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A PHARMACOKINETIC/MECHANISM-BASED ANALYSIS
OF THE CARCINOGENIC RISK OF BUTADIENE

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16. Abstract (Limit: 200 words) Rats and mice were exposed to butadiene (106990) for 6 hours/day, 5 days/week, in an effort to establish a pharmacokinetic model for this compound. Direct projections of human risks from models incorporating different blood/air partition coefficients were compared. Predictions were also given of different rat and mouse models for the overall production of butadiene epoxides in the rat and mouse bioassays. After corrections for pharmacokinetic differences, there were wide differences in the apparent carcinogenic susceptibility between the two species. Use of results from the mouse study in making an assessment of human risk was considered. A comparison was made of the results of the current study and those obtained for similar pharmacokinetic model studies for perchloroethylene and ethylene-oxide. All three compounds, even at the lowest currently proposed (1 part/per million) standard levels, should be expected to produce appreciable cancer risks if the exposures are maintained over an entire working lifetime. The authors suggest that future research should include direct measurements of the relevant blood/air and tissue/air partition coefficients. Also, existing occupational cohorts of butadiene exposed workers should be followed to add to the mortality experience data.			
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SUMMARY

In recent years there has been considerable discussion of opportunities to improve the state of the art of carcinogenesis risk assessment by making use of more detailed information on the biological processes involved--including the dynamics of absorption, elimination, and metabolic processing. This report is the third in an integrated series of pharmacokinetic models for small molecular weight alkylating agents and their precursors (earlier reports covered perchloroethylene and ethylene oxide). Such models should allow (1) better assessment of the "biologically effective dose" of activated metabolites that is delivered to target tissues by different external exposure levels, routes, and time patterns of exposure, and (2) more appropriate translation of dose units among species.

Earlier risk assessments for butadiene [EPA (1985) and ENVIRON (1986)] have used as their measure of delivered butadiene "dose" in animals, the amount of butadiene retained at the end of six-hour exposures in experiments by Bond et al. (1986). As noted by Bond et al. (1986), however, their absorption information

"is based only on the amount retained at the end of the 6-hr exposure and does not take into account any butadiene or metabolites that were absorbed and exhaled during the exposure."

To the degree that butadiene was processed and active metabolites effectively lost from the animals before being measured at the end of the six-hour exposures, "delivered" dosages in the animal bioassays would be underestimated by the EPA/ENVIRON approach. On the other hand, to the degree that some of the butadiene retained at the end of the six-hour

exposure period is later exhaled unchanged (and therefore escapes activating metabolism) the "delivered" dose would be overestimated. Our pharmacokinetic models of butadiene are designed to account for these different possibilities, and estimate the effective "dose" as the total amount of butadiene that eventually undergoes at least the first step of metabolic activation to 3,4-epoxy-1-butene.*

The summary below first discusses the basic pharmacokinetic modeling approach and results. Then we discuss the implications of the modeling and other data for human risk. A final section outlines opportunities for further research.

Pharmacokinetic Modeling Approach

Development of appropriate pharmacokinetic models for butadiene required modifications of the methods used for earlier compounds:

- o The absence of any applicable measurements of blood/air and tissue/blood partition coefficients required us to estimate the values of these parameters from other data. The method of Leo (Lyman et al., 1982) was used to estimate an octanol/water partition coefficient from chemical structural information, and a water/air partition coefficient was derived from aqueous solubility information as a function of temperature. From these primary data, blood/air and tissue/blood partition coefficients were derived using relationships developed in earlier regression analyses [Hattis et al. (1986a) and Fiserova-Bergerova and Diaz, (1986)]. Alternative models were developed incorporating different values of the key blood/air partition coefficient and checked for

 *If additional data were to become available on subsequent steps in the metabolism and detoxification of butadiene metabolites, it would be desirable to extend the modeling and further refine the butadiene "dose" in terms of a concentration X time product of the various active metabolites, perhaps weighted according to their activities in reacting with DNA and/or producing relevant mutations.

compatibility with the blood butadiene concentration data of Bond et al. (1986). Models with values reasonably compatible with the Bond et al. observations were retained throughout the risk analysis to assess the sensitivity of the final results to the determinations that were made. The final results showed only modest differences in projected risk from different reasonable assumptions in this area (see Table S-1).

- o Low dose butadiene metabolism rates measured by Kreiling et al. (1986) in animals were found to be incompatible with an assumption that all butadiene metabolism takes place in the liver. Additionally, other data (Schmidt and Loeser, 1985) directly demonstrated appreciable rates of metabolism in at least one extrahepatic organ (the lung). In the light of this, the pharmacokinetic models were structured to allow butadiene metabolism throughout the "vessel-rich group" (kidneys, viscera, brain) in addition to the liver.
- o Maximal metabolic rates for humans were scaled from data of Kreiling et al. (1986) for rats and mice. The rat and mouse data were found to be in good accord with an assumption that butadiene metabolism scales with general metabolic rates--(body weight)^{.75}--as expected from the theory of Boxenbaum (1982). Human michaelis constants were assumed to be similar to those in rats and mice. Because the human models were appreciably flow-limited, however, we found that the final risk analysis results were not very sensitive to the detailed assumptions made in this area (see Table 5.2, p. 53).

Pharmacokinetic Modeling Results

Tables S-2 and S-3 show the metabolized doses projected by our rat and mouse models for the 6 hour/day 5 day/week exposure regimens used in the carcinogenesis bioassays for butadiene. The 6-hr retained doses used EPA (1985) and ENVIRON (1986) are shown at the bottoms of the tables for comparison. It can be seen that our best estimates of rodent metabolized doses are 2 - 4.5 times the absorbed doses used in the earlier risk assessments. The larger metabolized dosage we estimate to have been available in the animal experiments tends to reduce the apparent potency of butadiene in causing tumors, relative to the earlier risk assessments.

Table S-1
Comparison of Direct Projections of Human Risks from Models
Incorporating Different Blood/Air Partition Coefficients

Exposure for 10 Years, 8 hours/day, 5 days/week

Probability per Individual of Developing at Least One Additional Cancer

Animal Data Set	--MLE Projections*--		-UCL Projections**-	
	1 ppm	10 ppm	1 ppm	10 ppm
<u>Blood/Air Partition Coefficient = .2552 (Alternative Model):</u>				
Male Rats	5.61E-9	5.61E-7	6.22E-5	6.22E-4
Female Rats	8.49E-5	8.50E-4	2.41E-4	.00241
Male Mice	.00252	.0249	<u>.00683</u>	<u>.0662***</u>
Female Mice	.00112	.0111	.00502	.0491
Geometric Mean	<u>3.40E-5</u>	<u>6.02E-4</u>		

Blood/Air Partition Coefficient = .35 (Best-Estimate Model):

Male Rats	3.55E-9	3.55E-7	3.46E-5	3.46E-4
Female Rats	1.08E-4	1.08E-3	2.66E-4	.00265
Male Mice	.00270	.0266	<u>.00731</u>	<u>.0707***</u>
Female Mice	.00104	.0103	.00546	.0533
Geometric Mean	<u>3.22E-5</u>	<u>5.69E-4</u>		

*These are derived from the MLE coefficients on the right side of Tables 5.29 and 5.30, assuming equal risk for humans and rodents per umole butadiene metabolized/(kg body weight)^{.75}.

**Calculations are similar to those for the MLE's, except that in this case we assume equal risks for humans and rodents per umole butadiene metabolized/(kg body weight)^{2/3}.

***The underlining signifies the largest of the four sets of UCL values.

Table S-2
Predictions of Different Rat Models for the Overall Production of
Butadiene Epoxides in the Rat Bioassay

(Alveolar ventilation is .15 l/min and $V_{\max}$ is 1.47×10^{-6} for all runs)

Based on 400 g rats

Blood/Air Partition Coefficient	K_m	exposure (ppm) 6 hr/day	umoles butadiene 5 exposure days	metabolized per day average of 7 days (including weekend)
"Alternative" Model:				
.2552	1×10^{-5}	1000	274.7	196.2
		8000	347.1	247.9
"Best Estimate" Model				
.35	5×10^{-6}	1000	359.6	256.8
		8000	607.7	434.1
EPA (1985) and ENVIRON (1986) Assumptions for Comparison*:				
		1000	78	55.7
		8000	274	195.7

*The EPA projections based on the Bond et al. (1986) net absorption data were 10.5 mg/kg (195 $\mu\text{mol/kg}$) at the 1000 ppm exposure level and 37.1 mg/kg (685 $\mu\text{mol/kg}$) at the 8000 ppm exposure level. The numbers shown in the table were determined by multiplying the $\mu\text{mol/kg}$ values by the .4 kg weight of the rats in our models.

Table S-3

Predictions of Different Mouse Models for the Overall Production of Butadiene Epoxides in the Mouse Bioassay

(Alveolar ventilation is .0233 l/min and $V_{\max}$ is 1.87×10^{-7} for all runs)

Based on 28 g mice

Blood/Air Partition Coefficient	K_m	exposure (ppm) 6 hr/day	umoles butadiene metabolized per day 5 exposure days	average of 7 days (including weekend)
"Alternative" Model:				
.2552	2×10^{-5}	625	23.79	16.99
		1250	41.42	29.58
"Best Estimate" Model				
.35	5×10^{-6}	625	28.08	20.06
		1250	45.76	32.68
EPA (1985) and ENVIRON (1986) Assumptions for Comparison*:				
		625	13.3	9.48
		1250	20.1	14.38

 *The EPA projections based on the Bond et al. (1986) net absorption data were 25.7 mg/kg (474 umol/kg) at the 625 ppm exposure level and 38.9 mg/kg (719 umol/kg) at the 1250 ppm exposure level. The numbers shown in the table were determined by multiplying the umole/kg values by the .028 kg weight of the rats in our models.

A second factor leading to discrepancies in the same direction appeared when we ran the human models. Both EPA (1985) and ENVIRON (1986) assumed that in the neighborhood of 50% of total inhaled butadiene would be absorbed by humans at low doses. Our models, for relatively light occupational effort (11.4 liters per minute alveolar ventilation, corresponding to about 16.25 liters per minute of total ventilation), predict that net absorption will represent only about 11-15% of the butadiene reaching the alveoli over an eight hour period, and only 8-10.5% of total inhaled butadiene. This causes our estimates of human delivered dose (and ultimately, risk) to be further reduced relative to the earlier risk assessments.

Conclusions for Human Risk

The case of butadiene also posed substantial challenges for the development of final risk estimates from the available data. It can be seen in Table S-1 above, that even after correction for pharmacokinetic differences between mice and rats as far as we have gone (through the first step of metabolism), very wide differences in apparent carcinogenic susceptibility remain between these two species.

Representatives of the affected industry association (the International Institute of Synthetic Rubber Producers--IISRP) have argued strongly that at least the mouse results should be disregarded in making a final assessment of human risk. Based on a comparison of the EPA (1985) risk projections with the human mortality study of Matanoski et al. (1982) [later published as Matanosky and Schwartz, (1987)], they conclude that

"man is approximately 100 to 1,000 times less sensitive than the mouse" (Tasher and Kaufman, 1986a).

The calculations that led the IISRP to this conclusion are unfortunately not fully detailed in the documents we have obtained from the OSHA hearing record. However what appears to have been done is to compare the total expected tumor risk from the EPA risk assessment, based on tumors at several sites (a minority of which were lymphoid), with the number of deaths from a narrow category of human tumors--non-leukemia lymphoid cancers. Two other considerations further weaken the comparison:

- o They did not correct for the "healthy worker effect".* An appropriate comparison would require that a control group be constructed of workers who were employed at similar times as the study cohort in industries that imposed similar selection criteria.
- o It is not clear that they made appropriate corrections for length of followup. This problem is better documented in the analogous calculations presented by ENVIRON (1986) for rats. To account for the average 18 years of followup in the Matanoski et al. (1982) data, ENVIRON made the assumption that 18/50 of the total tumor deaths should already have occurred by the end of the followup period. This implies a linear appearance of tumors with time of followup. It is well known, however (e.g. the case of asbestos) that often there is a considerable delay--a decade or more--between the initiation of exposure and the appearance of the first occupationally-induced tumors.

 *The 1995 deaths at the time of the study cutoff for the Matanoski et al. (1982) report represent only 81% of the deaths that would be expected in a random sample of U.S. males of comparable ages. Similarly the 398 total cancer deaths represent 84% of the cancer deaths that would be expected among the general-population comparison group, and the 40 observed non-leukemia lymphoid tumor deaths represent 85% of the deaths that would be expected within that subcategory.

The explanations for this are usually considered to be (1) the general population contains people who are already ill and therefore unlikely to be employed in factory jobs requiring the passage of a pre-employment

(footnote continued on the following page)

In our view, the available human data are incomplete enough in followup, have great enough uncertainties in exposure, and pose such serious difficulties for appropriately controlling for the healthy worker effect in doing a necessarily multi-site comparison of the animal risk projections with the human experience, that they hold little promise for appreciably narrowing the uncertainties in human risk projections. We therefore believe that it is better for the present to base human risk projections exclusively on the animal results.

Our approach for using the basic multistage cancer dose response model* to analyze the animal tumor data differs from that of EPA (1985) and ENVIRON (1986) in two other important respects:

(footnote continued from the previous page)

physical, and some continuing exertion, and (2) the general population contains people who are down and out, incapacitated by alcoholism and other problems, and those who are institutionalized and under treatment for mental and physical conditions, all of whom may have appreciable carcinogenic exposures. The existence of the healthy worker effect, and the likelihood that butadiene-induced tumors are dispersed among a number of tumor sites means that quite a large number of butadiene-induced tumors could be concealed and undetectable amid the general deficit of tumors in working, as compared to general, population groups. In the case of the Matanoski et al. (1982) study, to bring the total cancer deaths just up to the expectation for the general population in this study would require about 76 deaths--or about 3.8% of the deaths so far observed. Additional increases would be required, of course, to produce a statistically significant excess of total tumor deaths over expectation.

*Based on Howe and Crump's (1983) "Global82" program. This calculates maximum likelihood estimates (MLE) for the "q" coefficients in the equation:

$$P(d) = 1 - e^{-(q_0 + q_1d + q_2d^2 + \dots q_kd^k)}$$

Where $P(d)$ is the expected tumor incidence at a particular dose (d). The program also calculates sets of "q" coefficients for predicting upper 95% confidence limits on induced tumor incidence (UCL), based solely on statistical sampling-error uncertainties in the experimental data. All the "q" coefficients are constrained to be positive or zero. We use this model because we believe it has by far the strongest basis in the biology of the cancer process.

- o Both EPA and ENVIRON chose to add up the tumor-bearing animals at all sites with statistically significant tumor increases before entering the data into the multi-stage model program. We believe this is odd in the light of the biological context of the multistage model--which represents a series of irreversible transitions (mutations, gene derepressions) within a single cell line. It is difficult to understand how mutations in one organ/tissue could be part of the same sequence leading to cancer in another organ/tissue. Therefore we believe it is more appropriate to treat the dose response relationships of specific organs and tissues separately for purposes of analysis, and then add up the overall expected risks from all sites at the end. The results in Table S-1 above were produced using this approach.
- o This site-by-site analysis, when combined with our delivered dose estimates in animals, led to nonlinear maximum likelihood estimates (finite q_2 's) for a number of tumor sites, including all sites analysed for the male rats. The direct MLE projections for male rats in Table S-1 show a pure (dose)² dependence of risk even at low doses. If we were to use this relationship unchanged as a component of our final "best estimate" of human risk from butadiene, this would imply an assumption that butadiene must act alone in producing cancer by mechanisms that are completely distinct from the mechanisms that lead to other cancers in the human population. Such an assumption does not represent our best judgment. However, arriving at a better judgment required an innovative analysis that will undoubtedly be controversial. As documented in Table 5.33 (p. 109) nearly 2/3 of tumor deaths from well-identified sites were accounted for by just four sites that each account for over 1% of total deaths from all causes (in other words, they each produce a lifetime mortality risk of approximately .01 or more). Similarly, 96% of all deaths from identified tumors are produced at sites that account for lifetime cancer risks of over .001. We therefore asked what effects there would be on our estimates of incremental butadiene-induced cancer deaths if the butadiene-induced transitions predicted from the male rat data were part of a two-stage cancer process that produced a "background" of lifetime cancer deaths in the range of 10^{-3} to 10^{-2} . Under the 10^{-2} assumption the geometric mean MLE risks for 10 year exposures in Table S-1 become:

1 ppm	10 ppm
2.45E-4	.00244

Assuming a cooperating lifetime background cancer risk of 10^{-3} leads to the following comparable geometric mean MLE estimates:

1 ppm	10 ppm
1.84E-4	.00183

These do not differ a great deal from each other, but they are several times the uncorrected estimates in Table S-1. We chose to adopt the latter set of estimates (10^{-3} lifetime cooperating background) as our "best estimates" of human cancer risk for butadiene.

Table S-4 gives our "upper confidence limit" projections of human risk from the different animal data sets, in comparison with those of EPA (1985) and ENVIRON (1986) for 45 year exposures to 1 ppm. [EPA (1985), as is its custom, does not choose to make projections of human risk based on its maximum likelihood fits of the animal data.] It can be seen that our upper confidence limit projections are about 3-7 times lower than EPA's. This very likely results from the difference between our estimates of metabolized dose in animals and people, and those used by EPA (1985).

ENVIRON (1986) used animal and human absorption assumptions that were comparable to those of EPA (1985). And in the case of male rats, we see a comparably higher estimate of upper-confidence-limit risk than determined from our analysis. For the female rat data, this tendency for ENVIRON (1986) to produce a higher estimate than ours was offset by their exclusion of the data on mammary carcinomas presented by EPA (1985). We chose to follow EPA's analysis in this respect. In the case of male mice, as noted in the table, the differences may result from the use of mg/body weight rather than mg/surface area interspecies projection and the Hartly-Sielken procedure for adjustment for less-than-lifetime exposure and observation of the mice.

For our final "plausible upper limit" estimates, we use the highest of the eight sets of upper confidence limit risks projected in Table S-1 (and, in more detail, in Table 5.28):

Table S-4
 Comparison of 95% Upper Confidence Limit Projections From Our
 Pharmacokinetic-Based Analysis with Those Derived from EPA (1985)
 and ENVIRON (1986)--45 Year 8 Hour/Day Exposure to 1 ppm

Data Set	EPA (1985)*	ENVIRON (1986)**	This Study (Best Estimate Model)
Male Rats	6.43E-4	7.98E-4	1.55E-4
Female Rats	.00853	9.29E-4	.00119
Male Mice	.131	.00644***	.0325
Female Mice	.0665	-	.0243

 *Derived from EPA's upper 95% q_1 coefficients after correcting for 45-year, 8 hours/day, 5 days/week exposures.

**ENVIRON's projections have been multiplied by (45/40) and (52/50) to place them on a comparable basis with our assumed 45 year 52 week exposures.

***These calculations excluded malignant lymphomas. Some of the rest of the difference in this line may be accounted for by (1) mg/kg interspecies projection, in contrast to $\text{mg/kg}^{2/3}$ used by ourselves and EPA (1985) and (2) the use by ENVIRON (1986) of the Hartley-Sielken procedure for adjusting for less than lifetime exposure and observation.

-10 Year Exposures-		-45 Year Exposures-	
1 ppm	10 ppm	1 ppm	10 ppm
.00731	.0707	.0325	.282

The approximately 50-fold difference between our "best" (least unlikely) and "plausible upper limit" estimates of human risk serve to emphasize the considerable uncertainty in these risk projections. This uncertainty largely results from the differences in sensitivity observed between rats and mice, and our inability to say from first principles which species is likely to provide a better predictor for estimating human risk.

Finally, Table S-5 places our current results in perspective by comparing them with the results of our earlier pharmacokinetic-based risk projections for perchloroethylene (Hattis et al., 1986a) and ethylene oxide (Hattis, 1987). It can be seen that our rather uncertain "best estimate" of human risks from working lifetime exposure to 1 ppm butadiene is comparable to that for similar exposure to perchloroethylene, and about eight times less than that for ethylene oxide. The "plausible upper limit" of estimated risk for butadiene is a little higher than that for ethylene oxide. The wider range between the "best estimate" and "plausible upper limit" risks for butadiene results from the great difference between the responses of rats and mice, even after correction for pharmacokinetic differences up through the first step in butadiene metabolism. No such major interspecies differences were observed for ethylene oxide and perchloroethylene.

All three compounds, even at the lowest currently proposed (1 ppm) standard levels, should be expected to produce appreciable cancer risks if the exposures are maintained over an entire working lifetime.

Table S-5
"Best Estimates" and "Plausible Upper Limits" for Overall Cancer
Risk Based on Pharmacokinetic-Based Analyses

(Exposure to 1 ppm for 8 Hours/Day, 5 Days/Week for 45 Years)

Probability Per Individual of Developing at Least One Additional Cancer

Chemical	"Best Estimate"	"Plausible Upper Limit"
Ethylene Oxide	.0065	.019
Butadiene	7.9E-4	.032
Perchloroethylene	6.7E-4	.013

Opportunities For Further Research

Because there are a number of subsequent steps in butadiene metabolism and the detoxification of activated metabolites, there is reason for hope that the apparent differences between mice and rats in susceptibility to butadiene carcinogenesis could be narrowed by further analysis, using more extensive pharmacokinetic data.

Building a full pharmacokinetic model for butadiene in humans would be facilitated if there were direct measurements of the relevant blood/air and tissue/air partition coefficients. As we found in the current study, however, the modeling does not appear to be very sensitive to reasonable differences in estimates of this set of parameters.

Finally, the existing occupational cohorts of butadiene-exposed workers should continue to be followed for their mortality experience. For this information to have direct bearing on risk analysis, however, much better data is generally needed on past worker exposures. Additionally, controls for the "healthy worker effect" must be done by constructing comparison groups from comparable employed populations without butadiene exposure, rather than either the general U.S. population

1. INTRODUCTION

This is the third in a series of carcinogenic risk assessments for small molecular weight alkylating agents based on pharmacokinetic modeling of the likely internal dosage of DNA-reactive substances. Earlier reports (Hattis et al., 1986a; Hattis, 1987) studied perchlorethylene and ethylene oxide. It is hoped that models of this kind can improve upon standard risk assessment approaches by (1) better representing high-dose nonlinearities produced by enzyme saturation and other processes, and (2) facilitating better translation of effective dosages between species and between different patterns of exposure. By applying pharmacokinetic principles and data to 1,3-butadiene, we will test the potential helpfulness of the approach both for risk assessment and for identifying additional types of experimental and epidemiological information that can reduce uncertainties in risk assessment.

1.1 Choice of 1,3 Butadiene for Analysis

Approximately 2.3 billion pounds of 1,3-butadiene (referred to hereafter simply as butadiene) was produced in the United States in 1985 (U.S. ITC, 1986). The Office of Toxic Substances of the Environmental Protection Agency has estimated that up to 10,000 workers are exposed to butadiene (primarily through inhalation) mostly in plants manufacturing butadiene or using it to produce polymers such as synthetic rubber (EPA, 1985).

Like other small molecular weight olefins, the double bonds in butadiene can be oxidized by P450 enzymes to epoxides (Figure 1.1) that can react with DNA.* These epoxides are thought to be the activated intermediates that are directly responsible for the carcinogenic activity of the parent olefins. Pharmacokinetic modeling to determine the dosage of butadiene epoxide derivatives that are likely to be produced in different species holds the promise to provide another data point for the activity of carcinogenic epoxides for comparison with our earlier work on ethylene oxide and perchloroethylene.

Because butadiene has two double bonds, however, its activation is somewhat more complicated than that of the other compounds in Figure 1.1. Figure 1.2 shows the metabolic pathways that have been described by Malvoisin and Roberfroid (1982). The state of available information is not yet sufficient to assess the likely relative contributions of the different epoxide derivatives to the overall carcinogenic activity of butadiene. Both 3,4-epoxy-1-butene and 1,2:3,4-diepoxibutane are mutagenic in a number of systems (EPA, 1985) and both have shown carcinogenic activity in early skin painting experiments (Van Duuren et al., 1963). For purposes of this report, we will simply attempt to determine the overall amount of butadiene converted to the initial epoxide--3,4-epoxy-1-butene.

This at least will be a more proximate indicator of "delivered dose" than available raw information on the absorption of butadiene. The risk assessment by the Environmental Protection Agency (EPA, 1985) assessed dosage in rodents as the amount of butadiene retained in the animals at the

*For example, Citti et al. (1984) have measured the reactivity with DNA of the initial epoxide formed from butadiene--3,4-epoxy-1-butene.

Figure 1.1
Metabolism of Some Small-Molecular-Weight Olefins

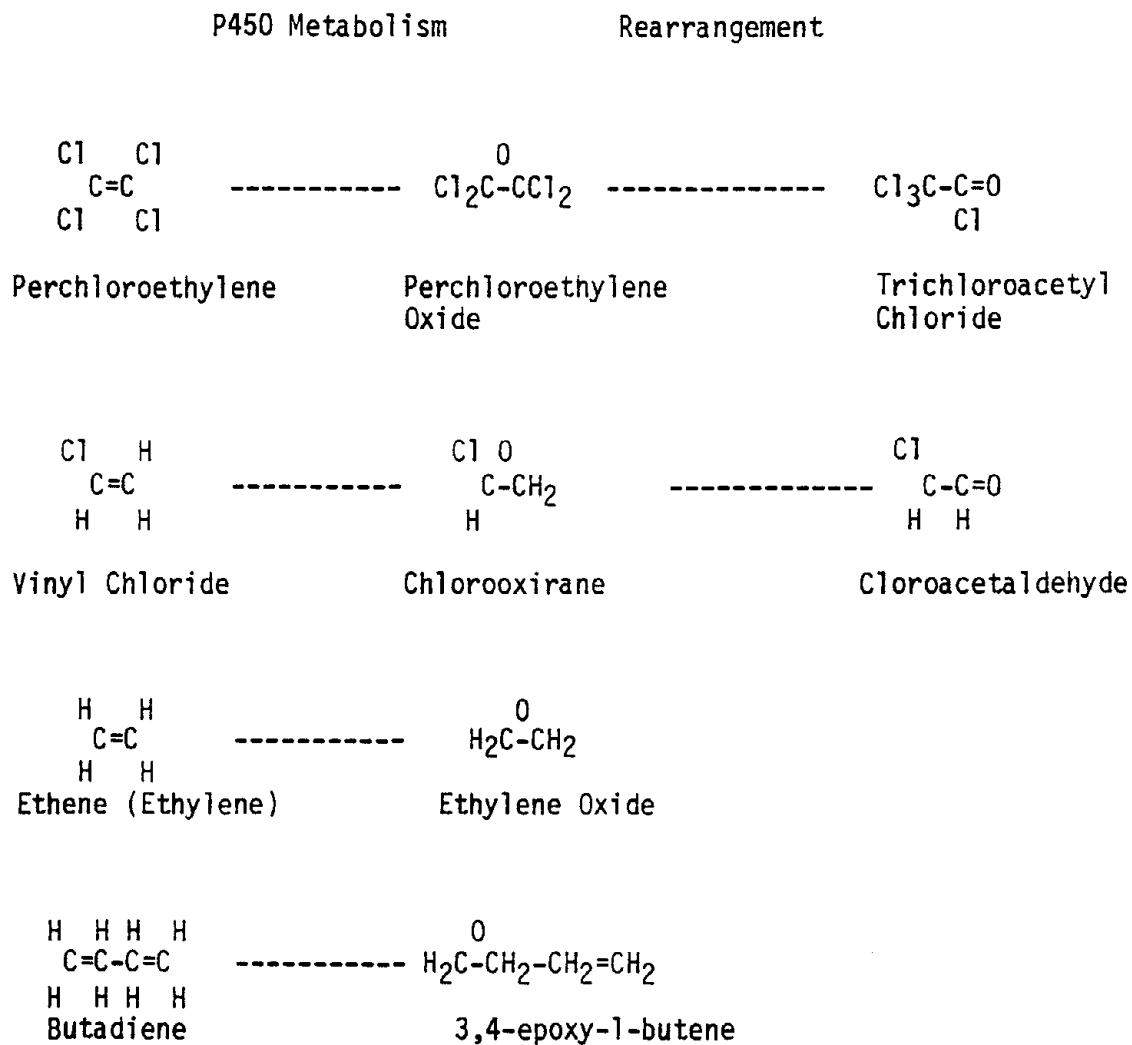
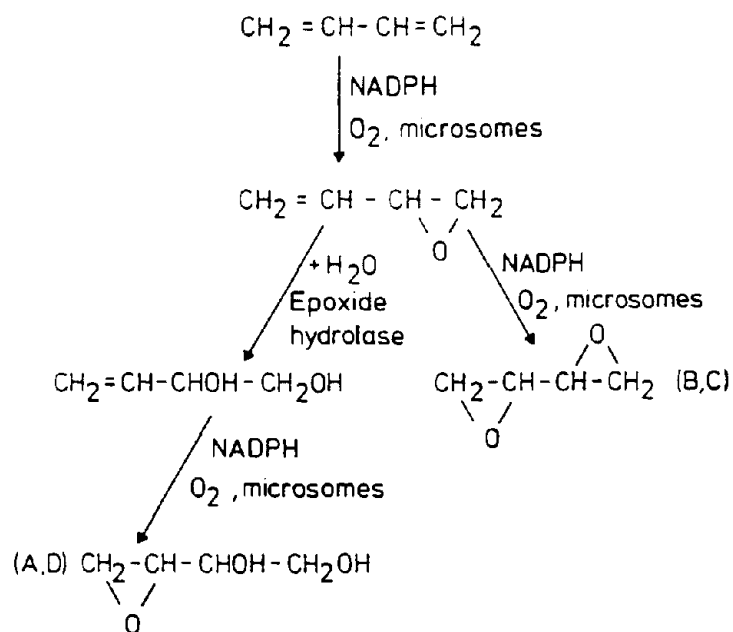


Figure 1.2
Metabolic Pathways for 1,3-Butadiene



Source: Malvoisin and Roberfroid, 1982

end of six-hour exposures in experiments later published by Bond et al. (1986). As noted by Bond et al. (1986), however, their absorption information

"is based only on the amount retained at the end of the 6-hr exposure and does not take into account any butadiene or metabolites that were absorbed and exhaled during the exposure."

To the degree that butadiene was processed and active metabolites effectively lost from the animals before being measured at the end of the six-hour exposures, "delivered" dosages in the animal bioassays would be underestimated by the EPA approach. On the other hand, to the degree that some of the butadiene retained at the end of the six-hour exposure period is later exhaled unchanged (and therefore escapes activating metabolism) the "delivered" dose would be overestimated.

EPA (1985) recognized the desirability of constructing a physiologically-based pharmacokinetic model to better estimate delivered doses. However, they did not proceed to develop such models because of the lack of published blood/air and tissue/blood partition coefficients for butadiene. In this report, we fill this gap by first estimating the octanol/water partition coefficient from basic structural information using the method of Leo (Lyman et al., 1982). Together with a water/air partition coefficient (inferred from basic solubility information available for butadiene) this allows us to make at least a crude set of estimates of blood/air and tissue/blood partition coefficients based on previously-established regression analyses by Fiserova-Bergerova and Diaz (1986) and ourselves (Hattis et al., 1986).

Our final models also incorporate key information on the rates of metabolic processing of butadiene in rats and mice, as determined by

Kreiling et al. (1986). As it happens, these rates are so large that they are incompatible with an allocation of all metabolic activity to the liver*, as was done in our perchloroethylene model. Additionally there are data that directly indicate that at least the lung metabolizes butadiene at rates that are of similar magnitude as those of the liver per unit of tissue (Table 1.1). In the light of this, in our models we have merged the liver and vessel-rich-group to allow the greater blood flow to the tissues of the vessel-rich-group to be exposed to metabolizing enzymes.

1.2 Structure of the Report

This report is divided into five sections. Section 2 describes the basic structure of our pharmacokinetic models for butadiene. Section 3 discusses the basic physical and physiological data for rodents. This includes a discussion of the rodent tissue volumes, flows, and the partition coefficients employed in the models. Section 4 details the fit

*Under low-dose conditions, Kreiling et al. (1986) find that rats metabolize the total amount of butadiene in 4.5 liters of air per kilogram of body weight per hour. With an estimated blood/air partition coefficient of .25 to .5, the rats would have to completely process the butadiene in 9 to 18 liters per hour per kg body weight in full equilibrium with air in order to achieve the observed air clearance rate. However, with a total cardiac output of about 22.6 liters per hour per kg body weight, and 25% of cardiac output allocated to the liver, less than six liters of blood can get to the liver per hour for processing. Therefore there must be at least a modest amount of butadiene metabolism outside of the liver in rats.

For mice the calculation is similar. The low dose metabolism rate is 7.3 liters of air per kilogram of body weight per hour, corresponding to an estimated 14.6 to 29.2 liters of fully saturated arterial blood. With a total cardiac output of 45.6 liters/hour-kg, 28% allocated to liver, liver blood flow is somewhat less than 13 liters per hour.

Table 1.1
Butadiene Oxidizing Activity of Liver and Lung Tissue Homogenates

Enzyme activity in nanomoles of butadiene processed per gram of tissue in 45 minutes (Observed under saturating conditions, *in vitro*)

Species, Sex	Liver Activity	Lung Activity
Mice, male	125 +/- 49*	251 +/- 28
Mice, female	217 +/- 33	198 +/- 14
Rats, male	82 +/- 45	41 +/- 11
Rats, female	67 +/- 11	27 +/- 12
Monkey, male	32 +/- 3	n.d.**
Monkey, female	34 +/- 6	n.d.
Human	79 (one determination)	n.d.

*Standard deviation, based on five animals for rat and mouse liver determinations, but based on 2-4 replicates of studies on 5 pooled organs in the case of the rat and mouse lung experiments. For monkeys, the results are based on 2-3 animals. The human data reflects only one determination (tissue was obtained in the course of unspecified surgery).

**Not detected, sensitivity not stated.

Source: Schmidt and Loeser, 1985

of our rodent models with inhalation experiments investigating the uptake and distribution of butadiene in rodents. Finally Section 5 draws the inferences from the models developed in Section 4 for metabolite dosage in chronic bioassays. Based on this measure of "delivered dose" we then project expected human risks for occupational exposure.

2. BASIC STRUCTURE OF THE PHARMACOKINETIC MODELS

With the exception of the merger of the liver and vessel-rich groups discussed above, the pharmacokinetic models developed here for butadiene are very similar to the models developed in our earlier report on perchlorethylene (Hattis et al., 1986a). Figure 2.1 shows the general schematic diagram for the models, and Table 2.1 gives the equations for the best-estimate model for rats.

To briefly review, our models are roughly patterned after those described by Fiserova-Bergerova (1983). Essentially the body is divided into a number of compartments representing organs with similar ratios of blood flow to tissue volume. Each compartment is represented as a well-mixed pool. In our models tissues are divided into the following three groups:

- o Liver and Vessel-Rich-Group (LVRG) containing the liver, brain, kidney, coronary, adrenals, and thyroid tissues as well as additional small viscera,
- o Muscle Group (MG) containing the lean body tissue: muscle, skin, and tongue
- o Fat Group (FG) containing the perirenal and subcutaneous fat and the marrow.

The concentration of butadiene in blood leaving each compartment is assumed to have reached equilibrium with the concentration of butadiene in the compartment. This leads to the following equation for the increase or decrease in the moles of butadiene stored in each compartment:

Figure 2.1
Flow Diagram for the Pharmacokinetic Models

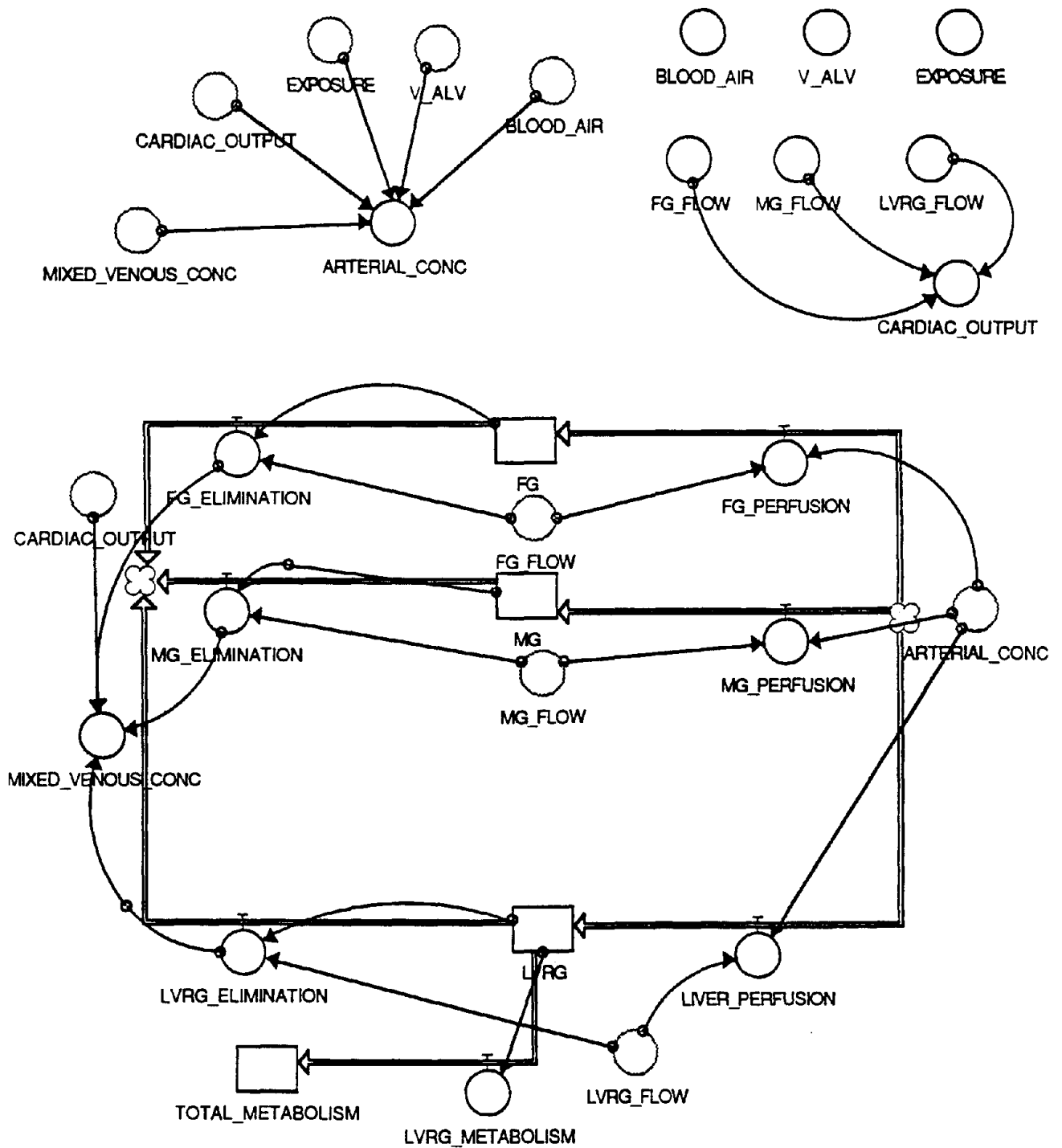


Table 2.1
Equations for the Best-Estimate Rat Model

- ☐ $FG = FG + FG_PERFUSION - FG_ELIMINATION$
 $INIT(FG) = 0$
- ☐ $LVRG = LVRG + LIVER_PERFUSION - LVRG_ELIMINATION -$
 $LVRG_METABOLISM$
 $INIT(LVRG) = 0$
- ☐ $MG = MG + MG_PERFUSION - MG_ELIMINATION$
 $INIT(MG) = 0$
- ☐ $RECOVERY = RECOVERY + EXHALATION$
 $INIT(RECOVERY) = 0$
- ☐ $TOTAL_ABSORBED = TOTAL_ABSORBED + FLOW_RATE$
 $INIT(TOTAL_ABSORBED) = 0$
- ☐ $TOTAL_METABOLISM = TOTAL_METABOLISM + LVRG_METABOLISM$
 $INIT(TOTAL_METABOLISM) = 0$
- ☐ $ARTERIAL_CONC = BLOOD_AIR * (CARDIAC_OUTPUT * MIXED_VENOUS_CONC +$
 $V_ALV * (EXPOSURE * 10^{(-6)/25.45}))$
 $/ (CARDIAC_OUTPUT * BLOOD_AIR + V_ALV) \quad \{moles/liter\}$
- ☐ $BLOOD_AIR = .35$
- ☐ $CARDIAC_OUTPUT = FG_FLOW + MG_FLOW + LVRG_FLOW$
 $\{liters/minute\}$
- ☐ $EXHALATION = IF (NET_MOLES < 0) THEN$
 $NET_MOLES * (-1) ELSE 0$
- ☐ $EXPOSURE = IF (0 \leq TIME) AND (TIME \leq 0 + 360) THEN 8000 ELSE 0$
- ☐ $FG_ELIMINATION = (FG * FG_FLOW) / (.028 * 118.2)$
 $\{moles/minute\}$
 $\{ ([FG] * fF[FG]) / (fV[FG] * fL[FG\BLOOD]) \}$
- ☐ $FG_FLOW = .0136 \{liters/minute\}$
- ☐ $FG_PERFUSION = ARTERIAL_CONC * FG_FLOW$
 $\{moles/minute\}$
 $\{ [ARTERIAL_BLOOD] * fF[FG] / fV[arterial\ blood] \}$
- ☐ $FLOW_RATE = IF (EXPOSURE > 0) THEN NET_MOLES$
 $ELSE 0$
- ☐ $FRACT_ABSORB =$
 $NET_MOLES / (V_ALV * (EXPOSURE * (10^{(-6)}) / 25.45))$
- ☐ $LIVER_PERFUSION = ARTERIAL_CONC * LVRG_FLOW \{moles/minute\}$
 $\{ fF[LIVER] * ARTERIAL_BLOOD / fV[arterial\ blood] \}$
- ☐ $LVRG_ELIMINATION = (LVRG * LVRG_FLOW) / (.036 * 5.40)$
 $\{moles/minute\}$
 $\{ (LIVER * fF[LIVER]) / (fV[liver] * fL[LIVER\BLOOD]) \}$
- ☐ $LVRG_FLOW = .1042 \{liter/minute\}$
- ☐ $LVRG_METABOLISM = (1.47E-6) * (LVRG/.036)/$
 $((5e-6) + (LVRG/.036))$
 $\{moles/minute\}$
- ☐ $MG_ELIMINATION = (MG * MG_FLOW) / (.300 * 5.26)$
 $\{moles/minute\}$
 $\{ ([MG] * fF[MG]) / (fV[MG] * fL[MG\BLOOD]) \}$
- ☐ $MG_FLOW = .0226 \{liter/minute\}$
- ☐ $MG_PERFUSION = ARTERIAL_CONC * MG_FLOW$
 $\{moles/minute\}$
 $\{ [ARTERIAL_BLOOD] * fF[MG] / fV[arter]$

Table 2.1 (continued)

- $MIXED_VENOUS_CONC = \frac{(FG_ELIMINATION + MG_ELIMINATION + LVRG_ELIMINATION)}{CARDIAC_OUTPUT}$
- $NET_ABSORPTION = IF (NET_MOLES < 0) THEN$
 $(NET_MOLES * (-1) / V_ALV) * 25.45 * 10^6$
 $ELSE 0$
- $NET_MOLES =$
 $CARDIAC_OUTPUT * (ARTERIAL_CONC - MIXED_VENOUS_CONC)$
- $V_ALV = 0.15 \text{ \{liters/minute\}}$

$$\frac{d(\text{BUT})}{dt} = \text{Input from arterial circulation} - \frac{Q_{\text{tissue}}[\text{BUT}]_{\text{art}} - Q_{\text{tissue}}[\text{BUT}]_{\text{tissue}}}{V_{\text{tissue}}L_{\text{tissue/blood}}} - \text{metabolism}$$

moles/min Input from output via venous
arterial circulation blood

where Q_{tissue} is the rate of blood flow to the compartment in liters/minute, $[\text{BUT}]_{\text{art}}$ and $[\text{BUT}]_{\text{tissue}}$ are the concentrations of butadiene in arterial blood and tissue, V_{tissue} is the volume of the compartment, and $L_{\text{tissue/blood}}$ is the equilibrium ratio of the tissue and blood butadiene concentrations. The values for the tissue volumes and flows used for rats and mice can be found in Section 3.1 below.

The metabolism term (which is included only for the Liver/Vessel-Rich Group compartment) is very similar to that used in the perchlorethylene report--a simple Michaelis-Menten enzyme equation:

$$\text{metabolism} = \frac{V_{\text{max}} * [\text{But}]_{\text{tissue}}}{K_m + [\text{But}]_{\text{tissue}}}$$

V_{max} is the maximum velocity of the reaction in moles/min. (This occurs at high concentrations of substrate when all active sites on the enzyme molecules are actively engaged in processing the substrate as rapidly as possible.) $[\text{But}]_{\text{tissue}}$ again is the concentration of butadiene in the metabolizing tissue in moles/liter. Finally K_m , the Michaelis constant, is the concentration of butadiene at which the reaction proceeds at half its maximum velocity. This is the major nonlinear equation that makes the system as a whole respond nonlinearly with external exposure.

3. BASIC PHYSICAL AND PHYSIOLOGICAL DATA FOR THE MODEL

3.1 Tissue Volumes and Flows in Rodents

Tables 3.1 and 3.2 show the volumes and perfusion rates for the various groups of tissues used in our basic rat and mouse models. The values for rats have changed in one significant way from our previous perchloroethylene report. The values shown in Table 3.1 are based on a rat of 400 grams while the values originally given in the perchloroethylene report were for 250 gram rats. This change was necessitated because the primary experimental rat study we will use for modeling (Bond et al. 1986) used 400 gram rats. The mouse data required a smaller increase from 25 to 28 grams. In correcting for these increased weights all flows and volumes were scaled up proportionately.

3.2 Partition Coefficients

The determination of appropriate blood/air, oil/air and tissue/blood partition coefficients for butadiene proved to be particularly difficult due to the lack of any published values for any of these coefficients. This section, which is divided into four parts, will describe how we generated alternative estimates for these parameters. Later, in the course of our modeling of the absorption data of Bond et al. (1986) in Section 4, we will be able to narrow somewhat the uncertainties represented here.

Table 3.1

Rat Tissue Group Volumes and Flows

Compartment	Volume (V) (milliliters)	Blood Flow (Q) (ml/min)	Intrinsic Clearance Rate (Q/V) (min ⁻¹)
Vessel-Rich-Group	20	76.6	3.83
Liver	16	37.6	2.35
Liver + Vessel-Rich-Group	36	104.2	2.89
Muscle Group	300	22.6	0.0753
Fat Group	28	13.6	0.486
Total	364	150.4	

Table 3.2
Mouse Tissue Group Volumes and Flows

Compartment	Volume (V) (milliliters)	Blood Flow (Q) (ml/min)	Intrinsic Clearance Rate (Q/V) (min ⁻¹)
Vessel-Rich-Group	1.40	10.85	7.75
Liver	1.68	5.32	3.17
Liver + Vessel-Rich Group	3.08	16.17	5.25
Muscle Group	19.6	3.19	0.163
Fat Group	2.8	1.92	0.684
Total	25.5	21.3	

3.2.1 Estimation of the Oil/Water Partition Coefficient

In the absence of published measurements for this coefficient we used the method of Hansch and Leo to determine the octanol/water partition coefficient (Lyman et al., 1982). This method uses empirically derived atomic or group fragment constants (f) and structural factors (F) to estimate values of $\log K_{ow}$:

$$\log K_{ow} = \text{sum of fragments (f) + factors (F)}$$

The only input information required for this is the structure of the chemical, since the fragment values and factors are known from studies of a wide variety of other compounds. Typical factors include molecular flexibility (e.g., possible rotation about bonds), unsaturation, multiple halogenation, branching, and interactions with H-polar fragments. Because of the large number of f and F values available, this method is fairly powerful, and there are relatively few man-made chemicals for which a value of $\log K_{ow}$ cannot be calculated. Lyman et al. (1982) contains all the fragment constants and structural factors along with the rules for calculating the partition coefficients.

Using this method, we calculate the butadiene octanol/water partition coefficient as follows:

$$\begin{aligned}
 \text{Log } K_{ow} &= 4*f_C + 10*f_H + (3-1)*F_b + 2*F_{=} \\
 &\quad \begin{array}{l} \text{(4 carbons)} \\ \text{(10 hydrogens)} \\ \text{(chain of 3 carbon-carbon bonds)} \\ \text{(2 conjugated double bonds)} \end{array} \\
 &= 4*(.20) + 10*(0.23) + 2*(-.12) + 2*(-.38) \\
 &= 2.10 \pm .1 \text{ (SD)}
 \end{aligned}$$

(.1 is the stated standard deviation of calculated vs. measured oil/water partition coefficients). Taking the antilog of 2.1, the resulting value of the octanol/water coefficient for butadiene is 125.9, with an indicated one standard deviation range of 100 - 158. In the above calculation f_C and f_H are the fragment constants for the carbon and hydrogen molecules found in butadiene. F_b accounts for the carbon-carbon bonds and $F_{=}$ accounts for the conjugated double bonds present in butadiene.

Existing regression analyses for blood/air and tissue/blood partition coefficients are not based on octanol/water partition coefficients, but rather are based on oil/air (Hattis, et al. 1986a) or fat/blood (Fiserova-Bergerova and Diaz, 1986) coefficients. We therefore need to use our estimated octanol/water coefficients into these latter forms. To explore this we calculated the octanol/water partition coefficient for a number of aliphatic and aromatic compounds using the method of Hansch and Leo and compared them to published values of the same compounds' oil/water partition coefficient. Table 3.3 shows the results.

These data suggest that octanol/water and oil/water partition coefficients may be approximately equal for aliphatic chemicals in butadiene's approximate range of lipophilicity. The three aliphatic compounds all show ratios of less than 1.7 and decrease with increasing octanol/water partition coefficient. Given that our best estimate of the

Table 3.3
Calculated Octanol/Water and Measured Oil/Water Partition
Coefficients for Various Aliphatic and Aromatic Compounds

Aliphatics			
Compound	Calculated Octanol/Water	Published Oil/Water	Ratio Octanol to Oil
Diethylether	8.31	5.0	1.66
Methane	10.33	13.2	1.27
Cyclopropane	54.76	63.1	1.15
Aromatics			
Compound	Calculated Octanol/Water	Published Oil/Water	Ratio Octanol to Oil
Benzene	175.71	134.9	0.77
Toluene	668.6	616.6	0.922
Styrene	931.8	891.25	0.957
Ethylbenzene	2235.9	2398.8	1.02

octanol/water partition coefficient for butadiene is 125.9, it seems plausible that the ratio of the octanol to oil/water partition coefficient could be one. Lacking any experimental values of the oil/gas partition coefficient for butadiene, we will assume the ratio is in fact one, recognizing that this assumption carries appreciable uncertainty given the limited data.

3.2.2 Calculation of the Air/Water and Oil/Air Partition Coefficients

We also found no published values for the air/water partition coefficient for butadiene. However, this parameter can be estimated from the published value of the solubility of pure butadiene gas in water at 37° C. This essentially involves assuming that one atmosphere of pure gas is in equilibrium with the saturated solution. The calculation takes the following form:

$$K_{\text{water/gas}} = \frac{(0.00052 \text{ g but/g sol})(1 \text{ g/ml})(1000 \text{ ml/l})(1 \text{ mol}/54.09 \text{ g but})}{1 \text{ mole}/25.42 \text{ liter gas}}$$

$$K_{\text{water/gas}} = 0.2444$$

Based on this water/air partition coefficient, and the earlier estimated oil/water coefficient of 125.9 (1 std. range 100-158), the oil/air coefficient is $.2444 * 129.2 = 30.77$ (1 std. range 24.4 - 38.6).

3.2.3 Estimation of Blood/Air and Tissue/Blood Partition
Coefficients Based on a Regression Analysis Used Earlier for
Perchloroethylene (Hattis et al., 1986a)

As for perchloroethylene, no one seems to have directly measured butadiene's tissue/blood or tissue/air partition coefficients. We therefore used the same generalized regression analysis of human tissue partition coefficients in relation to oil/air and water/air coefficients that was developed earlier for perchloroethylene. This analysis used data on 24 chemicals. Table 3.4 shows the regression equations and Table 3.5 shows the partition coefficients it would indicate for butadiene -- based on the water/air partition coefficient and the estimated range of the oil/air partition coefficient calculated in the previous section.

It can be seen that the resulting partition coefficients carry appreciable uncertainty. Particularly in the case of the key blood/air partition coefficient, the lower end of the range appears unlikely. In the previous section we showed that the water/air coefficient for butadiene was approximately 0.2444. According to this regression, the blood/air coefficient is essentially the same with a value of 0.2552. We might expect a best estimate of the blood/air coefficient to be larger than this because the blood contains fatty compounds not found in water that should lead to an increased partitioning of butadiene between blood and air as compared to water and air. Given that this value of the blood/air coefficient is also strongly influenced by the small negative intercept, we will consider it a minimal value of the blood/air coefficient for our analysis. In undertaking our modelling we will also use a blood/air partition of 0.35 which, as it turns out, seems to be more

Table 3.4
Regression Analysis Results for Human Tissue/Gas
Partition Coefficients

Dependent Variable	Oil/Gas Coefficient	Water/gas Coefficient	Intercept	R ²
*fat/gas	0.7354 (.0311)**	----	18.72	0.962
fat/gas	0.7304 (.0343)	2.61 (6.80)	16.94	0.962
*muscle/gas	0.0249 (.0010)	----	1.073	0.965
muscle/gas	0.02443 (.0011)	0.2429 (.2142)	0.9071	0.967
*kidney/gas	0.01382 (0.0012)	0.680 (.2338)	1.282	0.908
*liver/gas	0.03329 (.0012)	0.5212 (.2386)	0.7657	0.978
*blood/gas	0.007025 (.0008)	1.091 (.1576)	-0.228	0.906

*Used for calculation of 1,3-butadiene partition coefficients

**The numbers in parentheses are the standard errors of the oil/gas and water/gas coefficients given immediately above.

Table 3.5
1,3-Butadiene Tissue/Gas and Tissue/Blood Partition
Coefficients Calculated from the Equations in Table 3.4

Tissue	L _{Tissue/Gas}		L _{Tissue/Blood}	
	Best Estimate	Plausible Range*	Best Estimate	Plausible Range**
Fat	41.36	35.9-48.31	162.1	94.5-322
Muscle	1.84	1.66-2.32	7.21	4.37-15.5
Kidney (VRG)	1.87	1.70-2.08	7.33	4.47-13.8
Liver	1.92	1.62-2.28	7.53	4.26-15.2
Liver + VRG***	1.89	1.66-2.17	7.40	4.37-14.5
Blood/Gas	0.2552	0.15-0.38		

*The ranges in this column were calculated by adding or subtracting one standard error from each of the oil/gas and water/gas coefficients in Table 3.4, and then using a 1 standard deviation high or a 1 standard deviation low estimate of the octanol/water partition coefficient in the resulting equation. More sophisticated determination of appropriate uncertainties would require a Monte Carlo simulation.

**The low ends of the ranges in this column were computed by dividing the tissue/gas best estimate value - 1 SEM by the blood/gas best estimate value + 1 SEM. The high end of these values were computed by dividing the tissue gas best estimate value + 1 SEM by the blood/gas best estimate value - 1 SEM. More sophisticated determination of appropriate uncertainties would require a Monte Carlo Simulation.

***Weighted average.

compatible with the data of Bond et al. (1986). Using a blood/air partition coefficient of .35 implies the following best estimates of tissue/blood partition coefficients:

Fat/blood	118.2
Muscle/blood	5.26
Kidney(VRG)/blood	5.34
Liver/blood	5.49
Liver + VRG/blood	5.4

3.2.4 Calculation of Tissue/Blood Partition Coefficients Based on the Regression Analyses of Fiserova-Bergerova and Diaz (1986)

Fiserova-Bergerova and Diaz (1986) used published tissue-blood and blood-gas coefficients for 35 chemicals (including 7 relatively hydrophilic compounds) to develop a generalized linear regression for determining the tissue/blood coefficient for any chemical. Their regression took the following form:

$$L_{\text{tissue/blood}} = a(L_{\text{fat/blood}}) + b$$

where the values for a and b are reproduced in Table 3.6. Table 3.7 shows the resulting partition coefficients implied by this model assuming that the fat/blood partition coefficient is the same as the oil/water coefficient. It can be seen that these coefficients are quite similar to those calculated in the previous section assuming a blood/air partition coefficient of .35.

Table 3.6
Fiserova-Bergerova and Diaz (1986) Regression Analysis Results
for Human Tissue/Gas Partition Coefficients

Dependant Variable	Fat/blood Coefficient	Intercept	R ²	SD
Muscle/blood	0.02855	0.6017	.837	1.042
Kidney/blood	0.0211	0.6442	.944	.421
Brain/blood	0.0372	0.5199	.940	.779
Liver/blood	0.0425	0.5776	.919	.997

Table 3.7
Butadiene Tissue/Blood Partition Coefficients Calculated from the
Equations in Table 3.6, Assuming a Fat/Blood Partition
Coefficient of 125.9

Tissue	L _{Tissue/Blood}
Muscle	4.25
Brain	5.20
Kidney	3.30
Liver	5.93

4. ARTICULATION OF THE RODENT MODELS

4.1 Summary and First-Order Implications of the Data of Bond et al. (1986)

Bond et al. (1986) have investigated the uptake and disposition of butadiene in rats and mice. Male Sprague-Dawley rats and B6C3F₁ mice were exposed nose only to concentrations in the range of 0.078 to 7076 ppm butadiene (.14 to 13,000 micrograms per liter) for 6 hours. Blood samples were taken during exposure at time intervals of 2, 4 and 6 hours and butadiene concentrations measured. Urine, feces, and expired air were also collected for up to 65 hours after exposure. Tables 4.1 - 4.3 reproduce the primary data, and do some first-order analysis of where nonlinearities seem to be occurring.

This study is exemplary in its attempt to measure so many parameters of pharmacokinetic interest over such an extended range of dosage. And commendably, the authors report their raw observations in numerical form in tables. This allows us to assess the uncertainty of the experimental data and the fit of our models with the data. Unfortunately, there are appreciable ambiguities in some of the key measurements the authors made. Additionally the experimental variability seen in the data is large enough to cause considerable additional difficulties for interpretation.

We begin with the absorption data (Table 4.1). Taken at face value, the decrease in absorption per unit of exposure at the higher exposure levels indicates that the primary metabolism of butadiene is approaching saturation, although the strength of this conclusion is somewhat weakened by the nearly fourfold variability in absorption per unit of exposure seen

Table 4.1
Net Uptake of Butadiene During 6 Hour Inhalation Exposures

Exposure Level (ppm)	Alveolar Ventilation* (liter/min.)	Net Uptake During 6 hr Exposure (umole/animal +/- std. error)	Net Uptake (umole) per ppm-liter/min
<u>Observations in Rats</u>			
.078	.135	.03 +/- .002	2.84
.78	.167	.13 +/- .06	1.00
6.74	.154	.8 +/- .1	.772
74.7	.130	17 +/- 2	1.76
		Average, 4 "low" doses	1.40
958.2	.185	65 +/- 5	.429
7076	.130	240 +/- 40	.262
Average	.15		
<u>Observations in Mice</u>			
.078	.026	.006 +/- .001	2.96
.78	.022	.06 +/- .01	3.50
6.74	.022	.4 +/- .09	2.70
74.7	.024	3.2 +/- .1	1.78
		Average, 4 "low" doses	2.74
958.2	.022	19 +/- .5	.90
7076	not done		
Average	.0233		

*Bond et al. (1986) measured total ventilation. These data on alveolar ventilation were calculated assuming that alveolar ventilation is 2/3 of total ventilation.

Data Source: Bond et al., 1986

Table 4.2
Disposition of Absorbed Butadiene in Rats and Mice
65 Hours After a Six Hour Exposure

Data in Micromoles (Standard Errors in Parentheses)

Exposure Level (ppm)	Urine	Feces	CO ₂	Butadiene and other volatile metabolites*	Carcass	Total
<u>Observations in Rats</u>						
74.7	8 (.4)	.8 (.2)	2 (.5)	3 (.3)	3 (.4)	17
958	21 (4)	1 (.2)	16 (.2)	15 (1)	13 (.4)	66
7076	19 (2)	4 (.8)	124 (4)	62 (12)	35 (1)	244
<u>Observations in Mice</u>						
6.74	.4 (.1)	.06 (.01)	.2 (.01)	.2 (.02)	.09 (.02)	.95 **
74.7	1.6 (.3)	.4 (.05)	.1 (.01)	.6 (.2)	.5 (.05)	3.2
958	7 (.4)	.6 (.2)	3 (.1)	6 (1)	2 (.1)	19

*Except CO₂.

**Note: there is some discrepancy between this total of the reported disposition and the reported total absorption of .4 micromoles in Table 4.1.

Table 4.3

Concentration of Butadiene in the Blood of Rats and Mice During
Exposure (Blood Concentrations in Micromoles Per Liter)

Exposure Level (ppm)	Hours After the Start of Exposure			Average	Ave. Blood Conc. per ppm
	2	4	6		

Exposure

Observations in Rats

74.7	.1 (.02)*	.2 (.04)	.1 (.02)	.13**	.0017
958	1 (.08)	4 (.6)	4 (.3)	3**	.0031

Observations in Mice

6.74	.6 (.2)	.9 (.5)	.2 (.06)	.57	.084
74.7	.3 (.1)	.2 (.1)	.8 (.1)	.43**	.0058
958	7 (.3)	8 (2)	3 (1)	6**	.0063

*The numbers in parentheses are reported standard errors, based on three animals per data point.

**These values were used as "target" criteria for adjustment of model parameters (see Tables 4.5 and 4.7 below).

in the different experiments at low doses. (In this as in other cases below, the experimental variability that must be inferred from the data seems much larger than would be expected from the standard errors that the authors report in their tables).

As was discussed in the introduction (pp. 2-5 above), these were the data that EPA (1985) used for the primary assessment of delivered dose for their risk assessment. The major ambiguity for interpretation of these data for modeling purposes is that they represent the amounts of butadiene retained at the end of the six hour exposure periods--implicitly excluding any butadiene that was metabolized and excreted during the exposure. The reason why this is critical is that in order to assess the total delivered dosage of butadiene epoxides, we require the total amount of butadiene metabolized by the animals. Fortunately, as will be seen in the sections 4.2 and 4.3 below, the independent data of Kreiling et al. (1986) provide a means for assessing the magnitude of this putative loss of metabolized butadiene.

Ideally, the data on disposition of absorbed butadiene following exposure (Table 4.2) would provide a key input for modeling. In particular, the amount of butadiene excreted unchanged at low doses should provide us with a measure of the ratio of the rate of exhalation to the rate of metabolism. This could be used either to assess the ratio of V_{max} to K_m (as was done for perchloroethylene) or, given information about this ratio, as a check on the blood/air partition coefficient (which, together with alveolar ventilation, determines the rate of exhalation). At higher doses, approaching saturation of butadiene metabolism should produce a greater-than-linear increase in the amount of butadiene excreted unchanged, providing a way of assessing the absolute

value of K_m . Unfortunately, the measurement made by Bond et al. (1986) is not solely unchanged butadiene, but is listed as "butadiene and other volatile metabolites". Moreover, instead of increasing in proportion to other routes of loss at high exposure levels, if anything the micromoles excreted by this route increase less than proportionately with increasing exposure--suggesting that the "other volatile metabolites" indeed make up a significant fraction of the total. Not knowing the proportion of unchanged butadiene in this fraction at different doses renders the data practically unusable.

As it turns out, the only measurements we can really use without substantial interpretive difficulties are the blood measurements of unchanged butadiene in Table 4.3.* Even these, however, show large experimental variability which indicates that the analytical quantification should be regarded with some skepticism. In particular, we must ignore the the impossible result at the lowest dose level in mice (the blood butadiene level for the lowest 6.74 ppm exposure must not be larger than the blood level for 74.7 ppm exposure).

4.2 Use of the Metabolism Data of Kreiling et al. (1986) as a Key Input for Model Construction

Kreiling et al. (1986) provide metabolic data based on measurements of the uptake of butadiene from closed chambers. This continues a long

*Bond et al. (1986) also made a series of measurements of labelled butadiene epoxides in blood. These measurements could be helpful in later work formally modeling the elimination of the epoxides and the formation of DNA adducts.

series of experiments by Filser and Bolt in what by now is a well established system (Bolt et al., 1984; Filser and Bolt, 1981; Filser and Bolt, 1984). In contrast to Bond et al. (1986) what is being measured is the decline in the concentration of butadiene in the chamber over time in sequential measurements, rather than the appearance of C₁₄ from butadiene in the animals at a single time point (Figure 4.1). Other experiments were done measuring the appearance of butadiene and butadiene epoxide in the chamber air after intraperitoneal injection of butadiene.

A less favorable comparison is that unlike Bond et al. (1986), the Filser/Bolt laboratory does not usually provide raw numerical data in tabular form for us to work with. Rather, they interpret all data in terms of their own simple pharmacokinetic model, which allows only a single dynamic compartment for the animal--and they provide no estimates of the uncertainty of their metabolic parameters. The use of a single-compartment model is potentially hazardous because there might be at least some contribution to uptake over a several hour period from ongoing slow storage of butadiene in fat. In this case, however, they have done some experiments after inhibition of the animals' P450 metabolism that indicate that at least after several hours of exposure, ongoing storage does not seem to be very important (Figure 4.2).

Kreiling et al. (1986) report the following metabolism parameters for male Sprague-Dawley rats, and male B6C3F1 mice* per kilogram of body weight:

*These are the same strains used in the experiments of Bond et al. (1986)] and in the carcinogenesis bioassays.

Figure 4.1

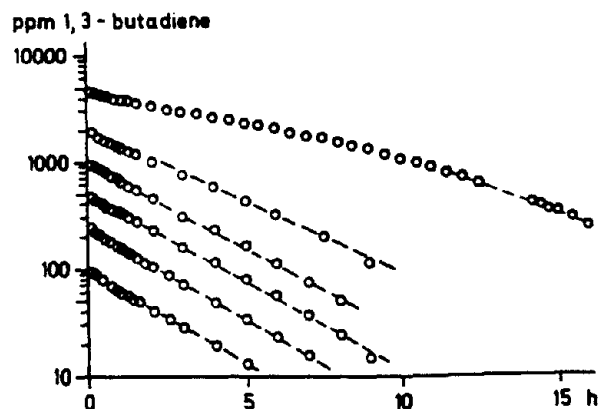


Fig. 1. Time-course of 1,3-butadiene concentrations in the gas phase of a closed 6.4-l desiccator jar chamber, occupied by eight animals (B6C3F1 mice untreated). Individual experiments with different initial butadiene concentrations show a first order decline at concentrations below 1000 ppm

Figure 4.2

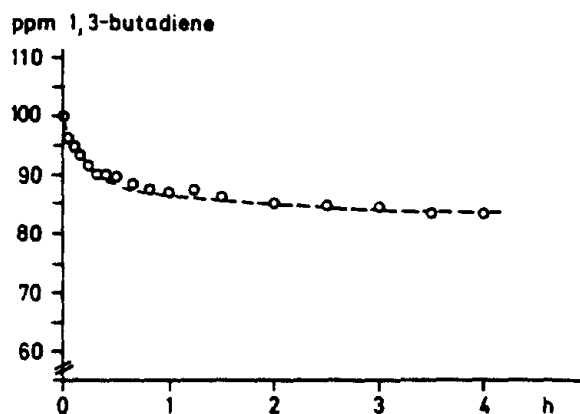


Fig. 2. Time-course of 1,3-butadiene concentrations in the gas phase of a 6.4-l desiccator jar chamber, occupied by eight B6C3F1 mice pretreated with dithiocarb. The data were fitted with a calculated equilibration curve (*dotted line*)

Source: Kreiling et al., 1986

	Low Dose Clearance (liters air/kg-hour)	Maximum High-Dose Metabolism (micromoles/kg-hour)
Rats	4.5	220
Mice	7.3	400

The second set of numbers ($V_{\max}$) can be incorporated directly into our models after conversion to appropriate units of moles/minute. This yields 1.47×10^{-6} moles/min. for rats and 1.87×10^{-7} moles/minute for mice.

Then, because low-dose metabolism is simply related to the ratio of $V_{\max}$ to K_m , all we need to do is to find the appropriate value of K_m for each model that at low (far below saturating) exposure levels will lead to metabolism of the amount of butadiene in the appropriate volume of air per hour several hours after the beginning of simulated exposure. For convenience in coordinating with the simulations of the Bond et al. (1986) data, we choose as our "low" dose 74.7 ppm. At this air level, the requirement that 400 kg rats clear 4.5 liters of air per kg body weight translates into the following number of moles of butadiene metabolised per hour:

$$(4.5 \text{ liters/kg-hour})(.4 \text{ kg})(74.7 \text{ ppm})(10^{-6} \text{ mole}/25.45 \text{ liter-ppm}) \\ = 5.28 \times 10^{-6} \text{ moles/hour}$$

Similarly, the requirement that .028 kg mice clear 7.3 liters of air per kg body weight translates into:

$$(7.3 \text{ liters/kg-hour})(.028)(74.7 \text{ ppm})(10^{-6} \text{ mole}/25.45 \text{ liter-ppm}) \\ = 1.54 \times 10^{-7} \text{ moles/hour}$$

To compare the model simulation results with these "target" criteria, we chose to observe our models' performance in the sixth hour of a continuous exposure--in reasonable parallel with the length of exposures needed to attain reasonably constant rates of butadiene consumption in the Kreiling et al. (1986) experiments (see Figure 4.1 above).

At the same time, we would like the blood concentration of butadiene as a function of exposure to correspond reasonably with the data of Bond et al. (1986) reproduced in Table 4.3 above. To achieve both of these goals, in addition to K_m , we varied the blood/air partition coefficient within the range established in Section 3 (in doing this, we maintained the tissue/air coefficients at their "best estimate" levels, leading to the changes in tissue/blood coefficients described on p. 24 above. This process is detailed in the following two subsections--first for rats and then for mice.

4.2.1 Articulation of the Rat Model

Table 4.4 shows the simulation runs made to adjust K_m and the blood/air partition coefficient for rats. For these purposes we used the exposure levels and alveolar ventilation rates as a function of exposure level as measured by Bond et al. (1986) (see Table 4.1). Table 4.5 compares key results from each of the models with the observed "target" values derived above.

It can be seen in Table 4.5 that the precise level of K_m makes very little difference. This is because with these blood/air partition coefficients, the model is essentially limited by the blood flow to the

Table 4.4a
Rat Model Simulations Used for Adjustment of K_m
and the Blood/Air Partition Coefficient

(V_{max} set at 1.47×10^{-6} for all runs)

ppm	Minutes after start of 360 min exposures	Butadiene Absorption* (umoles)	Butadiene Metabolism (umoles)	Butadiene Exhalation (umoles) **	Butadiene Venous Blood Concentration (umoles/liter)
blood/air = .2552, $K_m = 1 \times 10^{-5}$					
74.7	120	8.68	6.89		.129
	240	16.60	13.93		.164
	300	20.44	17.48		
	360	24.22	21.03	0	.180
	660		21.41	1.949	
	projected final		21.55	2.670	
958	120	111.8	87.2		1.74
	240	212.5	175.8		2.68
	360	308.5	264.9	0	2.90
	720		269.2	29.93	
	1080		269.9	35.51	
	projected final		270.2	38.26	
blood/air = .2552, $K_m = 5 \times 10^{-6}$					
74.7	120	8.94	7.17		.109
	240	17.14	14.49		.143
	300	21.11	18.18		
	360	25.03	21.88	0	.159
	720		22.29	2.051	
	projected final		22.40	2.625	
958	120				1.77
	240				2.24
	360	327.0	283.9	0	2.45
	1020		288.8	34.46	
	1980		289.2	37.53	
	projected final		289.2	37.77	

*Net after subtraction of butadiene that was exhaled unchanged during exposure, but including all butadiene metabolized.

**The numbers in this column reflect exhalation only after the end of exposure.

Table 4.4b
Rat Model Simulations Used for Adjustment of K_m
and the Blood/Air Partition Coefficient (continued)

(V_{max} set at 1.47×10^{-6} for all runs)

ppm	Minutes after start of 360 min exposures	Butadiene Absorption* (umoles)	Butadiene Metabolism (umoles)	Butadiene Exhalation (umoles) **	Butadiene Venous Blood Concentration (umoles/liter)
blood/air = .35, $K_m = 1 \times 10^{-5}$					
74.7	120	10.70	8.63		.207
	240	20.43	17.52		.248
	300	25.17	22.00		
	360	29.85	26.48	0	.265
	660		27.04	2.145	
projected final			27.18	2.672	
958	120				3.95
	240				4.55
	360	364.9	317.5	0	4.79
	720		324.1	33.86	
	1080		324.7	38.53	
projected final			324.9	40.01	
blood/air = .35, $K_m = 5 \times 10^{-6}$					
74.7	120	11.12	9.10		.171
	240	21.30	18.43		.211
	300	26.26	23.13		
	360	31.17	27.85	0	.228
	720		28.45	2.209	
projected final			28.56	2.611	
958	120	143.3	114.6		3.19
	240	271.7	231.4		3.77
	360	395.3	348.8	0	4.00
	720		355.2	33.02	
	960		355.9	36.73	
projected final			356.3	38.98	

*Net after subtraction of butadiene that was exhaled unchanged during exposure, but including all butadiene metabolized.

**The numbers in this column reflect exhalation only after the end of exposure.

Table 4.5
Fit of Rat Models With Various Values of K_m and the Blood/Air
Partition Coefficient to Data-Based "Target" Criteria

(V_{max} set at 1.47×10^{-6} for all runs)

Blood/Air Partition Coefficient	K_m	ppm expo- sure	Criterion from Bond et al., 1986--ave. venous blood Butadiene conc. at 2, 4, and 6 hours of exposure (see Table 4.3) (umoles/liter)	Criterion from Kreiling et al., 1986--Micromoles metabolized in the 6th hour of exposure (see Text)
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Target Criteria

74.7	.13	5.28
958	3	-----

Results of Model Simulations

.2552	1×10^{-5}	74.7	.158	3.55
		958	2.44	
.2552	5×10^{-6}		.137	3.70
		958	2.15	
.35	1×10^{-5}	74.7	.24	4.48
		958	4.43	
.35	5×10^{-6}	74.7	.203	4.72
		958	3.65	
.5	1.5×10^{-5}	74.7	.436	5.36
		958	8.82	

liver/vessel-rich-group. Even if we were to push K_m down to very low levels, we would not be able to get metabolism quite up to the criterion level of 5.28 umoles/hour. In this regard, however, the disparity is clearly much less with the higher blood/air partition coefficient.

Turning to the venous blood concentration criterion, we see that in pushing the blood/air partition coefficient up to .35 we cause the models to somewhat over-predict butadiene blood concentrations. This effect is not too great (particularly considering the variability of the underlying data) with the lower value of K_m (5×10^{-6}). Clearly, however, if we were to seek a better fit with the low-dose metabolism rate data by pushing the blood/air partition coefficient up any further (to .5, as shown at the bottom of Table 4.5), we would worsen the fit of the model to the blood concentration data. Our final "best estimate" parameters (blood/air partition coefficient = .35 and $K_m = 5 \times 10^{-6}$) are therefore a compromise between fitting our two main criteria based on observations.

To show the sensitivity of our results to this conclusion, we will also do some analyses based on a blood/air partition coefficient of .2552 and a K_m of 1×10^{-5} . As can be seen in Table 4.4, assuming the lower blood/air partition coefficient and the higher K_m leads to less metabolism and therefore a greater estimate of carcinogenic potency per unit of metabolized butadiene.

4.2.2 Articulation of the Mouse Model

Table 4.6 shows the simulation runs made to adjust K_m and the blood/air partition coefficient for mice. For these purposes we used the average alveolar ventilation rate seen in the Bond et al. (1986) data (.0233 l/min from Table 4.1). In parallel with Table 4.5, Table 4.7 compares key results from each of the model runs with the observed "target" values.

As with the rat model, it can be seen in Table 4.7 that it is possible to achieve a much better fit to the data if we assume a blood/air partition coefficient of .35 than one of .2552. Additionally, it is clear that if we place any credence in the butadiene blood concentration data, the blood/air partition coefficient cannot be as high as .5.

One difference with the rat results is that the mouse model is not entirely flow-limited under these conditions. The best fit was achieved only after raising the K_m (thus slowing low-dose metabolism somewhat) to 2×10^{-5} .

Again, in subsequent sections we will do some sensitivity analysis with a blood/air partition coefficient of .2552.

4.3 Results of Some Other Comparisons with the Data of Bond et al. (1986)

We can now return to the original question about the Bond et al. (1986) absorption observations. If our models using the Kreiling et al. (1986) metabolism rates are correct, how much of the metabolized butadiene must have been lost during the periods of exposure in the Bond et al.

Table 4.6
Mouse Model Simulations Used for Adjustment of K_m
and the Blood/Air Partition Coefficient

(V_{max} set at 1.87×10^{-7} for all runs)

ppm	Minutes after start of 360 min exposures	Butadiene Absorption* (umoles)	Butadiene Metabolism (umoles)	Butadiene Exhalation (umoles) **	Butadiene Venous Blood Concentration (umoles/liter)
blood/air = .2552, $K_m = 2 \times 10^{-5}$					
74.7	120	1.198	.995		.210
	240	2.289	2.007		.232
	300	2.822	2.515		
	360	3.350	3.024	0	.241
	720		3.061	.2367	
projected	final		3.068	.2819	
958	120	13.73	11.05		3.52
	240	25.98	22.28		3.82
	360	37.82	33.54	0	3.95
	720		34.03	3.103	
	1020		34.09	3.531	
projected	final		34.11	3.706	
blood/air = .35, $K_m = 5 \times 10^{-6}$					
74.7	120	1.679	1.459		.207
	240	3.239	2.946		.231
	300	4.007	3.693		
	360	4.769	4.449		.241
blood/air = .35, $K_m = 2 \times 10^{-5}$					
74.7	120	1.453	1.223		.328
	240	2.777	2.471		.354
	300	3.425	3.098		
	360	4.068	3.752	0	.365
	780		3.778	.2674	
projected	final		3.780	.2880	
958	120	15.64	12.55		5.85
	240	29.40	25.31		6.22
	360	42.70	38.10		6.38
	2580		38.86	3.838	

*Net after subtraction of butadiene that was exhaled unchanged during exposure, but including all butadiene metabolized.

**The numbers in this column reflect exhalation only after the end of exposure.

Table 4.7
Fit of Mouse Models With Various Values of K_m and the Blood/Air
Partition Coefficient to Data-Based "Target" Criteria

(V_{max} set at 1.87×10^{-7} for all runs)

Blood/Air Partition Coefficient	K_m	ppm expo- sure	Criterion from Bond et al., 1986--ave. venous blood Butadiene conc. at 2, 4, and 6 hours of exposure (see Table 4.3) (umoles/liter)	Criterion from Kreiling et al., 1986--Micromoles metabolized in the 6th hour of exposure (see Text)
<u>Target Criteria</u>				
		74.7	.43	.60
		958	6	
<u>Results of Model Simulations</u>				
.2552	2×10^{-5}	74.7	.227	.528
		958	3.76	
.35	5×10^{-6}	74.7	.227	.747
		958	Not done	
.35	2×10^{-5}	74.7	.35	.627
		958	6.15	
.5	4×10^{-5}	74.7	.738	.614
		958	11.92	

(1986) experiments? Table 4.8 makes this comparison for our "best estimate" rat and mouse models.

Because of the variability in the underlying experimental data, clearly one should not try to make too much of these numbers. However, it can be seen that our predicted loss of metabolites during the six hour exposure is, in general, quite significant. Moreover the loss appears to change appreciably with exposure level. This suggests that some process leading to retention of butadiene metabolites (perhaps reaction with glutathione) is approaching saturation at the higher exposure levels. The data in Table 4.2 suggest further that the less saturable process that consumes more butadiene metabolites at high exposures may lead to excretion in the form of CO_2 .

One final comparison with the Bond et al. (1986) observations should be noted. If Bond et al. (1986) succeeded in capturing essentially all exhaled butadiene in their fraction labelled "Butadiene and other metabolites" in Table 4.2, then these data should form a set of maximum values for the exhalation of unchanged butadiene after the end of the 6 hour exposures. Table 4.9 compares our "best estimate" model predictions of butadiene exhalation with these data.

It can be seen that there is an apparent two-fold discrepancy for the higher exposure level for rats, although all three of the other model predictions fall are within the data. Failing a better explanation, we attribute the one discrepancy to experimental error.

Table 4.8
Comparison of Storage and Total Metabolism Predicted by Models with
Bond et al. (1986) Observations of Net Uptake During 6 Hour Exposures

Exposure Level (ppm)	Observed Net Uptake During 6 hr Exposures (umole/animal +/- std. error)	-----Model Predictions----- Total Metab- olism During 6 hr Exposures (umole/animal)	Butadiene Stored Unchanged at 6 hr (umole/animal)	Indicated* % Metabolized Butadiene Lost During 6 hr Exposure
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Observations in Rats

74.7	17 +/- 2	27.85	3.32	51
958.2	65 +/- 5	348.8	46.5	95

Observations in Mice

74.7	3.2 +/- .1	3.752	.316	23
958.2	19 +/- .5	38.10	4.60	62

*The numbers in this column were calculated by subtracting the stored butadiene (4th column) from Bond's observed total uptake (2nd column), dividing the result by the total metabolism during the 6 hour exposures (3rd column), and subtracting that result from 100%.

Table 4.9
Comparison of Model Predictions of Butadiene Exhalation with Bond et al.'s Observations of Exhalation of "Butadiene and Other Metabolites"

Amounts in Micromoles Per Animal

Exposure	Observed exhalation of "butadiene and other volatile metabolites**"	Model predictions of exhalation of unchanged butadiene
<u>Observations in Rats</u>		
74.7	3 (.3)**	2.61
958	15 (1)	39.0
<u>Observations in Mice</u>		
74.7	.6 (.2)	.288
958	6 (1)	3.84

*Except CO₂.

**The numbers in parentheses are the authors' reported standard errors.

5. CONCLUSIONS AND RISK ASSESSMENT

5.1 Interspecies Comparison of Metabolite Production, and Development of Human Models

There are unfortunately no direct observations of butadiene metabolism in humans. We therefore need to estimate plausible ranges of $V_{\max}$ and K_m from the animal data.

5.1.1 Interspecies Comparison and Projection of Maximum Metabolic Rates for Butadiene

The basic comparison of $V_{\max}$ in rats and mice is already explicit in the data Kreiling et al. (1986) reproduced at the top of page 35. The maximum rate of butadiene metabolism per kilogram of body weight was found to be 220 $\mu\text{moles/kg-hr}$ in rats and 400 $\mu\text{moles/kg-hr}$ in mice. From these data, and our standard body weights for the two species of 400g and 28 g it is a simple matter to calculate an exponent (m) for the allometric scaling equation:

$$V_{\max} = K (\text{body weight})^m$$

On this basis, the m exponent comes out to .775. This is quite close to the value of .75 that would be expected if butadiene metabolism simply scaled with general metabolic rates, like the primary metabolism of ethylene oxide (Hattis, 1987) and a number of other substances (Boxenbaum, 1982). If we use the .775 exponent to project the human

$V_{\max}$ we obtain

$$1.47 \times 10^{-6} (70/.4)^{.775} = 8.05 \times 10^{-5} \text{ moles/minute}$$

(where 1.47×10^{-6} moles/minute is the absolute value of the rat $V_{\max}$). If, on the other hand, we choose to use the .75 exponent--scaling more directly with metabolic rates--then the projection from rat to human $V_{\max}$ is

$$1.47 \times 10^{-6} (70/.4)^{.75} = 6.6 \times 10^{-5} \text{ moles/minute}$$

The indicated range of 6.6 to 8.0×10^{-5} is not very large. In the units used by Kreiling et al. (1986), it corresponds to 56 - 69 $\mu\text{moles/kg-hr.}$

5.1.2 Interspecies Projection of the Michaelis Constant for Butadiene Metabolism

The Michaelis constant (K_m) is the concentration of substrate in the metabolizing tissues that is needed to half saturate the butadiene metabolizing enzyme system (presumably one or more variants of the P-450 group). It is related to the affinity with which the enzyme(s) bind the substrate. Other things being equal, the tighter the binding, the lower the K_m . In the absence of direct information about the affinity of the human enzyme(s), we have no reason to suppose that it binds butadiene with either greater or lesser affinity than the corresponding enzymes in rodent

tissues. In section 4.2 our "best estimates" for K_m in the rat and mouse systems were 5×10^{-6} and 2×10^{-5} moles/liter, respectively (although it should be recalled that the determination of the rat K_m was quite arbitrary, because the models were essentially flow limited--almost any value for K_m under 1×10^{-5} would be in reasonable accord with the observations). For purposes of sensitivity analysis we will construct human models using K_m 's in the range of 5×10^{-6} to 2×10^{-5} representing our "best estimate" values in rats and mice.

5.1.3 Construction of Human Models, and Projection of Butadiene Metabolite Formation for Occupational Exposures in Humans

The framework for the human occupational exposure model is borrowed directly from our earlier work on perchlorethylene (Hattis et al., 1986a). Alveolar ventilation and blood flows change according to a diurnal cycle of 16 waking hours and 8 hours of sleep. Alveolar ventilation during the waking hours is assumed to be 11.38 liters/minute--the average observed by Brugnone et al. (1980) in a group of workers doing light work in an artificial leather factory. Exposure occurs during eight of the waking hours, five days per week. The model equations (for the blood/air .35, high-metabolism variant) are presented in Table 5.1.

To assess the sensitivity of our analysis to (1) different values of the enzyme kinetic parameters V_{max} and K_m and (2) different values of the blood/air partition coefficient, we have explored the implications of three different human model variants. Using our best estimate for the blood/air partition coefficient of .35 there are two variants:

Table 5.1

Equations for the Best-Estimate Human Model (High Metabolism Variant)

- ☐ $FG = FG + FG_PERFUSION - FG_ELIMINATION$
INIT(FG) = 0
- ☐ $LVRG = LVRG + LVRG_PERFUSION - LVRG_ELIMINATION -$
LIVER_METABOLISM
INIT(LVRG) = 0
- ☐ $MG = MG + MG_PERFUSION - MG_ELIMINATION$
INIT(MG) = 0
- ☐ $RECOVERY = RECOVERY + EXHALATION$
INIT(RECOVERY) = 0
- ☐ $TOTAL_ABSORBED = TOTAL_ABSORBED + FLOW_RATE$
INIT(TOTAL_ABSORBED) = 0
- ☐ $TOTAL_METABOLISM = TOTAL_METABOLISM + LIVER_METABOLISM$
INIT(TOTAL_METABOLISM) = 0
- ☐ $ARTERIAL_CONC = BLOOD_AIR * (CARDIAC_OUTPUT * MIXED_VENOUS_CONC$
 $V_ALV * (EXPOSURE * 10^{(-6)}/25.45))$
 $/ (CARDIAC_OUTPUT * BLOOD_AIR + V_ALV)$ (moles/liter)
- ☐ $AWAKE_TIME = 960$ (minutes)
- ☐ $BLOOD_AIR = .35$
- ☐ $CARDIAC_OUTPUT = LVRG_FLOW + FG_FLOW + MG_FLOW$
- ☐ $DAY = DAY1_5 + RESTDAY + DAY9_12 - 1$
- ☐ $DAY1_5 = IF (TIME \geq 0) AND (TIME < 1440) THEN 1 ELSE IF (TIME \geq 1440) AND ($
 $TIME < 2880) THEN 2 ELSE IF (TIME \geq 2880) AND (TIME < 4320) THEN 3 ELSE$
 $IF (TIME \geq 4320) AND (TIME < 5760) THEN 4 ELSE IF (TIME \geq 5760) AND (TIME <$
 $7200) THEN 5 ELSE 0$
- ☐ $DAY9_12 = IF (TIME \geq 11520) AND (TIME < 12960) THEN 9 ELSE IF (TIME \geq$
 $12960) AND (TIME < 14400) THEN 10 ELSE IF (TIME \geq 14400) AND (TIME <$
 $15840) THEN 11 ELSE IF (TIME \geq 15840) AND (TIME < 17280) THEN 12 ELSE 0$
- ☐ $EXHALATION = IF (NET_MOLES < 0)$
 $THEN NET_MOLES * (-1) ELSE 0$
- ☐ $EXPOSURE = IF (DAY * 24 * 60 \leq TIME) AND (TIME < DAY * 24 * 60 + EXP_TIME)$
 $AND (DAY \leq 4) THEN 10 ELSE IF (DAY * 24 * 60 \leq TIME) AND (TIME < DAY * 24 *$
 $60 + EXP_TIME) AND (DAY \geq 7) AND (DAY < 12) THEN 10 ELSE 0$ (ppm)
- ☐ $EXP_TIME = 480$ (minutes)
- ☐ $FG_ELIMINATION =$
 $(FG * FG_FLOW) / (15.024 * 118.2)$
(moles / minute)
- ☐ $FG_FLOW = IF (DAY * 24 * 60 \leq TIME) AND$
 $(TIME < DAY * 24 * 60 + AWAKE_TIME)$
 $THEN .69 ELSE .35$
- ☐ $FG_PERFUSION = ARTERIAL_CONC * FG_FLOW$
(moles / minute)
- ☐ $FLOW_RATE = IF (EXPOSURE > 0) THEN NET_MOLES ELSE 0$
- ☐ $FRACT_ABSORB =$
 $NET_MOLES / (V_ALV * (EXPOSURE * (10^{(-6)}) / 25.45))$
- ☐ $LIVER_METABOLISM = (8 * 10^{(-5)} * (LVRG/8.513)) /$
 $(.5 * 10^{(-5)} + (LVRG/8.513))$
(moles/minute)

Table 5.1 (continued)

- $LVRG_ELIMINATION = (LVRG * LVRG_FLOW) / (8.513 * 5.4)$
{moles/minute}
- $LVRG_FLOW = IF (DAY * 24 * 60 \leq TIME) AND (TIME < DAY * 24 * 60 + AWAKE_TIME)$
THEN 5.094 ELSE 4.35
- $LVRG_PERFUSION = ARTERIAL_CONC * LVRG_FLOW$ {moles/minute}
- $MG_ELIMINATION = (MG * MG_FLOW) / (34.756 * 5.26)$ {moles / minute}
- $MG_FLOW = IF (DAY * 24 * 60 \leq TIME) AND (TIME < DAY * 24 * 60 + AWAKE_TIME)$
THEN 2.61 ELSE 1.1
- $MG_PERFUSION = ARTERIAL_CONC * MG_FLOW$
{moles / minute}
- $MIXED_VENOUS_CONC = (FG_ELIMINATION + MG_ELIMINATION + LVRG_ELIMINATION) /$
CARDIAC_OUTPUT
- $NET_MOLES = CARDIAC_OUTPUT * (ARTERIAL_CONC - MIXED_VENOUS_CONC)$
{moles/minute}
- $PPM_EXHALED = IF (NET_MOLES < 0) THEN (NET_MOLES * (-1) / V_ALV) * 25.45 * 10^6$
ELSE 0
- $RESTDAY = IF (TIME \geq 7200) AND (TIME < 8640) THEN 6 ELSE IF (TIME \geq 8640)$
AND $(TIME < 10080)$ THEN 7 ELSE IF $(TIME \geq 10080) AND (TIME < 11520)$ THEN
8 ELSE IF $(TIME \geq 17280) AND (TIME < 18720)$ THEN 13 ELSE IF $(TIME \geq 18720)$
THEN 14 ELSE 0
- $V_ALV = IF (DAY * 24 * 60 \leq TIME) AND (TIME < DAY * 24 * 60 + AWAKE_TIME)$
THEN 11.38 ELSE 4.8

- o A Low Metabolism variant uses the low end of the estimated range of $V_{\max}$ (6.6×10^{-5} moles/min.) and the high end of the estimated range of K_m (2×10^{-5} moles/liter. (It should be noted that at low doses, the rate of butadiene metabolism is directly related to the ratio of $V_{\max}$ to K_m . Therefore using a low estimate of $V_{\max}$ and a high estimate of K_m provides a particularly low estimate of low-dose metabolism, as constrained by other characteristics of the model)).
- o A High Metabolism variant similarly uses the high end of the estimated range of $V_{\max}$ (8×10^{-5} moles/min.) and the low end of the estimated range of K_m (5×10^{-6} moles/liter).

A third model, based on a blood/air partition coefficient of .2552, uses a $V_{\max}$ of 8×10^{-5} and a K_m of 1×10^{-5} (see p. 40 above for background discussion).

Table 5.2 shows the expected weekly production of butadiene metabolites for various doses using the different model variants.

The first conclusion from the data in the upper portion of Table 5.2 is that there is surprisingly little difference between the butadiene metabolism predicted by our "high metabolism" and "low metabolism" model variants. The more than fourfold difference in the ratio of $V_{\max}$ to K_m between the model variants produces only about a 20% difference in metabolite formation at low (non-saturating) doses. This is basically because, as was true in rats, the rate of metabolite formation is substantially limited by the flow of blood to the liver/vessel rich group. At low doses, we can calculate the rate constants for metabolism of butadiene vs elimination of butadiene with venous blood flowing out of the metabolizing compartment as follows:

Table 5.2
Butadiene Metabolism Indicated by the Human Model Variants
8 Hour/Day, 40 Hour/Week Occupational Exposures

Exposure Level (ppm)	umoles butadiene metabolized per day* "Low Metabolism" Model**	"High Metabolism" Model***	Average
<u>Human Models Based on a Blood/Air Partition Coefficient of .35</u>			
1	17.0	20.1	18.6
10	170	201	186
100	1,670	2,000	1,840
250	4,080	4,970	4,520
500	7,780	9,800	8,790
1000	13,600	18,500	16,000
<u>Human Model Based on a Blood/Air Partition Coefficient of .2552****</u>			
1	15.0		
10	150		
100	1,490		
1000	14,100		

*All data shown are 7-day averages based on simulation of 5 days of 8 hour exposure at the stated air level and 2 days of no exposure. A small amount of butadiene remaining stored at the end of the 2 days of no exposure was allocated to metabolized and exhaled fractions in proportion to the the total amounts that were metabolized and exhaled following exposure during the week.

** $V_{max} = 6.6 \times 10^{-5}$, $K_m = 2 \times 10^{-5}$

*** $V_{max} = 8 \times 10^{-5}$, $K_m = 5 \times 10^{-6}$

**** $V_{max} = 8 \times 10^{-5}$, $K_m = 1 \times 10^{-5}$

Model	Metabolism Rate $V_{max}/(K_m * Vol.)$	Rate of Elimination with with Venous Blood $Q/(Vol.*L_{Tissue/bl})$	Extraction Ratio
Blood/air=.35 Low Metabol.	.388	.111	.778
Blood/air=.35 High Metabol.	1.88	.111	.944
Blood/air=.2552	.940	.111	.894

Where Q is the blood flow to the tissue (5.1 liters per minute during waking hours), "Vol." is the tissue volume (8.5 liters), and $L_{tissue/blood}$ is the tissue/blood partition coefficient (5.4). The extraction ratio in the final column is the fraction of butadiene entering the compartment that is metabolized--that is, $K_{metabolism}/(K_{metabolism} + K_{blood\ elimination})$. It can be seen that even for the "low metabolism" variant, nearly 78% of the material entering the liver/vessel rich group is metabolized. Starting from this high level, the "high metabolism" variant has relatively little room to increase metabolite formation. Therefore in our further calculations, we will simply use the average numbers in the final column of table 5.2 to represent the metabolite formation for our best estimate blood/air partition coefficient of .35.

A second conclusion from Table 5.2 is that non-linearities in metabolism can be essentially ignored below about 100 ppm. Third, the effect of the lower blood/air partition coefficient (.2552) is to somewhat reduce expected metabolism. This is because there is somewhat less tendency for butadiene to be absorbed, and once absorbed, there is a somewhat greater tendency for butadiene to be exhaled unchanged before it can be metabolized.

A final conclusion that is not obvious from the numbers presented so far is that the models indicate that human absorption and metabolite formation from butadiene at low doses is likely to be less than was assumed by both the EPA (1985) risk analysis and the risk analysis that has been done for the affected industry (ENVIRON, 1986). At the 1 ppm exposure level, the net absorption for each day's eight hour exposure for the three models represented in Table 5.2 was 32.1, 27.9 and 24.5 umoles*. At an alveolar ventilation rate of 11.38 liters/minute, and a corresponding total ventilation of 16.25 liters/minute, the indicated daily absorption represents 11-15% of the butadiene reaching the alveoli, and 8-10.5% of total inhaled butadiene**. By contrast, for both mice and humans EPA (1985) assumed that 54% of total inhaled butadiene would be absorbed. In projecting human risks for comparison with existing epidemiological data ENVIRON (1986) (p. 94) seems to have made a similar assumption of 50% absorption, based on a somewhat larger total ventilation rate than we use (10 cubic meters per eight hour occupational exposure, corresponding to 20.8 liters/minute total ventilation). Because this factor could give rise to a fivefold difference between our projected human delivered doses and doses used to project risks in earlier analyses, it is worth tracing the source of the earlier assumptions of low dose absorption.

The ENVIRON (1986) assumption of 50% absorption of total inhaled butadiene is not explicitly justified. It is used in a brief 1 paragraph

*Of these average daily absorbed amounts, the three models project that eventually 87.7%, 85.4%, and 85.1% would be converted to butadiene metabolites (data are for the blood/air .35 high metabolism, blood/air .35 low metabolism, and blood/air .2552 model variants, respectively).

**Total inhalation in 8 hours corresponds to $(16.25 \text{ l/min}) \times (10^{-6} \text{ moles/25.45 liters for 1 ppm at } 37^{\circ}\text{C.}) \times (480 \text{ min}) = 306.5 \text{ umoles.}$

"Exposure Assessment" section that appears to be based on standardized assumptions used in the absence of other data.

The EPA (1985) estimate of 54% absorption at low doses was based on the following data as quoted (in their Table 4-3, p. 4-9) from a 1985 quarterly report from the Bond et al. group:

Species	Exposure Concentration ppm	ug/liter	umol Butadiene Inhaled	umol Butadiene Retained	Percent Retained
Rats	70	125	235	16.3	7.1
	930	1,700	3,100	64.7	3.1
	7,100	12,800	17,000	243	1.5
Mice	7	13	1.7	0.9	54
	80	145	34.7	3.2	9.6
	1,040	1,900	435	19.1	4.7

The 54% number appears for the 7 ppm exposure concentration for mice.

Unfortunately, both the numerator of retained butadiene (.9 umole) and the denominator of inhaled butadiene (1.7 umole) for the 54% calculation differ from the version of the table later published by Bond et al. (1986), (from which we derived our Table 4.1). The published version of the Bond et al. (1986) table is reproduced as Table 5.3. It can be seen that even in mice, at the three lowest doses Bond et al. (1986) report no more than about 20% absorption of butadiene. However, for the dose level used for calculation by EPA there is a notation that the data come from three separate experiments. A later table in the Bond et al. (1986) paper, which was the basis of our Table 4.2 (p. 29 above) shows disposition data that do sum approximately to the .9 umoles total absorption reported in the EPA table. Moreover, the other data in this

Table 5.3

RESPIRATORY TRACT RETENTION OF INHALED BUTADIENE IN RATS AND MICE^a

Exposure concentration ($\mu\text{g/l}$)	Total volume inhaled/animal (l) ^b		Butadiene inhaled/animal (μmol)		[¹⁴ C]Butadiene equivalents retained at 6 hr/animal (μmol) ^c		Percentage of inhaled [¹⁴ C]butadiene equivalents retained at 6 hr	
	Rats	Mice	Rats	Mice	Rats	Mice	Rats	Mice
0.14	73 $\pm$ 5	14 $\pm$ 2	0.2 $\pm$ 0.01	0.04 $\pm$ 0.002	0.03 $\pm$ 0.002	0.006 $\pm$ 0.001	17 $\pm$ 2 ^h	16 $\pm$ 1 ^h
1.4	90 $\pm$ 4	12 $\pm$ 2	2 $\pm$ 0.1	0.3 $\pm$ 0.06	0.13 $\pm$ 0.003	0.06 $\pm$ 0.01	6 $\pm$ 0.3 ^g	20 $\pm$ 5
13	83 $\pm$ 7	12 $\pm$ 1 ^d	19 $\pm$ 2	3 $\pm$ 0.4 ^d	0.8 $\pm$ 0.1	0.4 $\pm$ 0.09 ^d	4 $\pm$ 0.5 ^g	20 $\pm$ 5 ^d
130	70 $\pm$ 8 ^e	13 $\pm$ 2	170 $\pm$ 20 ^e	35 $\pm$ 5	17 $\pm$ 2 ^e	3.2 $\pm$ 0.1	8 $\pm$ 1 ^e	8 $\pm$ 0.7
1800	100 $\pm$ 10	12 $\pm$ 2	3,100 $\pm$ 360	440 $\pm$ 80	65 $\pm$ 5	19 $\pm$ 0.5	2.5 $\pm$ 0.2 ^g	4 $\pm$ 0.3
13,000	70 $\pm$ 15	f	17,000 $\pm$ 3100	f	240 $\pm$ 40	f	1.5 $\pm$ 0.1	f

^a Values are the mean $\pm$ SE.

^b Values corrected for rat and mouse body temperature, barometric pressure, inspired gas temperature, and relative humidity.

^c Data obtained from radioanalysis of rats and mice which were maintained in plethysmograph tubes during the 6-hr exposure.

^d Individual values pooled from three separate experiments.

^e Individual values pooled from two separate experiments.

^f Mice were not exposed to this concentration of butadiene.

^g Significantly different ($p < 0.05$) from mice exposed to the same butadiene concentration using Student's *t* test.

^h A linear regression analysis of the log transformed data vs the log of the exposure concentration indicated that there was a significant ($p < 0.001$) concentration-related decrease in the percent retained at 6 hr with increasing exposure concentration.

Source: Bond et al., 1986

Bond et al. (1986) table correspond well with the EPA report.

What seems to have happened is that EPA based its estimate of low dose mouse and human absorption on the results of only one of the three mouse experiments that gave rise to the data reported in Table 5.3. (Perhaps only one of the three experiments had been reported at the time the EPA did its calculations.) In any event, the two other experiments must have shown dramatically less absorption than the experiment used for calculation. The low dose absorption data for rats reported in Table 5.3 also suggest that the experiment used by EPA was aberrant.

5.1.4 Delivered Doses for the Rat and Mouse Carcinogenesis Bioassays

Tables 5.4 and 5.5 show the metabolized doses projected by our rat and mouse models, for the 6 hour/day 5 day/week exposure regimens used in the carcinogenesis bioassays for butadiene (NTP, 1984 and Hazelton Laboratories Europe, 1981, both cited by EPA, 1985). The implications of our "delivered doses" based on the pharmacokinetic analysis for animal carcinogenesis dose response relationships and human risk will be fully explored in Section 5.2. At this point, however it is possible to note the difference between our projected metabolized doses and the 6-hour net absorption data used by EPA (1985) and ENVIRON (1986) in their analyses. The latter are reproduced at the bottoms of Tables 4.4 and 4.5 for comparison. It can be seen that for rats, our best estimates of metabolized doses are 2 - 4.5 times the absorbed doses used by EPA and

Table 5.4
Predictions of Different Rat Models for the Overall Production of
Butadiene Epoxides in the Rat Bioassay

(Alveolar ventilation is .15 l/min and $V_{\max}$ is 1.47×10^{-6} for all runs)

Based on 400 g rats

Blood/Air Partition Coefficient	K_m	exposure (ppm) 6 hr/day	umoles butadiene metabolized per day 5 exposure days	average of 7 days (including weekend)
"Alternative" Model:				
.2552	1×10^{-5}	1000	274.7	196.2
		8000	347.1	247.9
"Best Estimate" Model:				
.35	5×10^{-6}	1000	359.6	256.8
		8000	607.7	434.1
EPA (1985) and ENVIRON (1986) Assumptions for Comparison*:				
		1000	78	55.7
		8000	274	195.7

*The EPA projections based on the Bond et al. (1986) net absorption data were 10.5 mg/kg (195 umol/kg) at the 1000 ppm exposure level and 37.1 mg/kg (685 umol/kg) at the 8000 ppm exposure level. The numbers shown in the table were determined by multiplying the umole/kg values by the .4 kg weight of the rats in our models.

Table 5.5
Predictions of Different Mouse Models for the Overall Production of
Butadiene Epoxides in the Mouse Bioassay

(Alveolar ventilation is .0233 l/min and $V_{\max}$ is 1.87×10^{-7} for all runs)

Based on 28 g mice

Blood/Air Partition Coefficient	K_m	exposure (ppm) 6 hr/day	umoles butadiene metabolized per day 5 exposure days	average of 7 days (including weekend)
"Alternative" Model:				
.2552	2×10^{-5}	625	23.79	16.99
		1250	41.42	29.58
"Best Estimate" Model				
.35	5×10^{-6}	625	28.08	20.06
		1250	45.76	32.68
EPA (1985) and ENVIRON (1986) Assumptions for Comparison*:				
		625	13.3	9.48
		1250	20.1	14.38

*The EPA projections based on the Bond et al. (1986) net absorption data were 25.7 mg/kg (474 umol/kg) at the 625 ppm exposure level and 38.9 mg/kg (719 umol/kg) at the 1250 ppm exposure level. The numbers shown in the table were determined by multiplying the umole/kg values by the .028 kg weight of the rats in our models.

ENVIRON*. For mice, our best estimates of metabolized doses are somewhat over twice the EPA and ENVIRON values. Depending on exactly how they interact with the tumor data in the dose response modeling, these differences could cause our best estimates of the carcinogenic potency of butadiene in animals to be 2-5 fold lower than those derived by the earlier risk assessors. This is in addition to the potential difference of 5-fold or so from the difference in estimated human dosage discussed in Section 5.1.1. These examples of differences in estimated delivered doses illustrate the potential of pharmacokinetic analysis to make testable predictions about parameters that could influence the calculation of expected risks.

5.2 Risk Assessment for Human Occupational Exposures

5.2.1 Controversies Among the Authors of Previous Risk Assessments

[ENVIRON (1986)/International Institute of Synthetic Rubber Producers
(Tasher and Kaufman, 1986ab) vs. EPA (1985)]

As is often the case for risk assessments that are done in the context of serious consideration of regulatory actions, the assessment of butadiene risk by EPA (1985) has been the subject of some dispute. In this subsection we review a number of specific issues that are raised in commentaries by ENVIRON Corporation (1986) and an affected industry association--the International Institute of Synthetic Rubber Producers

*The basic explanation for these differences is that our models indicate substantial loss of metabolized butadiene in the course of the 6 hour Bond et al. (1986) experiments.

(IISRP) (Tasher and Kaufman, 1986a and 1986b). The ENVIRON (1986) report was prepared for the Chemical Manufacturers Association and was submitted to the OSHA hearing record as part of the IISRP's documentation.

5.2.1.1 Choice of Data from Mice vs. Rats vs. Humans for Human Risk Projection

The cancer bioassays done in rats and mice suggest quite different cancer risks. The bioassay done in mice at exposure levels of 625 and 1250 ppm had to be terminated early, at 60-61 weeks because of high tumor-related mortality. In contrast the rat bioassay, conducted at 1000 and 8000 ppm went to completion, and found generally less intense (though still significant) overall tumor responses. The EPA (1985) summarization of these data is reproduced as Tables 5.6 and 5.7. Following its usual procedure for calculating a "conservative" upper-limit estimate of human risk, EPA (1985) based its risk projection on data from the most sensitive sex and species--male mice in this case.

IISRP Arguments

The IISRP documents are written in legal language and are clearly intended to lay the groundwork for possible future judicial review of any standard that may eventually result. The third paragraph of the executive summary of the May, 1986 position paper begins:

Before OSHA can promulgate a new standard dealing with butadiene it must determine that the standard will protect workers from any significant health risk attributed to butadiene exposure and that it

Table 5.6
Mouse Bioassay Results as Summarized by EPA (1985)

TABLE 6-1. SUMMARY OF THE STATISTICALLY SIGNIFICANT INCIDENCE OF TUMORS
IN MICE EXPOSED FOR 60-61 WEEKS TO 1,3-BUTADIENE

Tumor type and site	Sex	Airborne concentration (ppm)		
		0 ^a	625 ^b	1,250 ^b
Hemangiosarcomas (heart)	M	0/50 p=0.032	16/49 p<0.001	7/49 p=0.006
	F	0/49 p=0.001	11/48 p<0.001	18/49 p<0.001
Malignant lymphomas (hematopoietic system)	M	0/50 p<0.001	23/50 p=0.001	29/50 p=0.001
	F	1/50 p<0.006	10/49 p=0.003	10/49 p=0.003
Alveolar/bronchiolar adenoma	M	2/50 p=0.10	12/49 p=0.003	11/49 p=0.007
	F	3/49 p<0.001	9/48 p=0.056	20/49 p<0.001
adenoma/carcinoma	M	2/50 p<0.001	14/49 p<0.001	15/49 p<0.001
	F	3/49 p<0.001	12/48 p=0.010	23/49 p<0.001
Acinar cell carcinoma (mammary)	F	0/50 p=0.007	2/49 p=0.242	6/49 p=0.012
Granulosa cell tumor or carcinoma (ovary)	F	0/49 p<0.001	6/45 p=0.010	13/48 p<0.001
Forestomach (All papilloma and carcinoma)	M	0/49 p=0.354	7/40 p=0.037	1/44 p=0.473
	F	0/49 p<0.001	5/42 p=0.018	10/49 p<0.001
Hepatocellular adenoma	F	0/50 p=0.025	1/47 p=0.485	4/49 p=0.056
	F	0/50 p=0.016	2/47 p=0.232	5/49 p=0.015

^aThe p-values were calculated with the Cochran-Armitage Trend Test.

^bThe o-values were calculated with the Fisher Exact Test.

Table 5.7
Rat Bioassay Results as Summarized by EPA (1985)

TABLE 6-2. SUMMARY OF THE INCIDENCE^a OF TUMORS IN RATS
EXPOSED TO 1,3-BUTADIENE (100 RATS PER SEX PER DOSE GROUP)

Tumor type and site	Sex	Airborne concentration (ppm)		
		0 ^b	1,000 ^c	8,000 ^c
Multiple mammary gland tumors	F	50 p<0.001	79 p<0.001	84 p<0.001
Thyroid follicular (adenoma and carcinoma)	F	0 p<0.001	4 p=0.06	11 p<0.001
Uterine cervical/stromal sarcoma	F	1 p=0.115	4 p=0.184	5 p=0.106
Leydig cell (adenoma and carcinoma)	M	0 p<0.003	3 p=0.12	8 p<0.001
Pancreatic exocrine Carcinoma	M	0	0	1
Adenoma	M	3 p=0.019	1 p=0.879	10 p=0.041
Zymbal gland (carcinoma)	M	0 p=0.384	1 p=0.5	1 p=0.5
	F	0 p=0.037	0	4 p=0.061

^aComplete information on the number of animals examined was not available.

In calculating incidences, it is assumed that 100 animals per sex per group survived the first year and were histologically examined.

^bThe p-values were calculated using the Cochran-Armitage Trend Test.

^cThe p-values were calculated using the Fisher Exact Test.

will be economically and technologically feasible for the affected industry. These determinations must be supported by substantial evidence available to OSHA.

Later on in that paragraph, and in the body of the document, IISRP seems to dispute the propriety of basing human risk projections on the animal data at all, in the light of available data from human epidemiological studies:

EPA's risk assessments were based on long-term animal studies which showed evidence of BD carcinogenicity in rodents, but failed to take into account species differences in response to BD and epidemiologic data which shows no link between BD and human carcinogenicity. EPA failed to recognize that a more reasonable prediction of the actual risk posed to man by BD can be made when all the evidence is conserved as a whole.

The current animal data shows that there are pronounced differences in species response to BD. Long-term inhalation studies of BD using Sprague-Dawley rats and B₆C₃F₁ mice found that BD produced a potent response in the mouse but only a weak carcinogenic effect in rats. Numerous other studies have indicated that the mouse is more sensitive because of high rates of metabolism and production of the ultimate carcinogen, absence of metabolic saturation at doses as high as the MTD, and changing patterns of elimination...

On page 9, the argument continues:

As we will demonstrate below, the evidence of carcinogenicity in animals relied upon by EPA is not sufficient to predict carcinogenicity in humans. Due to differences in species response to BD, the results of rodent bioassays cannot be directly extrapolated to humans. Moreover the results from the rodent studies are in direct conflict with epidemiological studies reflecting the experiences of human populations exposed to BD. We maintain that all of the scientific information should be taken into account in the risk assessment.

The language about "substantial evidence" and taking all available scientific information into account flows directly from the commonly used standard for judicial review of rule-making actions by executive agencies.

To be fair, EPA did consider essentially all of the evidence cited by IISRP in its critique. Most of the evidence did not influence the final potency number because of either (1) perceived technical inadequacies (especailly the human epidemiological information) or (2) because it was not relevant for calculation of EPA's usual "conservative" upper-confidence-limit estimate of human risk.

Our own view is that risk assessors can provide decision-makers with a richer understanding of the uncertainties in risk estimates by deriving "best" or "most likely" estimates of risk in addition to "upper-confidence-limit" or "worst-case" estimates. Providing this additional information does indeed require analysis of all reasonably relevant data, by a range of procedures that reasonably reflect the current state of scientific understanding of cancer mechanisms. In the final risk analysis sections (5.2.2 and 5.2.3) below, we therefore use both the mouse and the rat data in formulating our "best estimates" of human risk. By utilizing delivered dose estimates derived from our pharmacokinetic models, moreover, we explicitly take into account at least the species differences in overall metabolism of butadiene that can be inferred from the Filser and Bolt data. We have already presented the very limited in vitro primate and human data on butadiene metabolism in various tissues (See pp. 6-7 above). We believe that these limited data provide no basis to suppose that projections from animal data in line with species metabolic rates will greatly mislead us in the projection of human risk.

Perhaps the most remarkable technical claim in the IISRP's documents is that a comparison of human risks predicted by EPA from the mouse data with human epidemiological experience indicates that humans are 100 to 1000

times less sensitive to butadiene than mice. We will discuss this in more detail below after presenting some of ENVIRON's views.

ENVIRON (1986) Arguments

ENVIRON (1986) takes positions that are on the whole similar to those of IISRP, although there are some subtle differences, and ENVIRON generally provides more detail and quantification in their arguments. ENVIRON seems to be amenable in principle to using animal data to project human risk, although they have substantial doubts about the suitability of the mouse bioassay as a basis for human risk projection. Their executive summary states:

First, the nature of the mouse response to 1,3-butadiene is likely to be substantially different than humans since it appears to depend on the presence of an endogenous virus present only in mice*. Second, there is evidence that mice metabolize relatively more 1,3-butadiene to putative toxic metabolites than rats and possibly humans. Third, from the data, it appears that the processes involved in the carcinogenic response were saturated at the lower dose making estimation of the dose response curve at low doses very uncertain. Lastly, the mice were exposed for only about one half of their lifetimes. Sole reliance on the mouse bioassay in the assessment of risk of 1,3-butadiene, as was done by the EPA, must therefore be seriously questioned. Instead, an adequate risk assessment for 1,3-butadiene must include a complete evaluation of both the mouse and rat bioassays together with a full description of the

*Environ's contention that the murine leukemia virus somehow interacts in the production of some butadiene-induced tumors may well prove correct. However, just because humans may not have this particular virus does not mean that butadiene could not similarly interact with other viruses that are present in humans. In the absence of good knowledge of the butadiene/virus interaction mechanisms involved, and fairly complete understanding of the relationship of human viruses to the processes causing human tumors, we think it is an open question whether the human response will prove to be more like the rat or mouse responses. Moreover, it is clear from Table 5.6 that lymphoid tumors do not represent the sole tumor site in mice, and in aggregate account for less than half of the total induced tumors.

uncertainties involved in extrapolating them to humans. Further, primary emphasis must be placed on the available epidemiologic data in assessing the existence of a risk to humans.

...
...When extrapolated to low dose levels, mice appear to be at greater risk (by a factor of 5-fold to 40-fold) than rats. Some of this difference (a factor of three-fold to five-fold) may be due to the faster rate of metabolism of 1,3-butadiene to, and higher blood levels of, epoxide derivatives in mice than in rats.

If humans, rats, and mice were at equal risk from equal average lifetime daily doses of retained 1,3-butadiene, the mouse and rat data would predict risks to occupationally-exposed humans that are statistically inconsistent at the 95% level with the results of the available human data, unless human exposure in the past was very much lower than is believed to have been the case. An exception to this is that the slight excesses of lymphopoietic cancer in the Downs et al. (1985) and Meinhardt et al. (1982) studies are numerically, but not biologically, consistent with the excess risk predicted on the basis of the Hazelton rat study. The lack of consistency among the epidemiologic studies, and between rats and mice in the target site affected, lead us to conclude that any apparent numerical consistency between the rat study and some of the epidemiologic studies is spurious.

There are a number of interesting contrasts here. ENVIRON (1986) points to the inconsistency between the EPA projections of human risk based on the mouse data and the negative Matanoski et al. (1982) human epidemiological observations,* and even reproduces the IISRP's table in this regard (Table 5.9). However, ENVIRON does not repeat the claim that this shows humans to be 100 to 1000 times less sensitive than would be indicated by the EPA mouse-based projections. Instead, they imply that projections from the rat data (with potencies reportedly 5 to 40 fold less than those based on the mouse data) would be "numerically" (although not "biologically") consistent with the two equivocal/positive human epidemiological studies (Meinhardt, 1982 and Downs, 1985).

*Matanoski (1982) found 40 non-leukemia lymphopoietic cancer deaths, compared to 47.1 expected from general U.S. population rates among 13,920 male employes at 8 styrene butadiene rubber plants who had worked at least 1 year (See Table 5.8).

Table 5.8
Matanoski et al. (1982) Results As Cited by Tasher and Kaufman, 1986a

SMRs for Specific Cancers for Styrene-Butadiene Rubber Workers

<u>Cause of Death</u>	<u>Observed</u>	<u>Expected</u>	<u>SMR</u>
All Cancer	398	473.8	84
Oral Cancer	4	16.0	25
Esophageal Cancer	14	13.0	108
Stomach Cancer	27	28.4	95
Liver Cancer	9	9.5	95
Pancreatic Cancer	22	25.9	85
Lung Cancer	126	148.2	85
Bone Cancer	2	2.5	79
Bladder Cancer	9	13.2	68
Kidney Cancer	11	11.5	96
Brain Cancer	11	14.1	78
Leukemia	17	18.7	91
Lymphopoietic Cancer	40	47.1	85

Table 5.9

Excess Lymphopoietic Cancer Deaths Predicted Among Study Cohort
of Matanoski et al. 1982 by EPA Modeling of NTP Mouse Data^a
(IISRP 1986)

<u>Assumed Exposure Level (ppm)^b</u>	<u>Extra Deaths Predicted</u>	<u>Total Deaths Predicted</u>	<u>Probability of Observing 40 Deaths Given EPA's Predictions</u>
1	5.5	52.6	0.09
5	27.5	74.6	1.8×10^{-5}
10	54.9	102.0	6.4×10^{-12}
25	137.3	184.4	4.1×10^{-36}

^aAssumes all excess risk to humans is due to lymphopoietic cancer.

^bSince no good estimates of exposure level exist, the effect of assuming different exposure levels is examined.

Source: ENVIRON, 1986

In our view, the implied distinction between "numerical" and "biological" consistency is an evasion that is unworthy of serious consideration. It is well known that the sites where a particular chemical can be observed to induce statistically significant increases in tumors often differs greatly from species to species. And the very rat and mouse data that are being used for the interspecies projections show dramatic differences in the sites involved. So the implication that we should disregard results in a particular cell type in humans if it does not correspond exactly to the transformed cell types observed in animal systems must be regarded as completely specious.

Moreover, the same observation that the tumor sites are quite diverse in both rats and mice, and different between these species, invalidates the comparisons made by IISRP and ENVIRON of animal-based predictions and the Matanosky et al. (1982) results. On what basis do they presume that the total tumor tumor risk seen in animals must be manifested within the single group of non-leukemia lymphopoietic tumors?

As is common in cohort studies of occupational populations, the Matanoski et al. (1982) data displays a "healthy worker effect." The 1995 deaths at the time of the study cutoff represent only 81% of the deaths that would be expected in a random sample of U.S. males of comparable ages. Similarly the 398 total cancer deaths represent only 84% of the cancer deaths that would be expected among the general-population comparison group. The explanations for this are usually considered to be (1) the general population contains people who are already ill and therefore unlikely to be employed in factory jobs requiring the passage of a pre-employment physical, and some continuing exertion, and (2) the general population contains people who are down and out, incapacitated by

alcoholism and other problems, and those who are institutionalized and under treatment for mental and physical conditions, all of whom may have appreciable carcinogenic exposures. In addition, because of the presence of illegal aliens and other groups of people who are difficult to count fully in the decennial census, the true denominator of people at risk of death in the general population is likely to be somewhat greater than the official figures indicate. [The illegal alien population may well be particularly large in the Texas gulf region where the plants studied were located. This may contribute to the difference in local vs. national general population mortality rates that affected the conclusion of the Downs et al. (1985) study]. The existence of the healthy worker effect, and the likelihood that butadiene-induced tumors are dispersed among a number of tumor sites means that quite a large number of butadiene-induced tumors could be concealed and undetectable amid the general deficit of tumors in working, as compared to general, population groups*.

In addition to this, there is one additional problem in the calculations used by ENVIRON (and presumably IISRP, though the latter do not give their detailed calculation methods). To account for the average 18 years of followup in the Matanoski et al. (1982) data, ENVIRON made the assumption that 18/50 of the total tumor deaths should already have

*Quantitatively, if we take the raw Matanoski et al. (1982) data as given, bringing 40 observed lymphopoietic cancers just up to the expectation for the general population of 47 deaths would require an increment of 7 deaths--or about .35% of the total deaths observed in the study. In order to bring the observed total of 398 cancer deaths up to the general population expectation would require about 76 deaths--about 3.8% of the total deaths observed. Further increases would be required, of course, to produce statistically significant excesses of tumors over the general population expectation. This illustrates the insensitivity of existing epidemiological studies for occupational cancer.

occurred by the end of the followup period*. This implies a linear appearance of tumors with time of followup. It is well known, however (e.g. the case of asbestos) that often there is a considerable delay--a decade or more--between the initiation of exposure and the appearance of the first occupationally-induced tumors. Failure of ENVIRON to acknowledge this problem and use a more reasonable model of tumor appearance times in relation to exposure further undermines the validity of their comparisons of expected and observed human tumors.

In any event, because of the differences in animal and human metabolite exposures as a function of external exposure levels (see section 5.1) our own projections of human risk from the animal data are likely to be less than those derived from EPA (1985). This reduces the opportunities for testing these projections with the available human data. To conclude, we believe that the available human data are incomplete enough in followup, have great enough uncertainties in exposure, and pose such serious difficulties for appropriately controlling for the healthy worker effect in doing an appropriate multi-site comparison of the animal risk projections with the human experience, that it is impossible to appreciably narrow the uncertainties in human risk projections with available human data. We therefore believe that it is better for the present to base human risk projections exclusively on the animal results.

*This same rat-data risk calculation of ENVIRON'S (on their page 100) is odd in another way. The expected excess cancer cases are derived by multiplying the individual excess lifetime risk at a particular dose by 18/50 and also by 13,920--the latter number being the total number of workers at risk in the Matanosky study. This seems to imply that 36% (18/50) of both induced cancer deaths, and total deaths from all causes should have occurred by the followup period. In fact, however, it is known that deaths in the cohort were only 1995, or about 14% of the total workers at risk. When applied to the mouse-based risk numbers, this calculation method appears to yield expected numbers of cases that are much larger even than those claimed by IISRP.

5.2.1.2 Detailed Tumor Results in Rats

Another issue raised by ENVIRON (1986) is the accuracy of EPA's summarization of the tumor results found in the study by Hazleton Laboratories (1981). ENVIRON reports results based on its own examination of pathology reports for individual animals. ENVIRON also determined the numbers of animals at risk for specific neoplasms based on the numbers of animals alive at the time the first tumor of each type was observed (this is the procedure usually used by EPA and others for this kind of analysis). Tables 5.10 and 5.11 summarize the differences between the EPA and ENVIRON tumor counts and animals at risk as reported by ENVIRON (1986) for male and female rats, respectively. For the bulk of this, we are inclined to credit ENVIRON's more detailed examination of the data. Although we will do calculations using both summarizations, our best estimates of tumor risk will use the ENVIRON rat figures, with one exception. ENVIRON disputes the appropriateness of EPA's summary category of "multiple mammary gland tumors" and says that EPA does not report the number of malignant mammary carcinomas in female rats. It is the case that EPA's table 6-2 (reproduced as our Table 5-7 on p. 64 above) does not break out the mammary carcinomas. However the text on the page facing EPA's table (p. 6-8) does list the incidence of mammary carcinomas in their reading of the data--reportedly 8% in controls, 42% at 1000 ppm, and 38% at 8,000 ppm. We are reluctant to believe that these data were made up out of whole cloth by EPA, and in this case we will provisionally resolve the discrepancy between EPA and ENVIRON's report in favor of the

Table 5.10

COMPARISON OF TUMORS IN MALE RATS AS
REPORTED BY EPA (1985) AND ENVIRON

EPA (1985) ¹	Exposure Level (ppm)		
	0	1000	8000
Numbers of rats with:			
Leydig cell adenoma/carcinoma*	0	3	8
Pancreatic exocrine adenoma/ adenocarcinoma*	3	1	11
Zymbal gland adenoma/carcinoma	0	1	1
Total number of tumor-bearing animals reported as having any of the above tumors (number in denominator is estimated number of animals at risk):	4/100	4/100	21/100
ENVIRON			
Numbers of rats with:			
Leydig cell adenoma/carcinoma*	0	3	8
Pancreatic exocrine adenoma/ adenocarcinoma*	2	1	11
Zymbal gland adenoma/carcinoma	0	1	2
Total number of tumor-bearing animals having any of the significantly increased tumors above i.e., not including Zymbal gland tumors (number in denominator is number of survivors at time of 1st tumor; pancreatic exocrine adenoma at 78 weeks):	2/87	4/80	18/74
Total number of tumor-bearing animals having any of the above tumors including Zymbal gland tumors (number in denominator is number of survivors at time of 1st tumor; Zymbal gland adenoma at 66 weeks)	2/96	4/96	20/87

¹ Numbers of rats with specific tumors was reported on page 6-9, Table 6-2 of the EPA report (USEPA 1985); total number of tumor bearing animals was reported on page 6-51. No explanation is provided regarding the derivation of the total number of tumor bearing animals.

* Significant difference between control and high exposure group by Fisher Exact test.

Table 5.11

COMPARISON OF TUMORS IN FEMALE RATS AS
REPORTED BY EPA (1985) AND ENVIRON

EPA (1985) ¹	Exposure Level (ppm)		
	0	1000	8000
Numbers of rats with:			
mammary gland carcinoma	NR	NR	NR
multiple mammary gland tumors*	50	79	84
thyroid follicular tumors*	0	4	11
uterine cervical/stromal sarcoma	1	4	5
Zymbal gland tumors	0	0	4
Total number of tumor-bearing animals reported as having either mammary gland carcinomas, thyroid follicular tumors or Zymbal gland tumors (number in denominator is estimated number of animals at risk):	8/100	46/100	50/100
ENVIRON			
Numbers of rats with:			
mammary carcinoma	18	15	26
mammary fibroadenoma*	40	75	67
thyroid follicular adenoma/carcinoma*	0	4	11
uterine/cervical stromal sarcoma*	1	5	7
Zymbal gland squamous carcinoma**	0	0	4
Total number of tumor-bearing animals having any of the significantly increased tumors above except mammary fibroadenomas (number in denominator is number of survivors at time of 1st tumor; uterine/cervical stromal sarcoma at 62 weeks)	1/94	8/95	21/92
Total number of tumor-bearing animals having any of the significantly increased tumors above including mammary fibroadenomas (number in denominator is number of survivors at time of 1st tumor; mammary fibroadenoma at 56 weeks)	41/99	77/97	72/96

¹ Numbers of rats with specific tumors was reported on page 6-9, Table 6-2 of the EPA report (USEPA 1985); total number of tumor bearing animals was reported on page 6-51. No explanation is provided regarding the derivation of the total number of tumor bearing animals.

NR - Not reported

* Significant difference between control and high exposure group by Fisher Exact test ($p < 0.05$)

** Nearly significant ($p = 0.055$)

Source: ENVIRON, 1986

EPA data.

5.2.1.3 Choice of Dose Response Model

ENVIRON (1986) uses both the Weibul and probit models to make tumor risk calculations in addition to the conventional multi-stage model used by EPA. The probit model is based on an assumption that population dose response relationships result from differences in individual thresholds for cancer. As we have discussed at length elsewhere (Hattis and Smith, 1986) the notion of individual thresholds for genetically-acting carcinogens is inconsistent with applicable theory for the biological mechanisms of cancer and relevant pharmacokinetics. This model has no place in a respectable analysis of cancer risks for a mutagenic agent. The Weibul model is without significant biological justification, and in our view, should not be used when a model with some reasonable basis in cancer mechanisms (the multistage) is available.

ENVIRON (1986) also faults EPA for making a relatively crude adjustment of tumor risk for the less-than-lifetime period of exposure and observation in the mouse NTP study. Because of high tumor-related mortality, the experiment was terminated at 60 weeks for male mice and at 61 weeks in female mice. EPA (1985) adjusted their risk projections assuming that tumors appear at a rate proportional to the third power of age, leading to a $(104/60)^3 = 5.21$ fold adjustment for male mice and a $(104/61)^3 = 4.96$ fold adjustment for female mice.

ENVIRON (1986) suggests using the Hartley-Sielken general product model, where $P(T,d)$ [the probability of tumor appearance as a function of time

(age) and dose (d) is equal to

$$1 - e^{-[a_0 + a_1 d + \dots + a_k d^k][b_1 T + \dots + b_m T^m]}$$

This is quite similar to the multi-stage model used by EPA, with the exception of the addition of the "b" terms. Fitting the latter requires detailed time-of-appearance data for specific tumors, which are not reproduced in either the EPA (1985) or ENVIRON (1986) reports. The EPA correction appears similar to what would be obtained in the Hartley-Sielkin model if m were set at 3 and if b₁ and b₂ were zero. Calculations reported by ENVIRON (1986) (on their page 86) indicate about a 2-fold difference between risk estimates using m = 2 and m = 3. In the absence of other information, we will use the EPA procedure for adjustment for less-than-lifetime risks.

5.2.2 Measures of "Dose" and Dose Response Relationships for Carcinogenesis in Animals

Tables 5.12 through 5.26 show the results of using our different estimates of "dose" along with the various tumor response data in the standard "Global82" multistage carcinogenesis modeling program (Howe and Crump, 1982). This program calculates maximum likelihood estimates (MLE) for the "q" coefficients in the equation:

$$P(d) = 1 - e^{-(q_0 + q_1 d + q_2 d^2 + \dots + q_k d^k)}$$

Table 5.12
Dose Response Models for Leydig Cell Adenomas and Carcinomas in Male Rats

Expression for "Dose"	Model Coefficients			Measures of Model Fit		
	q0	q1	q2	"chi ² "	degrees freedom	p*
<u>EPA Denominators for Tumor Data</u>						
EPA "mg/kg internal dose" (from retention in Bond expts.)	MLE**	0	.00335	0	.14	1 over .25
	UCL***	0	.00530	-		
umol metab./rat blood/air = .2552	MLE	0	0	1.14E-6	.65	1 over .25
	UCL	0	3.78E-4	-		
umol metab./rat blood/air = .35	MLE	0	1.22E-5	4.14E-7	No test possible--no degrees of freedom	
	UCL	0	2.48E-4	-		

<u>Environ Denominators for Tumor Data</u>						
EPA "mg/kg internal dose" (from retention in Bond expts.)	MLE	0	.00450	0	.06	1 over .25
	UCL	0	.00712	-		
umol metab./rat blood/air = .2552	MLE	0	0	1.50E-6	.89	1 over .25
	UCL	0	4.93E-4	-		
umol metab./rat blood/air = .35	MLE	0	0	5.99E-7	.05	1 over .25
	UCL	0	3.22E-4	-		

*For notes, see p. 94

Table 5.13
Dose Response Models for "Pancreatic Exocrine Adenomas and Carcinomas
In Male Rats

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	P*
<u>EPA Tumor Counts and Denominators</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	.019	0	1.30E-4	1.70	1	.1 - .25
	UCL	.018	.00356	-			
umol metab./rat blood/air = .2552	MLE	.023	0	8.51E-7	6.11	1	.01 - .025
	UCL	.019	2.97E-4	-			
umol metab./rat blood/air = .35	MLE	.020	0	3.74E-7	3.94	1	.025 - .05
	UCL	.020	1.60E-4	-			
<u>Environ Tumor Counts and Denominators</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	.016	0	1.96E-4	.92	1	over .25
	UCL	.014	.00477	-			
umol metab./rat blood/air = .2552	MLE	.018	0	1.34E-6	6.35	1	.01 - .025
	UCL	.015	4.16E-4	-			
umol metab./rat blood/air = .35	MLE	.016	0	5.67E-7	3.61	1	.05 - .1
	UCL	.016	2.03E-4	-			

*For notes, see p. 94

Table 5.14
Dose Response Models for Thyroid Adenomas and Carcinomas in Female Rats

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	p*
<u>EPA Denominators for Tumor Data</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	.00464	0	.13	1	over .25
	UCL	0	.00689	-			
umol metab./rat blood/air = .2552	MLE	0	0	1.57E-6	1.02	1	over .25
	UCL	0	4.80E-4	-			
umol metab./rat blood/air = .35	MLE	0	3.87E-7	6.18E-7	No test possible--No degrees of freedom		
	UCL	0	3.16E-4				
<u>Environ Denominators for Tumor Data</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	.00502	0	.09	1	over .25
	UCL	0	.00747	-			
umol metab./rat blood/air = .2552	MLE	0	0	1.69E-6	1.16	1	over .25
	UCL	0	5.13E-4	0			
umol metab./rat blood/air = .35	MLE	0	0	6.69E-7	.04	1	over .25
	UCL	0	3.37E-4	-			

*For notes, see p. 94

Table 5.15
Dose Response Models for Uterine Sarcomas in Female Rats

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	P*
<u>EPA Tumor Counts and Denominators</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	.015	.00167	0	.88	1	over .25
	UCL	.0074	.00371	-			
umol metab./rat blood/air = .2552	MLE	.010	1.20E-4	1.84E-7	No test possible--no degrees of freedom		
	UCL	.0057	3.09E-4	-			
umol metab./rat blood/air = .35	MLE	.010	1.02E-4	0	.07	1	over .25
	UCL	.0059	1.97E-4	-			
<u>Environ Tumor Counts and Denominators</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	.016	.00285	0	1.00	1	over .25
	UCL	.0081	.00532	-			
umol metab./rat blood/air = .2552	MLE	.011	1.22E-5	1.06E-6	No test possible--no degrees of freedom		
	UCL	.0064	4.25E-4				
umol metab./rat blood/air = .35	MLE	.011	1.61E-4	0	.09	1	over .25
	UCL	.0065	2.75E-4	-			

*For notes, see p. 94

Table 5.16
Dose Response Models for Zymbal Gland Tumors in Female Rats

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	p*
<u>EPA Denominators for Tumor Data</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	0	5.37E-5	.33	1	over .25
	UCL	0	.00201	-			
umol metab./rat blood/air = .2552	MLE	0	0	4.05E-7	2.56	1	.1 - .25
	UCL	0	1.70E-4	-			
umol metab./rat blood/air = .35	MLE	0	0	1.60E-7	1.43	1	.1 - .25
	UCL	0	1.00E-4	-			
<u>Environ Denominators for Tumor Data</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	0	5.84E-5	.34	1	over .25
	UCL	0	.00216	-			
umol metab./rat blood/air = .2552	MLE	0	0	4.35E-7	2.65	1	.1 - .25
	UCL	0	1.81E-4	-			
umol metab./rat blood/air = .35	MLE	0	0	1.72E-7	1.48	1	.1 - .25
	UCL	0	1.07E-4	-			

*For notes, see p. 94

Table 5.17
Dose Response Models for Mammary Carcinomas in Female Rats

Expression for "Dose"	Model Coefficients			Measures of Model Fit		
	q0	q1	q2	"chi ² "	degrees freedom	p*
<u>EPA Denominators for Tumor Data</u>						
EPA "mg/kg internal dose" (from retention in Bond expts.)	MLE	.162	.0175	0	21.2	1 under .001
	UCL	.119	.0250	-		

rerun, excluding high dose point because of poor fit:

	MLE	.083	.0615	-	No test possible--No	
	UCL	.072	.0827	-	degrees of freedom	
umol metab./rat blood/air = .2552	MLE	.086	.00190	0	2.08	1 .1 - .25
	UCL	.068	.00240	-		
umol metab./rat blood/air = .35	MLE	.0946	.00118	0	6.22	1 .01 - .025
	UCL	.0735	.00151	-		

rerun, excluding high dose point because of poor fit

	MLE	.0834	.00179	-	No test possible--No	
	UCL	.0715	.00242	-	degrees of freedom	

Environ Denominators for Tumor Data

(Not done--Environ considered that the tumor results at this site did not indicate a statistically significant increase when malignant and benign tumors were separated)

*For notes, see p. 94

Table 5.18
Dose Response Models for "Total Significant Tumors" in Rats
(EPA Compilation of Tumor Data)

Expression for "Dose"		Model	Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	P*	
<u>Males</u>								
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	.035	0	2.81E-4	.29	1	over .5	
	UCL	.028	7.05E-3	-				
umol metab./rat blood/air = .2552	MLE	.033	0	2.12E-5	7.77	1	under .01	
	UCL	.032	5.05E-4	-				
umol metab./rat blood/air = .35	MLE	.030	0	8.76E-6	3.63	1	.05 - .10	
	UCL	.029	2.26E-4	-				
<u>Females</u>								
EPA "mg/kg internal dose" (from reten- tion in Bond expts.) experiments)	MLE	.140	2.84E-2	0	18.3	1	under .001	
	UCL	.106	3.69E-2	-				
umol metab./rat blood/air = .2552	MLE	.084	2.57E-3	0	.17	1	over .5	
	UCL	.069	3.14E-3	-				
umol metab./rat blood/air = .35	MLE	.089	1.63E-3	0	2.61	1	.1 - .2	
	UCL	.072	2.01E-3	-				

*For notes, see p.

Table 5.19
Dose Response Models for Hemangiosarcomas of the Heart in Mice

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	P*

Males

(Note: The high dose point was excluded because of poor fit--P less than .01

EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	.0215	-	No test possible--No degrees of freedom
	UCL	0	.0317	-	

umol metab./mouse blood/air = .2552	MLE	0	.0233	-
	UCL	0	.0343	-

umol metab./mouse blood/air = .35	MLE	0	.0197	-
	UCL	0	.0290	-

Females

(The high dose point did not need to be excluded here.)

EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	.00960	2.47E-4	No test possible--No degrees of freedom
	UCL	0	.0206	-	

umol metab./mouse blood/air = .2552	MLE	0	.0151	1.26E-5
	UCL	0	.0207	-

umol metab./mouse blood/air = .35	MLE	0	.0113	8.20E-5
	UCL	0	.0182	

*For notes, see p. 94

Table 5.20
Dose Response Models for Malignant Lymphomas in Mice

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	p*
<u>Males</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	.0322	0	.61	1	over .25
	UCL	0	.0404	-			
umol metab./mouse blood/air = .2552	MLE	0	.0321	0	.55	1	over .25
	UCL	0	.0402	-			
umol metab./mouse blood/air = .35	MLE	0	.0283	0	.26	1	over .25
	UCL	0	.0354	-			
<u>Females</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.) experiments)	MLE	.022	.00891	0	.70	1	over .25
	UCL	.014	.0132	-			
umol metab./mouse blood/air = .2552	MLE	.023	.00874	0	1.26	1	over .25
	UCL	.014	.0130	-			
umol metab./mouse blood/air = .35	MLE	.022	.00776	0	.98	1	over .25
	UCL	.014	.0116	-			

*For notes, see p. 94

Table 5.21
Dose Response Models for Lung Adenomas and Carcinomas in Mice

Expression for "Dose"		Model	Coefficients			Measures of Model Fit		
			q0	q1	q2	"chi ² "	degrees	p*
						freedom		
<u>Males</u>								
EPA "mg/kg internal dose" (from retention in Bond expts.)	MLE	.043	.0133	0	.56	1	over	.25
	UCL	.030	.0188	-				
umol metab./mouse blood/air = .2552	MLE	.045	.0130	0	1.16	1	over	.25
	UCL	.031	.0186	-				
umol metab./mouse blood/air = .35	MLE	.044	.0116	0	.86	1	over	.25
	UCL	.030	.0164	-				
<u>Females</u>								
EPA "mg/kg internal dose" (from retention in Bond expts. experiments)	MLE	.058	0	5.53E-4	.39	1	over	.25
	UCL	.048	.0163	-				
umol metab./mouse blood/air = .2552	MLE	.062	0	5.22E-4	.85	1	over	.25
	UCL	.046	.0170	-				
umol metab./mouse blood/air = .35	MLE	.060	0	4.16E-4	.11	1	over	.25
	UCL	.047	.0146	-				

*For notes, see p. 94

Table 5.22
Dose Response Models for Forestomach Tumors in Mice

Expression for "Dose"	Model Coefficients			Measures of Model Fit	
	q0	q1	q2	"chi ² " degrees freedom	p*

Males

Note: Highest dose point excluded because of poor fit

EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	.0104	-	No test possible--No degrees of freedom
	UCL	0	.0184	-	
umol metab./mouse blood/air = .2552	MLE	0	.0113	-	
	UCL	0	.0199	-	
umol metab./mouse blood/air = .35	MLE	0	.00959	-	
	UCL	0	.0169	-	

Females

Note: The highest dose point did not need to be excluded in this case

EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	.00430	1.41E-4	No test possible--No degrees of freedom
	UCL	0	.0114	-	
umol metab./mouse blood/air = .2552	MLE	0	.00711	2.04E-5	
	UCL	0	.0113	-	
umol metab./mouse blood/air = .35	MLE	0	.00526	5.28E-5	
	UCL	0	.0100	-	

*For notes, see p. 94

Table 5.23
Dose Response Models for Hepatocellular Adenomas and Carcinomas
in Female Mice

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	P*
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	0	1.36E-4	.01	1	over .25
	UCL	0	.00555	-			
umol metab./mouse blood/air = .2552	MLE	0	.00110	8.57E-5	No test possible--No degrees of freedom		
	UCL	0	.00560	-			
umol metab./mouse blood/air = .35	MLE	0	3.79E-4	8.92E-5	No test possible--No degrees of freedom		
	UCL	0	.00491	-			

*For notes, see p. 94

Table 5.24
Dose Response Models for Mammary Acinar Cell Carcinomas
in Female Mice

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	P*
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	0	1.54E-4	.15	1	over .25
	UCL	0	.00586	-			
umol metab./mouse blood/air = .2552	MLE	0	0	1.48E-4	.002	1	over .25
	UCL	0	.00592	-			
umol metab./mouse blood/air = .35	MLE	0	0	1.17E-4	.04	1	over .25
	UCL	0	.00517	-			

*For notes, see p. 94

Table 5.25
Dose Response Models for Ovary Granulosa Cell Tumors
in Female Mice

Expression for "Dose"		Model Coefficients			Measures of Model Fit	
		q0	q1	q2	"chi ² " degrees freedom	P*
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	0	7.61E-4	3.81E-4	No test possible--No degrees of freedom	
	UCL	0	.0136	-		
umol metab./mouse blood/air = .2552	MLE	0	.00538	1.79E-4		
	UCL	0	.00138	-		
umol metab./mouse blood/air = .35	MLE	0	.00311	2.00E-4		
	UCL	0	.0121	-		

*For notes, see p. 94

Table 5.26
Dose Response Models for Total Significant Tumors in Mice
(EPA Compilation of Tumor Data)

Expression for "Dose"		Model Coefficients			Measures of Model Fit		
		q0	q1	q2	"chi ² "	degrees freedom	p*
<u>Males</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	.042	.0935	0	1.91	1	.1 - .2
	UCL	.038	.117	-			
umol metab./mouse blood/air = .2552	MLE	.042	.0941	0	3.77	1	.05 - .1
	UCL	.038	.118	-			
umol metab./mouse blood/air = .35	MLE	.042	.0825	0	2.82	1	.05 - .1
	UCL	.038	.103	-			
<u>Females</u>							
EPA "mg/kg internal dose" (from reten- tion in Bond expts.)	MLE	.086	0	.00297	.14	1	over .5
	UCL	.081	.0603	-			
umol metab./mouse blood/air = .2552	MLE	.087	.0212	.00205	No test possible (no degrees of freedom)		
	UCL	.077	.0769	-			
umol metab./mouse blood/air = .35	MLE	.087	.00512	.00211	No test possible (no degrees of freedom)		
	UCL	.080	.0627	-			

*For notes see p. 94

Notes to Tables 5.12 - 5.26

*This is the probability that the data would be expected to depart from the model by as large an amount as was observed purely by chance, on the basis of statistical sampling error, even if the model were a completely accurate description of the underlying dose response relationship. Higher P values indicate better fits than lower P values, although the reader should be cautioned that only very large differences are likely to be "statistically significant" by usual standards. P values below .01 are conventionally taken to indicate an unsatisfactory fit between the model and the data.

**Maximum likelihood estimates.

***Coefficients for calculating the 95% upper confidence limit on induced risk at low doses. To arrive at these numbers a specific low dose was selected--one thousandth of the lowest dose used in the animal bioassay.

Where $P(d)$ is the expected tumor incidence at a particular dose (d). The " q_0 " term determines the background tumor incidence, the " q_1 " determines the size of the linear dose response term, and q_k 's determine the contributions of higher powers of dose. In all cases we have limited the analysis to a second degree polynomial, so we can have only q_0 , q_1 and q_2 . The program also calculates sets of "q" coefficients for predicting upper 95% confidence limits on induced tumor incidence (UCL), based solely on statistical sampling-error uncertainties in the experimental data. All the "q" coefficients are constrained to be positive or zero.

One difference between our dose response modeling procedure and those of EPA (1985) and ENVIRON (1986) should be noted at the outset. It can be seen in the tables that we perform separate model fits for each tumor site within each animal group. By contrast EPA and ENVIRON summed up the tumor results at all sites that showed statistically significant increases in tumor incidence, and fit the multistage model only once for each species and sex. (For purposes of comparison, Tables 5.18 and 5.26 show "total tumors" treatments for the rat and mouse data using our estimates of metabolized doses.)

If one thinks of the multistage model as representing a sequence of somatic mutations within a cell line, it is difficult to understand how mutations in one organ/tissue could possibly be part of the same sequence leading to cancer in another organ/tissue. Therefore we believe it is more appropriate to treat the dose response relationships of specific organs and tissues separately for purposes of analysis, and then add up the overall expected risks from all sites at the end.

Tables 5.27 through 5.29 sum up the "q" coefficients for individual tumor sites for different dose metrics. In left hand portions of 5.28 and 5.29 (for our "metabolized dose" estimates), the "q" coefficients are expressed in the same units as were used in Tables 5.12 through 5.26--the q_1 's are in $(\text{umoles metabolized/animal})^{-1}$ and the q_2 's are in $(\text{umoles metabolized/animal})^{-2}$. The right hand columns normalize the "maximum likelihood" q's for the rat and mouse body weights according to the same procedure that was found to reconcile the best estimates of overall tumor risks per delivered dose among rats and mice for perchloroethylene and ethylene oxide (Hattis et al., 1986; Hattis, 1987). For this purpose the "maximum likelihood estimates" of q_1 are expressed on a metabolic energy density basis $[\text{umoles metabolized}/(\text{body weight})^{.75}]^{-1}$, and correspondingly the "maximum likelihood estimates" of q_2 are expressed in terms of $(\text{metabolic energy density})^2$ --i.e. $[\text{umoles metabolized}/(\text{body weight})^{.75}]^{-2}$ *. In recognition of standard practice for interspecies projection of "upper confidence limit" estimates, the "upper confidence limit" estimates of q_1 are adjusted to a slightly different basis--"body surface area". The units of the UCL q_1 's are therefore $[\text{umoles metabolized}/(\text{body weight})^{2/3}]^{-1}$.

 *For these calculations, we used our standard rat and mouse body weights of .4 and .028 kg, respectively. To obtain the q_1 's in the right half of Tables 5.28 and 5.29, the corresponding q_1 's in the left half were multiplied by (body weight) raised to the appropriate power. Because the q_2 's are in units of $(\text{dose/animal})^{-2}$, however, the appropriate multiplier is $(\text{body weight})^{.75 \times 2}$.

Table 5.27
Overall Carcinogenic Dose Response in Rats and Mice--Expressing "Dose" in
Mg/Kgⁿ Absorption After 6 Hour Exposures (Data of Bond et al., 1986)

Sex, Species and Site	---Risk Coefficients---			Overall "Normalized" Coefficients		
	MLE**	UCL***		MLE	UCL q1	
	q1 Kg/Mg	q2 (Kg/Mg) ²	q1 Kg/Mg	q1 Kg ^{.75} /Mg	q2 (Kg ^{.75} /Mg) ²	q1 Kg ^{2/3} /Mg
<u>Male Rats</u>						
EPA Compilation of Tumor Data:						
Leydig Cell	.00335	0	.00530			
Pancreatic	0	1.30E-4	.00356			
Total	.00335	1.30E-4	.00886	.00421	2.06E-4	.0120
Environ Compilation of Tumor Data:						
Leydig Cell	.00450	0	.00712			
Pancreatic	0	1.96E-4	.00477			
Total	.00450	1.96E-4	.01189	.00566	3.09E-4	.0161
<u>Female Rats</u>						
EPA Compilation of Tumor Data:						
Thyroid	.00464	0	.00689			
Uterine Sarc.	.00167	0	.00371			
Zymbal	0	5.37E-5	.00201			
Mammary Carc.	.0615	0	.0827			
Total	.0678	5.37E-5	.0953	.0852	8.49E-5	.129
Environ Compilation of Tumor Data:						
Thyroid	.00502	0	.00747			
Uterine Sarc.	.00285	0	.00532			
Zymbal	0	5.84E-5	.00216			
Total	.00787	5.84E-5	.01495	.00990	9.23E-5	.0203
+ EPA Mam. C.	.0694	5.84E-5	.0976	.0873	9.23E-5	.132
<u>Male Mice</u>						
Heart Hemang.	.0215	0	.0317			
Malig. Lymph.	.0322	0	.0404			
Lung Ad/Carc.	.0133	0	.0188			
Forestomach	.0104	0	.0184			
Total	.0774	0	.1093			
X 5.21*	.403	0	.569	.985	0	1.87
<u>Female Mice</u>						
Heart Hemang.	.00960	2.47E-4	.0206			
Malig. Lymph.	.00891	0	.0132			
Lung Ad/Carc.	0	5.53E-4	.0163			
Forestomach	.00430	1.41E-4	.0114			
Hepat. Ad/Ca	0	1.36E-4	.00555			
Mammary	0	1.54E-4	.00586			
Ovary	7.61E-4	3.81E-4	.0136			
Total	.0236	.001612	.0865			
X 4.96*	.117	.00800	.429	.286	.0478	1.41

*See p. 100 for notes

Table 5.28

Overall Carcinogenic Dose Response in Rats and Mice--Expressing "Dose" in Umol of Butadiene Metabolites per Animal (or per Body Weightⁿ) Per Day Using The Alternative Models (Blood/Air Partition Coefficient = .2552)

Sex, Species (Umol/Animal) ⁻¹ Coefficients and Site	MLE**			UCL***			Overall "Normalized" Coefficients		
	q1 Kg/Mg	q2 (Kg/Mg) ²	q1 Kg/Mg	q1 Kg/Mg	q2 (Kg/Mg) ²	q1 Kg/Mg	q1 Kg ^{.75} /Mg	q2 (Kg ^{.75} /Mg) ²	UCL q1 Kg ^{2/3} /Mg
Male Rats									
EPA Compilation of Tumor Data:									
Leydig Cell	0	1.14E-6	3.78E-4						
Pancreatic	0	8.51E-7	2.97E-4						
Total	0	1.99E-6	6.75E-4						
Environ Compilation of Tumor Data:									
Leydig Cell	0	1.50E-6	4.93E-4						
Pancreatic	0	1.34E-6	4.16E-4						
Total	0	2.84E-6	9.09E-4				0	7.18E-7	4.93E-4
Female Rats									
EPA Compilation of Tumor Data:									
Thyroid	0	1.57E-6	4.80E-4						
Uterine Sarc.	1.20E-4	1.84E-7	3.09E-4						
Zymbal	0	4.05E-7	1.70E-4						
Mammary Carc.	.00190	0	.00240						
Total	.00203	2.16E-6	.00336						
Environ Compilation of Tumor Data:									
Thyroid	0	1.69E-6	5.13E-4						
Uterine Sarc.	1.22E-5	1.06E-6	4.25E-4						
Zymbal	0	4.35E-7	1.81E-4						
Total	1.22E-5	3.18E-6	.00112						
+ EPA Mam. C.	.00191	3.18E-6	.00352				9.61E-4	8.04E-7	.00191
Male Mice									
Heart Hemang.	.0233	0	.0343						
Malig. Lymph.	.0321	0	.0402						
Lung Ad/Carc.	.0130	0	.0186						
Forestomach	.0113	0	.0199						
Total	.0797	0	.113						
X 5.21*	.415	0	.589				.0284	0	.0543
Female Mice									
Heart Hemang.	.0151	1.26E-5	.0207						
Malig. Lymph.	.00874	0	.0130						
Lung Ad/Carc.	0	5.22E-4	.0170						
Forestomach	.00711	2.04E-5	.0113						
Hepat. Ad/Ca	.00110	8.57E-5	.0056						
Mammary	0	1.48E-4	.0059						
Ovary	.00538	1.79E-4	.0138						
Total	.0374	9.68E-4	.0873						
X 4.96*	.1855	.00480	.433				.0127	2.25E-5	.0399

*See p. 100 for notes

Table 5.29

Overall Carcinogenic Dose Response in Rats and Mice--Expressing "Dose" in Umol of Butadiene Metabolites per Animal (or per Body Weightⁿ) Per Day Using the Best Estimate Models (Blood/Air Partition Coefficient = .35)

Sex, Species (Umol/Animal) ⁻¹ Coefficients and Site	Coefficients			Overall "Normalized" Coefficients		
	MLE**	UCL***		MLE	UCL q1	
	q1 Kg/Mg	q2 (Kg/Mg) ²	q1 Kg/Mg	q1 Kg ^{.75} /Mg	q2 (Kg ^{.75} /Mg) ²	UCL q1 Kg ^{2/3} /Mg
<u>Male Rats</u>						
EPA Compilation of Tumor Data:						
Leydig Cell	1.22E-5	4.14E-7	2.48E-4			
Pancreatic	0	3.74E-7	1.60E-4			
Total	1.22E-5	7.88E-7	4.08E-4			
Environ Compilation of Tumor Data:						
Leydig Cell	0	5.99E-7	3.22E-4			
Pancreatic	0	5.67E-7	2.03E-4			
Total		11.66E-7	5.25E-4	0	2.95E-7	2.21E-4
<u>Female Rats</u>						
EPA Compilation of Tumor Data:						
Thyroid	3.87E-7	6.18E-7	3.16E-4			
Uterine Sarc.	1.02E-4	0	1.97E-4			
Zymbal	0	1.60E-7	1.00E-4			
Mammary Carc.	.00179	0	.00242			
Total	.00189	7.78E-7	.00303			
Environ Compilation of Tumor Data:						
Thyroid	0	6.69E-7	3.37E-4			
Uterine Sarc.	1.61E-4	0	2.75E-4			
Zymbal	0	1.72E-7	1.07E-4			
Total	1.61E-4	8.41E-7	7.19E-4			
+ EPA Mam. C.	.00195	8.41E-7	.00314	9.81E-4	2.13E-7	.00170
<u>Male Mice</u>						
Heart Hemang.	.0197	0	.0290			
Malig. Lymph.	.0283	0	.0354			
Lung Ad/Carc.	.0116	0	.0164			
Forestomach	.0096	0	.0169			
Total	.0692	0	.0977			
X 5.21*	.360	0	.509	.0246	0	.0469
<u>Female Mice</u>						
Heart Hemang.	.0113	8.20E-5	.0182			
Malig. Lymph.	.00776	0	.0116			
Lung Ad/Carc.	0	4.16E-4	.0146			
Forestomach	.00526	5.28E-5	.0100			
Hepat. Ad/Ca	.00038	8.92E-5	.0049			
Mammary	0	1.17E-4	.0052			
Ovary	.00311	2.00E-4	.0121			
Total	.0278	9.57E-4	.0766			
X 4.96*	.138	.00475	.380	.00944	2.22E-5	.0350

*See p. 100 for notes

Notes to Tables 5.27 through 5.29

*The multipliers, 5.21 and 4.96, are a crude adjustment for the short duration of exposure and observation in the mouse experiments--see pp. 77-8 above for discussion

**Maximum likelihood estimates.

***Coefficients for calculating the 95% upper confidence limit on induced risk at low doses. To arrive at these numbers a specific low dose was selected--one thousandth of the lowest dose used in the animal bioassay.

There are a number of interesting results in Tables 5.27-5.29. First, the major significance of the difference between ENVIRON's and EPA's summarization of the rat tumor data results from the mammary carcinomas in female rats. The different data sets lead to similar results for male rats. As discussed previously, our own risk estimates from the rat data will use ENVIRON's data summary, with the addition of the mammary carcinomas from the EPA data. As can be seen in the tables, this generally results in an increase of several fold in the risk coefficients derived for female rats.

Second, if we compare the MLE q_1 's and q_2 's derived from the Bond et al. (1986) absorption results (Table 5.27) with the corresponding results derived using our estimated metabolized doses (Tables 5.28 and 5.29) we find that for the rats, there has been a shift in the direction of q_2 --that is, use of the metabolized doses has resulted in dose response relationships with more upward-turning nonlinearity in the carcinogenesis dose response relationship. For male rats, what were relationships with finite linear and dose² terms in Table 5.27 have been changed to pure dose² relationships in Tables 5.28 and 5.29. This suggests that either there are additional high-dose pharmacokinetic nonlinearities at stages in the transformation or detoxification of the active butadiene metabolites, or that at high doses the cancer responses studied are being appreciably enhanced at more than one mutagenic stage in the multi-step carcinogenic sequence.

Finally, comparing the spread of the final upper-confidence limit q_1 's in the three tables, it can be seen that the pharmacokinetic analysis has not narrowed the apparent interspecies differences in butadiene's carcinogenic potency. The ratio of the highest and lowest

q₁'s in the right hand side of Table 5.29 (with dose defined as butadiene metabolites estimated by our best-estimate model) is somewhat over 200, compared with slightly over 100 in Table 5.27.

5.2.3 Projection to Humans

5.2.3.1 Direct Projections of Human Risks From the Estimated Dose Response Relationships

Tables 5.30 and 5.31 show straightforward projections of human risks for 8 hour/day 5 day/week 10- and 45-year occupational exposures to 1 and 10 ppm butadiene from the relationships for lifetime exposure to butadiene metabolites in animals (Tables 5.28 and 5.29) and the human metabolized doses derived in Table 5.2 (p. 53 above). Aside from the large spread of the risk projections from the different species/sex combinations, one of the most striking implications from these tables is how little difference, in the end, is made by our choice of a blood/air partition coefficient of .35 vs. .2552. The largest differences in risk projections between Tables 5.30 and 5.31 are in the UCL projections for male rats, and even these are less than two-fold. The geometric means of the MLE estimates all differ by less than 20%. Clearly any uncertainties attributable to the pharmacokinetic modeling are trivial compared to the order of magnitude differences in the apparent sensitivities of rats and mice to butadiene metabolites.

Table 5.30
Direct Projections of Human Risks from the Alternative Model
--(Blood/Air Partition Coefficient = .2552)

Probability per Individual of Developing at Least One Additional Cancer

Animal Data Set	--MLE Projections*--		-UCL Projections**-	
	1 ppm	10 ppm	1 ppm	10 ppm
<u>Exposure for 10 Years, 8 hours/day, 5 days/week</u>				
Male Rats	5.61E-9	5.61E-7	6.22E-5	6.22E-4
Female Rats	8.49E-5	8.50E-4	2.41E-4	.00241
Male Mice	.00252	.0249	<u>.00683</u>	<u>.0662***</u>
Female Mice	<u>.00112</u>	<u>.0111</u>	.00502	.0491
Geometric Mean	3.40E-5	6.02E-4		

Exposure Over a 45 Year Working Lifetime, 8 hours/day, 5 days/week

Male Rats	1.14E-7	1.14E-5	2.83E-4	2.83E-3
Female Rats	3.88E-4	3.88E-3	.00109	.0109
Male Mice	.0114	.108	<u>.0307</u>	<u>.268</u>
Female Mice	<u>.00511</u>	<u>.0503</u>	.0226	.204
Geometric Mean	2.25E-4	3.94E-3		

*These are derived from the MLE coefficients on the right side of Table 5.29, assuming equal risk for humans and rodents per umole butadiene metabolized/(kg body weight)^{.75}, and a human daily dosage per ppm of occupational exposure of 15/(70)^{.75} = .62 umole butadiene metabolized/kg^{.75}. Also, to adjust for 10 and 45 year durations of exposure, the q₁'s from Table 5.28 were multiplied by 10/70 or 45/70 and the q₂'s were multiplied by (10/70)² or (45/70)² before calculation of the final projected risks as $1 - e^{-(q_1 d - q_2 d^2)}$.

**Calculations are similar to those for the MLE's, except that in this case we assume equal risks for humans and rodents per umole butadiene metabolized/(kg body weight)^{2/3}, and a human daily dosage per ppm of exposure of 15/(70)^{2/3} = .883 umole butadiene metabolized/kg^{2/3}.

***The underlining signifies the largest of the four sets of UCL values.

Table 5.31
Direct Projections of Human Risks from the Best Estimate Model
--(Blood/Air Partition Coefficient = .35)

Probability per Individual of Developing at Least One Additional Cancer

Animal Data Set	--MLE Projections*--		-UCL Projections**-	
	1 ppm	10 ppm	1 ppm	10 ppm
<u>Exposure for 10 Years, 8 hours/day, 5 days/week</u>				
Male Rats	3.55E-9	3.55E-7	3.46E-5	3.46E-4
Female Rats	1.08E-4	1.08E-3	2.66E-4	.00265
Male Mice	.00270	.0266	<u>.00731</u>	<u>.0707***</u>
Female Mice	<u>.00104</u>	<u>.0103</u>	.00546	.0533
Geometric Mean	3.22E-5	5.69E-4		

Exposure Over a 45 Year Working Lifetime, 8 hours/day, 5 days/week

Male Rats	7.20E-8	7.20E-6	1.55E-4	.00155
Female Rats	4.90E-4	4.90E-3	.00119	.0119
Male Mice	.00947	.0908	<u>.0325</u>	<u>.282</u>
Female Mice	<u>.00471</u>	<u>.0466</u>	.0243	.218
Geometric Mean	1.99E-4	3.50E-3		

*These are derived from the MLE coefficients on the right side of Table 5.29, assuming equal risk for humans and rodents per umole butadiene metabolized/(kg body weight)^{.75}, and a human daily dosage per ppm of occupational exposure of $18.6/(70)^{.75} = .768$ umole butadiene metabolized/kg^{.75}. Also, to adjust for 10 and 45 year durations of exposure, the q1's from Table 5.28 were multiplied by 10/70 or 45/70 and the q2's were multiplied by (10/70)² or (45/70)² before calculation of the final projected risks as $1 - e^{-(q_1d - q_2d^2)}$.

**Calculations are similar to those for the MLE's, except that in this case we assume equal risks for humans and rodents per umole butadiene metabolized/(kg body weight)^{2/3}, and a human daily dosage per ppm of exposure of $18.6/(70)^{2/3} = 1.095$ umole butadiene metabolized/kg^{2/3}.

***The underlining signifies the largest of the four sets of UCL values.

5.2.3.2 Derivation of "Best Estimates" of Human Risk, Considering Possible Interactions of Butadiene-Induced Somatic Mutations with Background Processes Leading to Cancers in the U.S. Population

Ordinarily we would be inclined to treat the geometric mean risk estimates from our best estimate pharmacokinetic model (Table 5.31) as our "best estimates" of human risk at the dose rates shown. However, the fact that one of the four species-sex combinations had a pure quadratic (dose²) MLE relationship means that the geometric mean risk projections have some residual nonlinearity even at the limit of low dosage. If adopted as a "best estimate" without modification, this would violate a basic conclusion from some of our earlier theoretical work (Hattis and Smith, 1986; Hattis, 1982) that deserves recapitulation here:

First, whatever nonlinearities may be produced at high doses by the saturation of enzymes, the saturation of active transport processes, the depletion of cellular reactants for electrophilic agents, or changes in cell division rates to make up for cell killing due to overt toxicity, all of these nonlinearities must necessarily disappear as one approaches very low dose rates (Hattis, 1982). The slope of the line relating ultimate DNA lesions in replicating cells to external dose may well be very different at low doses than at high doses, but it must be linear. The basic reason for this is that at low doses the rates of the transport and transformation processes that lead to DNA damage and repair directly depend on the number of collisions between molecules of an "input" chemical (or activated intermediate or DNA adduct) and a resident cellular reactant (or hole in a membrane or repair enzyme molecule). At low doses the number of resident cellular reactant molecules does not change

appreciably as a function of the concentration of the "input". Therefore the number of relevant collisions and the rates of the reactions and side reactions in the causal sequence at low dosage must be direct linear functions of the amounts of input chemical and its activated derivatives. Some finite fraction of the ultimate DNA lesions must escape repair before the next cell replication so long as the cells affected have a non-zero turnover rate, there are a finite number of repair enzyme molecules, and the repair molecules operate at a finite rate.

There is, of course, a possibility that one could get non-linear behavior extending down to the limits of low dosage if several sequential mutation-like events are required to change a normal cell into a tumor cell. However, in that case the form of the dose response relationship at low doses depends critically on whatever "background" rates of transitions there may be along the carcinogenic sequence that can be contributed to by the added chemical. This is illustrated with a simple numerical example in Table 5.32. It can be inferred that so long as the carcinogenic process induced by the chemical is not totally distinct from the process(es) that causes "background" cancers*, contributions to that background will cause the incremental number of cancers induced in the presence of genetically active chemical to be a simple linear function of the number of mutagenic transitions induced at low levels. The consequence of all of this is that any function that we fit to a set of carcinogenesis data for purposes of deriving best estimates of incremental carcinogenic risk for a genetically-acting carcinogen should have the property that incremental risks are linear for individuals as a function of dose at the limit of low dosage (interindividual variability will in

*Total separation of "background" from "induced" processes seems relatively unlikely, as a general rule.

Table 5.32
The Effect of Background Mutation Rates on the Carcinogenesis
Dose-Response Curve at Low Doses,
Assuming a Two-Stage Carcinogenic Process*

Dose	Rate of 1st Transition	Rate of 2nd Transition	Relative No. Tumors	Induced Excess Over Back- ground
1000	1001	1001	1.002×10^6	1,002,000
100	101	101	1.02×10^4	10,200
10	11	11	1.21×10^2	120
1	2	2	4	3
0.1	1.1	1.1	1.21	.21
0.01	1.01	1.01	1.02	.02
0.001	1.001	1.001	1.002	.002

*If there are background rates for all of the mutagenic steps in the multi-stage process, the response at low doses will tend toward linearity in a way that is not represented in a maximum likelihood fit to the multi-stage model. This table illustrates the simple case of a two-step carcinogenic process where:

--there are background rates of 1 (arbitrary unit) for each transition,

--each of the transitions is induced at a rate of 1 extra unit per unit dose of a carcinogen.

As can be seen in the results, at high doses the number of extra cancers induced falls off with the square of the dose (a ten-fold reduction in dose results in a hundred-fold reduction in induced tumors). However, at low doses the induced tumor excess is linear with dose. A multi-stage fit using high doses would very likely fail to detect a small linear term and would give rise to the incorrect "best estimate" that the number of tumors induced would be dependent on the square of the dose at low doses.

general introduce some upward convexity in population dose/risk curves in the low dose range--see Hattis et al., 1986b).

Given this, how should we modify the MLE risk projection from the male rat data to obtain something closer to a "best" (most likely, or at any rate, least unlikely) estimate? The prime difficulties are that (1) we do not know which tumor sites are likely to be elevated by butadiene in humans, and (2) for the affected sites we do not know the degree to which butadiene induces transitions along the line to cancer that can effectively cooperate with the transitions producing the "background" tumors. As it happens, we can do some alternative calculations based on different possible assumptions on these points.

Table 5.33 shows a listing of the number of reported deaths from malignant neoplasms at different sites among U.S. caucasian males in 1983. It can be seen that nearly 2/3 of the deaths from tumors from well-identified sites were accounted for by just four sites--the lung, prostate, colon and pancreas. These four sites each account for over 1% of total deaths (in other words, they each produce a lifetime mortality risk of approximately .01 or more). If we proceed down the table, it can be seen that 96% of all deaths from identified tumors are produced at sites that account for lifetime cancer risks of over .001.

It therefore seems reasonable to ask what effects would there be from adding the cancer mutations projected for male rats to tumor processes producing a "background" of lifetime cancer deaths in the range of 10^{-3} to 10^{-2} . The results of these calculations are shown in Table 5.34. The outcome is that even if, on average, the butadiene-induced cancer transitions can effectively cooperate with background transitions leading

Table 5.33
Deaths Reported For Various Cancer Sites as a Proportion
of Total Deaths--White Males, 1983

Cause of Death (ICD No.)	Number of Deaths	Proportion of Total Deaths	
All Causes	931,779	1	
All Malignant Neoplasms	208,073	.223	
All Malignant Neoplasms With Specifically Identified Sites:	184,404	.198	Cum. % Identified Neoplasms
Lung, trachea, bronchus (162)	70,598	.0758	38.3
Prostate (185)	20,787	.0223	49.6
Colon (153)	20,228	.0217	60.5
Pancreas (157)	9,749	.0105	65.8
Stomach (151)	6,816	.00731	69.5
Bladder (186)	6,232	.00669	72.9
Brain (191)	4,816	.00517	75.5
Esophagus (150)	4,595	.00493	78.0
Kidney, except pelvis (189.0)	4,527	.00486	80.4
Rectum/anus (154)	4,071	.00437	82.6
Myeloid leukemia (205)	3,609	.00387	84.6
Multiple myeloma (203)	3,049	.00327	86.3
Skin melanoma (172)	3,019	.00324	87.9
Liver/intrahepatic bile ducts (155)	2,977	.00319	89.5
Lymphoid leukemia (204)	2,565	.00275	90.9
Larynx (161)	2,492	.00267	92.2
Gall bladder/extrahepatic bile ducts (156)	2,491	.00267	93.6
Reticulosarcoma (200.0)	1,173	.00126	94.2
Connective/other soft tissue (171)	1,162	.00125	94.9
Skin non-melanoma (173)	1,161	.00125	95.5
Tongue (141)	1,052	.00123	96.0
Lymphosarcoma (200.1)	866	9.29E-4	
Oropharynx (146)	641	6.88E-4	
Bone (170)	542	5.82E-4	
Hypopharynx (148)	419	4.50E-4	
Testis (186)	414	4.44E-4	
Small intestine (152)	396	4.25E-4	
Nasopharynx (147)	367	3.94E-4	
Salivary glands (142)	323	3.47E-4	
Renal pelvis, other urinary (189.1-9)	298	3.20E-4	
Pleura (163)	297	3.19E-4	
Thyroid (193)	285	3.06E-4	
Retroperitoneum and peritoneum (158)	254	2.72E-4	

(continued on the following page)

Table 5.33, continued

Floor of mouth (144)	249	2.67E-4
Suprarenal gland (194.0)	245	2.63E-4
Nasal cavities, sinus, etc. (160)	241	2.59E-4
Breast (175)	226	2.42E-4
Penis, etc (187)	190	2.04E-4
Monocytic leukemia (206)	170	1.82E-4
Mediastinum (164.2-164.9)	136	1.46E-4
Erythrocytic leukemia (207.0)	129	1.38E-4
Eye (190)	126	1.35E-4
Lip (140)	106	1.14E-4
Burkitts tumor (200.2)	98	1.05E-4
Gum (143)	70	7.51E-5
Thymus (164.0)	59	6.33E-5
Megakaryocytic and other specified leukemia (207.2-8)	45	4.83E-5
Heart (164.1)	43	4.61E-5

Malignant Neoplasms With Incomplete Identification of Sites

Other/ill defined sites (195-199)	14,483	.0155
Other lymphoid/histiocytic (202)	4,535	.00486
Leukemia/unspecified (208)	1,943	.00208
Other/ill defined sites within the digestive organs/perit. (159)	898	9.64E-4
Other/ill defined sites within the lip, oral cavity and pharynx (149)	841	9.02E-4
Other/unspec. parts of mouth (145)	657	7.05E-4
Other lymphosarcoma/retic. (200.8)	151	1.62E-4
Other/unspec nervous system (192)	109	1.17E-4
Other/unspec. glands (194.3-9)	46	4.94E-5
Other/unspec. resp. tract (165)	6	6.44E-6

Source: Public Health Service (1987), Vital Statistics of the United States, Volume II--Mortality, Part A, Public Health Service Publication No. 87-1101

Table 5.34
The Effects of Assuming Cooperating Background Lifetime Tumor Risks of 10^{-2} and 10^{-3} on the Incremental Tumors Expected for Butadiene Based on the Male Rat Tumor Data, Assuming A Two-Stage Cancer Process

Exposure Level	Expected complete cancers from butadiene acting alone	Butadiene induced cancer transitions	Incremental cancer risk if cooperating background is 10^{-2} /lifetime**	Incremental cancer risk if cooperating background is 10^{-3} /lifetime***
<u>10 Year Exposures</u>				
1 ppm	3.55E-9	5.96E-5	1.19E-5	3.77E-6
10 ppm	3.55E-7	5.96E-4	1.20E-4	3.80E-5
<u>45 Year Exposures</u>				
1 ppm	7.20E-8	2.68E-4	5.37E-5	1.70E-5
10 ppm	7.20E-6	2.68E-3	5.44E-4	1.77E-4

*The numbers in this column are simply the square roots of the numbers in the previous column. This is because for a two stage process, both of which are significantly enhanced by butadiene, the number of completed tumors depends directly on the product of the lifetime transition rates for the two stages (see Table 5.33 for illustration).

**The numbers in this column are derived by first adding the butadiene-induced transitions from the previous column to .1 (the square root of .01--the lifetime mortality risk from the cooperating "background" cancers). Then the resulting sum of butadiene induced and background transitions is squared to derive a total tumor risk with butadiene exposure. Finally the background risk of .01 (no butadiene exposure) is subtracted to determine the increment to the total risk caused by the extra butadiene-dependent transitions.

***Similar to the previous column, only with the assumed cooperating background of 10^{-3} , the background number of transitions is .0316/lifetime.

to only a small fraction of human tumors, one should expect a dramatically larger incremental number of tumors to result than if the butadiene-induced transitions are totally unique in human carcinogenesis and unable to cooperate with transitions induced by any other agent (compare the last two columns with the second column of Table 5.34).

At the same time, for purposes of our "best estimate" risk calculations, it does not make a great deal of difference whether we choose the 10^{-2} or 10^{-3} assumptions of cooperating background cancer risks. Under the 10^{-2} assumption, the geometric mean MLE risks in Table 5.29 become:

-10 Year Exposures-		-45 Year Exposures-	
1 ppm	10 ppm	1 ppm	10 ppm
2.45E-4	.00244	.00104	.0103

The comparable geometric mean MLE risks in Table 5.29 assuming a cooperating lifetime background cancer risk of 10^{-3} is:

-10 Year Exposures-		-45 Year Exposures-	
1 ppm	10 ppm	1 ppm	10 ppm
1.84E-4	.00183	7.87E-4	.00782

We choose to adopt the latter as our "best estimates" of human cancer risk for butadiene.

For our "plausible upper limit" estimates, we will use the highest of the four sets of upper confidence limit risks projected in Table 5.31:

-10 Year Exposures-		-45 Year Exposures-	
1 ppm	10 ppm	1 ppm	10 ppm
.00731	.0707	.0325	.282

The approximately 50-fold difference between our "best" (least unlikely) and "plausible upper limit" estimates of human risk serve to emphasize the considerable uncertainty in these risk projections. This uncertainty largely results from the differences in sensitivity observed between rats and mice, and our inability to say from first principles which species is likely to provide a better predictor for estimating human risk. As will be discussed later, there is some hope that additional pharmacokinetic modeling of subsequent steps in the transformation, reaction, and detoxification of butadiene metabolites may be able to narrow the uncertainties indicated here, provided that some additional human or primate pharmacokinetic data are also produced. It is our understanding that some relevant studies in this regard are under way at NTP (L. Birnbaum, 1987, personal communication).

5.2.4 Discussion and Comparison With The Results of Earlier Risk Assessments

5.2.4.1 Comparison with EPA (1985) and ENVIRON (1986)

EPA (1985), as is its custom, does not choose to make projections of human risk based on its maximum likelihood fits of the animal data. We can, however compare upper confidence limit projections. Table 5.35 shows the risks that would be calculated from the different data sets for 45 year exposures to 1 ppm. It can be seen that our upper confidence limit projections are about 3-7 times lower than EPA's. This very likely

Table 5.35
Comparison of 95% Upper Confidence Limit Projections From Our
Pharmacokinetic-Based Analysis with Those Derived from EPA (1985)
and ENVIRON (1986)--45 Year 8 Hour/Day Exposure to 1 ppm

Data Set	EPA (1985)*	ENVIRON (1986)**	This Study (Best Estimate Model)
Male Rats	6.43E-4	7.98E-4	1.55E-4
Female Rats	.00853	9.29E-4	.00119
Male Mice	.131	.00644***	.0325
Female Mice	.0665	-	.0243

*Derived from EPA's upper 95% q_1 coefficients after correcting for 45-year, 8 hours/day, 5 days/week exposures.

**ENVIRON's projections have been multiplied by (45/40) and (52/50) to place them on a comparable basis with our assumed 45 year 52 week exposures.

***These calculations excluded malignant lymphomas. Some of the rest of the difference in this line may be accounted for by (1) mg/kg interspecies projection, in contrast to $\text{mg/kg}^{2/3}$ used by ourselves and EPA (1985) and (2) the use by ENVIRON (1986) of the Hartley-Sielken procedure for adjusting for less than lifetime exposure and observation.

results from the difference between our estimates of metabolized dose in animals and people, and those used by EPA (1985). As has been previously discussed, EPA's estimates of animal dose exclude butadiene that was inhaled, metabolized and then lost before the end of the 6-hour exposures of Bond et al. (1986). The underestimation of effective absorption and metabolism in animals has the effect of leading to an overestimation of butadiene's carcinogenic potency in animals. Additionally the formula that was used to estimate human absorption appears to overestimate human absorption and therefore human risk.

ENVIRON (1986) used animal and human absorption assumptions that were comparable to those of EPA (1985). And in the case of male rats, we see a comparably higher estimate of upper-confidence-limit risk than determined from our analysis. For the female rat data, this tendency for ENVIRON (1986) to produce a higher estimate than ours was offset by their exclusion of the data on mammary carcinomas presented by EPA (1985). We chose to follow EPA's analysis in this respect. In the case of male mice, as noted in the table, the differences may result from the use of mg/body weight rather than mg/surface area interspecies projection and the Hartly-Sielken procedure for adjustment for less-than-lifetime exposure and observation of the mice.

5.2.4.2 Comparison of Butadiene's Expected Carcinogenic Potency with Perchloroethylene and Ethylene Oxide

Table 5.36 places our current results in perspective by comparing them with the results of our earlier pharmacokinetic-based risk projections for perchloroethylene (Hattis et al., 1986a) and ethylene oxide (Hattis, 1987). It can be seen that our rather uncertain "best estimate" of human risks from working lifetime exposure to 1 ppm butadiene is comparable to that for similar exposure to perchloroethylene, and about eight times less than that for ethylene oxide. The "plausible upper limit" of estimated risk for butadiene is a little higher than that for ethylene oxide. The wider range between the "best estimate" and "plausible upper limit" risks for butadiene results from the great difference between the responses of rats and mice, even after correction for pharmacokinetic differences up through the first step in butadiene metabolism. No such major interspecies differences were observed for ethylene oxide and perchloroethylene.

5.3 Opportunities For Further Research

Because there are a number of subsequent steps in butadiene metabolism and the detoxification of activated metabolites, there is reason for hope that the differences between mice and rats in susceptibility to butadiene's carcinogenesis could be narrowed by further analysis, using more extensive pharmacokinetic data. To be really helpful, such data should include some observations in workers currently exposed to butadiene.

Table 5.36
"Best Estimates" and "Plausible Upper Limits" for Overall Cancer
Risk Based on Pharmacokinetic-Based Analyses

(Exposure to 1 ppm for 8 Hours/Day, 5 Days/Week for 45 Years)

Probability Per Individual of Developing at Least One Additional Cancer

Chemical	"Best Estimate"	"Plausible Upper Limit"
Ethylene Oxide	.0065	.019
Butadiene	7.9E-4	.032
Perchloroethylene	6.7E-4	.013

One parameter that might be relatively easily measured in workers is the rate of absorption of butadiene from alveolar air. This could be measured by techniques used by Brugnone et al. (1985). At the same time, if it were possible to measure the rate of exhalation of the initial epoxide metabolite of butadiene, this would allow inferences to be made about the rate of metabolism in humans. Alternatively, metabolism data could be collected in vitro--but this would need to be for a wide variety of human tissues, and preferably for at least 10 individuals (in the light of the notorious interindividual variability in metabolic activation--see Hattis et al. 1986b).

Building a full pharmacokinetic model for butadiene in humans would be facilitated if there were direct measurements of the relevant blood/air and (if autopsy material is available) tissue/air partition coefficients. As we found in the current study, however, the modeling does not appear to be very sensitive to reasonable differences in estimates of this set of parameters.

Finally, the existing occupational cohorts of butadiene-exposed workers should continue to be followed for their mortality experience. For this information to have direct bearing on risk analysis, however, much better data is generally needed on past worker exposures. Additionally, controls for the "healthy worker effect" must be done by constructing comparison groups from comparable employed populations without butadiene exposure, rather than either the general U.S. population

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