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Massachusetts Institute of Technology
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REPRODUCTIVE EFFECTS OF GLYCOL
ETHERS IN FEMALES--
A QUANTITATIVE ANALYSIS

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Mary Ballew, M.S.

Environment, Technology and Society Program, Clark University

Dale Hattis, Ph. D.

Center for Technology, Policy and Industrial Development
Massachusetts Institute of Technology

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SUMMARY

This report represents an experimental, pioneering analysis aimed at producing quantitative estimates of risk for female reproductive effects that are more traditionally treated using the NOEL/Uncertainty factor paradigm (USEPA, 1984a,b; Paustenbach, 1988). A parallel analysis of male reproductive effects (Hattis et al, 1988; Hattis and Berg, 1988) was published previously. In creating these analyses, we have not simply applied quantitative dose response models intended to treat carcinogenic risks, because it seems that the mechanisms of effect likely for the reproductive hazards of glycol ethers have little in common with the multiple mutation mechanisms of carcinogenesis. Rather, we have used our best judgment in choosing dose response models and other types of inferences that seem appropriate in the light of the distinctive features of the reproductive effects we studied, and the available data.

Reproductive effects caused by the glycol ethers EGEE and EGME include both a number of quantal effects--fetal mortality, and different types of malformations--and an apparently linear effect on a continuous variable, fetal weight (see Figures 17-19, pp. 68, 70). The fetal weight effect appears to reflect growth retardation in utero and may ultimately be reflected in changes in birth weights, and associated infant mortality in humans (which is very strongly associated with birth weight--see footnote to page 71).

Fundamentally different approaches were used to project risks for the quantal and continuous-variable categories of adverse reproductive effects. Risks for quantal effects were projected assuming a lognormal distribution* of effect thresholds in humans, and a degree of human interindividual variability inferred from experience with the variability of other types of parameters in people.** From the observed changes in the continuous variable, fetal weights, we projected possible effects on human infant mortality, assuming:

* A lognormal population distribution of thresholds would be expected to be produced if the factors causing individual differences in susceptibility are (a) relatively numerous, and (b) tend to act multiplicatively in modifying individual thresholds. While plausible, there is no direct evidence, however, that these assumptions are correct, either for fetotoxic/teratogenic effects in general or glycol ether effects in particular. Nevertheless the assumption of lognormal distributions of thresholds has been used by toxicologists for many years in the form of probit analyses for experimental data on lethality and many other effects (Finney, 1971).

** What was drawn from the animal data in this case was simply the level of exposure, and the percentage of individual animals or litters affected, at data points that showed statistically significant differences from concurrent controls. We did not attempt to infer the degree of interindividual differences to be expected in humans directly from the animal experiments because we believe that humans are likely to have much greater diversity, in both genetic endowments and environmental exposures, relative to experimental animals raised in controlled settings.

- An equal growth-retardation effect in animals and humans for a given rate of glycol ether exposure, expressed in ppm-hours per day,
- That changes in fetal weights observed in animals prior to birth would reflect similar percentage changes in birth weights,
- That agents that retard growth and affect birth weights will have similar effects on human infant mortality as are reflected in the overall associations of U.S. race-specific birth weights and infant mortality (in other words, the effects on birthweight are close enough to the causal pathway for changes in infant mortality, that we can predict the latter from the former), and
- That the pattern of birthweight change produced by glycol ethers in humans will resemble the pattern of change observed between the birth weights of full-term black and white infants--a relatively uniform difference in grams at all percentiles of the birthweight distributions (see Table 4-1, p. 75).

Using this general framework, Table S-1 summarizes the concentrations of EGEE and EGME that we project may cause various reproductive effects at a frequency of one in a million (upper portion of the table) and one in ten thousand (lower portion of the table). It should be emphasized that the risk calculations represented in this table are based on a series of assumptions, and carry considerable uncertainty. In addition to the assumptions mentioned above, the "best estimate" projections (right hand series of numbers) for the quantal reproductive effects (miscarriages and malformations) are based on:

- A litter-based definition of ED₅₀ levels for different quantal reproductive effects in animals.* (Relative to a fetus-based definition of ED₅₀'s, this reduces the doses associated with particular risks of effects by 5-8 fold, depending on the size of the litters in the experimental animals used.) ED₅₀'s were calculated only from individual data points showing statistically significant differences from concurrent controls. Where multiple points were available for specific endpoints, the overall ED₅₀ for each

* A fetus-based ED₅₀ is defined as the exposure level that causes a particular effect in half of the individual fetuses who would not otherwise have been affected. The litter-based ED₅₀, on the other hand, is the exposure level that causes a particular effect in at least one fetus per otherwise unaffected litter. In some cases the authors provided litter-based data that allowed us to calculate litter levels directly. However in other cases, where the data were only given in terms of the frequencies of individual fetuses affected, we calculated approximate litter-based ED₅₀'s from the fetus-based data.

Table S-1.

Summary of Doses of EGME and EGEE that are Projected to be Associated with Different Reproductive Risks in Humans at a Frequency of 10^{-6} or 10^{-4}

NOTE: PLEASE SEE TEXT FOR A SUMMARY OF THE SEVERAL ASSUMPTIONS UNDERLYING EACH OF THESE PROJECTIONS.

Reproductive effect	ppm EGEE lower limit, best est.	ppm EGME lower limit, best est.
<u>Doses Associated with Projected Risks of One in a Million for Each Effect:</u>		
miscarriages	0.00056 to 0.53	0.00026 to 0.067
minor skeletal defects	4.4×10^{-6} to 0.022	----
external malformations	0.0011 to 1.1	---
digit or limb malformations	----	0.00013 to 0.15
total malformations	----	4.6×10^{-5} to 0.042
infant mortality ^b (projected from fetal weight changes)	0.069	0.0085

Doses Associated with Projected Risks of One in Ten Thousand for Each Effect:

miscarriages	0.0061 to 1.8	0.0029 to 0.22
minor skeletal defects	4.8×10^{-5} to 0.073	----
external malformations	0.012 to 3.5	---
digit or limb malformations	----	0.0014 to 0.48
total malformations	----	5×10^{-4} to 0.14
infant mortality ^b (projected from fetal weight changes)	6.8	0.84

^aThe numbers on the left hand side represent the doses associated with the indicated level of effect under a more pessimistic assumption about the degree of interindividual variability in susceptibility of the human population (log probit slope of 1). The numbers on the right hand side represent calculations using our "best-estimate" assumption of the degree of interindividual variability in susceptibility for the quantal reproductive effects (log probit slope of 2).

^bDeath in the first year after birth. In the case of this hypothesized effect, we have only made "best estimate" projections.

species was calculated as the geometric mean of the available estimates, and the overall ED₅₀'s for each endpoint was calculated as the geometric mean of the similar ED₅₀'s calculated for each species.

- o An assumption that on a daily basis particular ppm-hour exposures in humans would pose the same risks as those observed in animals,
- o A log-probit dose response relationship in humans (i. e. human thresholds for effect are lognormally distributed) with a log probit slope of 2 (that is, 95% of humans would have thresholds for effect within a 100-fold range extending from 1/10th of the median threshold to 10 times the median threshold.)
- o No interactions between the processes by which the glycol ethers produce various quantal adverse effects, and the processes that are responsible for producing the "background" incidence of those same effects in humans.

The "plausible lower limit" doses given as the left hand series of numbers in each column of Table S-1 were calculated similarly, but with a different assumption for the degree of interindividual variability in human susceptibility for the quantal adverse reproductive effects of glycol ethers. For these calculations, we assumed a log probit slope of 1 (that is, 95% of humans would have thresholds for effect within a 10000-fold range extending from 1/100th of the median threshold to 100 times the median threshold.) This degree of interindividual variability, which was also used historically in the Mantel-Bryan (1961) "safety" calculations for carcinogens, is analogous to the degree of variability observed for the most variable individual parameters that are putatively related to susceptibility for carcinogenesis (see section 3.1.2, pp. 42-46). Similarly, the "best-estimate" interindividual variability assumption (log probit slope of 2) was drawn from an analogy with the overall degree of interindividual variability that is typically observed in the series of parameters that is likely to be involved in modifying individual susceptibility for carcinogenesis, for chemicals requiring metabolic activation.

After we had selected the log probit slopes of 2 and 1 for our best-estimate and plausible-upper-limit risk calculations respectively, we came across a series of human data that allowed us to calculate the apparent interindividual variability in effect thresholds for a variety of adverse effects of in utero methyl mercury exposure. Generally, the dose response relationships are reasonably well described by an assumption of lognormally-distributed effect thresholds (see Figures 12-16, pp. 51-53) but the data tend to indicate a larger amount of interindividual variability than we have assumed for our "best estimate" calculations. For these human methyl mercury effects, the

indicated log probit slopes range from about 0.85 to 1.8. All, in other words are less than our "best estimate" log probit slope of 2, with several being much closer to our "plausible upper limit" interindividual variability assumption of a log probit slope of 1. These findings, albeit for a completely unrelated fetal toxin, suggest that the risks projected using our "best estimate" assumption of human interindividual variability might eventually turn out to understate the actual risks.

We also did a series of calculations of what would happen to the quantal risks if it turned out that the processes by which glycol ethers caused various affects appreciably interacted with the "background" processes that produce those same effects in the general population. These results are presented in Tables 3-9 and 3-12 on pp. 63 and 66. In general, the effect of an interacting background is to make the dose response relationships for extra effects from glycol ethers linear at low doses, and, in the low dose region, considerably increase the expected frequency of effects.

Approximately 55,000 workers are potentially exposed to EGEE or EGME during the production of semiconductor chips (Ladou 1985). Recent data suggest that geometric mean exposure levels in this industry are 0.36 ppm for EGEE and 0.09 ppm for EGME--with considerable differences in exposure from worker to worker (Paustenbach, 1988). The projections represented in Table S-1 suggest that the risks of female reproductive effects at these exposure levels may be far from negligible, although they will undoubtedly be quite difficult to detect by direct epidemiological studies.*

The ACGIH TLV-TWA's for exposure to EGME and EGEE for an eight-hour workday are currently 5 ppm, and the OSHA standards are even higher at 25 ppm and 200 ppm, respectively. The present standards for allowable occupational exposures to EGEE and EGME clearly pose risks of fetal mortality and birth defects that are quite high (see Tables 3-8, 3-9, 3-11, 3-12 and 4-6, pp. 59, 63, 66 and 81) although fortunately it is likely that few pregnant workers have actually been exposed at these levels.

Several additional sources of uncertainty should be mentioned in connection with the risk estimates, including: 1) uncertainties about experimental data; 2) definitions of health effects; 3) validity of the data collected about background risks; and 4) lack of knowledge about the biological processes causing birth defects, miscarriages and infant mortality. Unfortunately, the researchers in the field have not arrived at clear and consistently applied definitions of what constitutes a

* By showing calculations of the doses associated with 10^{-4} and 10^{-6} risk levels for all of the endpoints in Table S-1, we do not wish to imply either that all the diverse reproductive effects represented in the table should be treated as equivalent for social policy decision-making, or that these specific numerical levels are necessarily those that policy makers will wish to define as the cutoffs for social preventive action. These policy questions are beyond the scope of our technical risk assessment study.

malformation in either humans or animals. The literature on experiments with animals contains such terms as "any skeletal defects", "minor skeletal defects", "major skeletal defects" "total malformations", "external malformations", and others. Seldom were these terms defined in the papers and different authors appear to attach different meanings to the same terms. Also it is uncertain whether the types of defects observed in one species will be reflected in exactly corresponding types of defects in other species. It is conceivable that under different biological processes, exposure to a given chemical could cause forepaw malformations in mice and heart defects in rabbits. The same questions apply when comparing malformations or post-implantation loss in animals to risk calculations for miscarriages and birth defects in humans.

It is also difficult to be sure about the extent of the background risk of miscarriages or birth defects in the human population, and the likelihood that these background processes would interact with the pathological effects of glycol ethers. Miscarriages, especially those occurring in the first three months of pregnancy, are undoubtedly underreported. Many hospitals do not collect information on miscarriages, or if the information is collected, it is not reported in a uniform manner. For this reason, we chose the background risk of miscarriages from a questionnaire administered to women as part of the National Survey of Family Growth conducted by the National Center for Health Statistics (Mosher and Pratt 1982). Again, the same issues arise when considering the background rate of birth defects. Although the standardization of the reporting of birth defects and birth weight has improved greatly compared to practices in the past, much work remains to be done, especially for reporting problems with low birth weight and very low birth weight infants. Even given the uncertainty in the data, when the risk is calculated assuming no interaction with background risks, the risks of malformations and miscarriages remains high. The potential interaction with background rates of miscarriages and birth defects most strongly affects risk at low exposures (0.001 to 0.1 ppm) to EGEE and EGME.

Perhaps the greatest unknown is lack of detailed information on the precise mechanisms by which the glycol ethers affect the developing organism. When these mechanisms are understood it may be possible to produce more quantitative dynamic models of the processes involved, and improved projections of the likely parallel processes that could occur in people.

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1. Introduction

1.1 Goals of the Analysis

This is the sixth in a series of research efforts to develop innovative methodology for risk assessment, based on available mechanistic information on the biological processes involved in producing damage. This report proposes a rationale and methodology for the analysis of the risk to the conceptus or fetus from in-utero exposure to toxic agents.

Ethylene glycol monoethyl ether (EGEE), ethylene glycol monomethyl ether (EGME), and other glycol ethers are widely used as solvents in inks, paints, and varnishes, as well as in the products used to service automobiles (Veulemans et al., 1987; USEPA, 1984). Exposure to glycol ethers produces testicular toxicity and infertility in male animals (Beattie et al., 1984; Chapin and Lamb, 1984; Creasy and Foster, 1984; Creasy et al., 1985; Foster et al., 1984, 1986, 1987; Hardin et al., 1984; Hurtt and Zenick, 1986; Moss et al., 1985; Oudiz and Zenick, 1986) as well as embryotoxicity and developmental anomalies in the offspring of females exposed during pregnancy (Andrew and Hardin, 1987; Doe 1984; Greene 1987; Hanley 1984b; Hardin and Eisenmann, 1987; Wier et al., 1987).

An earlier risk assessment effort of ours (Harris, 1988) dealt with the male fertility effects of EGEE and EGME. In this report we develop and apply a methodology for projecting the risks of a variety of reproductive effects associated with female exposures to these glycol ethers, including embryoletality, developmental anomalies, and reduced birth weight.

1.2 Data Available for Analysis

There is an extensive literature examining the developmental toxicity of glycol ethers (Andrew and Hardin 1984; Doe 1984; Wier et al., 1987; Hardin and Eisenmann 1987; Greene et al., 1987; Hanley et al., 1984b). Unfortunately, although the authors reported many experiments involving an assortment of parameters, we found that only a limited amount of the data would satisfy our requirements for an interpretable index of adverse effect,* and a demonstrated dose-response relationship, or a statistically significant effect due to exposure.

*Some of the data available was combined by the authors in a way that made the meaning of the index of exposure unclear. For example, data on fetal resorptions for dams that died was

One surprising feature of our initial survey of the available data was that, in some cases, a change in a continuous variable--reduction of birth weight--proved to be the most sensitive endpoint indicating glycol ether toxicity. For example, in experiments in mice by Weir et al. (1987) exposure to EGEE in utero produced a statistically significant reduction ($p \leq 0.05$) in fetal weight at 1,000 ppm, yet statistically significant changes in fetal resorption and malformations did not appear until exposure was increased to 1,800 ppm ($p \leq 0.05$).

An inhibition in the growth rate of exposed fetal animals, reflected by a lower fetal body weight in comparison with control animals, may well be related to the endpoint of growth retardation in newborn human infants. Consumption of tobacco (Kleinman et al. 1988; Abel 1980; Fried and O'Connell 1987) caffeine (Martin and Bracken 1987), or alcohol (Lemoine et al., 1968; Little et al., 1986) in pregnant women has been associated with reduced birth weight or slowed rate of growth in newborns.

Birth weight in humans is an important parameter that profoundly influences the risk of death in the first year of life (Wegman 1986; MMWR 1988), and perhaps more subtle developmental parameters. Reducing the incidence of low birth weight babies (defined as weights below 2.5 kg) is included as a national goal in the 1990 Federal Priority Objectives for Pregnancy and Infant Health.* An integral part of our analysis is to develop an innovative methodology to project changes in birth weight distributions to the human population, and interpret these changes in terms of likely changes in infant mortality.

1.3. Structure of the Report

This report is divided into separate analyses of quantal data (e.g. fetal deaths and deformities, which are either present or absent--covered in Sections 2 and 3) and continuous data (e.g. changes in birth weights--Covered in Section 4). Section 2 first discusses a number of methodological issues in the use of the quantal data for risk assessment, then presents the raw observations from animal studies, and then uses the animal data to project likely human ED50's for different effects for the different compounds under study. Based on these estimated ED50's,

sometimes combined with the data for dams that lived until their full term. When problems such as these occurred in the data set, it was excluded from our calculations.

* In 1985, low birth weight infants constituted 6.8% of all births in the U.S. and 12.4% of the births among blacks (MMWR 1988). In 1983, very low birth weight infants (those weighing less than 1500g at birth) constituted only 0.1% of all live births, yet contributed to 17% of neonatal mortality (Wegman 1986).

Section 3 utilizes a log probit analysis,** and assumptions about the human interindividual variability in thresholds for response, to produce a series of assessments of potential human risk for the quantal effects. (We limit our direct use of the animal dose response data to the ascertainment of ED50's in this way because we are skeptical that experimental animal populations, with relatively limited genetic diversity and relatively uniform environments, will be as diverse in their distributions of thresholds for adverse reproductive effects as the human population.)

The birthweight analysis in Section 4 begins with a compilation and comparison of the available fetal weight reduction information from the animal experiments. Then we examine the pattern of change in human birth weights in response to a variety of environmental stresses by comparing the distributions of birth weights in U.S. black and white infants. These two sources of information are then combined with data on the relationship between birth weight and infant mortality to project potential changes in infant mortality mediated by birth weight changes produced by different levels of exposure to EGEE and EGME.

** Probit analysis assumes (1) that effects occur in individual animals when specific thresholds of dose are exceeded and (2) that there is a lognormal distribution of thresholds in the exposed group of animals or humans. Probit analysis of quantal data is commonly used by biologists to find the dose at which the median of a population experiences an effect from a chemical or treatment (as known as the "median effective dose"). The classic description of probit analysis is Finney (1971).

2. Analysis of Animal Data for Quantal Effects and Projection of Human ED₅₀'s

2.1 Major Issues and Calculation Procedures for the Analysis of Quantal Animal Data

At the outset of our analysis of quantal reproductive effects of glycol ethers, we needed to address a number of fundamental questions:

- o What basic dose response relationship should be assumed in analyzing the data? What types of causal mechanisms are likely to be involved, and what tentative inferences should be drawn from those mechanism types to guide initial representations of the data for analysis?
- o How should effects be aggregated? For example, should each separate type of fetal anomaly be treated separately, or should apparently different effects be added together?
- o How should we deal with the background (control) incidence of different effects? Should it be assumed that the processes producing fetal death and anomalies in control animals interact with the processes by which glycol ethers cause these effects, or should the toxic mechanisms of the glycol ethers be treated as if they were independent of the background processes?.
- o What interspecies projection rule should be used in estimating human ED₅₀'s?
- o What assumptions should be made in analyzing the results of exposures by different routes (e.g. gavage vs. inhalation exposure)?

We do not know the exact mechanism(s) by which glycol ethers produce fetal death and developmental anomalies in the offspring of exposed pregnant animals. We have chosen to assume that these are individual threshold processes (similar to acute toxic effects) because (1) the classes of mechanisms we judge are likely to be involved (deaths of several key cells in a developing fetus, simultaneous failure of several cells to send or receive a signal for differentiation at a key time during development) are kinds of processes that are likely to produce relatively steep all-or-none threshold like dose response behavior, (2) there is no evidence in either chemical structure or existing experiments that the glycol ethers act by primary genetic mechanisms, which would be better represented as one- or multi-hit processes under "target" theory, and (3) the data

frequently show very steep upward-turning dose response relationships (e.g. see Tables 2.2 through 2.7 and the accompanying experimental descriptions in Section 2.2 below) which are reasonably well represented as log-probit or probit dose response relationships* (e.g., see Figures 2, 5, 10, and 11 below).

Because we have little specific information about likely mechanisms by which the glycol ethers produce different fetal effects, we also are unable to make very sophisticated choices about how to aggregate different effects for purposes of analysis. (Ideally, effects which are part of the same causal processes, such as a series of mutations in a tissue leading to cancer, should be grouped together for analysis, but effects with fundamentally different causal mechanisms, and hence likely differences in dose response relationships, should be kept separate.) We have therefore chosen to take a relatively pragmatic approach to the aggregation issue, adopting, for the most part, whatever groupings were used by the authors of different papers in summarizing their information. In a number of cases different authors chose different kinds of aggregates and that leads to some difficulty in comparing results across data sets. We examined each reported type of effect from each glycol ether separately. Because the background rate for specific reproductive effects varies from experiment to experiment, we did not attempt to average or combine the data at this stage.

The other issues listed above are best discussed in the context of a more detailed explication of our calculation procedures, and will be dealt with in a series of subsections which follow immediately.

2.1.1 Definition of the ED₅₀ and Treatment of "Background" Effects

For the purposes of our analysis, the ED₅₀ is defined as the dose of the chemical at which 50% of the otherwise unaffected proportion of the population is suffers a particular effect. This approach adjusts the ED₅₀ to take the often substantial "background" or "natural" rate of malformations into account.** This is adapted from Peto et al., (1984) who developed an index

* Probit analysis is a tool to normalize probabilities (or percentages) of response so the graphic distance between the units becomes uniform. A probit is simply the number of standard deviations from the mean (of an assumed distribution of individual lethal doses/susceptibilities—generally this distribution is assumed to be lognormal) plus 5; 5 was added to allow toxicologists to avoid having to deal with negative numbers—see Finney (1971). Probit analysis assumes (1) that effects occur in individual animals when specific thresholds of dose are exceeded and (2) that there is a lognormal distribution of thresholds in the exposed group of animals or humans.

** The background rate of specific endpoints (i.e., fetal mortality, malformations) is an important factor in any risk assessment. For example, litters of New Zealand White fetal rabbits exposed in utero only to air were affected by skeletal variations such as supernumerary ribs (95.8% of litters affected), sternebral variations (8.3%), or ossification defects (12.5%) (Andrew and Hardin

for tumorigenicity (TD₅₀) that takes into account the tumor rates of the control animals. For example, if the probability of developing a birth defect in the control animals is 40%, then the probability of being born without a defect is 60%. Thus, in this case, the ED₅₀ corresponds to the dose at which the probability of birth without a defect is halved, i.e.,:

$60/2 = 30\%$ "excess" over background to produce ED₅₀

40% background + 30% "excess" = 70% total incidence in experimental group at ED₅₀

The ED₅₀ provides an estimate of the dose which would be expected to produce an effect in the median otherwise unaffected individual in an exposed population.

Consistent with this definition for the ED₅₀, we calculate the percentage of otherwise unaffected individuals that show effects at other doses after subtracting the background incidence of the effect from the exposed animal groups. This treatment carries an implicit assumption that the processes producing the "background" incidence of the quantal effects do not interact appreciably with the processes by which the glycol ethers produce those same effects. More formally, according to *Abbott's formula*, one example of the standard rule for combining probabilities, if a proportion, C, of animals respond without the stimulus (the response among the control group), then a dose sufficient to induce a response rate P in the remaining animals has an expected rate of observed responses, P* (the response among the experimental group) (Finney 1971).

Therefore, the response attributable to the dose, (taking background into account) P, can be written as:

$$P = \frac{P^* - C}{1 - C}$$

The ED₅₀ is of course the dose at which P is 50%. Another way to look at this equation is that (1 - C) is the population at risk of getting a malformation, while (P* - C) is the risk attributable to that exposure.*

1984). Malformations are not uncommon among control animals, especially for inbred strains which are frequently used for experiments.

* For example, given that the proportion of animals affected by the fetotoxicant in the control group is 0.10 or 10%, and that the proportion of the animals affected in the experimental group exposed to 100 ppm of a chemical is 0.70, then the response due to exposure to this chemical equals P, where P equals:

$$P = \frac{0.7 - 0.10}{1.0 - 0.10} = \frac{.6}{.9} = 0.667$$

To calculate the ED₅₀, we used a simple log-probit analysis. Using a linear-regression program,* we plotted the logarithm of the dose on the x-axis and the probit of the response (calculated from the % of affected fetuses or litters) on the y-axis. We read the log dose-probit slope from the linear regression equation fitted to the points. Setting y equal to the probit of 5.0 and using the linear regression equation, $y = mx + b$, we solved for x. The antilogarithm of x is the ED₅₀ (based on either a fetus- or a litter-based measure--see below).

2.1.2 The Fetus vs the Litter as the Unit of Analysis

In analyzing the available quantal animal data, we had a choice of defining our ED₅₀'s in terms of either individual affected fetuses or affected litters. We chose to consider the litter as our principal unit of analysis for two reasons:

- (1) All of the pups within a litter are exposed to the same environment within a dam and thus receive exposure to the same dosage of a teratogenic or fetotoxic chemical.
- (2) Since multiple births are relatively rare among humans, when a litter of animals is used as an index for a teratogenic or fetotoxic effect, this index may better correspond to a single birth in humans. If one fetus or pup within a litter experienced a malformation, then this malformation reflected one "hit", i.e., a missed developmental signal or failure to complete an important process on time, such that the course of development was irreversibly affected.

Therefore, as our ultimate index for an effect on development, we chose:

the total litters "affected" (containing at least one affected offspring)
the total number of litters examined

As will be seen below, however, we have also done some parallel calculations for illustrative purposes using the fetus as the unit of analysis.

Unfortunately the studies we have available on glycol ether effects often present the data on abnormalities from exposure to fetotoxic chemicals in such a format that we can only directly calculate the ED₅₀ from the percentage of affected fetuses rather than the percentage of affected litters. To convert the data from a fetus- to a litter-based measure, we were forced to assume that the appearance of a specific defect in each fetus is an independent event.** At the ED₅₀, the

From Table 1 in Finney (1971), the probit at a probability of 66.7% is 5.4316. The ED₅₀ corresponds to that dose at which the probit is 5.0.

* Cricketgraph™ program for the MacIntosh computer.

** Of course, one could postulate situations in which this assumption would not hold. For example, because of the maternal circulation and the movement of the toxicant, certain fetuses in

individual fetus has a 50% probability of being affected by the toxicant--what is the chance that the litter, a group of individual fetuses, will be affected by the toxicant? To answer this question, we convert the probability of the fetus being affected into one for the litter being affected.*

2.1.3 Use of Probit Analysis to Estimate Fetotoxicity in a Population

As indicated earlier, the use of probit analysis to estimate fetotoxicity is based on an assumption that each individual has a threshold for toxicity, and that the thresholds for different individuals in the population are distributed lognormally (i. e., the logarithms of the threshold doses for each individual, k , have a "normal" Gaussian distribution).** The density of the

the uterus may have a greater likelihood of experiencing a toxic effect than others. For the purpose of this analysis, however, we shall neglect this possibility and assume independence.

* For example, if the average number of fetuses per litter is 11.6 and the probability of an individual fetus being affected is 0.5, then to affect half of the litters in a population,

$$0.5/11.6 = 0.0431$$

as few as 4.31% of the fetuses need to be affected.

From Table 1 in Finney (1971), a response of 4.31% in a population of fetuses corresponds to a probit of 3.2831. Using the linear regression equation fitted to the plot of the log dose versus the probits (as described above) and setting y equal to 3.2831, solve for x . The antilogarithm of x is the ED_{50} calculated on a litter-basis.

**A $\log_{10}$ probit slope is the number of geometric standard deviations of the log-normal distribution of susceptibilities per 1 $\log_{10}$ change in dosage. By contrast, the reciprocal of the probit slope is the number of $\log_{10}$ dosage units per 1 geometric standard deviation in the log-normal distribution of susceptibilities. Thus, a $\log_{10}$ probit slope of 2 implies a log-normal distribution of individual susceptibility with a $\log_{10}$ geometric standard deviation of 0.5. A convenient property of log-normal distributions is that geometric standard deviations and variances do not change when one takes the reciprocal of a parameter in this way:

$$\begin{aligned}\log(\text{threshold dose}) &= -\log(1/\text{threshold dose}) \\ &= -\log(\text{susceptibility})\end{aligned}$$

The variance of the $\log(\text{threshold dose})$ values is the sum of:

$$[\log(\text{dose}) - \text{mean } \log(\text{dose})]^2/N$$

whereas the variance of the $-\log(1/\text{dose})$ values is the sum of:

$$[\log(1/\text{dose}) - \text{average } \log(1/\text{dose})]^2/N$$

$$=[-\log(\text{dose}) + \text{average } \log(\text{dose})]^2/N$$

distribution of the logarithms of the threshold doses, k , is given by:

$$Z(x) = \{1/(2\pi)\}^{1/2} e^{-x^2/2}$$

where:

$$x = (\log k - \text{population mean of } \log k) + (\text{standard deviation of } \log k)$$

In section 3, we will make "best" ("least unlikely") and "plausible upper limit" estimates of the interindividual variability in susceptibility for fetotoxic effects in humans—stating these estimates in terms of log probit slopes, which correspond to the reciprocals of the corresponding estimates of the standard deviation of $\log k$.

2.1.4 Conversion Between Oral and Inhalation Routes of Exposure

The EGEE and EGME were administered via either of two routes: inhalation (with units in parts per million) or oral gavage (with units of mg/kg body weight). Provisionally, we assume that ppm in animals corresponds to ppm in humans. This is based on an analogy with the pharmacokinetic analysis performed on ethylene oxide—Harris 1987a. In this analysis we found that, like a number of other chemicals, overall elimination rates tend to scale approximately with overall metabolic rates, in proportion to (body weight)^{-0.75}. Because the scaling power is less 1, half-lives for elimination increase with body weight, approximately in proportion to (body weight)^{-0.25}, allowing a greater time for a given amount of ethylene oxide to react in the body before being eliminated. Thus, other things being equal, one would expect a greater amount of internal dose X time to result in humans than in rodents for a given administered dose, if the dose is expressed in mg/kg of actual uptake. However, when ethylene oxide is administered as a given concentration (ppm) in external air, there is a countervailing effect on uptake which almost exactly offsets the effect of the longer internal persistence of ethylene oxide in the body. Because general metabolic rates and therefore alveolar ventilation also scale approximately with (body weight)^{-0.25}, humans simply tend to breathe less air per body weight than rodents, and therefore have an opportunity to take up proportionately less ethylene oxide contained in the air. In contrast to the increased expected sensitivity of humans to similar doses expressed in mg/kg, therefore, a given external exposure in ppm X time in humans produces a similar internal ethylene oxide concentration X time as would be produced by the same external ppm X time in rats or mice.*

* The relationships may not hold true for high concentrations of a chemical. After exposure to approximately 1000 ppm ethylene oxide for 6 hours, rodents decrease their alveolar ventilation rates, so that under these conditions of exposure the above relationships may be significantly

In parallel with this result, we therefore choose to assume that a given ppm X time concentration of EGEE or EGME will have similar fetotoxic effects in rodents and people. Most of the data are from inhalation exposures and expressed in external ppm in any event. Where the dosage was given in mg/kg (following an oral gavage exposure), we converted the dose of EGEE and EGME, administered via oral gavage and measured in milligrams per kilogram body weight, to an equivalent dose administered via inhalation and measured in a concentration of parts per million. These calculations assume complete absorption of the chemicals from alveolar air.*

2.1.5 Conversion from an ED₅₀ Based on a Six -Hour Day to an ED₅₀ Based on an Eight -Hour Day

Most of the studies of chemicals administered during inhalation exposed the animals for six hours per day (Hanley et al., 1984b; Doe 1984) or 7 hours per day (Andrew and Hardin 1984).

altered. Also, at higher doses glutathione is appreciably depleted, so that ethylene oxide will be eliminated more slowly.

* We calculated the conversion in three steps: First, we calculated the amount of chemical administered on a daily basis, ie:

mg of the chemical administered per day = dose at the ED₅₀ for the litter (mg/kg body weight/day)
X reference body weight for the species (kg)

In the second step, we converted the same dose of the chemical administered via oral gavage into the dose of the chemical administered via inhalation, as shown for EGEE below:

mg EGEE/m³ air moved per 8 hour day =
 $\frac{\text{mg EGEE/day} \times 1000 \text{ l/m}^3}{\text{reference alveolar ventilation rate (liters/min)} \times 480 \text{ minutes/day}}$

Because the alveolar rate reflects the physiologically active movement of air, we chose to apply the alveolar rate, rather than the minute volume, in the calculations above. If the alveolar rate for a species was not available, we multiplied the minute volume by two-thirds, a procedure recommended in Arms and Travis 1987. The reference parameters for these calculations are listed in Table 2-1.

In the final step, we converted the dose from mg/m³ into ppm. To convert from mg/m³ into ppm, we assumed that the animals would be exposed to the chemical under the temperature and pressure conditions prevailing in a room. Following standard industrial hygiene practices for converting ppm to mg/m³ (NIOSH 1973):

ppm = mg/m³ of the chemical * (24.45 liters per gram-mole + molecular weight of the chemical)

where, at 25°C and 760 mm Hg, one gram-mole of a perfect gas or vapor occupies 24.45 liters

Table 2-1. Reference Respiration Parameters

species	body weight (kg)	alveolar ventilation rate (l/min)
mouse ^a	0.025	0.025
rat ^a	0.25	0.117
rabbit ^b	3.5 ^c	0.713
human		
males ^a	70.0	5.0
females ^d	54.0	4.5

a. Based on Arms AD, Travis CC. 1987. Reference physiological parameters in pharmacokinetic modeling. Office of Risk Analysis, Oak Ridge National Laboratory, April; Review draft, table 4-14.

b. Based on data for the European rabbit (*Oryctolagus cuniculus*); the minute volume was multiplied by 2/3 to yield the alveolar ventilation; Biology Data Book, table 74.

c. Based on the weight of New Zealand White female rabbits - data was not available for European rabbits.

d. Based on data in the Biology Data Book, table 74; minute volume during rest was multiplied by 2/3 to yield the alveolar ventilation.

For ease of later projection to 8 hour exposure regimens in human workers, we therefore converted the data to eight-hour equivalents:.

$$\text{ED}_{50} \text{ in ppm} * 6 \text{ hours} / 8 \text{ hours} = \text{ED}_{50} \text{ in ppm for an eight hour day}$$

2.2 Review of Basic Data, and Derivation of Individual Animal ED₅₀'s--

"Analysis 1" (Later Discarded)

Below we present a series of tables, divided by chemical and route of exposure, of the reproductive data for animals. Sections 2.2.1 and 2.2.2 present the details of the initial analysis, including the specifics about how we decided to edit the data used in the dose-response curves for the ED₅₀'s. In an approach called "analysis one" we calculate an ED₅₀ using the log dose-probit slope from linear fits to the animal data. Unfortunately, because of the scarcity of data at several concentrations of EGEE or EGME, this approach was not fully successful. Given experiments with clearly defined endpoints, more than five different exposure concentrations and limited fetal or maternal mortality, analysis one should work; for this reason we retained the technique in this report. After discovering the drawbacks of analysis one, we developed an alternative method of calculating the ED₅₀s, "analysis two", described in sections 2.3. through 2.5.

First below we will present the raw data, the linear plots based on this data, and the method of combining or selecting data used for analysis one. Next, we will present the rationale for analysis two, and the ED₅₀s based on this analysis. The risk calculations in Chapter 3 are solely based upon the best estimates for post-implantation loss, skeletal defects, and malformations derived from the ED₅₀'s of analysis two.

For analysis one, we estimated ED₅₀'s from the data sets that contained two or more points on a dose-response curve. Sometimes three data points were available, but we averaged the response of the lowest exposure group and the control group when the response of the exposure group was lower than that of the control. In other cases, the response to a series of exposure concentrations did not show a dose-response relationship so we noted any statistically significant responses, but did not derive an ED₅₀ from those data. For a number of other papers, an ED₅₀ could not be determined because the form of the data did not lend itself to this type of analysis.

2.2.1. Basic Observations for Ethylene Glycol Monoethyl Ether (EGEE)

2.2.1.1 Ethylene Glycol Monoethyl Ether via Inhalation

The raw data (Table 2-2) used to derive the ED₅₀'s for EGEE administered via inhalation came from articles by Andrew and Hardin (1984) and Doe (1984). Although we had access to other articles about EGEE, in our judgment the other data were not adequate for risk assessment.

The first data set (Table 2-2A) shows some of the limitations of the "Analysis 1" approach, where both ED₅₀ and probit slopes are derived directly from the animal data. Because all of the litters of the New Zealand White Rabbits were resorbed at 617 ppm (thus leaving only one dose level for the dose-response curve) and we had enough other data sets for analysis, we did not calculate an ED₅₀ for these animals for purposes of "Analysis 1". Even exposure to 160 ppm, however, caused a significant increase ($p < 0.05$) in resorptions among the rabbits. Also, the total number of live fetuses was significantly decreased ($p < 0.05$) after maternal exposure to 160 ppm and they all died after a 617 ppm exposure.

In the data from a study of Alpk/AP rats exposed to EGEE by Doe (1984) (Table 2-2B), because the data did not indicate a consistent dose-response relationship, we did not calculate the ED₅₀ for pre-implantation loss. At exposure to 50 ppm EGEE, however, the pre-implantation loss was significantly ($p < 0.05$) higher than that of the control group. Although no single dose produced a significantly higher rate of post-implantation loss, the percentage of implantations affected by post-implantation loss does increase with an increasing concentration of EGEE. Although mean fetal weight did not significantly change with exposure, the mean number of live fetuses was significantly decreased at 10 and 50 ppm. This latter finding, however, was not borne out by the result at 250 ppm (i.e., at this dose, the mean number of live fetuses was not significantly different from that of the controls). Because there was not a statistically significant effect from exposure, we also did not determine an ED₅₀ for external and visceral defects in the rats. With respect to minor skeletal defects, because the percentage of rats showing these effects was smaller in the group exposed to 10 ppm than in the control group, we averaged the two values (weighted by the number of fetuses in each group) to obtain the percent response due to the background rate of skeletal defects. From the remaining two points, at exposures of 50 and 250 ppm, we plotted the dose-response curve and calculated the ED₅₀ based on a measure of affected fetuses (Figure 1, Table 2-7). Almost all of the fetuses showed minor skeletal defects at 250 ppm, although even the background incidence of minor skeletal defects was high (46.3%).

For pre-implantation loss among the Dutch rabbits exposed to EGEE via inhalation (Doe 1984) (Table 2-2C), we calculated the background by averaging the responses of the group exposed to 10 ppm and the control group. From the remaining points at 50 and 175 ppm, we

Table 2-2
Ethylene Glycol Monoethyl Ether (EGEE) via Inhalation

A. Andrew and Hardin (1984) Data for New Zealand White Rabbits^{a,b}

OBSERVATIONS	EXPOSURE CONCENTRATIONS OF EGEE, ppm		
	0	160	617
litters with resorptions	6/24	17/23 ^c	22/22 ^c
(number affected/total litters; %)(25)		(74)	(100)
total live fetuses	214	167 ^c	0 ^c
mean body weight, g	47±7	44±11	--- ^d

a. Based on Andrew, F. and Hardin, B. (1984). "Developmental Effects after Inhalation Exposure of Gravid Rabbits and Rats to Ethylene Glycol Monoethyl Ether," Environmental Health Perspectives, vol. 57, p17, table 3, p19, table 5.

b. Pregnant female rabbits exposed to air or 160 or 617 ppm EGEE for 7 hr/day from 1 to 18 days of gestation. Maternal rabbits exposed to EGEE experienced decreased food intake and depressed weight gain.

c. $p \leq 0.05$ by chi-square compared with air group.

d. All of the fetuses exposed in utero to this concentration died.

B. Doe (1984) Data for Alpk/AP Rats^{a,b}

OBSERVATIONS	EXPOSURE CONCENTRATION OF EGEE, ppm			
	0	10	50	250
number pregnant	23	24	23	21
pre-implantation loss, %	2.4	9.7	14.3 ^c	6.2
post-implantation loss, %	5.5	7.6	8.9	12.6
fetus weight (mean, gms) (mean # of live fetuses)	5.1 (12.2)	5.2 (10.6) ^c	5.1 (10.8) ^c	4.7 (11.1)
<u>external and visceral defects</u>				
% showing any minor defects (# showing any minor defects)	11.7 (33/282)	16.1 (41/254)	11.6 (29/250)	18.4 (43/234)
<u>minor skeletal defects</u>				
% showing minor defects (# showing minor defects)	46.3 (68/147)	39.7 (52/131)	51.2 (66/129)	97.5 ^d (119/122)

a. Based on Doe, J (1984). "Ethylene Glycol Monoethyl Ether and Ethylene Glycol Monoethyl Ether Acetate Teratology Studies," Environmental Health Perspectives, vol. 57, p 36 tables 4-5.

b. Pregnant rats were exposed to EGEE for 6hr/day on days 6 to 15 (inclusive) of gestation

c. Significantly different from controls, $p < 0.05$

d. Significantly different from controls with a χ^2 test conducted by Ballew and Harris, $p < 0.05$.

Table 2-2, Continued
Ethylene Glycol Monoethyl Ether (EGEE) via Inhalation

C. Doe (1984) Data for Dutch Rabbits^{a,b,c}

OBSERVATIONS	EXPOSURE CONCENTRATION OF EGEE, ppm			
	0	10	50	175
number pregnant	21	21	16	22
pre-implantation loss, %	19.5	17.6	22.1	25.7
post-implantation loss, %	5.7	8.4	8.8	5.7
fetus weight (mean, gms)	34.1	34.2	35.7	36.1
(mean # of live fetuses)	6.5	6.6	6.0	6.1
<u>minor skeletal defects</u>				
% showing any minor defects	32.4	52.2 ^d	36.5	64.5 ^e
# showing any minor defects	44/136	72/138	35/96	87/135
% skeletal variants	51.5	60.0	64.6	79.1 ^d
# of skeletal variants	70/136	84/140	62/96	106/134

a. Based on Doe, J (1984). "Ethylene Glycol Monoethyl Ether and Ethylene Glycol Monoethyl Ether Acetate Teratology Studies," Environmental Health Perspectives, vol. 57, p37, tables 7-8.

b. Female rabbits exposed on days 6 to 18 inclusive of gestation to EGEE for 6 hr/day.

c. No evidence for a treatment-related, litter-based, effect on pre- and post- implantation loss or fetal weight at these concentrations. No evidence of maternal toxicity.

d. Significantly different from controls with a X^2 test conducted by Ballew and Hattis, $p < 0.05$.

e. Significantly different from the controls, $p < 0.05$.

Figure 1. Minor Skeletal Defects in ALPK/AP Rats Exposed to EGEE via Inhalation

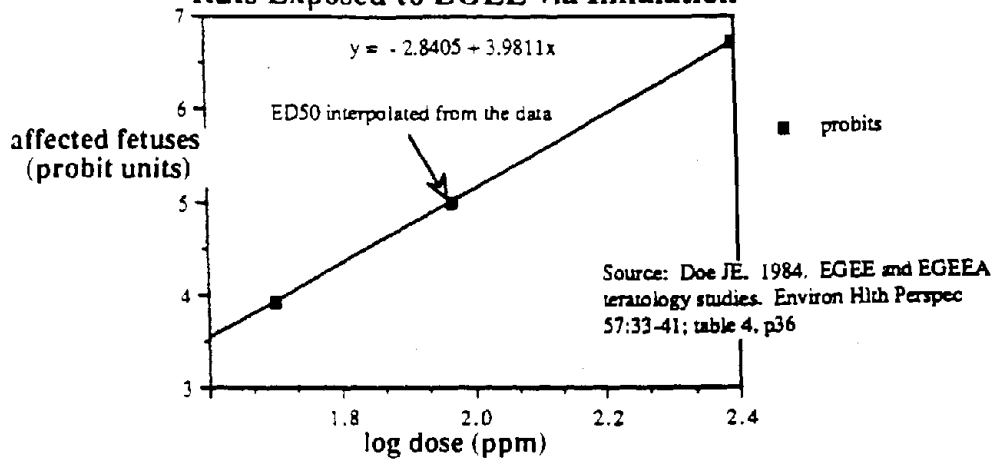
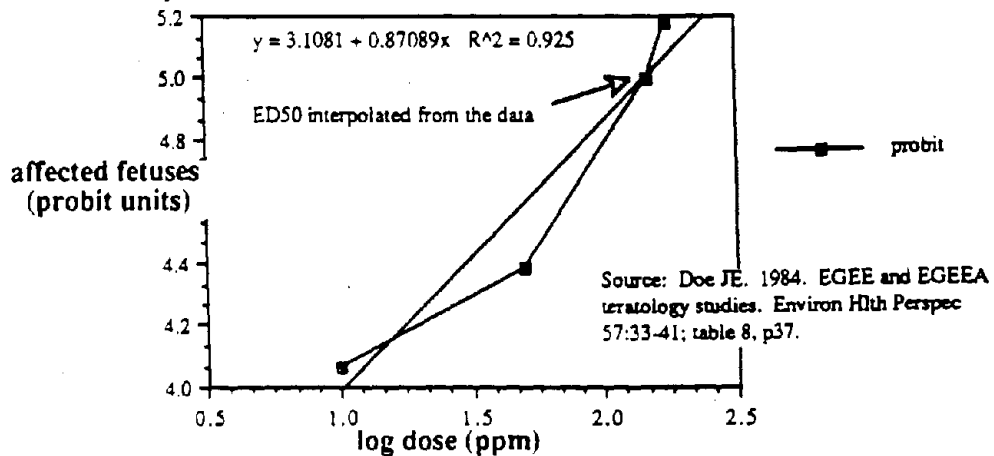


Figure 2. Skeletal Variants in Dutch Rabbits Exposed to EGEE via Inhalation



calculated the ED₅₀ (Table 2-7). The data from the post-implantation loss among the rabbits was too inconsistent to show a dose-response relationship. A slight gain in fetal weight with concentration of EGEE was not statistically significant. The percentage of rabbit fetuses showing minor skeletal defects was elevated significantly ($p < 0.05$) at 175 ppm; however the data was too irregular to plot a dose-response curve. The percentage of skeletal variants was elevated significantly ($p < 0.05$) among the offspring of Dutch rabbits exposed to 175 ppm. The fetus-based ED₅₀ for skeletal variants was interpolated along the linear regression line through the three data points (Figure 2; Table 2-7).

2.2.1.2 Ethylene Glycol Monoethyl Ether Acetate (EGEEA) via Inhalation

Ethylene glycol monoethyl ether acetate (EGEEA) is rapidly metabolized to form EGEE; thus, we consider an inhalation exposure to EGEEA to be the same as that to EGEE. Doe (1984) exposed pregnant Dutch rabbits to EGEEA via inhalation (Table 2-3). Rates of pre-implantation loss did not show any consistent dose-response relationship (Doe 1984). Post-implantation loss was elevated significantly ($p < 0.05$) among the offspring of maternal rabbits exposed to 400 ppm EGEEA; however, the rest of the data were too irregular to form a meaningful dose-response curve. Fetal weights were reduced significantly ($p < 0.05$) among animals exposed to 100 and 400 ppm EGEEA. The percentage of fetuses showing external and visceral defects after exposure to 400 ppm was increased significantly ($p < 0.05$) over the response among the controls (Table 2-3). We graphed the percentage of affected fetuses (showing minor external and visceral defects) against the dose in ppm (Figure 4). A linear plot of the dose versus the response fit the data better (i.e., had a higher correlation coefficient) than a linear plot of the log of the dose versus the response. Since one of the data points fell almost exactly on the ED₅₀, we did not need to extrapolate to estimate it (Table 2-7). Almost all (97.8%) of the fetuses showed a skeletal defect when exposed to 400 ppm EGEEA; this response was significantly higher ($p < 0.05$) than that found in the controls (Table 2-3). To graph the dose-response curve of this relationship, we removed the point at 25 ppm because the percentage of any skeletal defects in the exposed animals was lower than that in the control animals. We interpolated the ED₅₀ between the remaining two data points (Figure 5).

Table 2-3.
Ethylene Glycol Monoethyl Ether Acetate (EGEEA) via Inhalation

Dutch rabbit^{a,b,c}

OBSERVATIONS	EXPOSURE CONCENTRATION OF EGEEA, ppm			
	0	25	100	400
number pregnant	16	15	17	19
post-implantation loss, %	9.4	18.1	6.7	24.4 ^d
fetus weight (mean, gms)	34.7	35.2	30.1 ^d	30.8 ^d
(mean # of live fetuses)	6.6	5.1	7.4	4.9 ^d
<u>external and visceral defects</u>				
% showing minor defects	20.8	23.4	27	54.8 ^d
(# showing minor defects)	(22/106)	(18/77)	(30/111)	(51/93)
<u>skeletal defects</u>				
% showing any defects	21.7	18.2	33.3	97.8 ^d
(# showing any defects)	(23/106)	(14/77)	(37/111)	(91/93)
% showing major defects	0.9	0	0	6.5 ^d
(# showing major defects)	(1/111)	0	0	(6/92)
% skeletal variants	47.2	51.9	64.9 ^d	98.9 ^d
(# of skeletal variants)	(50/106)	(40/77)	(72/111)	(92/93)

- a. Based on Doe, J (1984). "Ethylene Glycol Monoethyl Ether and Ethylene Glycol Monoethyl Ether Acetate Teratology Studies," Environmental Health Perspectives, vol. 57, p38, tables 10-11.
b. Female rabbits exposed on days 6 to 18 inclusive of gestation to EGEEA for 6 hr/day.
c. No evidence for a treatment-related, litter-based, effect on pre-implantation loss at these concentrations. No evidence of maternal toxicity.
d. Significantly different from the controls, $p < 0.05$.

Figure 3. Minor External and Visceral Defects in Dutch Rabbits Exposed to EGEEA via Inhalation

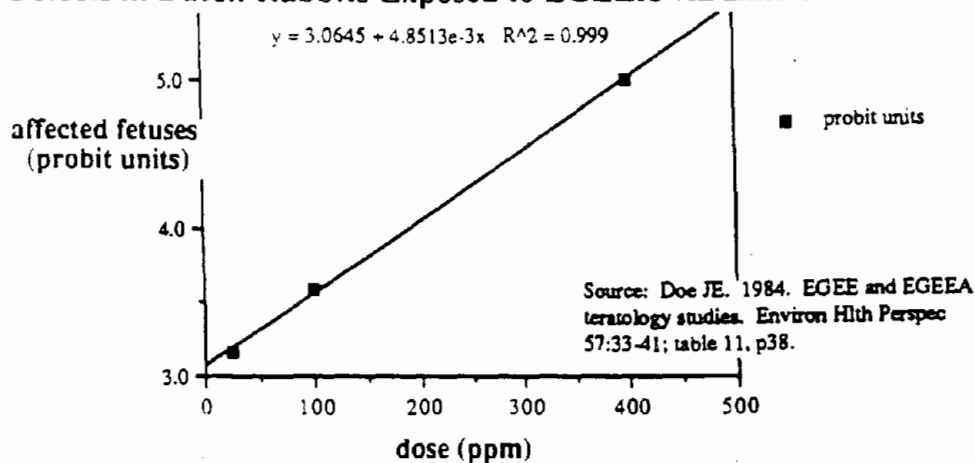


Figure 4. Any Skeletal Defects in Dutch Rabbits Exposed to EGEEA via Inhalation

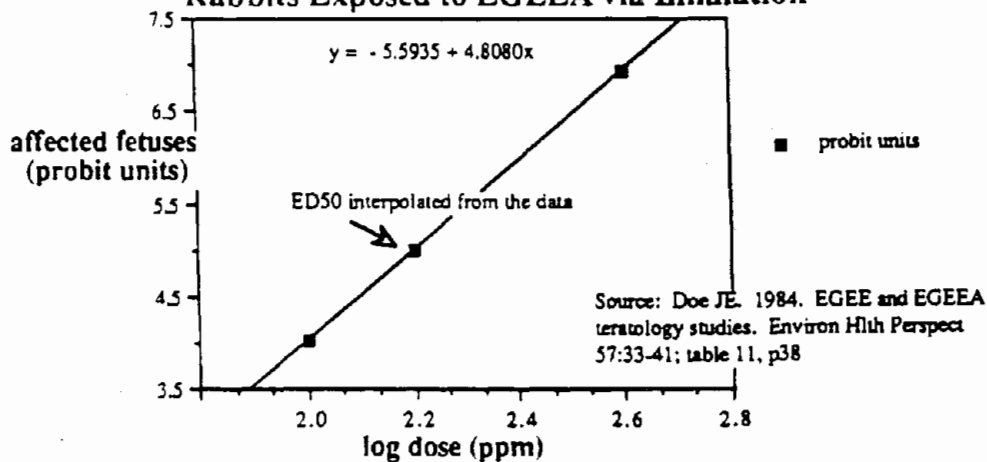
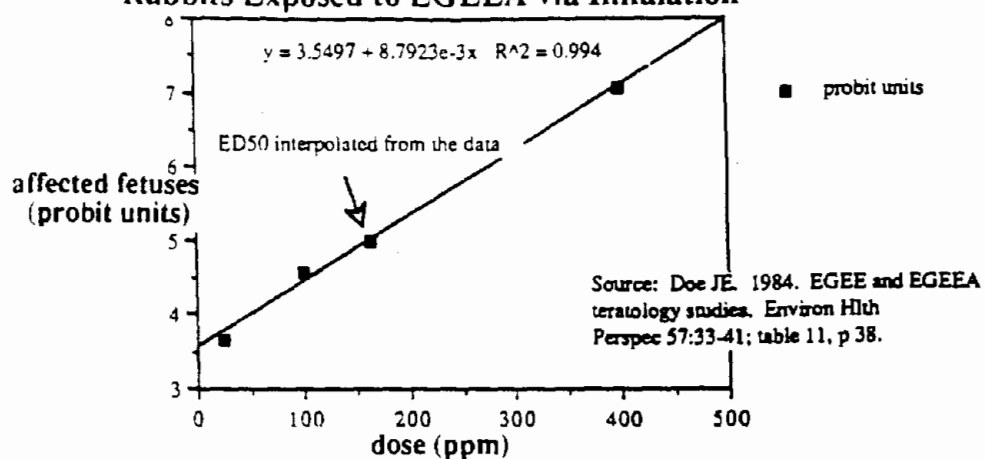


Figure 5. Skeletal Variants in Dutch Rabbits Exposed to EGEEA via Inhalation



2.2.1.3 Ethylene Glycol Monoethyl Ether via Oral Gavage

Wier, Lewis and Traul (1987) exposed CD-1 mice via oral gavage to 1,000, 1,800, 2,600, 3,400 and 4,200 mg EGEE per kilogram of body weight (Table 2-4). Fetal weight was significantly decreased ($p \leq 0.05$) by even the lowest concentration of EGEE. This weight loss followed a linear trend, a relationship discussed in Chapter 4. Resorptions were significantly increased ($p \leq 0.05$) at concentrations of EGEE above 1800 mg/kg. Although some fetal or maternal mortality occurred at the higher concentrations, the probit of the percentage of resorptions and malformations showed a linear relationship with the logarithm of the dose at moderate concentrations (Figures 6 and 7). These data were used to calculate ED50s for both analysis one and analysis two.

2.2.2 Basic Observations for Ethylene Glycol Monomethyl Ether (EGME)

2.2.2.1 Ethylene Glycol Monomethyl Ether via Inhalation

Hanley et al. (1984b) exposed rats, mice, and rabbits to EGME for 6 hours per day (Table 2-5). We calculated the percentage of litters of Fischer 344 rats affected by lumbar spurs; however, this data did not demonstrate a dose-response relationship for more than one dose. The number of litters affected by lumbar spurs was significantly higher in the group exposed to 50 ppm EGME than in the control group.

Next, we examined the percentage of litters affected by a delayed ossification of the vertebral centra. At exposure to 3 ppm EGME, the response observed among the exposed group was lower than that among the control group, so we combined the responses in the 3 ppm and control groups. We performed a linear regression on the two remaining points to calculate the fetus-based ED50 (Figure 8, Table 2-7).

After exposure to EGME, the percentage of CF-1 mice affected by extra lumbar ribs increased. The number of litters affected at 50 ppm was significantly higher ($p < 0.05$) than the number affected in the control group. The dose-response curve for extra lumbar ribs is plotted in Figure 9 and is used to calculate a fetus-based ED50. Similarly, the dose-response curve for the percentage of litters affected by unilateral testicular hypoplasia is used to calculate an ED50 (Table 2-7). The number of litters affected by unilateral testicular hypoplasia was significantly higher (α equals 0.05) in the group exposed to 50 ppm than in the control group.

Table 2-4
Ethylene Glycol Monoethyl Ether (EGEE) via Oral Gavage

CD-1 mice^{a,b}

OBSERVATIONS	DOSE EGEE (mg EGEE/kg body weight)					
	0	1,000	1,800	2,600	3,400	4,200
fetal weight (g) ^c	1.38	1.11 ^d	0.92 ^d	0.86 ^d	0.57 ^d	--- ^e
(# of fetuses)	(52)	(47)	(36)	(12)	(3)	--- ^e
resorptions (mean # per litter)	1.2	0.5	6.5 ^d	9.75 ^d	14.0 ^d	11.0 ^d
(# of litters)	(5)	(4)	(6)	(4)	(3)	(3)
implants (mean # per dam)	11.8	12.5	13.33	12.75	13.83	11.33
(# of litters; dead and alive dams)	(5)	(4)	(6)	(4)	(6)	(6)
malformed (mean # per litter)	0.2	0.0	4.17 ^d	5.50 ^d	1.0	--- ^e
(# of litters)	(5)	(4)	(6)	(2)	(1)	--- ^e

a. Based upon Wier, P. J., Lewis, S. C., and Traul, K. A. (1987). "A Comparison of Developmental Toxicity Evident at Term to Postnatal Growth and Survival Using Ethylene Glycol Monoethyl Ether, Ethylene Glycol Monobutyl Ether, and Ethanol," *Teratogenesis, Carcinogenesis, and Mutagenesis*, vol. 7, p58, table 1.

b. Mated females were given agents by oral gavage from day 8 through day 14 of gestation; dosing volume was 10 ml/kg/day based on the day 8 body weight.

c. Values given are mean weight per fetus.

d. Significantly different from the control ($p \leq 0.05$).

e. Complete fetal or maternal mortality.

**Figure 6. Resorptions in CD-1 Mice
Exposed to EGEE via Oral Gavage**

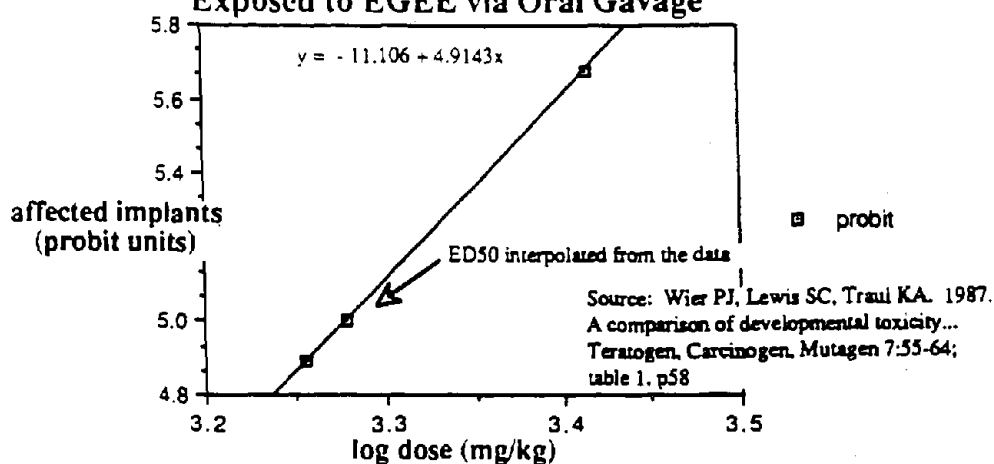


**Figure 7. Malformations in CD-1 Mice
Exposed to EGEE via Oral Gavage**

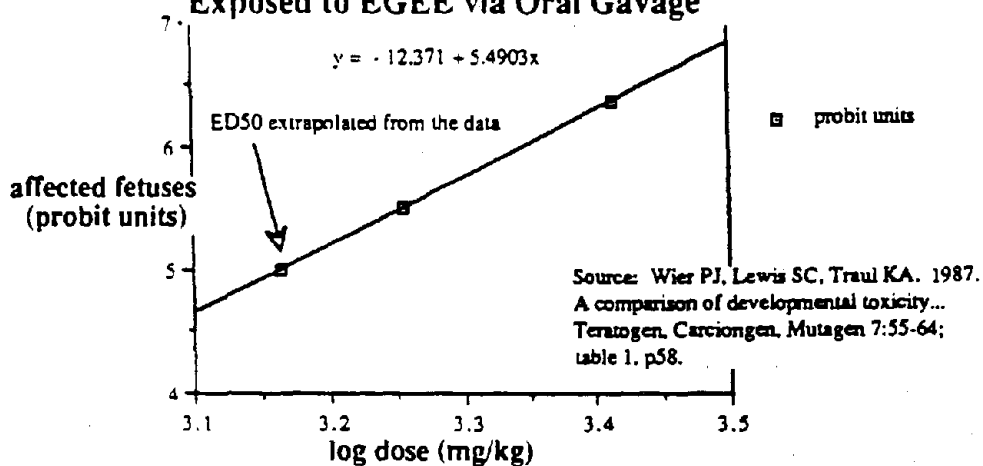


Table 2-5
Ethylene Glycol Monomethyl Ether (EGME) via Inhalation

A. Fischer 344 rats^{a,b,c}

OBSERVATIONS	EXPOSURE CONCENTRATION OF EGME, ppm			
	0	3	10	50
<u>skeleral examinations</u>				
fetuses	287	283	293	307
litters	29	28	28	30
<u>skeleral variations</u>				
<u>lumbar spurs</u>				
% of fetuses affected	6.27	4.59	6.83	18.6
(# of fetuses affected)	(18)	(13)	(20)	(57) ^d
% of litters affected	72.4	35.7	46.4	86.7
(# of litters affected)	(21)	(10)	(13)	(26) ^d
<u>delayed ossification of vertebral centra</u>				
% of fetuses affected	1.39	1.06	2.05	6.19
(# of fetuses affected)	(4)	(3)	(6)	(19) ^d
% of litters affected	13.8	10.7	17.9	43.3
(# of litters affected)	(4)	(3)	(5)	(13)

a. Based on Hanley, T. R. Jr., Yano, B. L., Nitschke, K. D., and John, J. A. (1984b).

"Comparison of the Teratogenic Potential of Inhaled Ethylene Glycol Monomethyl Ether in Rats, Mice, and Rabbits," Toxicology and Applied Pharmacology, vol. 75, pp. 414, table 4.

b. Pregnant Fischer 344 rats were exposed to EGME for 6 hr/day on days 6 through 15 of gestation.

c. Litter sizes, resorption rates, and fetal body measurements of exposed animals were comparable to the control values. There were no dose-related differences in the type or frequency of malformations observed in any of the exposure groups when compared to the controls.

d. Statistically significant difference from the control value (alpha equals 0.05).

(continued)

Table 2-5 (continued)
Ethylene Glycol Monomethyl Ether (EGME) via Inhalation

B. CF-1 mice^{a,b,c}

OBSERVATIONS	EXPOSURE CONCENTRATION OF EGME, ppm		
	0	10	50
<u>skeletal examinations</u>			
fetuses	317	260	251
litters	26	23	24
<u>skeletal variations</u>			
<u>extra lumbar ribs</u>			
% of fetuses affected	15.1	18.8	32.7
(# of fetuses affected)	(48)	(49)	(82) ^d
% of litters affected	53.8	60.9	87.5
(# of litters affected)	(14)	(14)	(21) ^d
<u>soft tissue examinations</u>			
fetuses	165	136	132
litters	26	23	24
<u>developmental variations</u>			
<u>unilateral testicular hypoplasia</u>			
% of fetuses affected	1.21	2.21	6.06
(# of fetuses affected)	(2)	(3)	(8) ^d
% of litters affected	14.3	21.4	25
(# of litters affected)	(2)	(3)	(6) ^d

- a. Based on Hanley, T. R. Jr., Yano, B. L., Nitschke, K. D., and John, J. A. (1984b). "Comparison of the Teratogenic Potential of Inhaled Ethylene Glycol Monomethyl Ether in Rats, Mice, and Rabbits," Toxicology and Applied Pharmacology, vol. 75, pp. 416, table 6.
- b. Bred CF-1 mice were exposed to EGME for 6 hr/day on days 6 through 15 of gestation.
- c. Litter sizes, resorption rates, and fetal body measurements of exposed animals were comparable to the control values. There were no dose-related differences in the type or frequency of malformations observed in any of the exposure groups when compared to the controls.
- d. Statistically significant difference from the control value (alpha equals 0.05).

(continued)

Table 2-5 (continued)
Ethylene Glycol Monomethyl Ether (EGME) via Inhalation

C. New Zealand White Rabbits^{a,b,c}

OBSERVATIONS	EXPOSURE CONCENTRATION OF EGME, ppm			
	0	3	10	50
% implantations resorbed	4 (7/180)	8 (14/186)	11 (23/210) ^d	24 (46/191) ^d
% litters with resorptions	22(5/23)	42 (10/24)	58 (14/24) ^d	67 (16/24) ^d
fetal body weight (mean of liter, g)	39.57	39.13	38.83	35.88 ^d
<u>external and skeletal examinations</u>				
feruses	173	172	187	145
litters	23	23	24	22
<u>total malformations</u>				
% of feruses affected (# of feruses affected)	3.47 (6)	2.32 (4)	1.60 (3)	62.8 (91) ^d
% of litters affected (# of litters affected)	26.1 (6)	17.4 (4)	12.5 (3)	90.9 (20) ^d

a. Based on Hanley, T. R. Jr., Yano, B. L., Nitschke, K. D., and John, J. A. (1984b).

"Comparison of the Teratogenic Potential of Inhaled Ethylene Glycol Monomethyl Ether in Rats, Mice, and Rabbits," Toxicology and Applied Pharmacology, vol. 75, pp. 417-419, tables 8,9,10.

b. Bred New Zealand White rabbits were exposed to EGME for 6 hr/day on days 6 through 18 of gestation.

c. Litter sizes of exposed animals were comparable to the control values. Numerous, statistically significant (alpha equals 0.05), minor skeletal and developmental variations occurred in rabbits exposed to 50 ppm.

d. Statistically significant difference from the control value (alpha equals 0.05).

Figure 8. Delayed Vertebral Ossification in Fischer 344 Rats Exposed to EGME via Inhalation

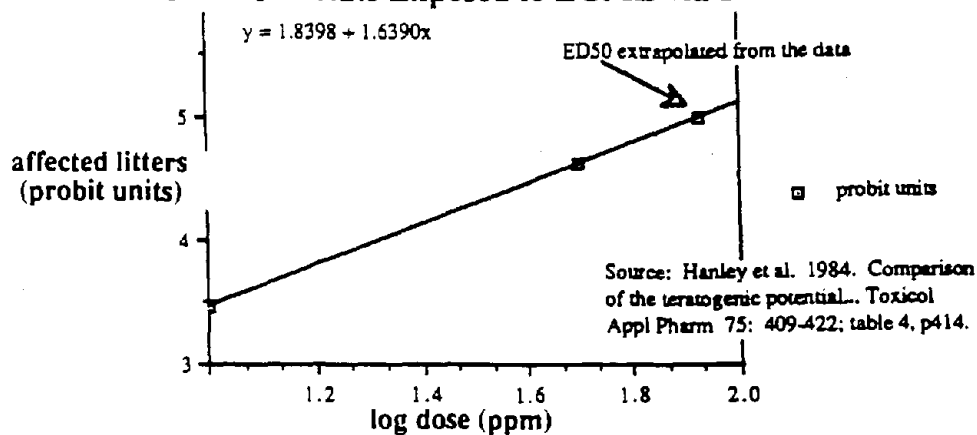
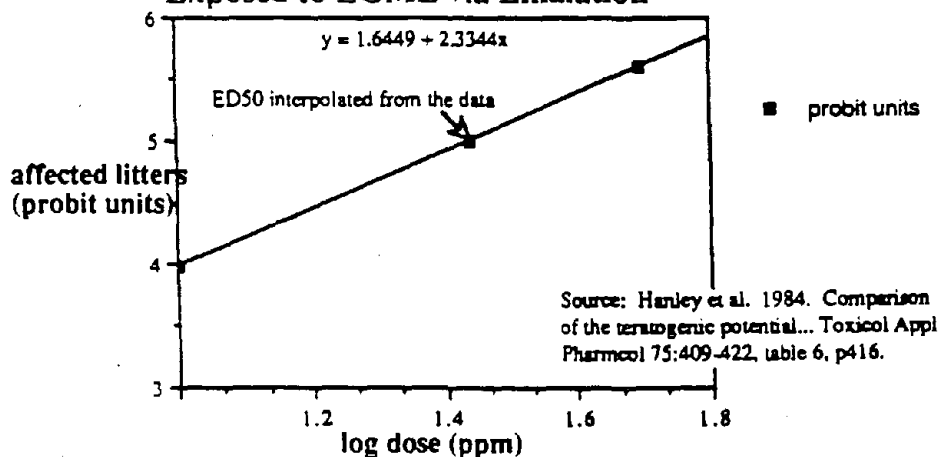


Figure 9. Extra Lumbar Ribs in CF-1 Mice Exposed to EGME via Inhalation



In the study of New Zealand White Rabbits exposed to EGME via inhalation, the percentage of litters affected by resorptions showed a dose-response relationship with EGME exposure (Figure 10). This information was used to calculate the ED₅₀ (Table 2-7). Groups exposed to either 10 or 50 ppm EGME showed significantly higher percentages of litters with resorptions than the control group.

2.2.2.2 Ethylene Glycol Monomethyl Ether via Oral Gavage

Hardin and Eisenmann (1987) exposed CD-1 mice to 304 mg/kg of EGME via oral gavage. These animals showed significantly higher numbers of litters with malformations in the exposed groups than in the control group (Table 2-6A). We did not, however, calculate a fetus-based ED₅₀ from these data because there was only one exposure concentration.

Greene et al., (1987) exposed CD-1 mice to EGME via oral gavage. They examined the percentage of fetuses affected with forepaw malformations at nine concentrations of EGME. The response at 100 mg/kg was indistinguishable from that of the control group (ie., both groups showed no fetuses with forepaw malformations). The remaining points are well described by a log-probit dose-response curve (Figure 11). From this graph we calculated the fetus-based ED₅₀ (Table 2-7).

2.2.3. Discussion of the ED₅₀'s Derived from Experiments with Animals: Analysis One

A visual inspection of Figures 1-11 reveals that the analyzed data sets generally fit a linear or log-linear dose-response curve reasonably well. Most often, the logarithm of the dose yielded a good fit to the data. This analysis, however, yielded some very high fetus-based ED₅₀'s (e.g., 9.5×10^5 and 2.1×10^4) (Table 2-7). Therefore, we distrusted these numbers and felt that this method used to calculate the ED₅₀'s does not work well in every situation. To fit the log probit dose-response curves, we sometimes used individual data points that were not significantly different from controls; in other cases, we rejected some data that had significant points within it because the data did not follow a consistent relationship of increasing response with an increasing dose. Some of the data sets displayed unusually low log-dose probit slopes and these same data exhibited the high estimates for individual fetus-based ED₅₀'s (Table 2-7). The log-dose probit slopes themselves ranged from 0.3362 to 5.7442. This variation in the log-dose probit slopes is greater than that found in our analyses of pharmacokinetic and carcinogenic data (section 4.1). By

Figure 10. Resorptions in New Zealand White Rabbits Exposed to EGME via Inhalation

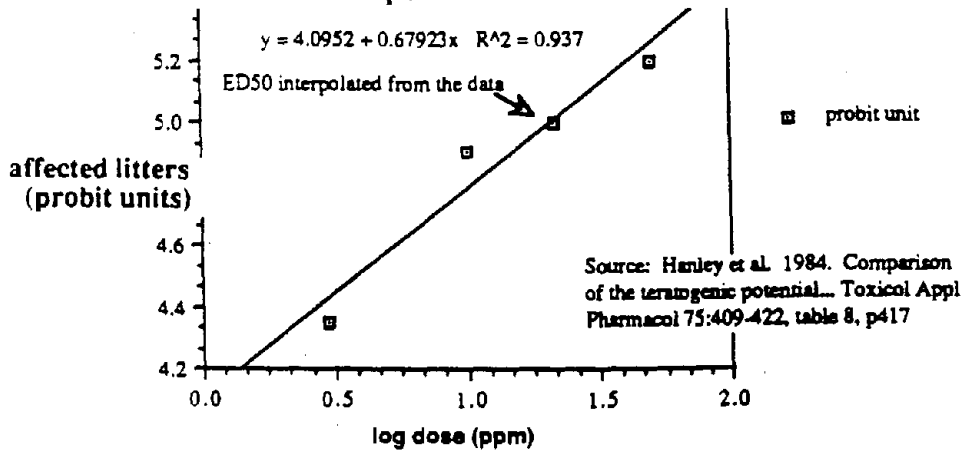


Figure 11. Digital Malformations in CD-1 Mice Exposed to EGME via Oral Gavage

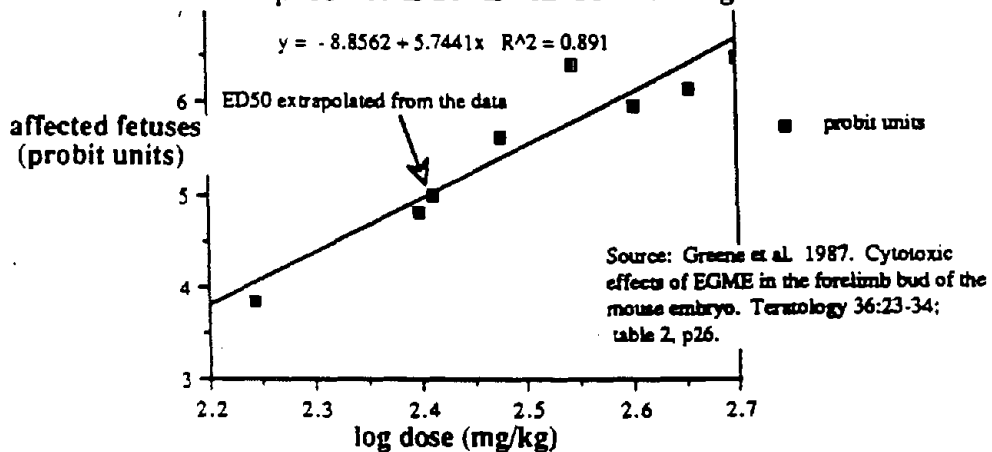


Table 2-6
Ethylene Glycol Monomethyl Ether (EGME) via Oral Gavage

A. Data of Hardin and Eisenmann (1987) for CD-1 mice^{a,b}

OBSERVATIONS	EXPOSURE CONCENTRATION OF EGME, (mg/kg)	
	0	304
# of litters examined	15	16
# of fetuses examined	169	176
# of malformations (litters)	2	14 ^c
% of malformations (litters)	13.3	87.5
malformations (fetuses)	3	112 ^c
% ^d of malformations (fetuses)	1.7	68.5

a. Based on Hardin, B. D., and Eisenmann, C. J. (1987). "Relative Potency of Four Ethylene Glycol Ethers for Induction of Paw Malformations in the CD-1 Mouse," Teratology, vol. 35, pp. 324, table 2.

b. Female mice were orally dosed on gestation day 11 (plug = 0) with 4 mmol/kg EGME.

c. The number of litters affected differs from the concurrent control ($p < 0.001$); although the authors failed to calculate the p value for the number of fetuses affected, the treated animals obviously have more malformations than the controls.

d. Percent of fetuses refers to the average % per litter.

B. Data of Greene et al. (1987) for CD-1 mice^{a,b}

OBSERVATIONS	DOSE OF EGME, (mg/kg)								
	0	100	175	250	300	350	400	450	500
# of fetuses examined	186	125	120	200	116	117	124	125	99
# of litters examined	16	11	10	18	10	11	11	10	10
% fetuses with forepaw malform..	0	0	12	42	73	92	83	87	93

a. Based on Greene, J. A., Sleet, R. B., Morgan, K. T., and Welsch, F. (1987) "Cytotoxic Effects of Ethylene Glycol Monomethyl Ether in the Forelimb Bud of the Mouse Embryo," Teratology, vol. 36, pp.26, table 2.

b. Female mice were orally dosed on gestation day 11 with a volume of 5.2 μ l/g body weight.

Table 2-7

Analysis One: Derivation of the Fetus-Based ED₅₀'s from Experiments on Rats, Rabbits and Mice

EGEE via inhalation

response	species	dose(ppm)	probit	log dose- probit slope	ED ₅₀ (ppm)	ref
minor skeletal defects	ALPK/AP rats	50 250	3.9233 6.7060	3.9811	93	Doe 1984
post-implantation loss		10 50 250	2.9896 3.2009 3.5612	0.4089	9.5 x 10 ⁵	
pre-implantation loss	Dutch rabbits	50 175	.04358 .08778	0.6542	2.1 x 10 ⁴	
skeletal variants		10 50 175	4.0666 4.3875 5.1741	0.8709	149	

EGEEA via inhalation

response	species	dose(ppm)	probit	log dose- probit slope	ED ₅₀ (ppm)	ref
minor external and visceral defects	Dutch rabbits	25 100 400	3.1588 3.5834 4.9983	0.0049 ^a	399 ^a	
any skeletal defects		100 400	4.0226 6.9173	4.8080	160	
skeletal variants		25 100 400	3.6531 4.5744 7.0375	0.0088 ^a	165 ^a	

a. Based upon a linear fit to the data. We tested the fit of a logarithmic transformation of the dose, but based on the r square it did not fit as well as the untransformed data.

(continued)

Table 2-7 (continued)

Analysis One: Derivation of the ED₅₀ from Experiments on Rats, Rabbits and Mice

EGEE via oral gavage

response	species	dose (mg/kg)	probit	log dose- probit slope	ED ₅₀ (mg/kg)	ref
% implants resorbed	CD-1 mice	1800	4.8913	4.9143	840	Wier 1987
		2600	5.6761			
malformations		1800	5.5012	5.4903	1459	
		2600	6.3781			

EGME via inhalation

response	species	dose(ppm)	probit	log dose- probit slope	ED ₅₀ (ppm)	ref
delayed ossification of vertebral centra; litter based	Fischer 344 rats	10	3.4788	1.6390	85	Hanley 1984
		50	4.6244			
extra lumbar ribs; litter based	CF-1 mice	10	3.9793	2.3344	27	
		50	5.6110			
unilateral testicular hypoplasia; litter based		10	3.6142	0.3362	1.3 x 10 ⁵	
		50	3.8492			
litters with resorptions	New Zealand White Rabbits	3	4.3455	0.6792	21	
		10	4.9034			
		50	5.1940			

EGME via oral gavage

response	species	dose (mg/kg)	probit	log dose- probit slope	ED ₅₀ (mg/kg)	ref
fetuses with forepaw malformations	CD-1 mice	175	3.8250	5.7442	259	Greene 1987
		250	4.7981			
		300	5.6128			
		350	6.4051			
		400	5.9542			
		450	6.1264			
		500	6.4758			

a test for parallelism* of the slopes, the smallest slopes were significantly different ($\alpha = 0.05$) from the weighted average of all the slopes. Many of the data sets contained too small a number of points to allow us to calculate the 95% confidence interval around the mean of the pooled slope. Based on the various log dose probit slopes (Table 2-7), we calculated fetus-based ED₅₀'s for several different reproductive effects.

2.3 Interpretation of the Data With a Fixed External Assumption of the Degree of Interindividual Variability--"Analysis 2"

2.3.1 Rationale for "Analysis 2"

Unfortunately, the values of the fetus-based ED₅₀'s vary widely even with relatively small changes in the log-dose probit slope (Table 2-7). In addition to the computational difficulty in determining appropriate log-dose probit slopes from these relatively noisy data, in any case the degree of interindividual variability reflected in the log-probit relationships for the animal data is likely to understate the interindividual variability that should be expected among free-living, genetically diverse humans. We therefore decided to adopt another approach, which we call "analysis 2." For this analysis, we assume a single fixed value for the log-dose probit slope for all data sets. This assumed value (2 for our best-estimate computations, 1 for later computations of upper confidence limit risks) is derived with the aid of an analogy from our previous analyses of the degree of interindividual variability observed in parameters that are putatively along the causal pathway to carcinogenesis (Hattis et al., 1987). In our judgment, the processes leading to fetotoxicity and teratogenicity are likely to be roughly similar in overall complexity** (and hence likely variability) as the processes leading to carcinogenesis for a precursor compound needing metabolic activation. Section 3.1 discusses in some detail the data on interindividual variability in parameters that are putatively along the causal pathway for carcinogenesis.

* Performed with the technique presented by Kleinbaum, Kupper, and Muller, 1988, p. 266.

** Steps in the process, each of which may occur at different rates and with different overall efficiencies in different individuals, are likely to include (1) absorption of the parent compound, (2) metabolic activation in the maternal liver and other organs, (3) metabolic detoxification in the maternal liver and other organs, (4) physical excretion of activated metabolites and the parent compound via the kidney, etc., (5) transfer of the parent compounds and/or active metabolites via the placenta to the fetus, (6) activating metabolism in the placenta and fetal organs, (7) detoxification of active metabolites in the fetus, (8) back-transfer of active metabolites from the fetus to the maternal circulation, and (9) action of the active metabolites in the fetus to produce fetal deaths or developmental abnormalities.

Analysis 2 uses exactly the same basic data sets used in analysis 1 (Tables 2-2 to 2-6); however analysis 2 is based solely on individual data points that were statistically significantly different ($p \leq 0.05$) from the concurrent control responses. Moreover, an independent ED₅₀ calculation is made from each of these significant data points, and the assumed log-dose probit slope of 2. The fetus--based ED₅₀'s are calculated by a method similar to the one noted in section 2.1.1 above:

$$ED_{50} = 10^{\left[\log_{10}(\text{dose}) - (\text{probit of the fetus-based response at this dose} - 5.0) / 2 \right]}$$

(where the probit of the response is corrected for background, as described in section 2.1.1.). The same formula is used for litter-based calculations when the original data are given in terms of the percentages of litters affected.

2.3.2 Derivation of Litter-Based ED₅₀'s, and Projection of Human Inhalational ED₅₀ Levels for "Analysis 2"

Since a litter-based measure is preferable to a fetus-based one (for the reasons noted in Section 2.1.2), where litter-based results were not given by the authors, we converted the data in analysis 2 into ED₅₀'s for a litter. In analysis two, this calculation can take place in two steps. First we calculate the "target" proportion of individual animals that would need to respond for half of the otherwise unaffected litters to contain an affected fetus. This is 0.5/average number of fetuses or implants per litter (based on the controls). For example, if the controls average 10 fetuses per litter, the target proportion of fetuses that need to be affected in order to affect just half the litters is 5%. Then the litter-based ED₅₀ is given by:

$$ED_{50} = 10^{\left[\log_{10}(\text{dose}) - (\text{probit of the fetus-based response at this dose} - \text{probit of "target" individual response}) / 2 \right]}$$

Table 2-8 shows this series of calculations performed for the ED₅₀'s from the fetus-, implant- or litter-based data in analysis 2. It can be seen that in general, when an ED₅₀ is converted from a fetus- or implant- into a litter-based measure, the value of the ED₅₀ is appreciably reduced, sometimes by an order of magnitude. Otherwise, it can be seen that there are examples in the table where these fetus-based calculations yield results that are both larger and smaller than the comparable results where litter-based data were available for direct analysis.

Later, in Section 3, we will use these results in projecting human risks at a variety of dose levels below the calculated human ED₅₀'s. The net effect of this will be the same as if we had projected human low-dose risks directly from the data without the intermediate step of calculating

Table 2-8
Relative Effectiveness of EGEE and EGME; Analysis 2

response ^a chemical, route of administration	species	data points ^a	ED50 fetus or implant 6hr (mg/kg or ppm)	ED50 litter 6hr (mg/kg or ppm)	ED50 ^b humans 8hr (ppm)
post-implantation loss					
EGEE					
EGEE-INH ^c	New Zealand White rabbits ^d	17/23 litters w/resorptions at 160 ppm	Not done	102 ppm	88.9
EGEEA-INH	Dutch Rabbits ^d	24.4% at 400 ppm; based on implants	1225 ppm	235 ppm	176.2
EGEE-GAV ^e	CD-1 Mice ^f	39 resorptions/51 implants at 2,600 mg/kg	1248 mg/kg	172 mg/kg	97.0
EGEE-GAV	CD-1 Mice	39 resorptions/80 implants at 1,800 mg/kg	2208 mg/kg	304 mg/kg	171.6
EGME					
EGME-INH	New Zealand White Rabbits ^g	16/24 litters w/resorptions (67%) at 50 ppm	Not done	40.3 ppm	30.2
"	"	14/24 litters w/resorptions (58%) at 10 ppm	Not done	11 ppm	8.3
skeletal defects					
EGEE					
EGEE-INH	Alpk/AP rats ^h	119/122 fetuses w/ minor skeletal defects; 250 ppm	36.2 ppm	4.9 ppm	3.6
EGEE-INH	Dutch Rabbits ^h	87/135 fetuses w/ minor skeletal defects; 175 ppm	188 ppm	36.4 ppm	27.3
"	"	72/138 fetuses w/ minor skeletal defects; 10 ppm	18.7 ppm (Caveat: apparent dose response inversion here)	3.6 ppm	2.7
EGEEA-INH	Dutch Rabbits ^h	91/93 fetuses w/ any skeletal defects; 400 ppm	43.9 ppm	8.6 ppm	6.3

(continued)

- a. Includes only those points which are statistically significant ($p \leq 0.05$), as tested either by the authors of the articles or by the chi-square.
- b. The dose at which 50% of an exposed human population will be affected by the chemical after an 8-hour day of exposure via inhalation (does not take sensitive subpopulations into account).
- c. The ending -INH stands for the inhalation route of exposure to the chemical used during the animal experiments.
- d. Andrew and Hardin 1984.
- e. The ending -GAV stands for the oral gavage route of exposure to the chemical used during the animal experiments.
- f. Wier 1987.
- g. Hanley et al 1984b
- h. Doe 1984

Table 2-8
Relative Effectiveness of EGEE and EGME; Analysis 2 (continued)

response ^a chemical, route of administration	species	data points ^a	ED50 fetus, implant or litter 6hr (mg/kg or ppm)	ED50 litter 6hr (mg/kg or ppm)	ED50 ^b humans 8hr (ppm)
malformations					
EGEE					
"external" malformations					
EGEE-GAV ^d	CD-1 Mice ^f	25/80 affected fetuses (31.3%) at 1,800 mg/kg	3280 mg/kg	451 mg/kg	255
EGME					
"total" malformations					
EGME-INH	New Zealand White Rabbits ^g	20/22 affected litters (90.9%) at 50 ppm	Not done	13.2 ppm	9.9
"forepaw" malformations					
EGME-GAV	CD-1 Mice ⁱ	14/16 affected litters (87.5%) at 304 mg/kg	Not done	89.6 mg/kg	59.9
EGME-GAV	CD-1 Mice ^j	14.4/120 affected fetuses (12%) at 175 mg/kg	677 mg/kg	93.8 mg/kg	62.8
"	"	84/200 affected fetuses (42%) at 250 mg/kg	315 mg/kg	43.7 mg/kg	29.2
"	"	84.68/116 affected fetuses (73%) at 300 mg/kg	148 mg/kg	20.5 mg/kg	13.7

a. Includes only those points which are statistically significant ($p \leq 0.05$), as tested either by the authors of the articles or by the chi-square.

b. The dose at which 50% of an exposed human population will be affected by the chemical after an 8-hour day of exposure via inhalation (does not take sensitive subpopulations into account).

c. The ending -INH stands for the inhalation route of exposure to the chemical used during the animal experiments.

d. Andrew and Hardin 1984.

e. The ending -GAV stands for the oral gavage route of exposure to the chemical used during the animal experiments.

f. Wier et al. 1987. The highest dose points in this series were omitted from the calculations because they would have required larger dose extrapolations to reach the ED₅₀ than the three doses whose data are shown.

g. Hanley et al 1984b

h. Doe 1984

i. Hardin and Eisenman 1987

j. Greene et al., 1987

ED50's. The ED50's, however, provide a convenient benchmark which can be used to compare the apparent potency of the different glycol ethers, and the relative sensitivities of different animals and endpoints for detecting effects.

2.3.3 Human Inhalational ED50's in Analysis 2: Interspecific Comparisons and Relative Potency of EGEE and EGME

Examination of the ED50's for on an 8-hour inhalation-equivalent basis (analysis 2, Table 2-8) shows that CD-1 mice, New Zealand White rabbits and Dutch rabbits show a similar sensitivity to EGEE's effect in increasing post-implantation loss. The ED50's based on CD-1 mice exposed to EGEE ranged from 97 to 171.6 ppm, while those for New Zealand White Rabbits were 88.9 ppm and Dutch Rabbits were 176.2 ppm. Thus three species tested under varying conditions show fairly consistent ED50s.

The ED50s for malformations, on the other hand, suggest that rabbits may be slightly more sensitive to the effect of EGME than are CD-1 mice. The ED50 for "external malformations" in CD-1 mice exposed to EGME was 255 ppm, and their ED50's for forepaw malformations ranged from 13.7 ppm to 62.8 ppm. In comparison, the ED50's for "total malformations" (including both external and visceral defects) of New Zealand White Rabbits exposed to EGME ranged from 4.6 to 9.9 ppm.

The two species tested for skeletal defects did not show large interspecies differences in their response to EGEE. The ED50 for the Alpk/AP rats was 3.7 ppm, while that for Dutch Rabbits ranged from 2.7 to 27.3 ppm.

By examining the ED50s for the same reproductive effect and species, we can compare the relative potency of EGEE and EGME (Table 2-8). To do this we first calculate the geometric mean of the post-implantation-loss ED50s for the rabbits and mice exposed to EGEE (127 ppm), then calculated the geometric mean of the similar ED50s for the rabbits exposed to EGME (16 ppm). Dividing the ED50 for EGEE by the ED50 for EGME, we obtain:

$$\frac{\text{ED}_{50} \text{ for EGEE}}{\text{ED}_{50} \text{ for EGME}} = \frac{127 \text{ ppm}}{16 \text{ ppm}} = 8.1$$

To estimate the upper limit for the relative potency, we similarly calculated the relative potency from the post-implantation-loss ED50s for EGEE and EGME based on a steeper log-dose probit slope of one. This yields a relative potency of 2.2. Thus, as a cause of early fetal death through post-implantation loss, EGME is at least 2 and probably about 8 times more effective than EGEE. Although there are a considerable amount of data on malformations in mice and rabbits exposed to EGEE and EGME, the studies counted different things under the term "malformations",

including external malformations, total malformations (both external and visceral) and forepaw malformations. Thus, the data are not strictly comparable for a determination of relative effectiveness.

3. Population Dose Response Relationships for Quantal Effects in Humans

In "Analysis 2" in Section 2.3 above we elected to impose a uniform assumption of a log probit slope of 2* based on an analogy with the results of an earlier study (Harris et al., 1986a) of human data for parameters that are putatively related to human susceptibility for carcinogenesis. In section 3.1 below, we review some of the data and analysis that leads us to this particular quantitative conclusion. First 3.1.1 examines data on the human variability of systemic pharmacokinetic parameters. 3.1.2 compares this relatively modest variability with the variability observed for parameters that could influence susceptibility to carcinogenesis in some cases. Finally 3.1.3 offers as a second point of comparison a first analysis of human data on the incidence of effects in human fetuses as a result of mercury exposure in utero. With all this as background, in Section 3.2, we proceed to carry on the projection of human risks to dose and risk-levels of potential interest for occupational health standard setting.

3.1 Observations of Human Interindividual Variability for Different Effects

3.1.1 Interindividual Variability in Systemic Pharmacokinetic Parameters

A standard toxicological practice within the U.S. government (EPA, FDA, and WHO) is to use a tenfold uncertainty factor to account for the variability in susceptibility among individual humans. This technique was first presented in a paper by Lehman and Fitzhugh (1954) of the FDA. It is unclear whether this analysis was based on any data. Dourson and Stara (1983) have defended this idea based on an analysis of data presented by Weil (1972). From 490 determinations of acute lethality in rats resulting from single oral doses of a toxicant, Weil calculated a series of log-probit dose-response slopes (Table 1 - Harris et al., 1987). Based on these data, Dourson and Stara observed that 92% of the chemicals had log probit slopes greater than 3. Or, in 92% of the cases, a tenfold lowering of dosage spans at least three standard deviations in the assumed lognormal distribution of thresholds for lethality. (For example, if a dose of 1 unit of the chemical produces 50% mortality, with a log probit slope of 3, a dose of 0.1 unit should therefore produce about 0.13% mortality.) Dourson and Stara caution, however, that a human population is more heterogeneous than a population of laboratory rats.

* For some upper limit calculations this value will be 1.

Our initial work for pharmacokinetic parameters (Hattis et al., 1987) sought to assemble and assess the variability of specific parameters in humans that might be predictive of susceptibility for either lethal or other (carcinogenic) effects. In this case, susceptibility is defined as the reciprocal of the dose that is needed to produce a standardized response in an individual or population. Specifically, we studied three particular types of pharmacokinetic parameters in humans, based primarily on studies of drugs in the basic scientific literature: elimination half lives ($T_{1/2}$), Area Under the Curve (AUC) and Peak Concentration (C_{max}) in blood:

- o Elimination half-lives ($T_{1/2}$) are defined as the time required for a twofold reduction in the concentration of the substance in some compartment (usually blood) after absorption is complete. The longer the elimination half-life, the higher the concentrations that will be attained in the body if exposure is continuous or repeated at a frequency that is short relative to the half-life. Therefore, elimination half-lives are likely to be key determinants of susceptibility to toxicity from chronic continuous exposures.
- o Area Under the Curve (AUC) is a plot of the plasma concentration versus time after exposure. The AUC integrates variability in the efficiency of absorption as well as metabolism and elimination.
- o Peak Concentration (C_{max}) in blood represents an effective dose in cases of acute toxicity from an isolated single exposure to a substance. Like AUC, it also integrates information about absorption; in this case, both the efficiency and dynamics of absorption.

Table 3-1 summarizes the variation we observed in the pharmacokinetic parameters $T_{1/2}$, C_{max} , and AUC in normal healthy adult human volunteers, in relation to the overall variability in lethal effect thresholds reported by Weil (1972).^{*} It can be seen that for purposes of this table, we

^{*} These data are crudely comparable and do not depart markedly from each other. Whereas Weil reported that 14% of the data sets from rats showed $\log_{10}$ geometric standard deviations over 0.25 (that is, probit slopes under 4), the percentage of chemicals showing this degree of variability in pharmacokinetic parameters in humans was about 5% for $T_{1/2}$, 20% for AUC measurements, and 8% for C_{max} . The type of variability discussed so far is purely due to physical transport and metabolic elimination mechanisms. It does not include that variability attributable to variation in exposure or in the response of people for a given concentration and time at the site of action of a toxicant. In addition, the contribution to variability from special subpopulations, such as the elderly, or children, is not considered in this approach. Given an exposed population of healthy adult volunteer subjects, the degree of interindividual variability in the population was comparable to that expected from analysis of the data on experimental animals.

Table 3-1

Comparison of Variability for Human Systemic Pharmacokinetic Parameters With the Distribution of Log Probit Dose-Response Slopes Observed in Oral LD₅₀ Determinations in Rats

Log ₁₀ Geometric Std. Deviation ^a	Cumulative % of Rat Observations ^b	Human Interindividual Variability in Pharmacokinetic Parameters					
		Elim. Half-Life		AUC		C _{max}	
		No. Obs.	Cum %	No. Obs.	Cum %	No. Obs.	Cum %
.5-.71	1.4						
.25-.5	13.7	2 ^c	5	3	20	1	8
.167-.25	32.0	5	16	3	40	2	25
.125-.167	51.8	13	45	2	53		
.1-.125	66.3	7	61	4	80	5	67
.085-.1	78	3	68	1	87		
.0625-.085	88.8	8	86	1	93	2	83
.045-.0625	95.7	5	98	1	100	2	100
.0333-.045	98.4	1	100				
.0154-.0333	100						
Total		44		15		12	
Overall Log Geom. Std. Dev.		.111		.145		.109	
95% Range of observations		.045-.269		.048-.439		.030-.399	

a. This is the reciprocal of the corresponding log probit slope. Thus a Log GSD of .5 corresponds to a log probit slope of 2.

b. Weil (1972) observations as quoted by Dourson and Starra (1983).

c. Each of these observations represents a single chemical. Where there were multiple observations of interindividual variability for a particular chemical, a combined geometric standard deviation was calculated by pooling the variances within the different experiments.

expressed interindividual variability as the $\log_{10}$ of the geometric standard deviation--which in turn would be the reciprocal of the corresponding log probit slope. In both the $T_{1/2}$ and $C_{\max}$ data, the median values of the $\log_{10}$ of the standard deviations fell just over 0.11. Where AUC or steady state concentrations were measured, there was a greater degree of interindividual variability. The median $\log_{10}$ of the standard deviation was about 0.145 and three of the 20 data sets exceeded 0.3. Since AUC data includes variability from both the absorption and elimination of a chemical, it is reasonable to expect to show somewhat more interindividual variability than $T_{1/2}$ data. Another way to look at the variability in the data is to examine the 95% ranges of the data; by this measure, both the $C_{\max}$ and AUC data appear more variable than the $T_{1/2}$ data. The ranges encompassing 2 standard deviations from the mean are 0.045 to 0.269 for $T_{1/2}$, compared to 0.048 to 0.439 for AUC data and 0.03 to 0.399 for $C_{\max}$ data. This means that, assuming the $\log_{10}$ geometric standard deviations are themselves lognormally distributed, in about 2% of the chemicals the $\log_{10}$ geometric standard deviations for $T_{1/2}$ will be above 0.27, whereas for AUC and $C_{\max}$, 2% of the chemicals will be above 0.4.

3.1.2 Interindividual Variability in Parameters Potentially Related to Carcinogenesis

Table 3-2 juxtaposes our observations of variability in systemic pharmacokinetic parameters with the variability we observed for a number of parameters that, under favorable circumstances, could affect individual susceptibility to carcinogenesis for specific carcinogens. In this case, each observation represents a separate experiment (covering at least four different people) but observations for individual chemicals were not combined. The upper part of the table includes only data from population samples that are putatively representative of "normal" populations, such as normal volunteers, fresh autopsy specimens from victims of head injuries, etc. The lower part of the data includes additional data from various "special subpopulations" to see if it was comparable to that shown in the "normal" populations.

It can be seen in Table 3-2 that this analysis covered two systemic pharmacokinetic parameters (AUC and $T_{1/2}$) and several parameters that measure processes distinctive to carcinogenesis (metabolic activation, enzymatic detoxification, DNA adduct formation, DNA repair, and others). A number of the parameters that measure processes that are more distinctive for carcinogenesis appear to have greater amounts of interindividual variability than the systemic pharmacokinetic parameters. The findings for each parameter studied are summarized below.

Table 3-2
Interindividual Variability for Various Categories of Parameters
(Numbers of Data Sets in Ranges of Log₁₀ Geometric Standard Deviation)

A. Putatively "Normal" Populations Only

Log ₁₀ Standard Deviation	Systemic Pharmacokinetics		Parameters Measuring Processes That are More Specific to Carcinogenesis					
	Elim. Half Life	AUC *	Metab. Activa- tion	Metab. Detox.	DNA Adduct Form.	DNA Repair	Chrom. Change	x-ray Sens.
0.5-0.999			5		1			
0.25-0.499	3	4	15	1	1			
0.125-0.249	21	7	1	2		1	3	
0.0625-0.1249	31	8	2			2	2	
0.03125-0.0624	6	1						
under 0.03125							1	1
Total	61	20	23	3	2	3	6	1

B. "Special" and Putatively "Normal" Populations Combined

Log ₁₀ Standard Deviation	Systemic Pharmacokinetics		Parameters Measuring Processes That are More Specific to Carcinogenesis					
	Elim. Half Life	AUC *	Metab. Activa- tion	Metab. Detox.	DNA Adduct Form.	DNA Repair	Chrom. Change	x-ray Sens.
0.5-0.999			6		7			
0.25-0.499	3	4	24	4	11			
0.125-0.249	21	7	3	4	4	1	3	
0.0625-0.1249	31	8	4			2	2	
0.03125-0.0624	6	1	1					
under 0.03125							1	1
Total	61	20	38	8	22	3	6	1

*Area Under the Curve in a plot of concentration X time, per mg/kg of dosage.

Source: Hattis et al. (1986a).

1. $T_{1/2}$ -- In general, the longer the half life, the higher the concentrations that will be attained in the body if exposure is continuous or repeated at a frequency that is short relative to the half life. Elimination half lives were found to have median values of $\log_{10}$ geometric standard deviation somewhat over 0.11.
2. AUC --in a plot of the plasma concentration of a chemical vs. time after exposure. This measure estimates the effective dose of the carcinogen that will directly react with the DNA. Because this measure integrates both the efficiency of absorption (per mg/kg of dose) and elimination kinetics, the AUC measurements show more interindividual variability than the $T_{1/2}$ measurements. For AUC measurements, the median $\log_{10}$ geometric standard deviation was about 0.145 and three of the 20 data sets were over 0.3 (compared with none of 61 data sets over 0.3 in the case of $T_{1/2}$).
3. Metabolic activation--provided by far the greatest interindividual variability out of all of the data studied. This data primarily consists of measurements of the activity of the P-450 groups of enzymes in reactions which added epoxide or hydroxyl groups to specific substrates, usually aromatic compounds, in cultured samples of human tissues. The median $\log_{10}$ geometric standard deviation for metabolic activation was about 0.4. The largest single value among 23 observations (for metabolic activation of benzo(a)pyrene by placental tissue in a mixed group of smoking and non-smoking mothers) was just under 1.0., and about 10% of the observations may be as high as 0.75. Observations of interindividual variation in this parameter among "special" populations was no greater, and possibly less than, that found in "normal" populations.
4. Enzymatic detoxification--the data was too limited to make any confident statements about the degree of interindividual variability for this parameter. Tentatively, the interindividual variability was less than that seen for metabolic activation: the median of the eight observations was 0.25 and no single observation was much over 0.3.
5. DNA adduct formation--contained few usable observations from "normal" populations. The interindividual variability was comparable to that seen in metabolic activation. The median of the 22 observations is just under 0.4.
6. DNA repair--the limited experimental data on "normal" tissues was conducted on white blood cells exposed to ultraviolet light; the light induced DNA synthesis and the rate of repair of the DNA was subsequently measured. In this case, the interindividual variability in DNA repair was low, less than that observed in metabolic activation and about the same order as that observed in $T_{1/2}$.
7. Chromosome aberrations, sister chromatid exchange(SCE) and radiation sensitivity--data was limited and came exclusively from studies of lymphocytes. The observations tentatively suggest less interindividual variability than in metabolic activation. The observations of chromosome aberrations fell in the range of 0.125 to 0.25 and the observations of SCE's ranged from 0.03 to 0.1.
8. Alveolar ventilation, ventilation per unit of body weight, % absorption in alveoli, uptake per unit body weight--the $\log_{10}$ geometric standard deviations for these parameters are displayed in (Table 3, Hattis 1986a). The variability in these parameters was relatively low, indeed, even lower than the variability in the systemic pharmacokinetic parameters.

Given the physiological and biochemical data presented above, it is possible to calculate an overall estimate of the likely interindividual variability in susceptibility for carcinogenesis, given a number of rather heroic assumptions. These are:

- o The interindividual variability in all of the parameters is truly lognormal
- o The log₁₀ geometric standard deviations of interindividual differences for specific chemicals are themselves lognormally distributed.
- o The individual parameters interact in a simple, multiplicative way (ie., a person who metabolizes a procarcinogen to an active metabolite three times faster and repairs DNA one-third as fast as the median person, will therefore be nine times more susceptible to a carcinogen compared to the median person, all other factors being equal.).
- o The individual parameters act independently of each other (for example, people with more rapid metabolic activation do not also tend systematically to have more rapid DNA repair). Also, the factors that alter susceptibility to the carcinogen and the factors that enhance susceptibility to "background" cancers act independently.
- o Simple measurement variability is small compared to the true long term interindividual variability in the parameters studied.

Given a simple multiplicative interaction between parameters, the variances of the lognormal distributions of different parameters can simply be added together. Therefore, an estimate of the overall interindividual variation in susceptibility can be calculated from the log₁₀ geometric standard deviations with the formula:

$$GSD_t = [(GSD_{\text{minute volume}})^2 + (GSD_{\text{AUC}})^2 + (GSD_{\text{Metabolic Act.}})^2 + (GSD_{\text{Metabolic Detox.}})^2 + (GSD_{\text{DNA repair}})^2]^{.5}$$

where GSD stands for the log₁₀ geometric standard deviation of the parameters.*

* In this type of calculation one must not use two parameters that are likely to be measuring individual susceptibility for the same portion of the carcinogenic pathway. For example, because much of the variability in DNA adduct formation appears to be caused by variability in the rate of metabolic activation, one should not use both a measure of variability in metabolic activation and a measure of variability in DNA adduct formation.

Each of the components in the above equation is uncertain for each carcinogen. To represent that uncertainty for each parameter, we can examine the spread of the log₁₀ geometric standard deviation measurements themselves in the different trials summarized in Table 3-2. We therefore calculated the geometric means and geometric standard deviations of the log₁₀ geometric standard deviations of the interindividual variability measurements (Table 3-3).

To calculate an overall estimate of GSD_t from the above equation and place confidence bounds on that estimate, one can perform a Monte Carlo simulation.* Sets of values for each of the log₁₀ geometric standard deviations for specific parameters are drawn at random, using the distributions specified in Table 3-3, and used to calculate a distribution of values of GSD_t . Four variations of the model were explored: 1) two that use metabolic activation or DNA adduct formation as the term for $GSD_{\text{Metabolic Act.}}$ and 2) two that use the data from either all of the populations studied in Table 3-2 or only from the "normal" populations. In each case, 200 values of GSD_t were calculated, and the range between the 10th and the 190th value became the 90% confidence interval. The results from the four runs are basically similar (Table 3-4). The geometric mean of the overall interindividual variability from these parameters is about 0.5 to 0.7 (corresponding to log probit slopes of 2 to 1.4); for about 95% of the samples, the log₁₀ geometric standard deviation should be less than 1. We therefore tentatively chose to use a log probit slope of 2 for our best estimate of interindividual variability for projecting human risks for teratogenic and fetotoxic risks, and a log probit slope of 1 for upper confidence limit projections.**

3.1.3 Observations of Apparent Interindividual Variability In Susceptibility for Fetal Effects from Dietary Methyl Mercury Exposure

We are fortunate to have available one other human data set that can be used to check the reasonableness of our uniform assumption of a log probit slope of 2 from interindividual variability in susceptibility for fetal effects. Recently Marsh et al. (1987) have published detailed information on the incidence of a variety of fetal mercury effects in relation to the maximal levels of mercury found in the hair of the mothers during gestation. (The observations come from an Iraqi mass poisoning incident which resulted from the distribution of methyl-mercury treated Green

* This was performed with an automated stochastic modeling system (DEMOS) developed by Henrion and Morgan (1985).

** A 95% upper confidence limit of one also corresponds to the log probit slope of one hypothesized in the old Mantel-Bryan (1961) model for "safety" calculations for carcinogens before it was recognized that the log probit model is inappropriate for carcinogenesis risk assessment.

Table 3-3
Summary of Lognormal Statistics Used to Represent Interindividual Differences
in Parameters that Affect Susceptibility for Carcinogenesis

<u>Parameter</u>	<u>Geometric Mean of Log₁₀ GSD</u>	<u>Geometric Standard Deviation of Log₁₀ GSD</u>
Minute volume or alveolar ventilation	.0744	1.459
Systemic Area Under the Concentration-time curve	.152	1.723
Metabolic activation	.322 (.377)*	1.843 (1.707)*
Metabolic detoxification	.233 (.229)*	1.310 (1.295)*
DNA adduct formation	.378 (.521)*	1.513 (1.591)*
DNA repair	.151	1.422

*Values in parentheses are the result of doing the analysis only on putatively "normal" populations

Table 3-4

Results of Demos Monte Carlo Simulation Modeling of Overall Interindividual Variability in Susceptibility from Studied Carcinogenesis-Related Parameters

Parameter Used to Represent Metabolic Activation	Data Restricted to Putatively "Normal" Groups?	-----GSD _t Results-----	
		Geometric Mean	Approx. 90% Confidence Interval
Metabolic Activation	No	0.53	0.33-0.98
DNA Adduct Formation	No	0.55	0.38-0.84
Metabolic Activation	Yes	0.56	0.37-1.02
DNA Adduct Formation	Yes	0.67	0.41-1.18

Revolution seed grain.) In all, 81 mother-infant pairs were studied. Table 3-5 shows the numerical results, grouped in four-fold ranges of dosage. Figures 12 through 16 show log probit dose response relationships for these effects, plotted similarly to the methods used for analysis of the animal data in Section 2. It can be seen that the log probit slopes for the five different fetal mercury effects range from 0.66 to 1.5. All, in other words, suggest greater interindividual variability (and hence greater low-dose risks) than implied by our best-estimate probit slope of 2.

One possible criticism of the fitting procedure represented in Figures 12-16 is that we have not weighted the individual points for their relative degree of statistical power. As a check on our procedure, therefore, we fit the data in Table 3-5 to a log-normal risk model in Clement Associates ToxRisk2 package--which essentially uses a log probit model with the addition of a background term, where indicated by the data. The model fit is given by

$$P(d) = A_0 + (1 - A_0)\Phi(A_1 + A_2 \log_{10} d)$$

Where $P(d)$ is the risk at a particular dose, A_0 is the "background" frequency of response, $\Phi(x)$ is the cumulative distribution function of the standard normal distribution, A_1 is a fitted term that essentially adjusts the equation to give the correct central tendency (ED_{50}) value, and A_2 is analogous to our log probit slope values. The table below allows a comparison between our fitted log probit slopes and the value of A_2 determined by the TOXRISK2 lognormal model system:

Effect	Clement TOXRISK2 Lognormal Model Parameters			Graphically-Determined Log Probit Slope (Fig 12-16)
	A_0	A_1	A_2	
Late Walking	0	-2.79677	1.211	1.037
Late Talking	.0744	-4.28124	1.793	1.520
Ment. Symptoms	.0253	-3.48317	1.112	1.023
Seizures	0	-3.4845	1.108	0.875
Neuro. Score >4	0	-2.5833	0.855	0.664

It can be seen that in general, the A_2 parameter calculated by the TOXRISK2 program, with its fine weighting of the points and maximum likelihood estimation methods, comes out a little bit higher (slightly less interindividual variability) than our simple procedure without weighting. Overall, however, there is reasonably close agreement, and in any case the conclusion appears unchanged that if anything the Marsh et al. (1987) data indicate greater interindividual variability than the log probit slope of 2 assumed by us for our best estimate calculations. The mean log probit slope for the five effects as determined by the TOXRISK2 lognormal model is about 1.22. Still the indication is that even for these mercury effects, our lower limit log probit value of 1 would still be

Table 3-5
Observations of Marsh et al. (1987) on the Incidence of Various Effects in
Children Following *In Utero* Methyl Mercury Exposure

<u>Observations of Various Effects</u>								
Maximum Hair Hg (ppm) During Gestation			Late Walking (After 18 Mo.)		Late Talking (After 24 Mo.)		"Mental" Symptoms	
Range	Geom. Mean	Number Subjects	No. Cases	Fract. Affect.	No. Cases	Fract. Affect.	No. Cases	Fract. Affect.
one to three	1.37	27	0	0.000	2	0.074	1	0.927
five to 19	10.00	14	2	0.143	1	0.071	0	0
20 to 79	52.53	13	2	0.154	3	0.231	1	0.077
80 to 319	163.38	12	3	0.250	4	0.333	3	0.250
over 319	436.60	15	12	0.800	11	0.733	4	0.267
		81						

Maximum Hair Hg (ppm) During Gestation			Seizures		Neurol. Score over 3		Neurol. Score over 4	
Range	Geom. Mean	Number Subjects	No. Cases	Fract. Affect.	No. Cases	Fract. Affect.	No. Cases	Fract. Affect.
one to three	1.37	27	0	0.000	3	0.111	0	0
five to 19	10.00	14	0	0.000	1	0.071	1	0.071
20 to 79	52.53	13	1	0.077	4	0.308	2	0.154
80 to 319	163.38	12	2	0.167	3	0.250	2	0.167
over 319	436.60	15	4	0.267	9	0.600	6	0.400

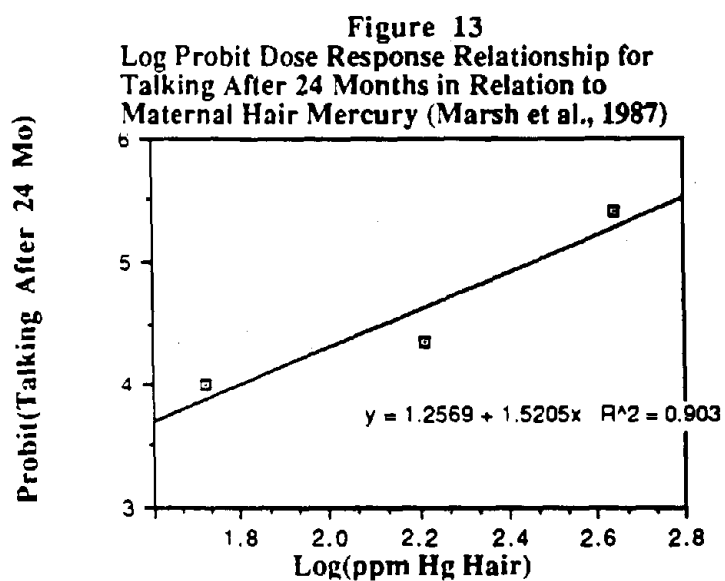
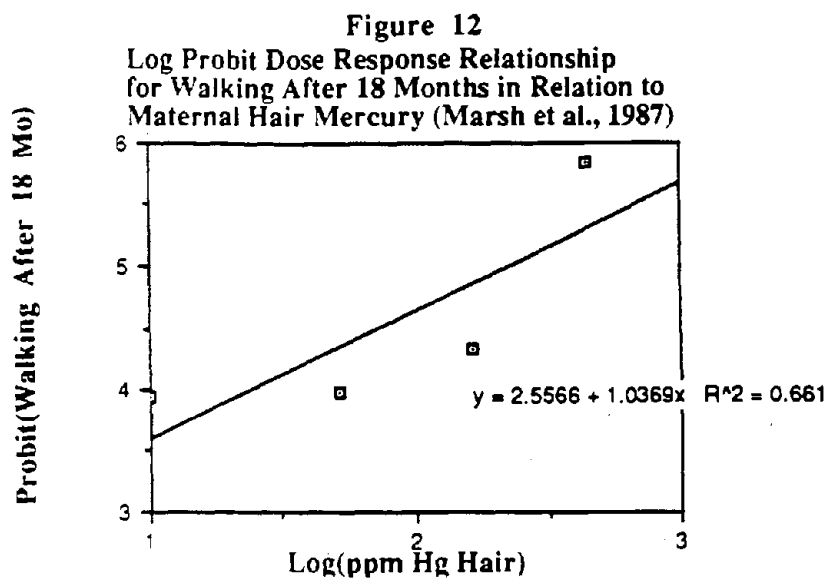


Figure 14
Log Probit Dose Response Relationship for
"Mental Symptoms" in Children in Relation
to Maternal Hair Hg During Pregnancy

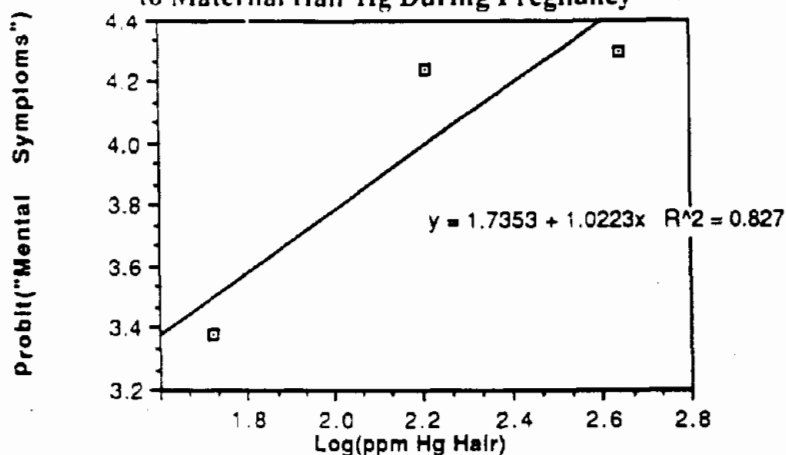


Figure 15
Log Probit Plot of Seizures in Children as a
Function of Maternal Hair Hg During
Pregnancy--Data of Marsh et al. (1987)

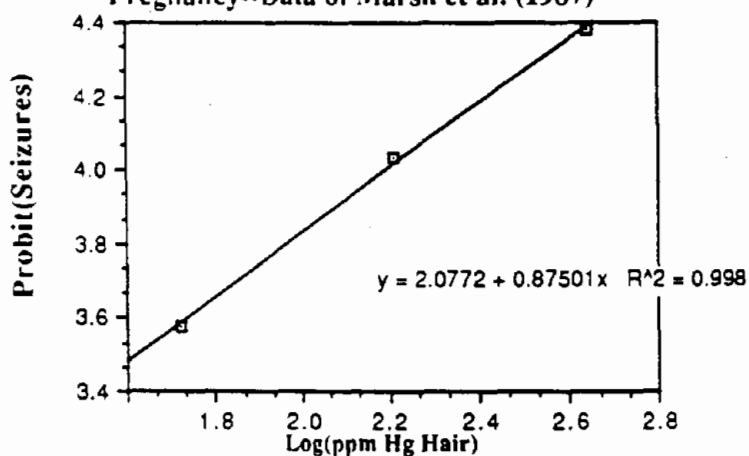
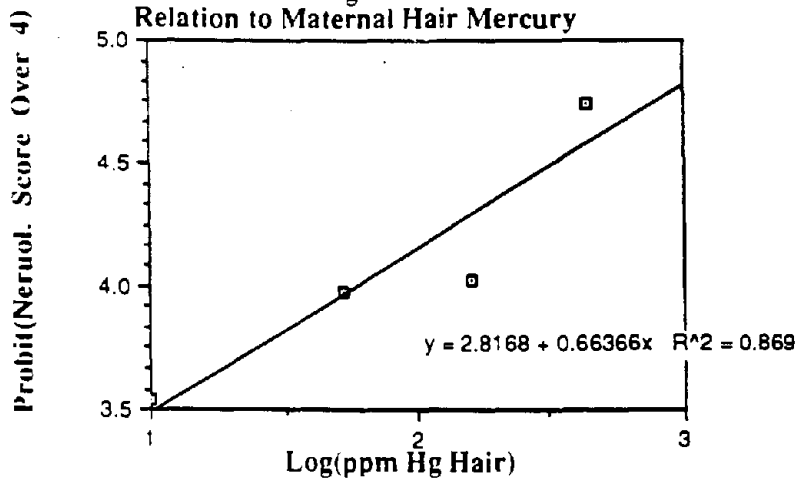


Figure 16
Log Probit Dose Response Relationship for
Adverse Neurological Scores in Children in
Relation to Maternal Hair Mercury



generally expected to somewhat overstate the actual amount of interindividual variability (although not by as comfortable a margin as we had imagined from our carcinogenesis results).

3.2 Quantal Population Dose Response Relationships, and Risk at Various Doses

3.2.1. "Best Estimates" of Quantal Reproductive Risks, Assuming No Interaction with Background Processes

After analyzing all of the ED₅₀ data for animals, sixteen 8-hour human ED₅₀ estimates resulted. By taking the geometric means of estimates for the individual chemicals for individual effects, six overall "best estimates" resulted for specific reproductive effects from either EGEE or EGME (Table 3-6).^{*} These values are used in the quantal estimates of reproductive risk presented in Tables 3-7 to 3-11.

The experiments on malformations had to be handled specially. The authors of the original articles chose different ways to count malformations. In small animals such as fetal mice, they counted all external malformations or malformations only in the external or skeletal structure of the forepaws. In the larger fetal rabbits, they counted external and visceral defects and reported the "total malformations" in the rabbits (unfortunately, they did not report the visceral or external defects separately). For these reasons, we used three estimates of human ED₅₀s for malformations. The best estimate for the ED₅₀ for "external malformations" in humans exposed to EGEE is based on one significant point in an experiment on CD-1 mice. Similarly, the ED₅₀ for "total malformations" in humans exposed to EGME is based on a single experiment in New Zealand White rabbits (the experiment in which the effect of the chemical was reported in the percentage of affected litters). There were eight significant data points in experiments examining the effect of EGME on forepaw malformations in CD-1 mice. We excluded four of these points

^{*} Generally where data were present for multiple experiments within species and among species, we first took geometric means for the ED₅₀ estimates within species, and then did geometric means for the values between species. To obtain the best estimate for post-implantation loss in humans exposed to EGEE, we calculated the geometric mean of the four ED₅₀s based on significant data points from the experiments on New Zealand White and Dutch rabbits, as well as CD-1 mice. Similarly, for the best estimate of the ED₅₀ for post-implantation loss in humans exposed to EGME, we calculated the geometric mean of the ED₅₀s derived from the two significant data points in the experiment on New Zealand White rabbits. For the best estimate of the human ED₅₀ for minor skeletal defects resulting from exposure to EGEE, we calculated the geometric mean of the ED₅₀s from three experiments on Dutch rabbits, then took the geometric mean of this result and averaged it with the ED₅₀ from an experiment on Alpk/AP rats. Generally, there were too few major skeletal defects among the experimental animals to provide a statistically significant increased incidence of these defects.

Table 3-6
Summary "Best Estimate" Values for Different Quantal Responses, Analysis 2

response ^a	chemical	species			ED50 ^b humans 8hr (ppm)
post-implantation loss	EGEE	New Zealand	Dutch	CD-1	127
		White rabbits ^c	rabbits ^c	mice ^d	
	EGME	New Zealand			16
		White rabbits ^e			
minor skeletal defects	EGEE	Alpk/AP rats ^f	Dutch rabbits		5.3
malformations	EGEE				
	"external"	CD-1 mice ^c			255
	EGME				
	"total"	New Zealand			9.9
		White rabbits ^e			
	"forepaw"	CD-1 mice ^{g,h}			35

a. Includes only those points which are statistically significant ($p \leq 0.05$), as tested either by the authors of the articles or by the chi-square.

b. The dose at which 50% of an exposed human population will be affected by the chemical after an 8-hour day of exposure via inhalation (does not take sensitive subpopulations into account).

c. Andrew and Hardin 1984.

d. Wier 1987.

e. Hanley et al., 1984b.

f. Doe 1984.

g. Hardin and Eisenman 1987.

h. Greene et al., 1987.

(from an experiment by Greene et al., 1987) because the percentage of affected fetuses at each exposure concentration was far above the ED₅₀ (83% or above) and would therefore have required a relatively large dose response interpolation. We took the geometric mean of the human ED₅₀s calculated from the remaining four points to obtain the best estimate of the human ED₅₀ for malformation of the hands, feet and limbs.

3.2.1.1. Method of Calculating Risks at Different Doses and Doses at Different Risks, Assuming No Interaction with Background

For the calculations in this section, we assume that the processes by which the glycol ethers cause the excess risk of reproductive effects do not interact with the biological processes causing the "background" risk of developmental abnormalities. Given the "best estimate" ED₅₀ values shown in Table 3-6, our assumed log probit slope of 2, and this assumption of independence, Table 3-7 shows estimates of the concentrations of EGEE and EGME associated with three different levels of risk--one in a hundred (1×10^{-2}), one in ten thousand (1×10^{-4}) and one in a million (1×10^{-6})., were estimated (Table 3-7).^{*} The doses corresponding to various risk levels were calculated by reducing the ED₅₀ levels by a factor proportional to the number of geometric standard deviations below the mean that represent a given level of risk, as shown below:

$$\text{dose at risk } x \text{ (ppm)} = \text{ED}_{50} \div (\text{reduction factor})$$

where:

the reduction factor

$$= 10^{(\text{geometric standard deviations below the mean to achieve risk } x \div \log \text{ dose-probit slope})}$$

For example, based on an ED₅₀ for post-implantation loss of 127 ppm:

$$\begin{aligned} \text{dose(ppm) at a risk of one in a hundred} &= 127 \text{ ppm} \div (10^{(2.3263 \text{ sd's below median} \div 2 \text{ standard} \\ &\quad \text{deviations per 10-fold change in dose)}) \\ &= 8.7 \text{ ppm} \end{aligned}$$

Similarly, the number of geometric standard deviations below the median that is needed to reduce risks to one in ten thousand are 3.719, and the number needed to reduce risks to one in a million is 4.7536. These values were calculated from the area under the curve of the standard normal

^{*} The risk level of one in a hundred was chosen because it represents a high risk, whereas a risk of one in a million was chosen because risks below this number are often accepted in the context of carcinogenic risk assessment. We do not here wish to imply, however, that any specific risk level is to be automatically deemed "acceptable" in general without reference to considerations of the feasibility and economic consequences of measures to reduce it.

Table 3-7

Doses (ppm) at "Best-Estimate" Risks of One in a Hundred, One in Ten Thousand, and One in a Million

Response	ED50 humans 8hrs (ppm)	Dose (ppm) at 10^{-2} risk	Dose (ppm) at 10^{-4} risk	Dose (ppm) at 10^{-6} risk
Chemical, species				
post-implantation loss				
EGEE				
NZW ^a rabbits, Dutch rabbits, CD-1 mice ^{b,c,d}	127	8.7	1.8	0.53
EGME				
NZW Rabbits ^e	16	1.1	0.22	0.067
minor skeletal defects				
EGEE				
Dutch rabbits, Alpk/AP rats ^c	5.3	0.36	0.073	0.022
malformations				
EGEE				
"external" CD-1 mice ^d	255	17	3.5	1.1
EGME				
"total" NZW rabbits ^e	9.9	0.68	0.14	0.042
"forepaw" CD-1 Mice ^f	35	2.4	0.48	0.147

a. New Zealand White rabbits

b. Andrew & Hardin 1984

c. Doe 1984

d. Wier 1987

e. Hanley et al., 1984b

f. Greene et al., 1987

distribution (for a one-tailed test), using a polynomial approximation (Texas Instruments, 1977; Abramowitz and Stegun, 1964).

Table 3-8 shows analogous calculations of the risk of adverse reproductive effects at different specific 8-hour ppm exposure levels of EGEE or EGME.* Again, these calculations are based on the assumption of a lack of interaction between the chemicals and the process causing the background rate of developmental abnormalities.

3.2.1.2 Discussion of Results: Risks at Different Doses and Doses at Different Risks. Assuming No Interaction with Background

The results suggest that even quite low exposures to EGEE and EGME carry substantial risks of embryoletality, skeletal defects or malformations in humans (Table 3-7). Exposure to as little as 0.68 ppm of EGME may produce a risk for any type of (both external and visceral) malformation that is as high as one in a hundred. For comparison, the recommendation of the American Conference of Governmental Industrial Hygienists (ACGIH), a time-weighted average (for an eight-hour work day) of the threshold limit value (TWA-TLV) for EGME, is 5 ppm and the OSHA standard is 25 ppm (based on exposure to the skin) (Paustenbach, 1988). Similarly, the exposure at which our data suggest that the population might experience a one in a hundred risk of a significant increase in some kind of malformation in a limb is 2.4 ppm. And the exposure at which the exposed population may experience a one in a hundred risk of embryoletality is about 1.1 ppm of EGME. These risks appear at concentrations far below the current OSHA standards. Exposure to the 5 ppm ACGIH TWA-TLV for EGME appears to involve substantial reproductive risks----on the order of 28% for overall malformations, and 16% for post-implantation loss (Table 3-8). The risks expected at the 25 ppm current OSHA TLV are even higher. The concentration of

* The risk at a given concentration of EGEE or EGME can be calculated with the following formula:

risk = area under a normal curve corresponding to 5 probits + (log dose-probit slope * log₁₀ (dose in ppm ÷ ED₅₀))

For example, given a log dose-probit slope of two, an ED₅₀ of 88.9 ppm and a dose of 1 ppm, the calculation becomes:

$$\begin{aligned}\text{risk} &= 5 \text{ probits} + (2 * \log_{10} (1 \text{ ppm}/88.9 \text{ ppm})) \\ &= 1.1022 \text{ probits}\end{aligned}$$

Looking at the probits table in Finney (1971) or a calculation of the area under the curve of the normal distribution (Abramowitz and Stegun 1964), 1.1022 probits represents a risk of about 3.3×10^{-5} .

Table 3-8.

"Best Estimate" Risks to Humans from Exposure to EGEE and EGME

Response	ED50 humans 8hrs(ppm)	risk at 0.001 to 25 ppm					
		0.001 ppm	0.01 ppm	0.1 ppm	1.0 ppm	5.0 ppm	25.0 ppm
post-implantation loss							
EGEE							
	127	9.4E-25	1.1E-16	2.7E-10	1.3E-05	0.0025	0.079
EGME							
	16	2.1E-17	7.4E-11	5.2E-06	0.008	0.16	0.65
minor skeletal defects							
EGEE							
	5.3	4.8E-14	2.6E-08	2.8E-04	0.074	0.48	0.92
malformations							
EGEE							
"external"	255	1.5E-27	6.2E-19	4.8E-12	7.4E-07	3.2E-05	0.022
EGME							
"total"	9.9	6.7E-16	1.0E-09	3.3E-05	0.023	0.28	0.79
"forepaw"	35	5.1E-20	6.8E-13	1.8E-07	0.001	0.046	0.39

EGME below which risks are small (i.e., less than one in a million) falls below about 0.042 ppm for any kind of malformation, 0.147 ppm for limb malformations and 0.067 ppm for embryoletality, in this best-estimate projection series.

For EGEE, the TLV-TWA (for an eight-hour work-day) of the ACGIH is 5 ppm and the OSHA standard is 200 ppm (based on exposure of the skin). Our projections suggest that exposures to EGEE in concentrations of about 17 ppm produce a risk of external malformations of one in a hundred. Best-estimate projected risks of skeletal defects might reach one in a hundred at concentrations of EGEE of 0.37 ppm. Projected risks of embryoletality reach one in a hundred at concentrations of about 8.7 ppm (Table 3-7) and are about 2.5 per 1000 at the 5 ppm ACGIH TLV-TWA. Risks become small (less than one in a million) at concentrations of EGEE of less than about 0.53 ppm for post-implantation loss, 0.022 ppm for skeletal defects, and 1.1 ppm for external malformations.

Clearly for both EGME and EGEE, our best-estimate risk projections suggest that there are very significant female reproductive risks at the current OSHA standards for these materials. Orders of magnitude reductions in current permissible level would seem to be desirable. Data recently reported by Paustenbach (1988) indicate geometric mean levels of 0.36 ppm for EGEE and 0.09 ppm for EGME, based on surveys in the semiconductor industry.

3.2.2 Population Dose Response Relationships, and Risk at Various Doses, Assuming Significant Background Interactions

What if our "best estimate" assumption of no interaction between EGME and EGEE with processes producing "background" reproductive risks (the "normal" population incidence of these effects due to miscellaneous causes) proves not to be correct? In this section we offer some illustrative calculations of the potential effects of background interactions on the risks projected in Tables 3-7 and 3-8 above.

A first step is determining what types of "background" reproductive effects are potentially eligible for interaction with EGME and EGEE. Since both EGEE and EGME have not demonstrated genotoxic effects, we subtracted out that component of the background risk of miscarriages or malformations in humans resulting from genetic damage. In the human population, the "background" risk of early miscarriages can be as high as about 30% in women who have had previous miscarriages and about 14% for all pregnancies (Mosher and Pratt 1982). Since 14% includes both high and low risk pregnancies, we chose this number to represent the background risk of miscarriages. Therefore, about 5% to 6% of all recognized conceptions in humans are chromosomally abnormal; most of them are lethal (Vogel and Motulsky 1979). They

lead to miscarriage or to delivery of a severely malformed child. The surviving infants generally suffer from X-chromosomal aneuploidies. Since the approximate incidence of all X-chromosomal aneuploidies in humans is about 0.6% (Vogel and Motulsky 1979), the risk of miscarriages due to chromosomal abnormalities varies from 4.4% to 5.4%. We chose the midpoint of this range, 4.9%, to represent the risk of miscarriages in humans due to chromosomal abnormalities. Subtracting this risk of a miscarriage due to chromosomal abnormality from the total risk of miscarriage in the population yields:

$$\begin{aligned} &\text{Eligible background risk of miscarriages} \\ &= (\text{total risk of miscarriages}) - (\text{risk of miscarriages from chromosomal abnormalities}) \\ &= 14\% - 4.9\% = 9.1\% \end{aligned}$$

About 24.1% of all infants' deaths in 1980 were due to congenital anomalies and the mortality rate from congenital anomalies was 2.45 deaths per thousand live births (Christianson et al., 1981). A "birth defect" is defined as any structural, functional, or biochemical abnormality, whether genetically determined or induced during gestation, that is not due to injuries suffered during birth. Recognized birth defects afflict 1 of every 14 live-born infants (about 7.1 percent), or more than a quarter-million in the U.S. each year (OTA 1985, March of Dimes 1984). Some component of the total birth defects is due to chromosomal abnormalities, and because EGEE and EGME are not genotoxic, the processes causing genotoxicity would not be expected to interact with these chemicals. Therefore, we subtracted out the risk of chromosomal abnormalities from the total risk of birth defects in the human population. Approximately 10 in 1,000 live born infants are born with a single gene disorder and an additional 6 in 1,000 live born infants are born with a major chromosomal abnormality (Office of Technology Assessment 1986), resulting in a risk of 1.6% for abnormalities of primary genetic origin. Thus, for the purposes of this study, the eligible background risk is:

$$\begin{aligned} &\text{Eligible background risk of malformations (or birth defects)} \\ &= (\text{total risk of birth defects in the human population}) - (\text{risk of chromosomal abnormalities in the human population}) \\ &= 7.1\% - 1.6\% = 5.5\% \end{aligned}$$

Given the appreciable background risks of different malformations, and assuming in this section that the glycol ether effects do interact with the processes causing the "background" teratogenic anomalies and fetal losses, how much of the excess risk can be attributable to exposure to glycol ethers? To estimate the excess risk, one performs a series of calculations. First, one estimates a dose of EGEE or EGME that would produce a risk equivalent to the observed "background" risk of birth defects in the human population. For example, for EGEE, with an estimated human ED50 of 127 ppm, our best estimate of the dose that would itself produce a 9.1%

(probit of 3.6654) is $127 \cdot 10^{(3.6654 - 5)/2} = 27.32$ ppm. Next, one calculates the risk attributable to an extra one ppm of exposure to EGEE, i.e., the risk attributable to 28.32 ppm--which calculates out to be 9.62% by methods detailed earlier. Now by subtracting out the background risk (9.1%) from the total risk projected for 28.32 ppm (9.62%), one can then estimate the excess risk (0.52%) due to exposure to one ppm of EGEE under conditions of background interaction. Comparing the corresponding value without background interaction in Table 3-8 (1.3×10^{-5}) it can be seen that the effect of the assumption of an interacting 9.1% background has been to increase the extra risk attributable to 1 ppm of EGEE by $(.0052/.000013) = 400$ fold.

Table 3-9 presents a full set of these calculations with background interactions according to the format used for Table 3-8. It can be seen that the multiplicative increment to risk produced by interaction increases as the concentration of EGEE or EGME decreases. With interaction, the effect of exposure is linear at the limit of lower dosage, whereas without interaction, the risk falls rapidly because of the rapidly decreasing level of the probit function with dose. For example, at about 0.001 ppm exposure to EGEE or EGME the excess risks of malformations or miscarriages exceed 1×10^{-6} with interaction but the risks without interaction are minimal (the largest risk at 0.001 ppm is about 4×10^{-14}). At the highest levels of exposure the effect of interaction declines, and even appears in the table to be a slight decrease in risk. However, this is due to an implicit change in the group considered to form 100% for purposes of the calculation--from the otherwise unaffected population (Table 3-8), to the total group of conceptuses or births whether affected in the absence of exposure or not (Table 3-9).

3.2.3 Uncertainty and the Upper estimates for Quantal Risks, With and Without Interaction with Background

What are the upper bounds of the risk estimates in Tables 3-7 to 3-9? To answer this question we used the same raw data and types of calculations as for the best estimates; however, we substituted a log-dose probit slope of one in all of the calculations of the ED₅₀s and their associated risks. The choice of this slope is supported by the analysis, described above (section 3.1), of the variability in biological processing shown by normal humans exposed to toxicants and carcinogens. And as the comparison with the risks of fetal exposure to methyl mercury shows (section 3.1.3), some real human fetal responses seem to be as variable among individuals as would be implied by a log probit slope of 1.

Table 3-9

Excess Reproductive Risks Due to EGEE and EGME, Assuming Interaction with the Processes Causing the Background Risk of Miscarriages (But "Best Estimate" Value for Log Probit Slope of 2)

Response	ED50 humans 8hrs(ppm)	excess risk at 0.001 to 25 ppm					
		0.001 ppm	0.01 ppm	0.1 ppm	1.0 ppm	5.0 ppm	25 ppm
post-implantation loss, assuming a 9.1% background rate of miscarriages							
EGEE							
	127	5.2E-06	5.2E-05	5.2E-04	0.0052	0.026	0.13
EGME							
	16	4.1E-05	4.1E-04	0.0041	0.041	0.198	0.60
minor skeletal defects, assuming a 5.5% background rate of birth defects							
EGEE							
	5.3	1.1E-04	0.0012	0.012	0.124	0.479	0.865
malformations, assuming a 5.5% background rate of birth defects							
EGEE							
"external"	255	2.4E-06	2.4E-05	2.4E-04	0.0024	0.012	0.064
EGME							
"total"	9.9	6.1E-05	6.2E-04	0.0062	0.066	0.306	0.75
"forepaw"	35	1.7E-05	1.7E-04	0.0017	0.018	0.094	0.40

In the upper estimates of risk the estimated ED50s are smaller and the associated risks higher than those shown by the best estimates. Most of the ED50s with a log-dose probit slope of one became 4 or 5 or more times smaller than the ED50s with a log-dose probit slope of two (Table 3-10). For the upper estimates, the inhalational doses that cause a given level of risk became one to three orders of magnitude lower for EGEE (Tables 3-10 and 3-7). A one in a million risk level might result from exposure to as little as 1.3×10^{-4} to 4.6×10^{-5} ppm EGME or 1.1×10^{-3} to 4.4×10^{-6} ppm EGEE (this does not include the effect of interaction with background).

The upper estimates of risk reflect the greater uncertainty in the risk estimates for low concentrations of EGEE or EGME. At 0.001 ppm, the upper and best estimates may differ by 11 to 20 orders of magnitude from each other (Tables 3-11 and 3-8). These differences decrease at higher concentrations of EGME or EGEE; for example, at 1 ppm the upper and best estimates differ by 1 to 5 orders of magnitude. At high concentrations such as 25 ppm the best and upper estimates differed by less than one order of magnitude and in some cases were very similar. In the case of miscarriages from exposure to 25 ppm EGME, the estimate calculated from the log-dose probit slope of one was actually slightly lower than that from the log-dose probit slope of two; probably the small differences in upper (15 ppm) and best (16 ppm) estimates of the ED50s explains this finding.

On the other hand, when the effect of interaction with background was considered (Table 3-12), the upper and best estimates consistently differed by one order of magnitude or less in the range of 0.001 to 25 ppm EGEE or EGME. Possibly the interaction of EGEE or EGME with the biological processes causing the background rate of birth defects accounts for some of the variability seen in the estimates of risk at low concentrations. When the interaction is taken into account, the risk estimates become more stable at low concentrations. Again, at high concentrations the upper and best estimates become very similar, reflecting the lower uncertainty in the estimates of risk from a high exposure (Tables 3-9 and 3-12).

Table 3-10

Upper Estimates for Doses (ppm) Associated with Risks of One in a Hundred, One in Ten Thousand, and One in a Million (Based on an Assumed Log Probit Slope of 1, but Without Background Interaction)

Response	ED50 humans 8hrs (ppm)	Dose (ppm) at 10 ⁻² risk	Dose (ppm) at 10 ⁻⁴ risk	Dose (ppm) at 10 ⁻⁶ risk
Chemical, species				
post-implantation loss				
EGEE				
NZW ^a rabbits, Dutch rabbits, CD-1 mice ^{b,c,d}	32	0.15	0.0061	0.00056
EGME				
NZW Rabbits ^e	15	0.071	0.0029	0.00026
minor skeletal defects				
EGEE				
Dutch rabbits, Alpk/AP rats ^e	0.25	0.0012	4.8E-05	4.4E-06
malformations				
EGEE				
"external" CD-1 mice ^d	64	0.3	0.012	0.0011
EGME				
"total" NZW rabbits ^e	2.6	0.012	5E-04	4.6E-05
"forepaw" CD-1 Mice ^f	7.3	0.034	0.0014	0.00013

a. New Zealand White rabbits

b. Andrew & Hardin 1984

c. Doe 1984

d. Wier 1987

e. Hanley et al., 1984b

f. Greene et al., 1987

Table 3-11

Upper Estimates of Risks to Humans from Exposure to EGEE and EGME (Based on a Log Probit Slope of 1, but Without Background Interaction)

ED50		risk at 0.001 to 25 ppm					
Responsehumans		0.001	0.01	0.1	1.0	5.0	25.0
8hrs(ppm)		ppm	ppm	ppm	ppm	ppm	ppm
post-implantation loss							
EGEE							
	32	3.3E-06	2.3E-04	0.0061	0.066	0.21	0.46
EGME							
	15	1.5E-05	7.5E-04	0.015	0.12	0.32	0.59
minor skeletal defects							
EGEE							
	0.25	0.0082	0.081	0.35	0.73	0.90	0.98
malformations							
EGEE							
"external"	64	7.7E-07	7.1E-05	0.0025	0.035	0.13	0.34
EGME							
"total"	2.6	3.2E-04	0.0079	0.079	0.34	0.61	0.84
"forepaw"	7.3	5.6E-05	0.0021	0.031	0.19	0.43	0.70

Table 3-12

Grand Upper Estimates of Excess Reproductive Risks Due to EGEE and EGME, Assuming A Log Probit Slope of 1, and Also Interaction with the Processes Causing the Background Rates of Reproductive Risks

ED50		excess risk at 0.00001 to 25 ppm							
Responsehumans		0.00001	0.0001	0.001	0.01	0.1	1.0	5.0	25.0
8hrs(ppm)		ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
post-implantation loss, assuming a 9.1% background rate of miscarriages									
EGEE	32	4.8E-07	4.8E-06	4.8E-05	4.8E-04	0.0047	0.042	0.15	0.38
EGME	15	1E-06	1E-05	1E-04	0.001	0.01	0.081	0.25	0.5
minor skeletal defects, assuming a 5.5% background rate of birth defects									
EGEE	0.25	7.7E-05	7.6E-04	0.0075	0.063	0.3	0.67	0.85	0.92
malformations, assuming a 5.5% background rate of birth defects									
EGEE									
"external"	64	3E-07	3E-06	3E-05	3E-04	0.003	0.027	0.11	0.3
EGME									
"total"	2.6	7.4E-06	7.4E-05	7.4E-04	0.0072	0.061	0.29	0.56	0.78
"forepaw"	7.3	2.6E-06	2.6E-05	2.6E-04	0.0026	0.024	0.16	0.39	0.65

4. Assessment of Human Risk from Continuous Fetal Weight/Birth Weight Data

4.1 Analysis of Continuous Data in Animals for Changes in Birth Weight with Dose

In at least two cases (Figures 17 and 18 on the following page), the average weight of exposed fetuses decreased with the concentration of glycol ether administered to the dam. Moreover this effect appeared to show a simple linear relationship with dose.* This finding was surprising to us in that it contradicted our general expectation that gross measures such as body weights tend not to begin to show effects until important body compensatory systems are overwhelmed, and there are other gross manifestations of toxicity. This expectation, however, is based on experience from studies in adult animals. Mechanistically, it seems possible that a rapidly growing organism, using essentially all the available metabolic energy it can muster to grow and differentiate, might well have little or no functional reserve capacity to absorb added insults to key systems without effect. Thus even marginal additional stresses might produce responses at the limit of low dose, if key limiting systems are affected by the stressors. Essentially, the toxic chemical could be acting as a tax on a limiting function for the organism (growth and differentiation) under circumstances where every available resource would ordinarily be mobilized to the cause of growth and development.

Beginning with the regression line shown for the Weir data in Figure 17, we can derive an expression for a percentage reduction in fetal weights as a function of dose by dividing by .013608:

$$\% \text{ normal weight} = 100 - .0164 * (\text{mg/kg EGEE exposure})$$

Because in eight hours a mouse breathes about 1.769 mg/kg for every ppm of exposure, this equation becomes

$$\% \text{ normal weight} = 100 - .029 * (\text{ppm EGEE exposure over 8 hours}).$$

* We also graphed the logarithm of the dose against the fetal weight, but this analysis did not fit the data as well.

Treated similarly, the body weight reduction relationship from the data of Hanley et al. (Figure 18) becomes:

Figure 17. Loss in Fetal Weight in CD-1 Mice Exposed to EGEE via Oral Gavage

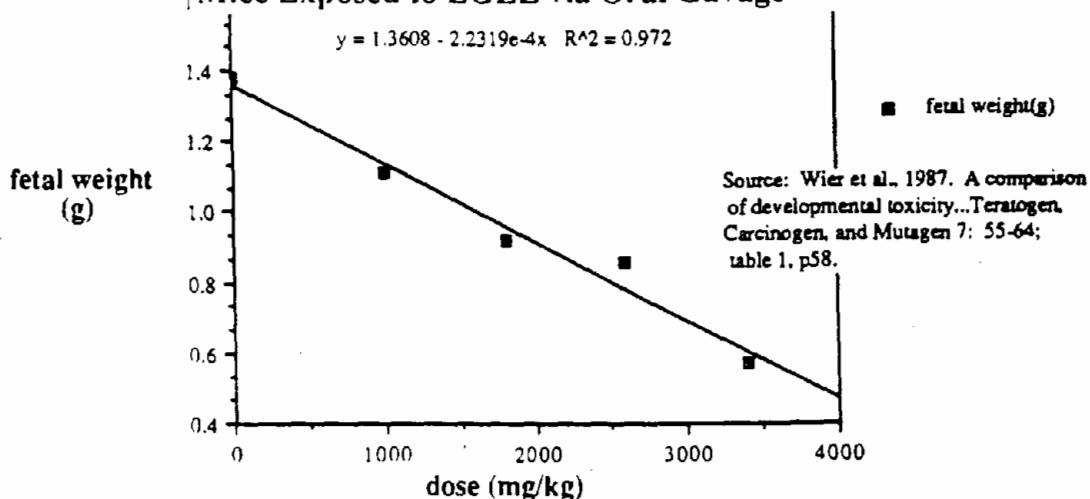
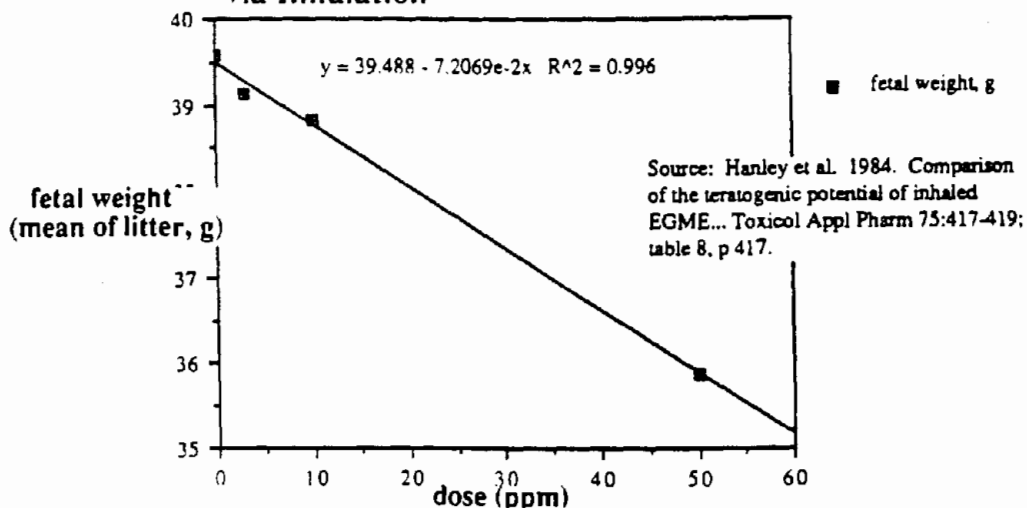


Figure 18. Loss in Fetal Weight in New Zealand White Rabbits Exposed to EGME via Inhalation



$$\% \text{ normal weight} = 100 - .1825 * (\text{ppm EGME exposure})$$

Converting from a 6-hour to an 8-hour period of exposure, this becomes:

$$\% \text{ normal weight} = 100 - .243 * (\text{ppm EGME exposure}).$$

Thus, the coefficients of these two data imply that EGME is $.243/.029 = 8.4$ times as potent in lowering fetal weights. This is almost identical with the ratio of 8.1 we derived for the relative potency of EGME and EGEE for inducing postimplantation fetal loss (see p. 37 above).

Some, but not all, of the results of Doe (1984) also imply some reductions in fetal weight at relatively high doses. For ALPk/AP rats, a regression line fit to the existing (nonsignificant) data points suggests

$$\% \text{ normal weight} = 100 - .0476 * (\text{ppm 8 hour EGEE exposure}).$$

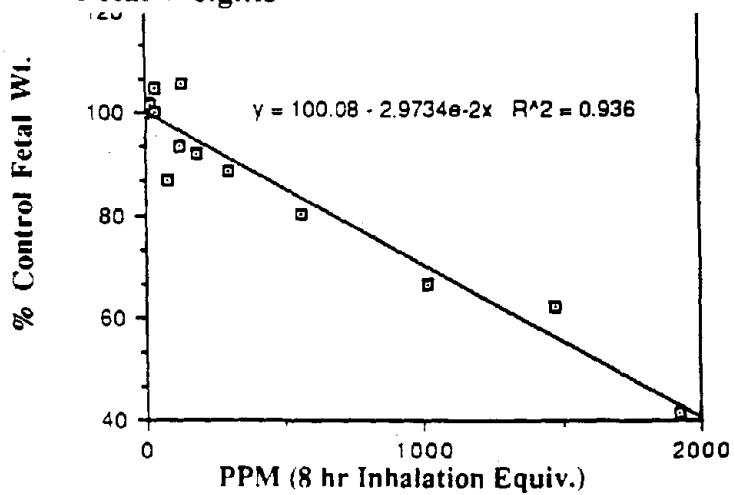
Clearly, these nonsignificant data if are not inconsistent with an effect of the magnitude suggested by the Weir et al. gavage experiments in mice. Working with EGEEA (but not EGEE) in dutch rabbits, Doe (1984) also found a statistically significant reduction in fetal weights at 100 and 400 ppm. The best fitting line to these data imply:

$$\% \text{ normal weight} = 100 - .037 * (\text{ppm 8 hour EGEEA exposure}).$$

This is also quite close the the .029 coefficient inferred from the mouse gavage experiments of Weir et al.

The result of combining all available EGEE and EGEEA equivalent data is shown in Figure 19. Although there is some scatter in the data, the overall coefficient indicated by the combined data is essentially unchanged, though it now might be rounded upward to .03 percent decline in fetal weight per ppm of 8-hour exposure.

Figure 19. Combination of All Data for Effects of EGEE (or EGEEA) Exposure on Fetal Weights



4.2 Implications for Low Birth Weight in the Human Population

4.2.1 General Form of U.S. Birth Weight Distributions, and Apparent Mode of Change of Birth Weight Distributions Between Populations with Different Exposures

A key question is how exactly we should apply the fetal weight change implied by the animal data reviewed in the previous section to predict a change in the distribution of human birth weights, and possible consequent changes in other parameters of interest. If the indicated change in fetal weights in animals were to be paralleled by a change in average birth weights in humans, there might well be an indirect effect on infant mortality, which is very strongly associated with birth weights (Hogue et al., 1987).^{*} However if we are willing to make this leap, it is still not entirely clear exactly how we should project birth weight changes to the human situation. Should the proposed change in birth weights be assumed to be a uniform multiplicative change throughout the birth weight distribution, or should the low end of the birth weight distribution (which suffers a large proportion of the infant mortality) be assumed to move a lesser or a greater amount?

The most straightforward model of birth weight change would be to simply multiply the expected percentage reduction in animal weights per ppm of exposure by the birth weights of humans, at all percentiles of the human birth weight distribution. To see whether this was a reasonable model of birth weight change in humans exposed to an array of different stresses, we have recently compared the population distributions of all black and all white singleton births from 1980 (Figure 20, Table 4-1). Overall, it can be seen in Figure 20 that both black and white birth weight distributions tend to be bimodal, with the lower mode including 2.5-5% of all births.

^{*} The basic data relating 1980 birth weights to infant mortality (death in the first year of life) were calculated from data given by Hogue et al (1987) for infants whose birth weights were known:

Weight Range	White Births	White Infant Deaths	White Infant Mort./1000	Black Births	Black Infant Deaths	Black Infant Mort./1000
Under 500	1630	1630	1000	1128	1128	1000
500-999	7775	5235	673.31	4815	3110	645.90
1000-1499	11364	2703	237.85	6090	1023	167.98
1500-1999	25046	1925	76.86	12093	698	57.72
2000-2499	92947	2486	26.746	40638	873	21.482
2500-2999	405910	3392	8.3565	138935	1366	9.832
3000-3499	1051300	4475	4.2566	224234	1488	6.636
3500-3999	897828	2734	3.0451	116282	649	5.581
4000-4499	286208	867	3.0293	25139	148	5.89
4500+	59908	296	4.941	4702	58	12.33
Totals (excluding unknown wt.)	2839916	25743	9.065	574056	10541	18.362

Figure 20

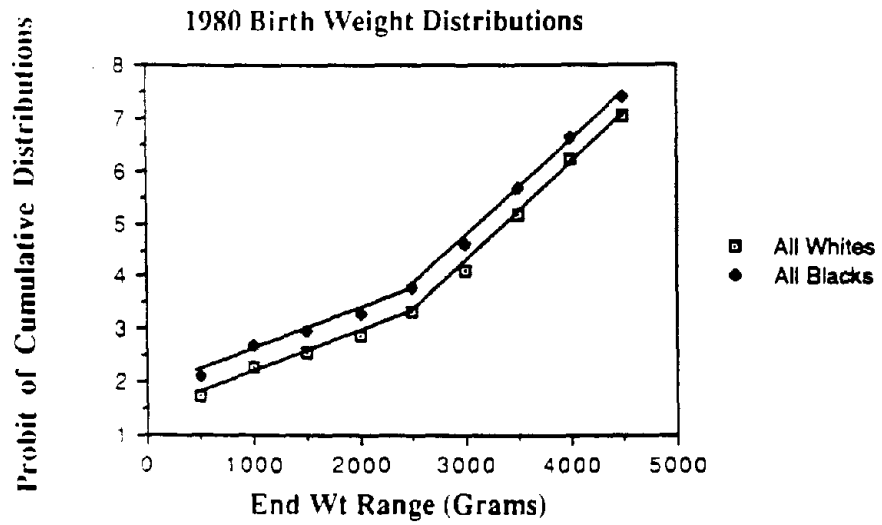


Table 4-1.
Differences in the Population Distributions of Birth Weights
Between Blacks and Whites

ANALYSIS WITHOUT CONTROLLING FOR GESTATIONAL AGE:

Percentile	<u>Weights at Different Percentiles</u>		Weight Difference(g)	% Weight Reduction	<u>g difference</u> average wt dif.
	1980 Single- ton Whites ^a	1980 Single- ton Blacks ^a			
1	1691.8	988.7	703.1	41.56	2.569
2	2090.1	1465.4	624.8	29.89	2.283
5	2507.1	2080.2	426.9	17.03	1.560
10	2738.5	2432.3	306.2	11.18	1.119
30	3168.7	2909.4	259.3	8.18	0.948
50	3424.0	3180.3	243.7	7.12	0.891
70	3673.7	3434.3	239.4	6.52	0.875
90	4040.0	3821.8	218.2	5.40	0.797
95	4263.1	4012.3	250.7	5.88	0.916
98	4514.1	4276.4	237.8	5.27	0.869
99	4523.2	4362.7	160.6	3.55	0.587
Overall Ave.	3411.9	3138.2	273.6	8.02	

ANALYSIS AFTER CONTROLLING FOR GESTATIONAL AGE:

Percentile	<u>Weights at Different Percentiles</u>		Weight Difference(g)	% Weight Reduction	<u>g difference</u> average wt dif.
	Full Term 1985 Whites ^b	Full Term 1985 Blacks ^b			
1	2257.1	2030.0	227.1	10.06	0.830
2	2438.2	2200.2	237.9	9.76	0.869
5	2667.0	2455.5	211.5	7.93	0.773
10	2859.1	2643.5	215.6	7.54	0.788
30	3225.3	3014.5	210.8	6.54	0.770
50	3466.0	3251.8	214.2	6.18	0.783
70	3717.6	3489.1	228.5	6.15	0.835
90	4098.3	3869.2	229.1	5.59	0.837
95	4305.3	4064.4	240.8	5.59	0.880
98	4538.2	4318.7	219.4	4.84	0.802
99	4588.0	4395.3	192.6	4.20	0.704
Overall Ave.	3477.6	3254.4	223.1	6.42	

^aData from Hogue et al. (1987)

^bData from National Center For Health Statistics (1987), Table 27, p. 40.

When the overall differences in birth weights at different percentiles of the black and white distributions are compared (upper part Table 4-1) it appears that there are more profound reductions at the lower end of the birth weight distribution than at the high end. Were this pattern to be produced by an environmental chemical, there would be considerably greater expected changes in infant mortality than if the agent caused a simple multiplicative reduction in birth weights at all percentiles of the distribution.

An additional set of comparisons--this time focusing only on term infants (37 weeks or more of reported gestation) is shown in the lower part of Table 4-1. Comparing the upper and the lower portions of Table 4-1 (without and with control for gestational age) it can be seen that restricting the analysis to full term infants produces a relatively uniform additive change in birth weights across the entire distribution. Because we have no evidence that glycol ethers lead to reductions in gestational age [unlike, for example, PCB's--(Taylor et al., 1989; Fein et al., 1984)], the uniform additive pattern seen in the lower portion of Table 4-1 represents our best judgment of the likely form of the change in human birth weights that might be induced by glycol ethers.

Our basic strategy for using these data further to project birth weight effects of glycol ethers will therefore be to (1) develop a race-specific base-line model of the population distribution of birth weights (and associated infant mortality) that can be easily shifted by various incremental amounts, according to the pattern we observe in the lower part of Table 4-1, and (2) project the birth weight changes for the mixed group of black and white infants, and the resulting potential changes in infant mortality, as a function of birth weight change and (by extension) glycol ether exposure.

4.2.2 Development of a Baseline Model of Black and White Birth Weight Distributions for the U.S.

In order to be able to easily manipulate the observed distribution of U.S. birth weights (e.g., subtracting a given amount in grams from all percentiles) we needed to have a simple formula or series of formulas that would adequately represent the raw data. We therefore first explored a number of bimodal distribution formulas (with different mean birth weights for high and low modes, and different proportions of the high and low mode infants) for representing U.S. birth weight distributions, using the chi-squared statistic as our criterion of goodness-of-fit. In all cases, this exploration was done keeping the overall arithmetic mean birth weights to the values

observed for the overall populations of 1980 white and black infants.* Table 4-2 shows our best final effort to fit the black and white 1980 birth weight distributions with simple bimodal models.

We were not entirely satisfied with the adequacy of the fits shown in Table 4-2. At the suggestion of a number of researchers in the reproductive effects area (including C. Hogue and D. Ozonoff) we introduced a third mode at a higher-than average range of weights (our impression is that there is an adverse syndrome of particularly heavy infants that is associated with maternal diabetes). The resulting distributions (Table 4-3) fit much better overall, and in particular, in the higher reaches of the white and black birth weight distributions. These trimodal fits were the basis of all our subsequent calculations of the potential effects of changes in birth weights on infant mortality.

4.2.3 Projection of the Possible Infant Mortality Response to Different Amounts of Change in Overall Birth Weights

Table 4-4 illustrates the effect of a 1% (33.66 g) overall shift in birth weights on the infant mortality expected in different ranges of birth weight for black and white infants. The second and third columns show the expected percentage distribution of white and black infants by birthweight, before and after the 33.66 g reduction. The fifth and sixth columns basically result from multiplying the second and third columns by the race- and weight-specific mortality risks per 1000 births shown in the fourth column. These data essentially show the contribution of infants in each weight category to the overall population risk of infant mortality. Finally, the last column--which is simply the difference between the fifth and sixth columns--shows the net change in population-attributable infant mortality for each weight category from the overall 1% change in birth weights. It can be seen in the bottom line of the table that because the black population has a larger number of relatively low-weight, high-risk infants in the absence of any additional exposure, blacks could be expected to suffer almost twice as large an incremental effect on infant mortality as whites from a 1% reduction in overall birth weights (about an additional .79 infant deaths per 1000 births for blacks, rather than about an additional .44 infant deaths per 1000 births for whites).

Using this same procedure Table 4-5 summarizes the expected effects of various amounts of birth weight change for infant mortality. The "total excess mortality/1000" in the final column represents the weighted average of effects in white and black subgroups.* It can be seen that the

* All optimizations of the parameters of the distributions (fractions of the population in different modes, the standard deviation associated with each mode, and the modal weights) were done by iterative trial and error with the aid of Excel spreadsheet programs.

* Assuming, as in 1980, about 2,839,916 births per year for whites and 574,056 births per year for blacks.

Table 4-2
Best-Fitting Bimodal Birth Weight Distributions

Characteristics of the 1980 White Birth Weight Distribution:

Subgroup	Mean Weight (g)	Standard Deviation (g)	Proportion of Population
low weight group	1876	719	0.0252
high weight group	3451.568	494	0.9748
Total group	3411.86359		

Birth Weight Range (grams)	Model--Expected Number of Infants	Data--Observed Number of Infants	CHI-SQUARED for Departure of Model
Under 500	1991.31	1630	65.56
500-999	5992.40	7775	530.28
1000-1499	13630.19	11364	376.78
1500-1999	23635.42	25046	84.18
2000-2499	87369.94	92947	356.00
2500-2999	433943.71	405910	1811.04
3000-3499	996426.39	1051300	3021.91
3500-3999	907351.95	897828	99.97
4000-4499	322766.68	286208	4140.88
4500+	46808.01	59908	<u>3666.25</u>
Overall Chi-squared statistic for departure of model from data--			14152.85

Characteristics of the 1980 Black Birth Weight Distribution:

Subgroup	Mean Weight (g)	Standard Deviation (g)	Proportion of Population
low weight group	1483	616	0.043
high weight group	3212.591	498	0.957
Total group	3138.218571		

Birth Weight Range (grams)	Model--Expected Number of Infants	Data--Observed Number of Infants	CHI-SQUARED for Departure of Model
Under 500	1364.30	1128	40.93
500-999	3982.15	4815	174.18
1000-1499	7427.94	6090	240.99
1500-1999	11048.43	12093	98.76
2000-2499	41520.76	40638	18.77
2500-2999	143061.81	138935	119.04
3000-3499	210754.82	224234	862.08
3500-3999	123623.51	116282	435.98
4000-4499	28598.62	25139	418.52
4500+	2673.66	4702	<u>1538.77</u>
Overall Chi-squared statistic for departure of model from data--			3948.03

Table 4-3
Best-Fitting Trimodal Birth Weight Distributions

Characteristics of the 1980 White Birth Weight Distribution:

Subgroup	Mean Weight (g)	Standard Deviation (g)	Proportion of Population
low weight group	2000	740	0.0324
middle weight group	3436.560	470	0.953
high weight group	4933	1004	0.0146
Total group	3411.86359		

Birth Weight Range (grams)	Model--Expected Number of Infants	Data--Observed Number of Infants	CHI-SQUARED for Departure of Model
Under 500	1962.83	1630	56.44
500-999	6163.31	7775	421.56
1000-1499	14906.75	11364	842.60
1500-1999	26076.43	25046	40.81
2000-2499	82903.19	92947	1220.46
2500-2999	430641.39	405910	1422.95
3000-3499	1029095.48	1051300	480.06
3500-3999	901644.16	897828	16.23
4000-4499	286824.42	286208	1.36
4500+	59698.03	59908	1.38
Overall Chi-squared statistic for departure of model from data--			4503.85

Characteristics of the 1980 Black Birth Weight Distribution:

Subgroup	Mean Weight (g)	Standard Deviation (g)	Proportion of Population
low weight group	1857	770	0.0669
middle weight group	3214.0914	461	0.9215
high weight group	4500	100	0.0116
Total group	3138.218571		

Birth Weight Range (grams)	Model--Expected Number of Infants	Data--Observed Number of Infants	CHI-SQUARED for Departure of Model
Under 500	1498.00	1128	91.39
500-999	3604.70	4815	406.36
1000-1499	7295.62	6090	199.23
1500-1999	11867.53	12093	4.28
2000-2499	38491.83	40638	119.68
2500-2999	142904.12	138935	110.22
3000-3499	219564.38	224234	99.31
3500-3999	118730.11	116282	50.49
4000-4499	25361.93	25139	2.26
4500+	4737.77	4702	.91
Overall Chi-squared statistic for departure of model from data--			1084.15

Table 4-4
Expected Infant Mortality Effects of a 1% (33.66g) Reduction in Birth Weights

Weight range (g)	----Fraction of Births---- orig. population	after 1% wt. reduct.	Mortality Risk per 1000 Births in Category	Fract. Births X Mortality Risks/1000 without birthwt reduction	after 1% birthwt reduction	net change
White Infants:						
Under 500	.0006912	.0007701	1000	.6912	.7701	.0789
500-999	.002171	.002335	673.31	1.4612	1.5720	.1107
1000-1499	.005249	.005488	237.85	1.2485	1.3053	.0568
1500-1999	.009182	.009575	76.86	.7057	.7360	.0302
2000-2499	.029192	.032804	26.746	.7808	.8774	.0966
2500-2999	.15164	.16568	8.3565	1.2672	1.3845	.1174
3000-3499	.36237	.37081	4.2566	1.5424	1.5784	.0359
3500-3999	.31749	.30337	3.0451	.9668	.9238	-.0430
4000-4499	.10100	.09027	3.0293	.3060	.2734	-.0325
4500+	.021021	.01890	4.941	.1039	.0934	-.0105
Total	1	1		9.0736	9.5142	.4406
Black Infants:						
Under 500	.0026095	.0028661	1000	2.6095	2.8661	.2566
500-999	.006279	.006666	645.90	4.0558	4.3058	.2500
1000-1499	.012709	.013154	167.98	2.1348	2.2096	.0748
1500-1999	.020673	.02165	57.72	1.1932	1.2495	.0563
2000-2499	.067052	.074351	21.482	1.4404	1.5972	.1568
2500-2999	.24894	.26444	9.832	2.4476	2.6000	.1524
3000-3499	.38248	.37936	6.636	2.5381	2.5174	-.0207
3500-3999	.20683	.19100	5.581	1.1543	1.0660	-.0883
4000-4499	.04418	.04029	5.89	.2602	.2373	-.0229
4500+	.008253	.006226	12.33	.1018	.0768	-.0250
Total	1	1		17.9358	18.7257	.7899

Table 4-5

Summary Implications for Infant Mortality of Reducing Birth Weights
by Equal Amounts in Grams at All Percentiles of the Black and
White Birthweight Distributions

% Birthwt reduction	White excess mort./1000	Black excess mort./1000	Total excess mort/1000
0.01	0.00426	0.00768	0.00484
0.1	0.0427	0.0770	0.0485
1	0.440	0.790	0.499
10	6.00	10.2	6.70
0.00207	0.000882	0.00159	0.0010009
0.0207	0.00882	0.0159	0.010015
0.205	0.0879	0.158	0.09977
1.95	0.877	1.58	1.0040

overall relationship between birthweight reduction and expected excess infant mortality is essentially linear up to 1% birthweight reduction, with a slight upward-turn (somewhat greater excess mortality per incremental birthweight reduction) in the region between 1% and 10% birthweight reduction. The 1% level of birthweight reduction represents a level that can be detected directly in reasonable sized groups of exposed workers with extensive control of confounding variables (e.g., see Taylor et al., 1989). However because infant deaths occur only in about 10 births per thousand or so, and incremental "excess" deaths associated with a 1% weight reduction would be expected to be about 0.5 per 1000, it is likely that even cohorts including 10,000 births--containing 5 "excess" cases in 100 or so "background" cases--would be too small to expect direct detection of the infant mortality effect itself. This illustrates the power of using the intermediate continuous variable--birthweight--to indirectly detect and project effects on a variable of even more social importance--infant mortality.

The lower portion of Table 4-5 shows the degree of birthweight reduction that is projected to be associated with specific excess infant mortality rates--placed in a more familiar risk form for comparison with other expressions of risk. Thus the bottom line suggests that about a 1.95% birthweight reduction may be associated with an excess overall infant mortality of $1/1000$ (10^{-3}). If one wished to hold the effects of an exposure below a level that would be expected to produce a 10^{-6} infant mortality risk ($.001/1000$), the projections indicate that it would be necessary to hold weight reductions below 0.002%.

Table 4-6 shows the application of these birth weight-infant mortality projections to the degree of fetal weight reduction observed in animals for EGEE and EGME. At the current TLV levels (5 ppm) the projected infant mortality risks approach 10^{-3} and 10^{-4} , respectively. Holding expected infant mortality risks to below 10^{-6} appears to require EGEE exposures below 0.1 ppm, and EGME exposures below 0.01 ppm.

Table 4-6
Projected Changes in Infant Mortality Due to Possible Changes in
Infant Birth Weights Associated with EGEE and EGME Exposure

PPM Exposure Level	Projected % Birthwt Reduct.	White Excess Mort./1000	Black Excess Mort./1000	Total Excess Infant Mortality ^a
EGEE:				
0.01	.0003	.000128	.000230	1.45E-7
0.1	.003	.00128	.00230	1.45E-6
1	.03	.0128	.0231	1.45E-5
5	.15	.064	.116	7.3E-5
25	.75	.33	.59	3.7E-4
EGME:				
0.01	.00243	.00104	.00187	1.18E-6
0.1	.0243	.0104	.0187	1.18E-5
1	.243	.104	.188	1.18E-4
5	1.215	.539	.966	6.1E-4
25	6.075	3.18	5.54	3.6E-3

^aNote: The numbers in this column, unlike the previous two columns are not expressed per 1000 births.

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