limited value of the common animal cancer experiments for quantitative extrapolations, and indicate the need for more biological information relevant to the identification of the parameters of multistage models of carcinogenesis.

1. Introduction

The coupling of physiological pharmacokinetic models with extended multistage models of carcinogenesis is potentially a tool of choice for performing human health risk assessment (Zeise, Wilson and Crouch, 1987). In this context the pharmacokinetic model provides an estimate of effective exposure, which the cancer model links to tumor incidence. It is assumed that the mechanistic basis of these models makes them more suitable than empirical formulae for inter-route, inter-species and low-dose extrapolations (Ritschel and

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Banerjee, 1986). While in theory their use is certainly beneficial, several difficulties are associated with their parametrization, especially when coupled.

Commonly, the pharmacokinetic component involves at least 15 parameters, most of which are fixed *a priori*, except for one or two (usually describing the metabolism) which are varied until an acceptable or best fit to the experimental data is obtained (Medinsky et al., 1989). The values of the fixed parameters are taken from the literature, where often little or conflicting information is available. Typically, the uncertainty affecting the parameter estimates is ignored because a classical estimation procedure is not usually possible with that many parameters. Consequently, the pharmacokinetic models may often be poorly parametrized and poorly fitted. While they may give some insight into the dynamics of dispersion of a chemical within the body, the information they provide is mostly qualitative, and the uncertainty affecting their prediction of effective exposure is unknown.

The situation is somewhat better for many of the multistage models, which contain fewer parameters: classical maximum likelihood estimates of the parameters may be obtained, together with their approximate confidence intervals (Crump and Howe, 1984; Portier and Bailer, 1989). A difficulty arises when these models are coupled to the previous ones, as the effective exposure used as input is a random variable of unknown distribution for the reasons mentioned above.

In this paper, in order to overcome the computational burden of the classical maximum likelihood approach, Monte Carlo simulations were used to optimize the adjustment of the pharmacokinetic and multistage models to experimental data. A new weighting procedure, based on likelihood estimation, was used to derive the distribution of predicted metabolic rates for the pharmacokinetic model and the distribution of predicted risk for the multistage model. Our approach is essentially pragmatic, as our main goal is not to describe or explain interactions between the model parameters, but to take them fully into account when using the models for extrapolations. Benzene, a carcinogen in animals and humans, was chosen to explore the benefits and limitations of the technique.

2. Physiological Pharmacokinetic Model

In the pharmacokinetic model developed, the mammalian body is divided into five compartments: the well perfused tissues, which include the heart, brain, kidneys and other viscera; the poorly perfused tissues, consisting mainly of muscles and skin; the liver; the fat; and the bone marrow. The evolution with time of the concentration of benzene in each compartment is described by a differential equation dependent on the blood flow through the compartment, the partition coefficient between the tissue and the blood, the volume of the compartment and, for the liver and bone marrow, metabolic parameters (Andersen et al., 1987; Dedrick, Forrester and Ho, 1972; Gerlowski and Jain, 1983). A diagram of the model is presented in Figure 1. The description of benzene metabolism is critical since the metabolites are suspected to be the actual cancer-causing agents (Eastmond, Smith and Irons, 1987; Kalf, 1987). The major site of metabolism is the liver (Cooper and Snyder, 1988). In addition, some metabolic activity, which may contribute to toxicity, occurs in the bone marrow (Irons et al., 1980). Michaelis-Menten terms, suitable for saturable kinetics (Gerlowski and Jain, 1983), are used to describe the enzymatic transformation of benzene into its metabolites in the liver and the bone marrow.

To take into account known structural dependencies among the parameters, many of them are scaled to the body weight or to the body surface area (Davidson, Parker and Beliles, 1986). For example the blood flows to the various organs are percentages of the total flow, which in turn is proportional to the body weight to a given power (i.e. proportional to the body surface area). Initial ranges were chosen for each of the scaling coefficients, one for each parameter of the model, and for the scaling powers (see Table 1). The ranges were selected to include various values reported in the literature for adult rats (Andersen et al., 1987; Bois, Tozer and Zeise, 1989; Bois, Zeise and Tozer, 1990; Fairchild, 1972; Fiserova-Bergerova and Hugues, 1983; Pathiratne, Puyear and Brammer, 1986; Sato et al., 1975). These initial ranges were made quite large to encompass the uncertainties affecting many of the published values.

3. Extended Multistage Model

A simple clonal two-stage model previously described by Moolgavkar (1983), Moolgavkar and Venzon (1979), and Portier (1987), was adapted to the case of benzene (Figure 2).

Let $Z(t,E)$ denote the number of malignant cells at time t, in animals subject to exposure E of benzene metabolites (or, equivalently, amount of benzene metabolized). In the model used, the probability that $Z(t,E) \geq 1$ is given by:

$$P[Z(t,E) \geq 1] = 1 - \exp\left[-(\mu_0 + \xi_0 E)(\mu_1 + \xi_1 E)\frac{\exp[(\beta_1 - \delta_1)t] - 1 - (\beta_1 - \delta_1)t}{(\beta_1 - \delta_1)^2}\right], \quad (3.1)$$

For a given animal this probability is taken as the probability of being cancerous at time t. The parameters correspond to the transition probability rates between the various events involved in the process (see Figure 2); for a non-zero exposure the transition rates between the stages are equal to $\mu_0 + \xi_0 E$ and $\mu_1 + \xi_1 E$. Only the variables $a = \mu_0\mu_1$, $b = \mu_0\xi_1 + \xi_0\mu_1$, $c = \xi_0\xi_1$,and $\gamma_1 = \beta_1 - \delta_1$ are identifiable, so that the following 4-parameter model was used:

$$P[Z(t,E) \geq 1] = 1 - \exp\left[\left(-a - bE - cE^2\right)\frac{\exp(\gamma_1 t) - 1 - \gamma_1 t}{\gamma_1^2}\right]. \quad (3.2)$$

The parameter γ_1 was not made dependent on E, as benzene can not be considered as a promoter on the basis of the available data. The ranges assigned to each of the parameters are given in Table 2. These ranges depend in part on the value of E, and their derivation is described in section 6.

4. Global Optimization of the Pharmacokinetic Model

Using uniform or log-uniform random sampling within each parameter range (log-uniform for the widest ranges), 10,000 Monte Carlo runs were performed to simulate experimental data published by Rickert et al. (1979) and Sabourin et al. (1987, 1988). These experiments provide extensive information about the kinetics of benzene in Fischer-344

rats, and the formation of its metabolites, after both gavage and inhalation exposure. Rickert et al. (1979) exposed rats to 1.28 mg/L of benzene in the air, for 6 or 8 hrs, and observed the blood, fat, and bone-marrow concentrations during and after exposure. These authors also report the quantity remaining to be exhaled after exposure. Sabourin et al. (1987) monitored the total amount of benzene exhaled, the quantity of metabolites excreted and left in the body, for 48 hrs after various gavage exposures (0.5, 5, 15, 50, 150 and 300 mg/kg), and for 56 hrs after 6 hrs of various inhalation exposures (0.033, 0.075, 0.34, 0.68, 2.26 mg/L). Sabourin et al. (1988) report blood and liver concentrations after 6 hrs of exposure to 0.12 mg/L of benzene by inhalation.

Assuming that the values for experimental results described above are normally distributed, the likelihood $L(\boldsymbol{\theta})$ of each parameter vector $\boldsymbol{\theta}$ was computed as follows (Edwards, 1972):

$$L(\boldsymbol{\theta}) = \prod_{i=1}^{N} \left(s_i^2 + \left(\bar{y}_i - \hat{y}_i \right)^2 \right)^{-n_i/2}, \tag{4.1}$$

where N = 101 is the number of experimental data points. s_i^2, $\hat{y}_i$, n_i, and $\bar{y}_i$, are the variance, mean, number of individual samples, and predicted mean for each point, respectively.

The best-likelihood vector, $\boldsymbol{\theta}_m$, out of 10,000 trial vectors, produces the fit shown in Figure 3. The mean ratio between predicted and observed values is 1.01, with a standard deviation of 0.63, and ranges from 0.16 to 4.0. Here, "best likelihood" does not refer to the absolute maximum of the likelihood function, but to the highest likelihood value obtained during the Monte Carlo simulations. The search for the maximum likelihood vector by classical optimization would be prohibitive in term of computing load. Monte Carlo simulations give a useful approximation of the global maximum, while being insensitive to the potential presence of multiple maxima.

In the course of the 10,000 runs several parameter ranges were modified, as it appeared that some extreme values would yield only very poor fits (with relative log-likelihood $r(\boldsymbol{\theta}) = \log(L(\boldsymbol{\theta})/L(\boldsymbol{\theta}_m))$, less than -50.0). Consequently, the ranges for these

parameters were reduced in the subsequent runs (Table 1, column "final range"). It appeared also that values outside the original ranges could be acceptable for two parameters poorly defined in the literature, thus their ranges were extended (Table 1).

The plots of the relative log-likelihood, $r(\theta)$, versus the values of the scaling coefficients for the parameters F_{alv} (alveolar ventilation rate) and V_{maxl} (maximum rate of metabolism in the liver) are given in Figure 4 (only the results for the last 1000 simulations are shown). The paucity of high $r(\theta)$ values indicates that most of the runs give poor fits. The parameters F_{alv} and V_{maxl} are among the few for which some information on location is available in such univariate analysis. For most of the parameters it was impossible to restrict the broad initial sampling range on the basis of the likelihood function (only 7 parameter ranges could be modified from their *a priori* definition during the global adjustment). One way to improve the performance of the Monte Carlo runs would be to stop computing the data points when the likelihood is certain to be below a given limit. On the other hand, a multivariate analysis – such as Classification and Regression Trees (Breiman et al., 1984) – of the results at an intermediate stage could point to regions of the parameter space where the likelihood is almost certain to be below a given limit; a parameter vector falling in such a region would be discarded and a new one sampled.

5. Weighting of the Parameter Vectors

A total of 10000 parameter vectors, with their respective likelihoods, were generated during the Monte Carlo simulations. Theoretically it should be possible to derive, from this information, a joint distribution function for the individual parameters of the model. Then, a resampling of the parameters out of this distribution could be performed in order to obtain the distribution of given model predictions (e.g. the rate of metabolic transformation at very low exposure). In fact, with 25 parameters the sampling of 10000 points is too sparse to make this approach feasible, and no realistic number of simulations will suffice. Here, instead, a weight was first given to each parameter vector θ, based on its relative log-

likelihood $r(\theta)$. The same 10000 vectors were used again to obtain a set of predictions, each of which carried the weight of its parent parameter vector.

The parameter vectors were assigned to 45 different classes i, on the basis of their relative log-likelihood. The classes were defined by reference to various α_i confidence regions for θ_m (α_i = 0.01, ..., 0.1, ..., 0.91, ..., 0.991, ..., 0.9991, ..., 0.9999). A vector θ would fall in class i if $-2r(\theta)$ was included between the α_{i-1} and α_i fractiles of the $\chi^2_{(n)}$ distribution (i.e. if θ was included in the α_i but not the α_{i-1} confidence region). For the pharmacokinetic model the number of parameters n = 25. Vectors falling outside the 0.9999 confidence region were not considered. Let Z_i denote the number of vectors in class i. For any given vector θ in class i, the weight W is:

$$W = \frac{\alpha_i - \alpha_{i-1}}{Z_i}. \qquad (5.2)$$

This ensures that each vector is weighted according to the inter-fractile region in which it falls, and the density of sampled vectors in that region. Each vector can be considered to represent its class, the representation of a class being equally divided among its vectors. In the following, the model predictions obtained using a parameter vector will be given the weight of that same vector. We will use the term distribution of the predictions when considering the graphs of the weights versus the values of these predictions.

Out of 10,000 sampled vectors, only 54 were contained in the 99.99% confidence region. They were each used to compute the total amounts (termed E_1, E_2, E_3 in the following) of benzene metabolized per week during the NTP cancer experiment, after exposure of male Fischer-344 rats to various doses of benzene (Huff et al., 1989). The exposures were to 50 mg/kg, 100 mg/kg and 200 mg/kg of benzene by gavage, 5 days per week, for 103 weeks. A non-exposed control group was observed in parallel. Only the amount metabolized during the first week was computed, as preliminary runs showed that for the 10 vectors of highest likelihood the relative difference between the first week and the following did not exceed 1 or 2 percent of the first week estimate and therefore was negligible. This indicates that an equilibrium was reached after one week of exposure. The

predicted amounts metabolized were given the weight of their respective generating parameter vectors. Figure 5 shows the distributions of these amounts E_1, E_2, and E_3. The height of the histogram bars corresponds to the sum of the relative weight, figured by vertical lines, in each class. These distributions are remarkably tight: estimates for E_1 range from 64 to 82 mg/week; for E_2 from 99 to 131 mg/week; and for E_3 from 139 to 204 mg/week. Although the experimental data available do not give adequate information for most of the individual parameter values for this model (see above and Figure 4), the parameter combinations controlling the amount metabolized appears to be quite well defined. With 25 parameters, and the complex data set used, combinations are likely involve many parameters. It should be stressed that the Monte Carlo/weighting procedure used allows for an implicit account of the global covariance structure imposed both by the model and the experimental data. Portier and Kaplan (1989) also used Monte Carlo simulations to estimate the distribution of "virtually safe doses" in the case of methylene chloride, but did not take into account any parameter covariance. Their parameter values were derived mostly *a priori*, as well as the variation bounds imposed. In a similar context, Bois, Zeise and Tozer (1990) take into account the covariance between the two most sensitive input parameters, but not among all of them.

Figure 6 shows the evolution of the estimated distribution for E_1, amount metabolized per week for a gavage dose of 50 mg/kg, during the course of the last 5000 simulations. The range did not change appreciably during the last 5000 runs and the 3 distributions formed after 8000, 9000 or 10,000 runs do not differ at $P = 0.1$ (using the Kolmogorov-Smirnov test). We therefore assume that a sufficient approximation of the distribution was obtained.

6. Results for the Multistage Model

The 54 exposure triplets (E_1, E_2, E_3) generated by the pharmacokinetic model were used as the input of 10,000 Monte Carlo simulations with the multistage model. Each triplet was used for a number of simulations proportional to the relative weight W of its generating

parameter vector in the pharmacokinetic model. At the same time, the four parameters of the multistage model (a, b, c, γ_1) were sampled log-uniformly from their respective intervals (Table 2), and the incidence of Zymbal gland carcinoma in male rats (given by 3.2), after two years of continuous exposure to benzene was simulated. The initial parameter ranges were chosen to be very large. Preliminary runs showed that above the upper bound of these ranges no acceptable fit could be obtained. The lower bounds appeared to be at zero, except for the parameter a. An arbitrary low value of 10^{-30} was taken for the other parameters. The likelihood $L(\theta')$ of each parameter vector θ' for the multistage model was computed, assuming a binomial distribution of the number of tumor-bearing animals. Note that each θ' vector has an associated θ vector of pharmacokinetic parameters used for computing the exposure triplet. The best-likelihood adjustment obtained is presented in Figure 7. The cancer incidence is related to the benzene gavage dose in Figure 7A, and to the amount of benzene metabolized per week in 7B. The model parameter values are indeed the same in the two cases. A saturation of the rate of benzene metabolism occurring within the gavage dose range explains the difference in shape of the two curves. Adjustments obtained using the gavage dose, rather than the amount metabolized, as the effective exposure for the multistage model would give different parameter values, likely to lead to biased predictions of cancer incidence at low exposure. The best-likelihood estimates for the parameters a, b, c, γ_1 (probabilities per week) were found to be 8.75×10^{-6}, 2.6×10^{-29}, 3.55×10^{-9}, and 2.10×10^{-12}, respectively. This best fit is nearly quadratic, b being negligible in the model.

Out of 10,000 sampled θ' vectors, 767 were contained in the 99.9% confidence region (i.e.with a relative log-likelihood greater that -9.233). The relative weights W' of these vectors were computed as described above, and the vectors were used to predict the incidence of Zymbal gland carcinoma, for gavage doses of 1 μg/kg and 100 μg/kg, using the NTP protocol. The amount metabolized per week at the same levels was first computed using the corresponding θ vector of pharmacokinetic parameters. The results of this low-dose extrapolation are presented in Figure 8A and B.

Both distributions are bimodal and extend over several orders of magnitude. The bimodality is an interesting effect which has been described, in a somewhat different context, by Portier and Hoel (1983). This arises because, in a linear-quadratic model, either the linear term, bE, or the quadratic term, cE^2, control the outcome of the simulation. Hence, in about 40% of the cases, the quadratic term is significant and the linear term negligible, and vice-versa in about 30% of the cases. The remaining 30% of the predictions fall at very low values, including zero, and have very little weight (they are not even visible on the Figures). The bimodality has no discernable influence within the range of the NTP exposure doses (50 to 200 mg/kg), as both alternatives lead to acceptable fits and high $L(\boldsymbol{\theta}')$ values. However, by contrast, larges differences are seen at low exposure levels. It should be noted that the parameter γ_1 cannot be properly identified with the NTP data, as its likely values are spread evenly in the range sampled. The best-likelihood estimate of the cancer incidences at low doses falls in the lowest peak of the bimodal distribution (5.8×10^{-11} for a dose of 1 μg/kg and 5.9×10^{-7} for 100 μg/kg). It would be very misleading to use only the best- or maximum-likelihood estimates, for example in risk assessment, without considering the fact that a large fraction (about 30%) of the possible risks lays at a much higher level.

7. Conclusions

A global adjustment of a pharmacokinetic model and of a multistage cancer model to benzene cancer data was obtained using Monte Carlo simulations. A weighting procedure, accounting for high-dimension covariance structure, was described and used to obtain the distribution of the effective exposure and of the cancer incidence at very low exposure.

While our pharmacokinetic model has 25 parameters, about 100 data points obtained in various exposure situations are available to fit it. Variation bounds for many of the parameters can be obtained *a priori*, when these parameters have an immediate physiological meaning. This explains that a set of the most likely parameter vectors gives fairly precise predictions of the quantity of benzene metabolized. It can be concluded that good

confidence can be placed in the inter-route or inter-dose extrapolations made with such models, even if empirical parameter fitting is used to parametrize the model. This conclusion should hold for models designed to describe the pharmacokinetics of compounds other than benzene, provided that data from properly designed experiments are available.

On the other hand, the parametrization of multistage models with currently available data is a much more delicate problem. There is not enough information in the usual cancer experiments to adjust the four parameters of the simple model considered here, which represents a gross simplification of the carcinogenic processes. As a result, very broad, bimodal distributions of low-dose risk estimates are obtained, implying that standard maximum-likelihood point estimation might be inadequate. As long as additional biological information is lacking, the types of uncertainty considered in this paper should not be ignored. If these uncertainties can not be reduced for now, at least they should be estimated. The modeling method presented provides a mean of analyzing such variabilities.

Beyond its obvious application to the specific problems of cancer risk assessment, the method developed here is useful in numerous cases where large models are considered.

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RÉSUMÉ

Une optimization globale des 29 paramètres d'un modèle pharmacocinétique physiologique, couplé à un modèle multi-étape de cancérogénese, est réalisée à l'aide de simulations Monte Carlo. Les vecteurs de paramètres sont pondérés sur la base de leur vraisemblances

relatives, et réutilisés afin d'obtenir la distribution de l'incidence de cancer estimée, pour de faibles niveaux d'exposition. Le cas de la cancérogénese par le benzène, chez le rat, est pris pour example. Cette approche originale montre que l'on peut avoir bien plus confiance dans les extrapolations faites à l'aide d'un modèle pharmacocinétique, que dans celles réalisées à l'aide d'un modèle multi-étape. Les distributions des risques de cancer estimés, pour de faibles expositions, sont bimodales, et s'étendent sur plusieurs ordres de magnitude. Ces résultats démontrent la valeur limitée des expérimentations de cancérogénese animale actuelles, et appelent à davantage d'informations biologiques susceptibles de conduire à l'identification des paramètres de modèles multi-étapes.

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Table 1: Sampling ranges of the parameter scaling coefficients[a] used for the model of benzene pharmacokinetics in male rats. The final ranges were derived from the initial ranges during the fitting process (see text).

Scaled Parameter	Multiplier	Scaling Coefficient Sampling Range					
		Initial Range			Final Range		
Body weight (Bw)	1	0.205	-	0.245 [b]	0.205	-	0.245 [b]
Scaling powers							
$sc1$	1	0.65	-	0.85	0.65	-	0.85
$sc2$	1	0.65	-	0.85	0.65	-	0.85
Total blood flow (F_{tot})	Bw^{sc1}	0.22	-	0.28	0.22	-	0.28
AlveolarVentilation rate (F_{alv})	F_{tot}	0.5	-	1.0	0.5	-	1.0
Blood flows							
Liver (F_l)	F_{tot}	0.23	-	0.33	0.23	-	0.33
Bone marrow (F_{bm})	F_{tot}	0.01	-	0.05	0.01	-	0.05
Fat (F_f)	F_{tot}	0.04	-	0.10	0.04	-	0.10
Poorly perfused tissue (F_{pp})	F_{tot}	0.10	-	0.18	0.10	-	0.18
Well perfused tissue (F_{wp}) [c]	F_{tot}		-			-	
Volumes							
Liver (V_l)	Bw	0.03	-	0.05	0.03	-	0.05
Bone marrow (V_{bm})	Bw	0.02	-	0.04	0.02	-	0.04
Fat (V_f)	Bw	0.07	-	0.13	0.07	-	0.13
Poorly perfused tissue (V_{pp}) [d]	Bw		-			-	
Well perfused tissue (V_{wp})	Bw	0.04	-	0.09	0.04	-	0.09
Blood/air partition coefficient (P_a)	1	14.0	-	26.0	10.0	-	26.0
Tissue/blood partition coefficients							
Liver (P_l)	1	0.5	-	3.0	0.5	-	3.0
Bone Marrow (P_{bm})	1	5.0	-	13.0	3.0	-	12.0
Fat (P_f)	1	24.0	-	34.0	24.0	-	33.0
Poorly perfused tissue (P_{pp})	1	0.5	-	2.0	0.6	-	2.0
Well perfused tissue (P_{wp})	1	0.5	-	3.0	0.5	-	3.0
Maximum rates of metabolism							
liver ($Vmax_l$)	Bw^{sc2}	0.05	-	0.18	0.05	-	0.17
bone marrow ($Vmax_{bm}$)	$Vmax_l \times Bw^{sc2}$	0.05	-	0.18	0.05	-	0.18
Vmax/affinity constant ratios							
liver (Km_l) [e]	1	0.02	-	1.0	0.02	-	0.8
bone marrow (Km_{bm}) [e]	1	0.001	-	0.2	0.001	-	0.2
Intestinal absortion rate (K_{ing})	1	0.003	-	0.03	0.003	-	0.03
Metabolite excretion rate (K_{ex})	1	0.0006	-	0.002	0.0009	-	0.002

[a] Scaled parameter = multiplier × scaling coefficient. Units: body weights in kg, flows (F) in L/min, volumes (V) in L, Vmax in mg/min, and Km in L/min.

[b] This range was used for simulating Rickert (1979) experiments. For Sabourin (1987,1988) experiments the range was 0.27 - 0.31 kg.

[c] Values for F_{wp} were computed at each run so that the sum of the blood flows was equal to the total flow.

[d] Values for V_{pp} were computed at each run so that the sum of the volumes was equal to 90% of the body volume.

[e] These parameters were sampled log-uniformly.

Table 2: Parameter ranges used for the multistage model of Zymbal gland carcinoma in Fischer-344 rats.

Parameter	Range
Background transition rate (a)	10^{-8} - 5×10^{-4}
Coefficient of the exposure (b)	10^{-30} - 8×10^{-6}
Coefficient of the squared exposure (c)	10^{-30} - 10^{-7}
Net growth rate (γ_1)	10^{-30} - 10^{-1}

Units: all parameters are probability rates per unit time (i.e. per week).

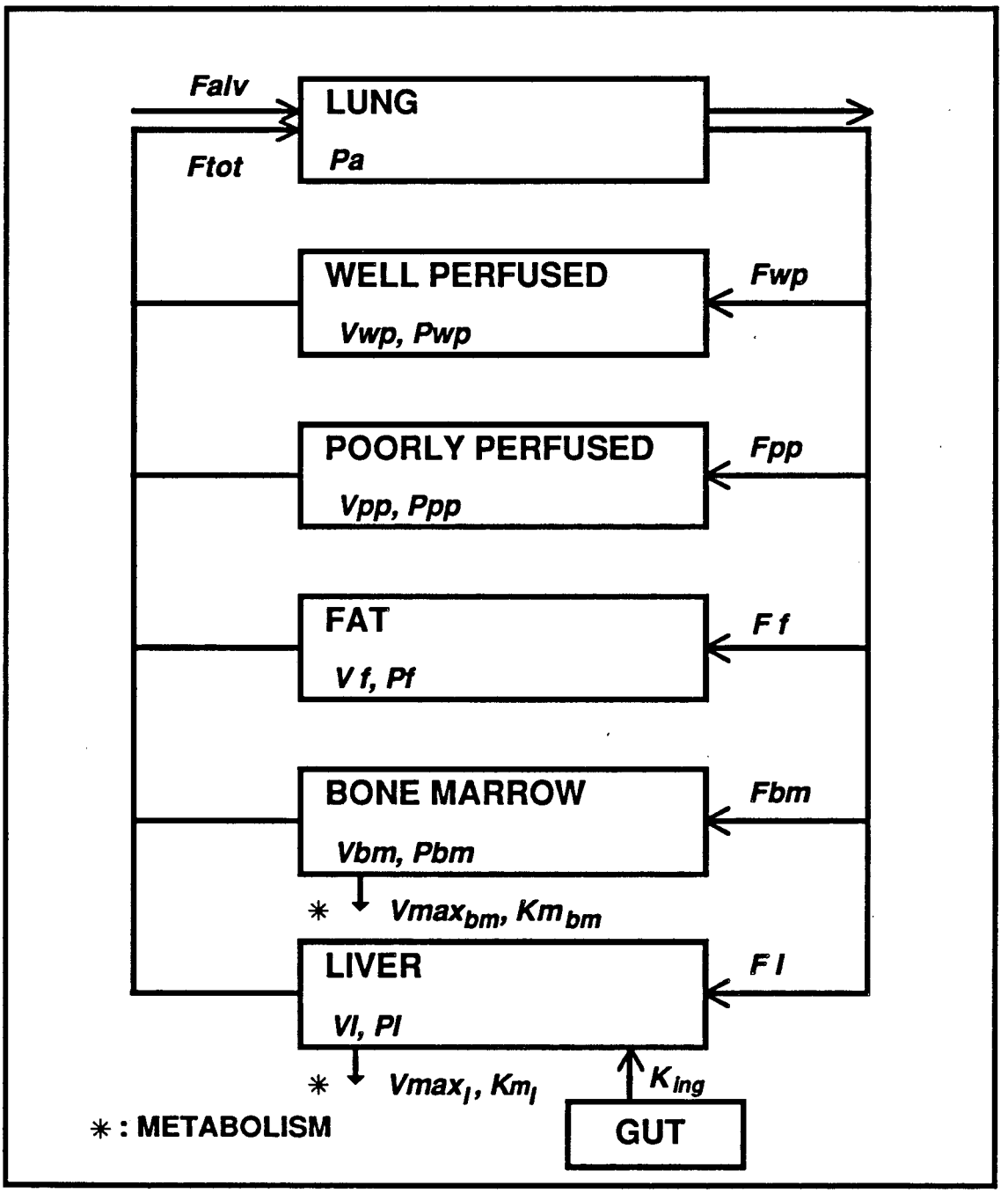

Figure 1: Schematic representation of the physiological model used to simulate the distribution and metabolism of benzene. The symbols are: F_{alv}, alveolar ventilation rate; P_a, blood over air partition coefficient; F_{tot}, total blood flow; Vmax, maximum rates of metabolism; Km, Vmax/affinity constant ratios. Subscripts representing the tissues are used to identify the blood flows to the tissues, F, the volumes, V, and the tissue over air partition coefficients, P. The units of the parameters are given in Table 1.

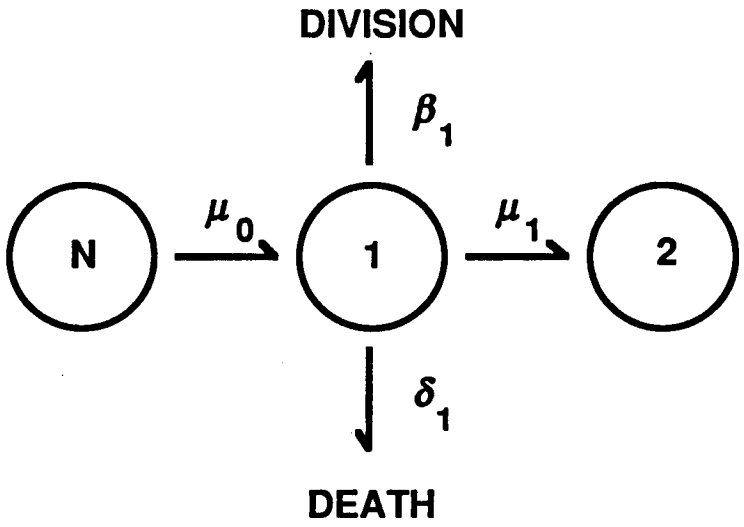

Figure 2: Schematic representation of the multistage model used to simulate the carcinogenicity of benzene. The symbols are: μ_0, background transition rate from normal to initiated cell stage; μ_1, background transition rate from initiated to cancerous; β_1, and δ_1 division and death rates, respectively, for the initiated cell stage.

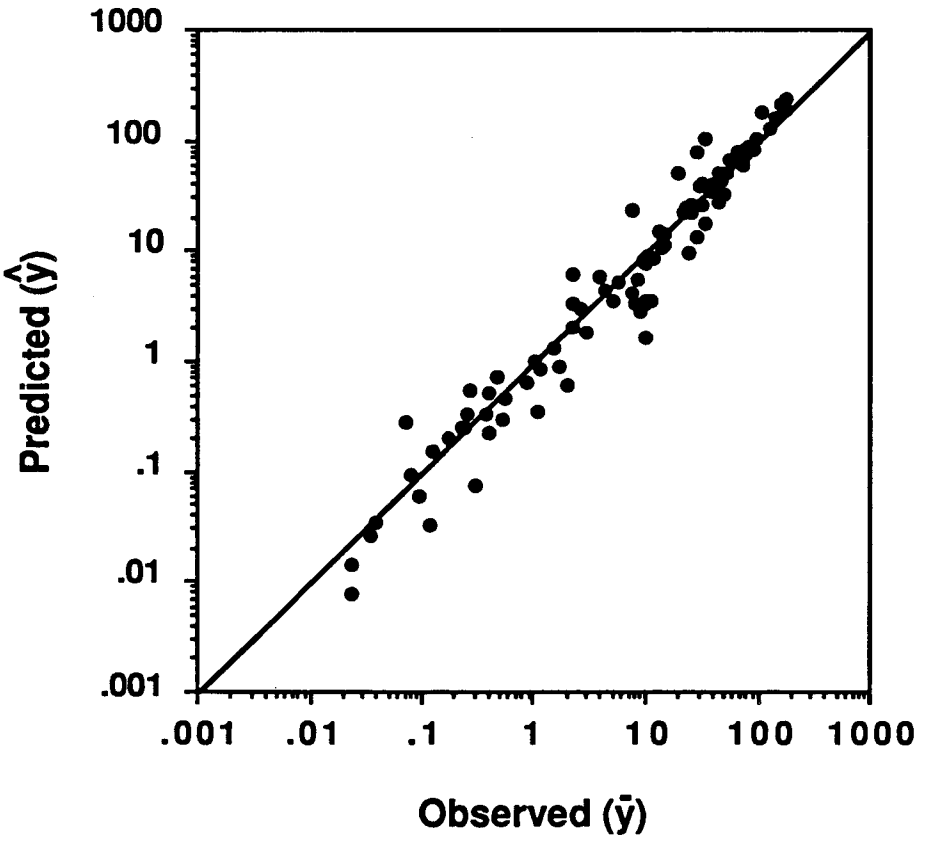

Figure 3: Best-likelihood predictions versus observed values for the experimental pharmacokinetic data of Rickert et al. (1979) and Sabourin et al (1987, 1988).

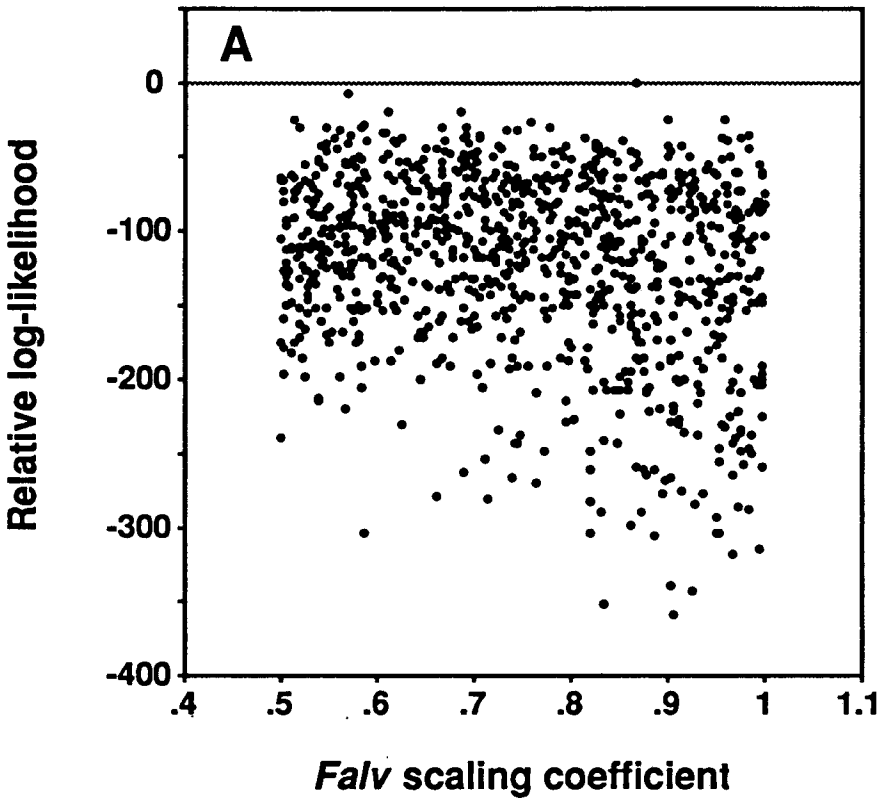

Figure 4A: Relationship between the value of the relative log-likelihood and.the value of the scaling coefficient of F_{alv}. Only the values for the last 1000 Monte Carlo simulations, out of 10,000, are presented.

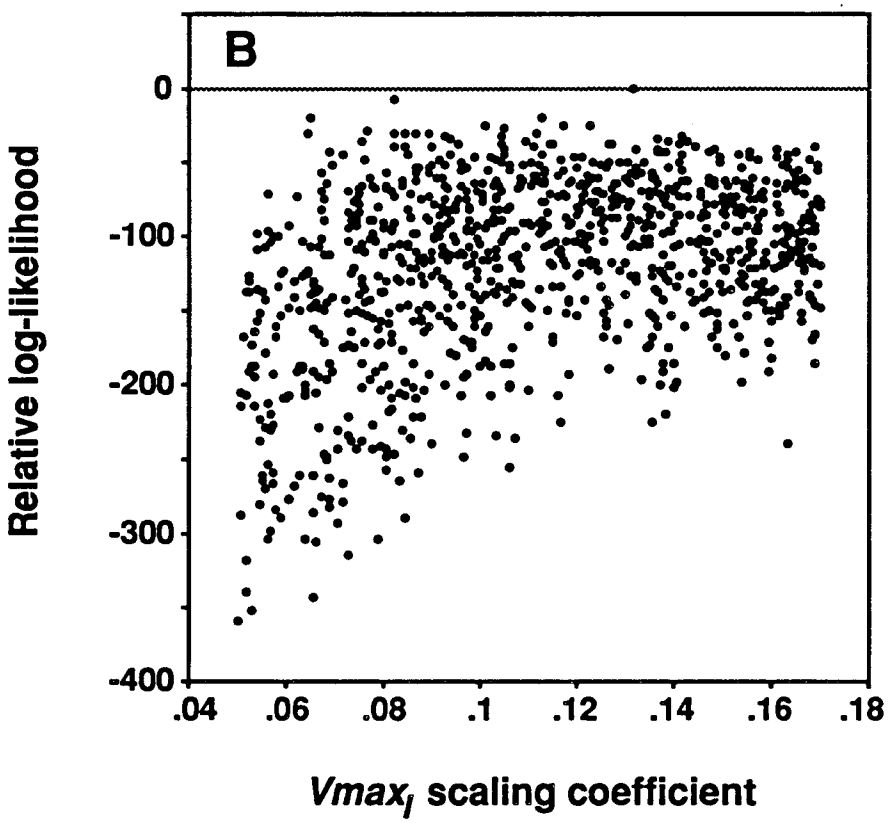

Figure 4B: Relationship between the value of the relative log-likelihood and.the value of the scaling coefficient of V_{maxl}. Only the values for the last 1000 Monte Carlo simulations, out of 10,000, are presented.

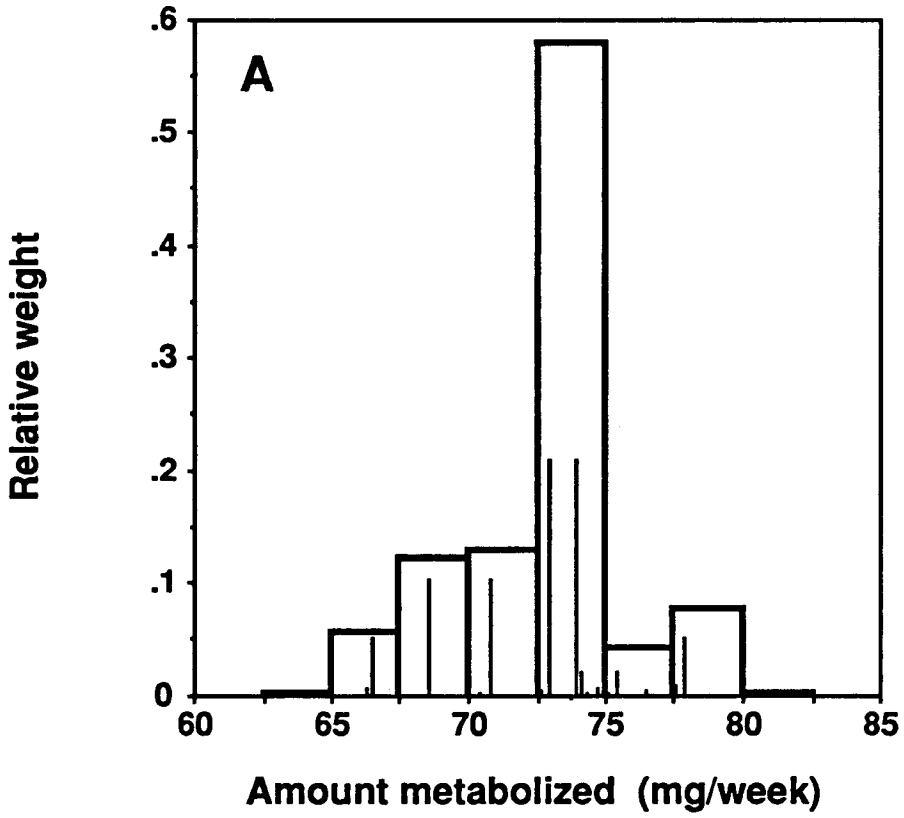

Figure 5A: Distribution of the predicted amounts, E_1, of benzene metabolized per week during the NTP cancer experiment, for Fischer-344 rats. The bars correspond to the sum of the individual weights (indicated by lines) within a given interval. The benzene gavage exposure simulated was 50 mg/kg.

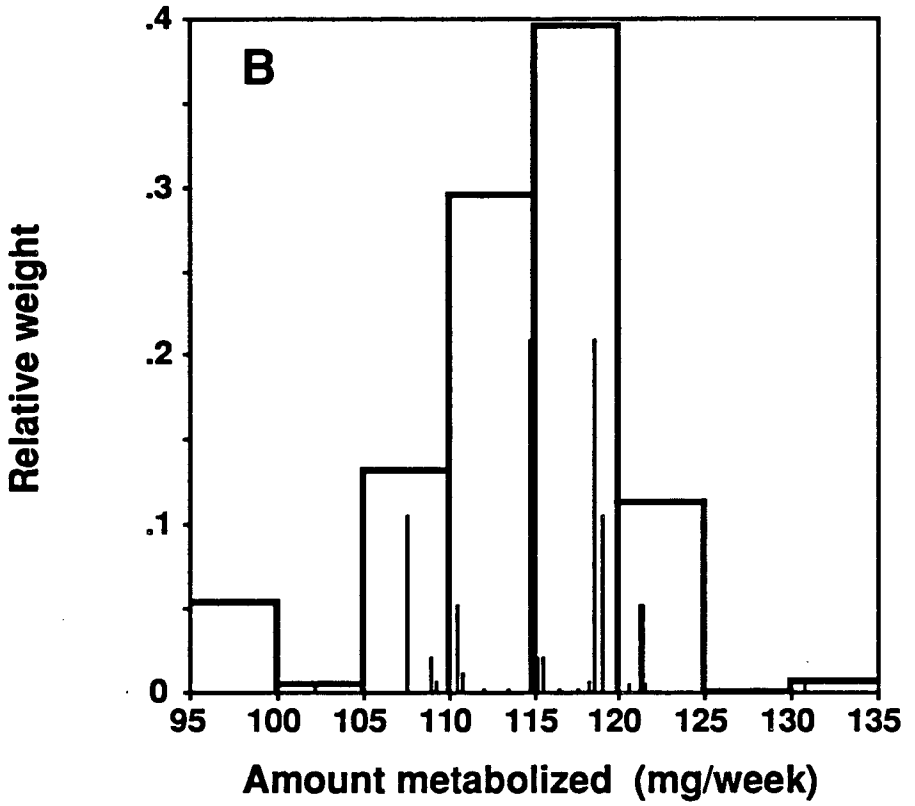

Figure 5B: Distribution of the predicted amounts, E_2, of benzene metabolized per week during the NTP cancer experiment, for Fischer-344 rats. The bars correspond to the sum of the individual weights (indicated by lines) within a given interval. The benzene gavage exposure simulated was 100 mg/kg

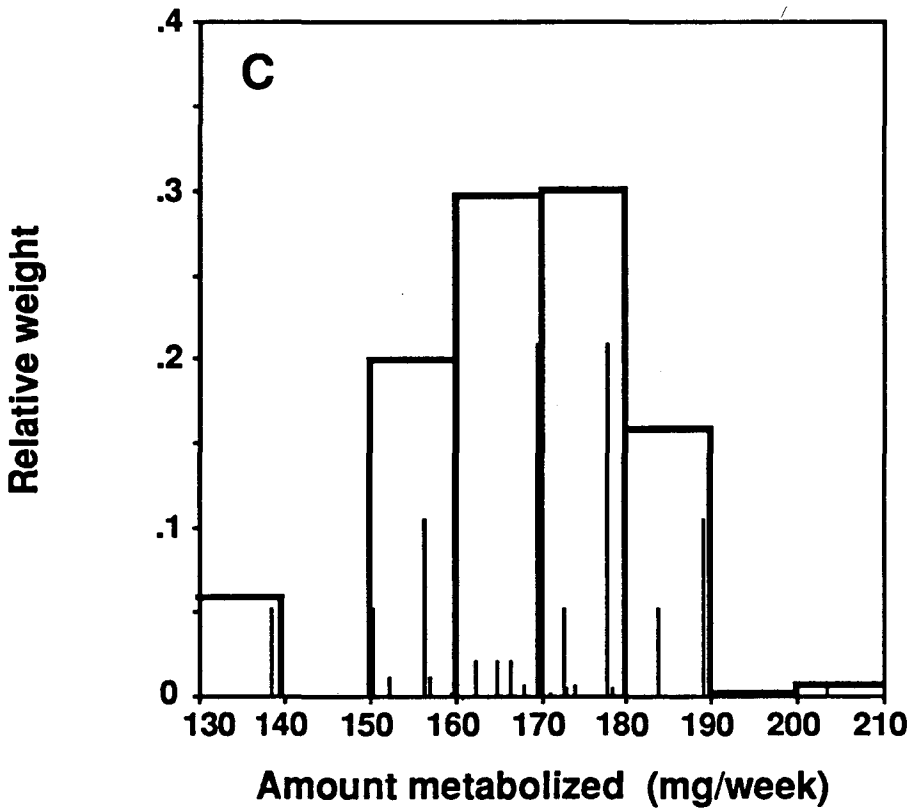

Figure 5C: Distribution of the predicted amounts, E_3, of benzene metabolized per week during the NTP cancer experiment, for Fischer-344 rats. The bars correspond to the sum of the individual weights (indicated by lines) within a given interval. The benzene gavage exposure simulated was 200 mg/kg.

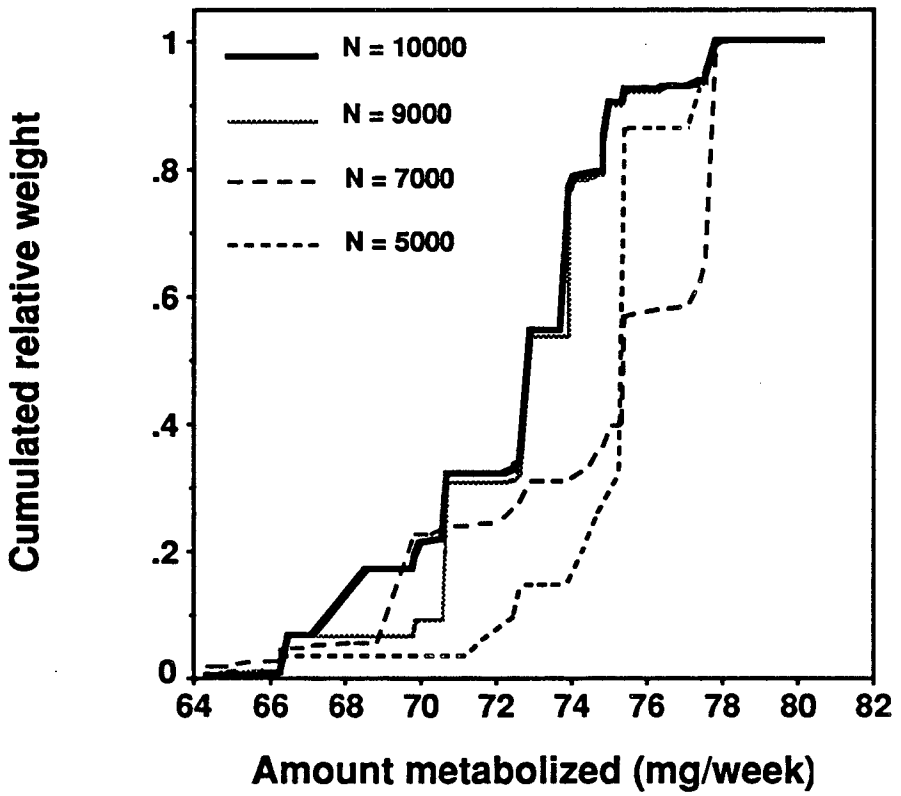

Figure 6: Distribution of the predicted amount of benzene metabolized per week, after $N = 5000$, etc., Monte Carlo runs of global optimization. The predicted amount is for the gavage dose of 50 mg/kg, during the NTP experiment.

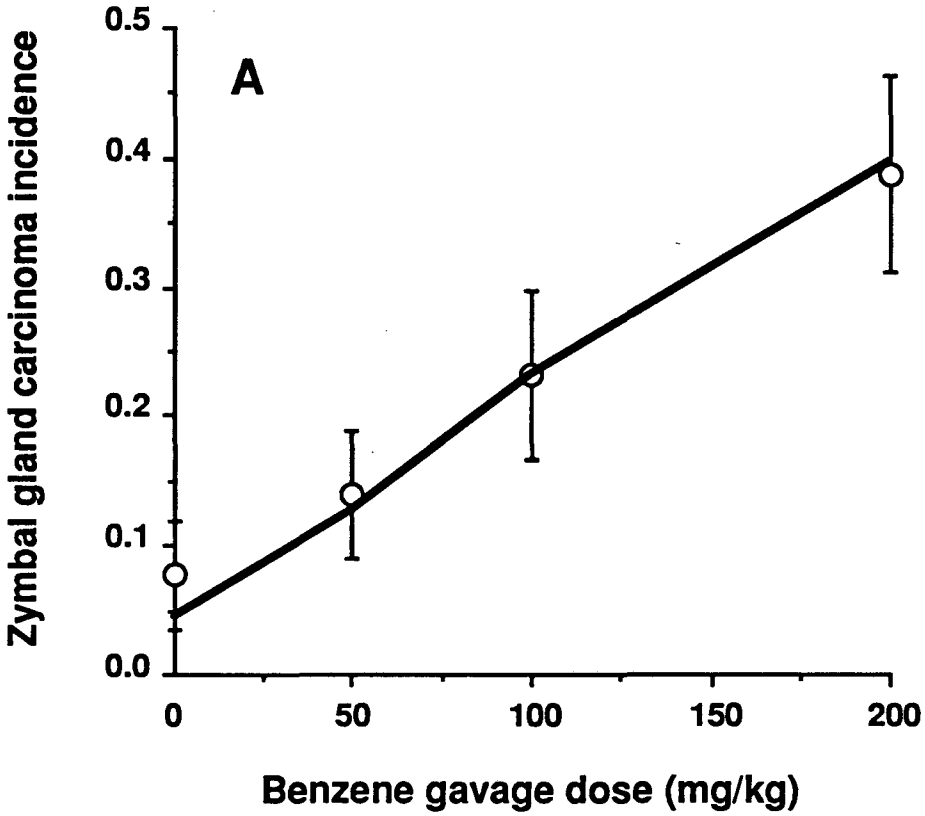

Figure 7A: Best-likelihood adjustment of the multistage model to the NTP cancer data for Zymbal gland carcinoma in male Fischer-344 rats. The circles mark the experimental incidence (with estimated standard deviation), the solid line corresponds to the model predictions. The cancer incidence after two years of exposure is plotted against the benzene gavage dose.

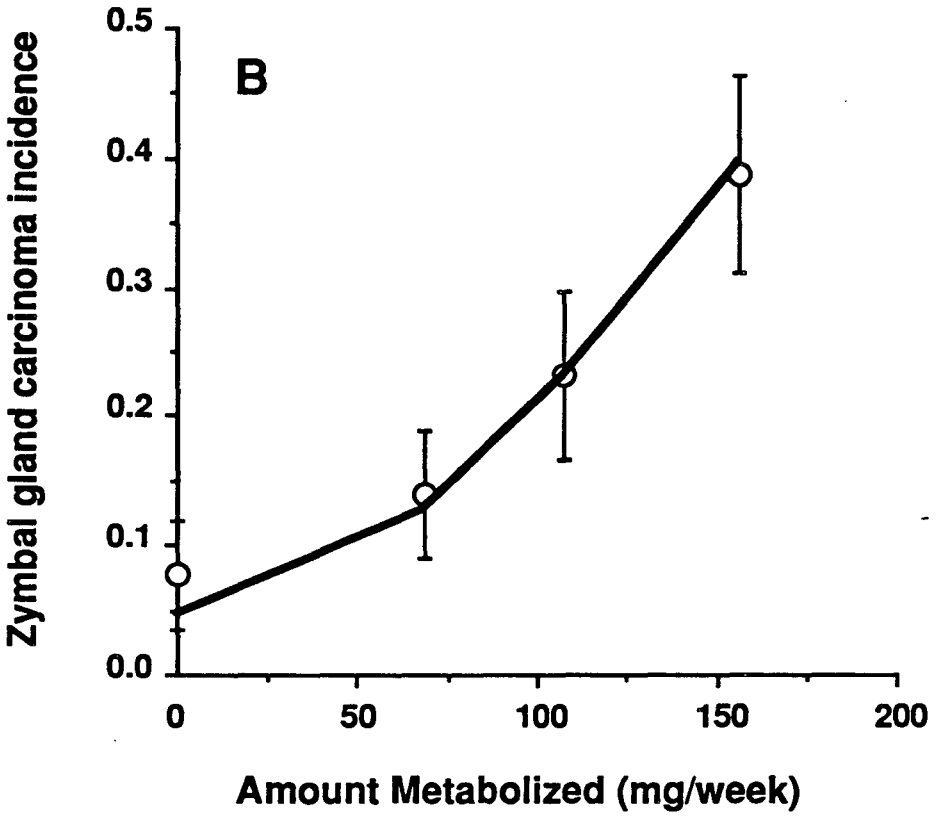

Figure 7B: Best-likelihood adjustment of the multistage model to the NTP cancer data for Zymbal gland carcinoma in male Fischer-344 rats. The circles mark the experimental incidence (with estimated standard deviation), the solid line corresponds to the model predictions. The cancer incidence after two years of exposure is plotted against the amount of benzene metabolized which was taken as a measure of effective exposure. The model parameter values are the same as in Figure 7A.

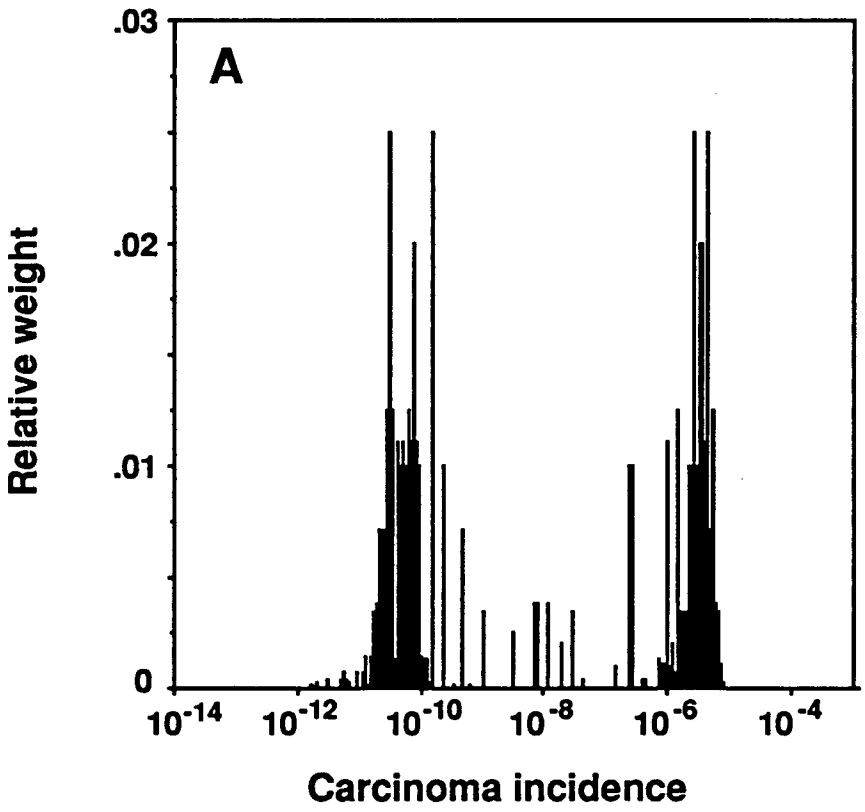

Figure 8A: Distribution of the predicted incidence of Zymbal gland carcinoma, for Fischer-344 rats, at low exposure. A benzene gavage dose of 1 μg/kg was simulated. The dosing schedule is the same as in the NTP experiment (Huff et al., 1989).

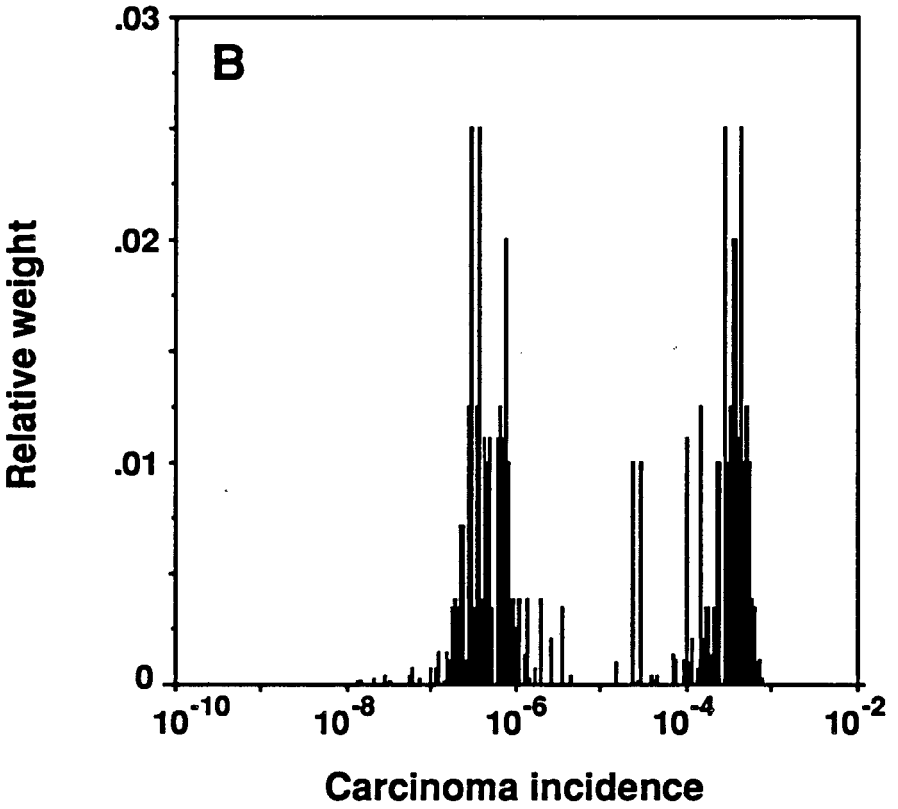

Figure 8B: Distribution of the predicted incidence of Zymbal gland carcinoma, for Fischer-344 rats, at low exposure. A benzene gavage dose of 100 μg/kg was simulated. The dosing schedule is the same as in the NTP experiment (Huff et al., 1989).

LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
INFORMATION RESOURCES DEPARTMENT
1 CYCLOTRON ROAD
BERKELEY, CALIFORNIA 94720