TOXICOLOGICAL SCIENCES **62,** 124–139 (2001)

Proposed Occupational Exposure Limits for Select Ethylene Glycol Ethers Using PBPK Models and Monte Carlo Simulations

L. M. Sweeney,*[,1] T. R. Tyler,† C. R. Kirman,‡ R. A. Corley,§ R. H. Reitz,¶ D. J. Paustenbach,‖ J. F. Holson,‖‖ M. D. Whorton,‖‖‖ K. M. Thompson,# and M. L. Gargas*

**The Sapphire Group, Inc., 2928 Idaho Falls Drive, Suite 100, Beavercreek, Ohio 45431; †Union Carbide Corporation, Danbury, Connecticut; ‡The Sapphire Group, Inc., Cleveland, Ohio; §Battelle, Richland, Washington; ¶RHR Toxicology, Midland, Michigan; ‖Exponent, Menlo Park, California; ‖‖WIL Laboratories, Ashland, Ohio; ‖‖‖M. Donald Whorton, M.D., Inc., Alameda, California; and #Harvard School of Public Health, Boston, Massachusetts*

Received October 18, 2000; accepted February 13, 2001

Methoxyethanol (ethylene glycol monomethyl ether, EGME), ethoxyethanol (ethylene glycol monoethyl ether, EGEE), and ethoxyethyl acetate (ethylene glycol monoethyl ether acetate, EGEEA) are all developmental toxicants in laboratory animals. Due to the imprecise nature of the exposure data in epidemiology studies of these chemicals, we relied on human and animal pharmacokinetic data, as well as animal toxicity data, to derive 3 occupational exposure limits (OELs). Physiologically based pharmacokinetic (PBPK) models for EGME, EGEE, and EGEEA in pregnant rats and humans have been developed (M. L. Gargas *et al.,* 2000, *Toxicol. Appl. Pharmacol.* 165, 53–62; M. L. Gargas *et al.,* 2000, *Toxicol. Appl. Pharmacol.* 165, 63–73). These models were used to calculate estimated human-equivalent no adverse effect levels (NAELs), based upon internal concentrations in rats exposed to no observed effect levels (NOELs) for developmental toxicity. Estimated NAEL values of 25 ppm for EGEEA and EGEE and 12 ppm for EGME were derived using average values for physiological, thermodynamic, and metabolic parameters in the PBPK model. The uncertainties in the point estimates for the NOELs and NAELs were estimated from the distribution of internal dose estimates obtained by varying key parameter values over expected ranges and probability distributions. Key parameters were identified through sensitivity analysis. Distributions of the values of these parameters were sampled using Monte Carlo techniques and appropriate dose metrics calculated for 1600 parameter sets. The 95th percentile values were used to calculate interindividual pharmacokinetic uncertainty factors (UFs) to account for variability among humans ($UF_{h,pk}$). These values of 1.8 for EGEEA/EGEE and 1.7 for EGME are less than the default value of 3 for this area of uncertainty. The estimated human equivalent NAELs were divided by $UF_{h,pk}$ and the default UFs for pharmacodynamic variability among animals and among humans to calculate the proposed OELs. This methodology indicates that OELs (8-h time-weighted average) that should protect workers from the most sensitive adverse effects of these chemicals are 2 ppm EGEEA and EGEE (11 mg/m^3 EGEEA, 7 mg/m^3 EGEE) and 0.9 ppm (3 mg/m^3) EGME. These recommendations assume that dermal exposure will be minimal or nonexistent.

[1]To whom correspondence should be addressed. Fax: (937) 431-3735. E-mail: lms@thesapphiregroup.com.

Key Words: **occupational exposure limit; ethoxyethyl acetate; ethoxyethanol; methoxyethanol; EGEEA; EGEE; EGME; PBPK models; Monte Carlo simulation.**

Short-chain alkyl groups attached to ethylene glycol by ether linkages (ethylene glycol ethers, EGEs) have found multiple uses as solvents because of their ability to form solutions with both water and many less polar organic materials. The ethylene glycol monoethers formed with methyl and ethyl groups (EGME and EGEE) and the acetate ester of EGEE (EGEEA) were used extensively in the past for various solvent applications including coatings applications, cleaning solvents and, EGME in particular, as a military jet fuel additive for deicing purposes. In the past 10 to 15 years, markets for these glycol ethers have greatly diminished, in part based on concerns about the health hazards. The use of EGME as a jet fuel additive has been largely replaced with the diethylene glycol analog. Producers of these glycol ethers warn against their use in consumer products. In the United States there has been an effort to replace EGME, EGEE, and EGEEA as components in photoresist formulations used in the microelectronics industry (D. S. Tornow, Union Carbide Corp., Danbury, CT, personal communication).

The primary use of EGME is as a process/extraction solvent in pharmaceutical production units and as a chemical intermediate in the production of glymes (dimethyl ethers of ethylene glycols; mono-, di-, and tri-). In addition, EGME is used as a process solvent for adhesive use in the manufacturer of circuit boards in some European and Asian countries. The primary use of EGEE is as a chemical intermediate in the manufacture of EGEEA. EGEE is sometimes used as an industrial coatings solvent primarily for original equipment manufacturer (OEM) types of applications. EGEEA's major end use is as an industrial solvent for coatings. It is a slow-evaporating solvent used primarily in Southeast Asia in automotive coatings. It is not recommended for use in consumer products, pesticides, pharmaceutical formulations, or photo-resist mixtures used in semi-

conductor fabrication processes (D. S. Tornow, Union Carbide Corp., Danbury, CT, personal communication).

The current Permissible Exposure Limits (PELs), 8-h time-weighted average (TWA_8) for occupational exposure, established by the Occupational Safety and Health Administration (OSHA) in 1971, are 25 ppm for EGME, 200 ppm for EGEE, and 100 ppm for EGEEA (each has a skin notation). These standards were established on the basis of blood, kidney, liver, and central nervous system toxicity in experimental animals. OSHA has proposed PELs of 0.1 ppm for EGME and 0.5 ppm for EGEEA and EGEE based on reproductive and developmental toxicity (OSHA, 1993), but these have not been promulgated to date. The proposed PELs were based upon determination of the NOAEL in animal studies, divided by an uncertainty factor of 100 in an attempt to account for inter- and intraspecies variability. In the setting of PELs for systemic toxicants, it is not unusual to apply UFs of this magnitude to animal data (Paustenbach, 2000).

The Threshold Limit Value (TLV) established for EGME by the American Conference of Governmental Industrial Hygienists (ACGIH) is 5 ppm (TWA_8), which was based on review of the relevant information in toxicology and epidemiology studies, with particular emphasis on testicular toxicity in shipyard workers applying EGME-containing paints (ACGIH, 1991, 1999). The TLV for EGEE is also 5 ppm, based on "analogy" to EGME, and evidence that EGEE is less potent in animals than EGME. Likewise, the TLV for EGEEA is 5 ppm, based on review of the relevant toxicity information in toxicology and epidemiology studies, with particular emphasis on testicular toxicity in rats and analogy to the EGEE TLV (ACGIH, 1991). These TLVs were all established in 1984, with the documentation revised in 1991. Since the publication of OSHA's proposed rule, additional animal toxicology research on the effects and disposition of EGME, EGEE, and EGEEA has been conducted (e.g., Davis *et al.,* 1997; Gargas *et al.,* 2000a,b; Terry *et al.,* 1994).

With increasing frequency, regulatory agencies are using physiologically based pharmacokinetic (PBPK) modeling and/or Monte Carlo analysis in setting permissible exposure values. These techniques attempt to account for species differences and variation in physiology and metabolism. For example, the OSHA PEL for methylene chloride was established based on the glutathione-S-transferase metabolites of methylene chloride, as calculated using a PBPK model and Monte Carlo simulation (OSHA, 1997). The U.S. Environmental Protection Agency (U.S. EPA) is also using PBPK modeling to convert external exposure concentrations to internals doses as a step in the derivation of cancer slope factors (CSFs), reference concentrations (RfCs), and reference doses (RfDs). Recently, the U.S. EPA in its Integrated Risk Information System (IRIS) database published CSFs, RfCs, and RfDs for vinyl chloride that were derived using PBPK models to calculate internal doses and assuming that equivalent toxicity between species results from equivalent target tissue concentrations of reactive metabolites (U.S. EPA, 2000). In addition, the U.S. EPA published in IRIS RfC and RfD values for ethylene glycol monobutyl ether (EGBE) using a PBPK approach for determining the human equivalent concentration (HEC) (U.S. EPA, 1999).

We reviewed the glycol ethers literature to identify the important and relevant toxicology and epidemiology studies. We then applied PBPK modeling and Monte Carlo simulation to perform interspecies extrapolation and assess intraspecies variation. Using this information we then calculated potential occupational exposure limits for EGME, EGEEA and EGEE. The methods used to derive the values presented here represent an alternative to methods that in the past relied on default assumptions, by necessity, to estimate occupational exposure limits. It is hoped that approaches such as are described here will be given careful consideration by regulatory organizations responsible for setting appropriate limits of exposure.

Selection of critical studies. The starting point for the understanding the published literature was an assessment of previous reviews of the EGME, EGEE, and EGEEA databases and online searches using MEDLINE. EGME and its acetate ester have been the subject of a recent review (Johanson, 2000). Additional studies were identified from citations within other papers. Studies were evaluated for suitability as the basis for occupational exposure limits using criteria such as identification of a NOEL or lowest observed effect level (LOEL) and the quality of the study. The studies with the lowest identified NOELs were deemed to be of high quality and were determined to be suitable for use as the critical studies in OEL derivation.

Animal data, EGEEA and EGEE. EGEEA is efficiently taken up by the body and rapidly hydrolyzed to EGEE, which in turn is metabolized to ethoxyacetic acid (EAA). EAA is considered to be the proximal toxicant derived from EGEEA and EGEE (Gargas *et al.,* 2000a). Thus, studies conducted with EGEEA or EGEE are considered equally appropriate for establishing occupational exposure limits for both compounds when the pharmacokinetics of EGEE production from metabolism of EGEEA are taken into account. Developmental toxicity (fetotoxicity and fetal defects) was considered the most sensitive endpoint for these glycol ethers; a total of 27 studies pertaining to the reproductive and developmental toxicity of EGEEA and EGEE were reviewed, and the study of Doe (1984) was found to be the critical study. OSHA (1993) also selected this study as the basis for the proposed PEL. Doe (1984) identifies 50 ppm EGEE (6 h/day, to pregnant rats on gestational days [GD] 6–15) as the NOEL for developmental toxicity. A LOEL of 100 ppm was identified by Tyl *et al.* (1988). Other reproductive or developmental toxic effects observed with higher doses of EGEEA or EGEE include testicular damage (Foster *et al.,* 1983; Samuels *et al.,* 1984). These effects were observed at higher exposure concentrations than

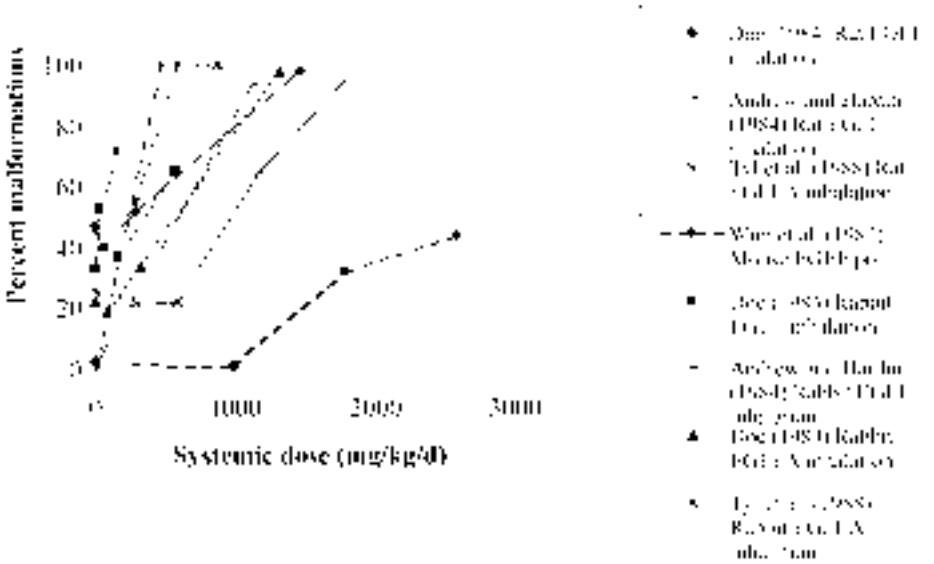

FIG. 1. Dose response for EGEEA- and EGEE-exposed animals. Daily systemic dose calculated as described in the text.

the fetotoxicity and fetal defects observed in the Doe (1984) study.

The Doe (1984) study in rats was selected as the critical study in our assessment because it provides the NOEL for the relevant route of exposure (inhalation) and was conducted with adequate numbers (24/group) of animals. To confirm that the findings are in accord with studies conducted by other routes, a dose-response analysis was conducted. The response selected was percent of litters with malformed animals, consistent with the NOEL/LOEL critical endpoint. Dose was expressed as daily systemic dose (mg/kg/day) on exposure days, calculated as the product of the exposure concentration, inhalation rate (calculated from body weight as in Gargas *et al.,* 2000a,b), the exposure duration, and the alveolar retention fraction (Groeseneken *et al.,* 1986) divided by body weight. There is good concordance in dose response among the inhalation studies of EGEEA and EGEE in rats and rabbits, but mice dosed orally with EGEE exhibit fewer malformations at the same systemic doses (Fig. 1). Given the lack of pharmacokinetic data in mice, it is not possible to say whether this difference in response is due to target tissue concentrations or a difference in susceptibility. While it would be desirable to evaluate dose response based on a measure of internal dose, the only validated pharmacokinetic model for EGEEA or EGEE is that of Gargas *et al.* (2000a), which addresses only one route of exposure (inhalation) in one species (rat).

Animal data, EGME. EGME is metabolized to methoxyacetic acid (MAA), which is considered to be the proximal toxicant (Gargas *et al.,* 2000b). A total of 50 studies pertaining to the reproductive and developmental effects of EGME were reviewed, and the critical study was found to be the study of Hanley *et al.* (1984) that identified 10 ppm (6 h/day on GD 6–15) as the NOEL for developmental effects (skeletal alterations) in rats. OSHA (1993) also identified Hanley *et al.* (1984) as the critical study. Toxic effects observed with higher doses of EGME include spermatocyte degeneration (Ku *et al.,* 1995), hematological effects and decreases in testes weight (Miller *et al.,* 1983), and immunosuppression in animals (Smialowicz *et al.,* 1991). Again, these latter effects were noted at higher exposure concentrations than the developmental effects identified in the Hanley *et al.* (1984) rat study.

The Hanley *et al.* (1984) study in rats was selected as the basis for this analysis because it provides the NOEL for the relevant route of exposure (inhalation) and was conducted with adequate numbers (24–32/group) of animals. To confirm that the findings are in accord with studies using other routes of exposure, a dose-response analysis was conducted. The response selected was percent of litters with malformed animals, consistent with the NOEL/LOEL critical endpoint. Dose was expressed in 3 ways: daily systemic dose of EGME, peak blood concentration of MAA, and average daily area under the blood concentration-time curve (AUC) of MAA on exposure days from GD 11–15. The daily systemic dose for inhalation studies was calculated as the product of the exposure concentration, inhalation rate (calculated from body weight as in Gargas *et al.,* 2000a,b), the exposure duration, and the alveolar retention fraction (Groeseneken *et al.,* 1989) divided by body weight. Peak concentration and average daily AUC of MAA were calculated using the PBPK model of Gargas *et al.* (2000b) for rat inhalation exposure, calculated using the PBPK model of Hays *et al.* (2000) for rat po and iv exposure, and taken from published pharmacokinetic data (Clarke *et al.,* 1992) for mice exposed via sc infusion or po dosing.

There is good concordance between systemic dose and malformation rate among mice and rats exposed to EGME by po, iv, ip, and sc infusion, but the response rate in mice and rats

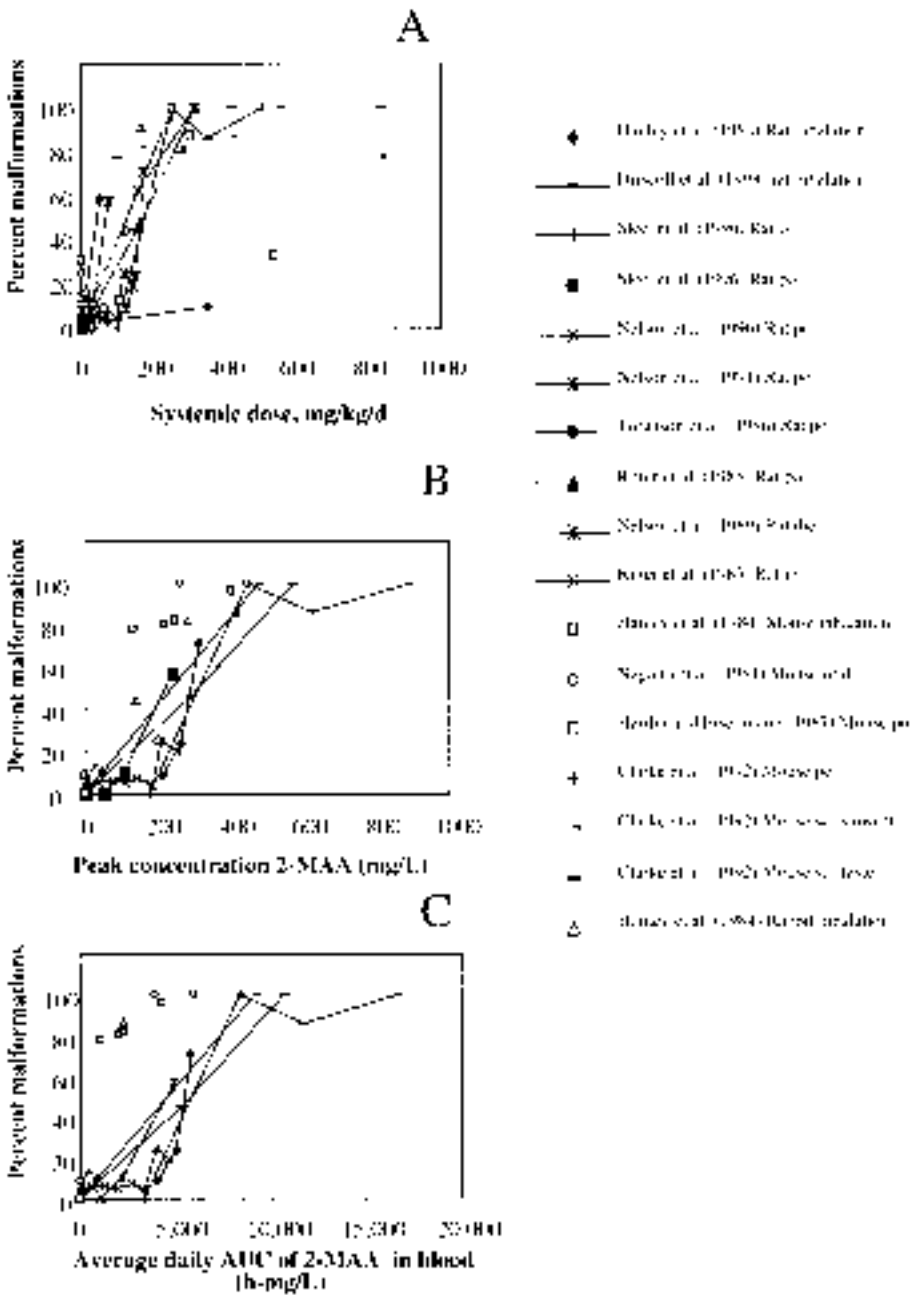

FIG. 2. Dose response for EGME-exposed animals, with dose expressed as (A) daily systemic dose, (B) peak blood concentration of MAA, and (C) average daily blood AUC of MAA (determined as described in the text).

exposed by inhalation is much lower for a given systemic dose (Fig. 2A). However, when blood concentrations of metabolite are considered (as peak concentration or average daily blood AUC), the inhalation response data are consistent with the po, sc infusion, and iv response data (Figs. 2B and 2C). This stresses the value of using a blood or tissue dose rather than an administered or systemic dose as the basis of comparisons and extrapolations among species and for different routes of exposure. Based on the peak blood concentration and average daily blood AUC for MAA, the Hanley *et al.* (1984) studies yield a NOEL lower than the lowest LOEL—that is, the lowest peak blood concentration and AUC associated with a statistically-significant increase in malformations, found in Driscoll *et al.* (1998). This finding increases our confidence that this is the most sensitive toxicologic endpoint for derivation of a human occupational exposure limit.

Human experience with EGME, EGEE, and EGEEA. The human data on developmental and reproductive outcomes for glycol ethers include both epidemiologic studies and case reports. Most of these data do not have sufficiently precise exposure assessments regarding the glycol ethers and/or other chemicals to which these persons were exposed to allow for inclusion in the risk assessment process. Chia *et al.* (1997)

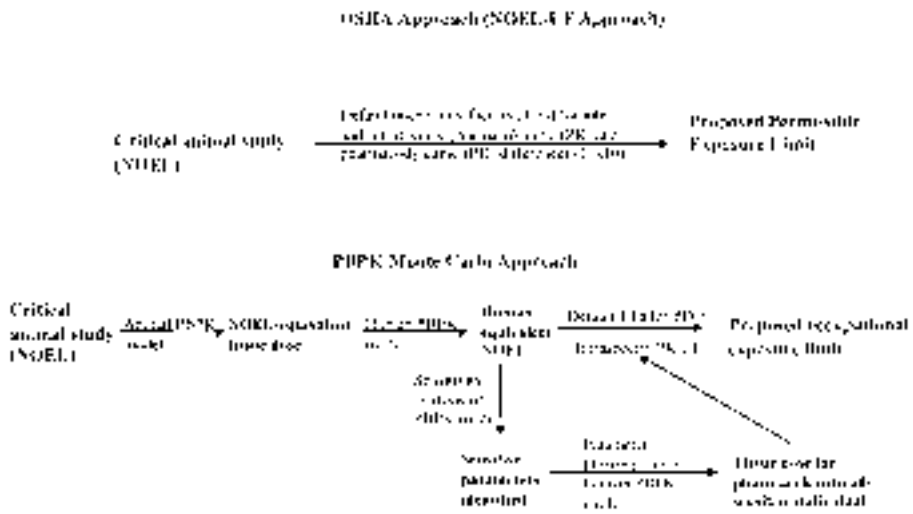

FIG. 3. Two approaches used to identify an occupational exposure limit.

found no differences in menstrual patterns in women with EGEEA exposures (by inhalation only; authors report no dermal contact) compared to nonexposed women. Ratcliffe *et al.* (1989), Veulemans *et al.* (1993), and Welch *et al.* (1988) all report a decrease in semen quality, primarily sperm density (count) among males exposed to EGEE. Cook *et al.* (1982), Shih *et al.* (2000a), and Veulemans *et al.* (1993) do not report such changes in men exposed to EGME, although the number of subjects was much smaller. None of the EGEE data are precise enough for inclusion in calculations for a risk assessment.

There are several difficulties in using case reports and human studies in the risk assessment process. The lack of information regarding the airborne exposure concentrations, as well as the probability of dermal contact, make the data from almost all of these studies difficult to use in the risk assessment process. The studies mentioned above provide quantitative exposure information, but no statistically significant reproductive effect. In the remaining quantitative study, Veulemans *et al.* (1993) demonstrate significant effects (infertility or subfertility), but the urinary EAA measurements cannot be converted to airborne exposure concentrations without additional information on the exposure (e.g., duration, pattern, time since exposure). Thus, even the quantitative studies cannot currently be used in risk assessment. In addition, these reports are difficult to interpret due to concurrent exposures to other agents. After careful consideration and review, we concluded that the human data were not acceptable for setting an OEL and chose to rely on the animal studies, which provide quantitative exposure and effect information.

METHODS

Calculation of an OEL. The approach used by OSHA and the approach used in this effort to calculate OELs are depicted in Figure 3. OSHA (1993) provides a detailed description of the derivation of its proposed PELs using the no observed effect level-uncertainty factor (NOEL-UF) approach. Briefly, critical studies were selected and the NOEL identified. A total uncertainty factor of 100 (10 for interspecies variability and 10 for intraspecies variability) was used for the each of these glycol ethers. This approach has been commonly employed in risk assessment (Dourson and Stara, 1983; Dourson *et al.*, 1996). It is assumed, for inhalation exposures, that each of these factors of 10 may be considered the composite of pharmacodynamic and pharmacokinetic variability. For intraspecies variability, it is assumed that each component contributes equally (Andersen *et al.*, 1995; Barton *et al.*, 1998; Renwick and Lazarus, 1998). That is, a default UF of $10^{0.5} = 3.2$ for intraspecies pharmacokinetic differences and a UF of 3.2 for intraspecies pharmacodynamic differences together result in a total intraspecies UF of 10. In general, because of the imprecision in toxicity data, fractional uncertainty factors (i.e., 3.2) are rounded to the nearest integer (i.e., 3) resulting in the use of quantized factors of 3 or 10 (i.e., $3 \times 3 = 10$). For interspecies variability, a subdivision of a factor of 4.0 for toxicokinetics and 2.5 for toxicodynamics has been recommended (Renwick, 1993).

The PBPK/Monte Carlo-based approach likewise begins with the identification of the critical study and NOEL. Internal dose metrics that are consistent with the mode of action are selected. Using the PBPK model for the animals in the critical toxicity study, the internal dose metrics corresponding to the NOEL exposure are calculated (using average parameter values for physiology, etc.). The PBPK model for a human with average parameter values is used to derive exposure concentrations at which the predicted internal doses are equal to those predicted for the animal NOEL study. Each different internal metric may correspond to a different external concentration. That is, the average tissue concentration based on the animal NOEL leads to one estimate for the human equivalent NAEL. The peak tissue concentration associated with this same animal NOEL may be associated with a different human equivalent NAEL. The lowest of these concentrations is considered the human-equivalent NAEL estimate, a health-protective practice. The use of PBPK models removes the need to use a default UF for interspecies pharmacokinetic differences as this conversion is done with reliable models. This approach has been described in detail by Barton *et al.* (1998) and applied to 2-butoxyethanol (U.S. EPA, 1999), vinyl chloride (Reitz *et al.*, 1996), and other chemicals.

As described by Gargas and coworkers (Gargas *et al.*, 2000a,b) the peak concentration (C_{max}) and average daily AUC of the alkoxyacetic acid metabolite in the blood were the dose metrics selected for EGME, EGEE, and its acetate ester. The relationship between dosimetry and toxic effect (developmental toxicity) has been closely evaluated for EGME. Correlations have been observed between total exposure (AUC) to MAA or peak MAA concentrations and developmental toxicity—the better choice of dose metric was dependent on the specific endpoint being considered (Clarke *et al.*, 1992; Terry *et al.*, 1994). As the mode of action of EGEE and EGEEA is expected to be similar,

AUC and peak concentration of EAA were considered appropriate dose metrics for EGEE and EGEEA-induced developmental toxicity. The model for human exposure was built to simulate an average pregnant woman exposed 8 h per day, 5 days per week for 38 weeks. For EGME, EGEEA, and EGEE, use of the average daily AUC provided more health-protective human-equivalent NAEL estimates, airborne concentrations of 25 ppm EGEEA or EGEE and 12 ppm EGME for pregnant workers, in the absence of dermal exposure.

To derive uncertainty factors for human pharmacokinetic variability, an assessment of human variability was integrated into the OEL derivation process. Uses of probabilistic methods in derivation of acceptable human exposures have previously been described by Baird *et al.* (1996), Clewell *et al.* (1999), Slob and Pieters (1998), and Swartout *et al.* (1998). In order to focus on the critical parameters, sensitivity analyses were conducted to determine those parameters for which small changes result in the greatest changes in the dose metric.

Monte Carlo simulation was used to replace the default UF for intraspecies pharmacokinetic sensitivity with a UF that reflects the known or expected variability of the population. The ratio of the values of the dose metric for the 95th percentile human (who receives a larger internal dose due to pharmacokinetic sensitivity) to that of the "average" human is proposed as an alternative to the default UF for intraspecies pharmacokinetic variability. Delic *et al.* (2000) have also used the 95th percentile human dose metric derived from Monte Carlo simulation and PBPK modeling in an assessment of the adequacy of existing occupational exposure standards for chloroform and carbon tetrachloride in the United Kingdom. Clewell *et al.* (1999) have demonstrated a similar approach (use of Monte Carlo simulation to develop the intraspecies pharmacokinetic uncertainty factor) for methylmercury, using hair mercury concentrations, a surrogate for ingestion rate, rather than blood or target tissue (fetal tissue) concentrations. In the present study, Monte Carlo simulation was also used to evaluate how well the average individual human or animal reflects the pharmacokinetics found in the population (i.e., does the "average" individual receive an internal dose that is larger or smaller than that of most of the population?).

Physiologically-based pharmacokinetic modeling. The PBPK models of Gargas and coworkers (Gargas et al., 2000a,b) were used either without modification (for sensitivity analysis and the impact of brief exposures to higher concentrations) or with minor modifications (see below, "Uncertainty Analysis"). Briefly, the disposition of inhaled EGME, EGEEA, and EGEE is described for pregnant rats and pregnant and non-pregnant humans. The models contain 5 perfusion-limited tissue compartments—liver, blood, adipose tissue (fat, including mammary), slowly perfused tissues (e.g., muscle), and a lumped compartment representing richly perfused tissues including the fetus(es) and placenta(e). Rapid hydrolysis of EGEEA to EGEE is modeled as taking place in the blood. Metabolism of EGEE and EGME to EAA and MAA, respectively, is assumed to take place in the liver. These alkoxyacetic acid metabolites are modeled as being eliminated unchanged in the urine; the first order rate constants for the elimination of MAA and EAA may be considered a composite of direct elimination of the compound or further metabolism.

Physiological parameters in the model vary with time throughout the course of the pregnancy. The pregnant rat and non-pregnant human models were parameterized and validated, using exhaled breath, blood concentrations, and urinary elimination of EGME, EGEE, and EGEEA, and their alkoxyacetic acid metabolites (rat data collected by Gargas *et al.* [2000a,b], human data from Groeseneken *et al.* [1987a,b, 1988, 1989]). Physiological parameters for an average pregnant woman were used to calculate human-equivalent NAEL estimates, based on internal concentrations in rats exposed at previously determined NOELs for developmental toxicity. All model simulations were performed using the Advanced Continuous Simulation Language (ACSL, AEgis Technologies Group, Austin, Texas).

Sensitivity analysis. Sensitivity analyses on the models were performed by increasing a single parameter value by 1% and noting the resulting change in average daily AUC of EAA or MAA in the blood ("internal dose" or "dose metric"). This test was done for all the parameters in each model. The sensitivity coefficient (SC) was defined as the percent change in the dose metric for a 1% change in the parameter.

For those parameters that changed over time and were described by "table functions" in ACSL (values at certain times are specified, with values at other times calculated by linear interpolation), new table functions were written with the parameters values at all times set 1% higher. Simulations were run with the new table function, and the results compared to the base case.

The baseline for the sensitivity analyses was the NOEL exposure described in the critical toxicology study or the human-equivalent NAEL estimate. For the rat, the baseline was an exposure at 50 ppm EGEEA or 10 ppm EGME for 6 h/day (on GD 6–15), and the average daily blood AUC of EAA or MAA during GD 13–15 was computed. The choice of GD 13–15 was based on the experimental conditions that maximize the occurrence of malformations and number of live embryos/litter (Sleet *et al.,* 1996) in rats dosed intravenously with 500 mg EGME/kg body weight. For humans, the baseline simulation was for a pregnant woman exposed to 25 ppm airborne EGEEA or EGEE or 12 ppm airborne EGME for 8 h/day, 5 days/week for the 38 weeks of pregnancy, and the average daily blood AUC of EAA/MAA was computed. As the blood concentration profile changes very little during pregnancy (based on comparisons of blood concentrations at various time points), the choice of a window of susceptibility (e.g., only during organogenesis) did not affect the average blood AUC (data not shown).

Uncertainty Analysis

Model structure. Based on the results of the sensitivity analysis, the rat models of Gargas *et al.* (2000a,b) were modified slightly as follows: The table function for exposure concentration was eliminated by assuming the concentration is the same every day. (Table functions were needed to describe day-to-day variation in exposure concentration in experiments reported by Gargas *et al.,* but were not necessary for the present analysis.) Rat body weight was split into a constant (body weight on GD 0) and time-sensitive multiplier. For the uncertainly analysis, body weight on GD 0 was allowed to vary, but the multiplier was not. These changes allowed sensitive parameter values to be easily varied in the simulations for the uncertainty analysis.

Parameter coefficients of variation. Parameter variation is reported as the percent deviation from the mean (standard deviation/mean value)—the coefficient of variation (CV). The coefficients CVs for physiological parameters were taken from Allen *et al.* (1996) and Cronin *et al.* (1995), with the exception of rat body weights, which were taken from the critical studies (Doe, 1984; Hanley *et al.,* 1984). The CV for the urinary elimination rate, a fitted parameter, was taken from Allen *et al.* (1996). The CVs for metabolism parameters were taken from the studies providing the *in vitro* data from which the rates were scaled (Green *et al.,* 1996; Tyson *et al.,* 1989). The variation in the alveolar retention of EGME and EGEEA/EGEE were taken from human inhalation studies conducted by Groeseneken *et al.* (1986 and 1989, respectively).

Selection of parameters for inclusion in uncertainty analysis. The expected impact of a parameter on dose variability is related to the product of CV and SC, (amount of variation of the input) $\times$ (change in dose when input changes). The absolute values of the SC $\times$ CV product were summed for all model parameters. To limit the computation time while capturing most of the variation, only those parameters that contributed to $>$ 1% of the sum were included in the uncertainty analysis (Monte Carlo simulation).

Parameter distributions. Although correlations are likely to exist between parameters, they were treated independently in the simulations conducted with the model in this study. This practice may be viewed as protective since it generally maximizes variation in the results. Distribution shapes (that is, lognormal or normal) for the baseline analysis were those used by Clewell *et al.,* (1999). The sensitivity of the results to the distribution shapes was tested by also performing the simulations with all parameters normally distributed or all lognormally distributed (to be discussed later). Normal parameter distributions were truncated at 0 as necessary (first order rate constants for EGME metabolism to MAA and ethylene glycol had to be truncated).

TABLE 1
Parameters Used in EGEEA and EGEE MODEL Uncertainty Analyses for Rat and Human

Parameter	Model sensitivity × parameter coefficient of variation		
	EGEEA (human)	EGEE (human)	EGEEA (rat)
KEX	−0.270	−0.270	−0.30
QPC	0.160	0.160	0.15
KEAAC	0.120	0.120	0.1
KEGC	−0.120	−0.120	−0.1
BW0/BWC	0.105	0.105	0.07
ALV1 or ALV2, or ALV	0.070	0.070	0.07
CONCC	—	—	0.05

Note. Parameters listed contribute >99% of the expected variation in the dose estimate. KEX, urinary excretion rate of EAA; QPC, pulmonary ventilation rate; KEAAC, rate of conversion of EGEE to EAA; KEGC, rate of conversion of EGEE to ethylene glycol; BW0, initial body weight; BWC, body weight (expressed in a table function); ALV1, alveolar retention of EGEEA; ALV2, alveolar retention of EGEE; ALV, alveolar retention of EGEEA and EGEE; CONCC, exposure concentration of EGEEA (expressed in a table function). Negatives indicate that an increase in the parameter value produced a decrease in the average daily blood AUC of EAA ("dose"). The dose was insensitive to other model parameters, such as cardiac output, tissue blood flow distribution, tissue volumes, and partition coefficients.

Monte Carlo simulation. Parameter values were randomly generated using Latin Hypercube sampling in Crystal Ball® (Decisioneering, Denver, CO) and sent to ACSL via Visual Basic® programming in Microsoft® Excel for Windows™. The input values of the parameters (e.g., urinary excretion rate, body weight) and the output (dose) used in each iteration were saved for additional analysis. For the human models, the time period simulated was reduced for computational reasons; only the first 8 weeks (rather than the full 38 weeks) were simulated. While the average daily blood AUC of EAA or MAA is slowly increasing at this point, it exceeds 95 % of the 38-week value for the EGEEA, EGEE, and EGME models for pregnant women. Sufficient trials were conducted to reduce the SE of the mean to less than or equal to 1% of the mean (1400–1600 trials).

Analysis of Monte Carlo simulation results. The model input and output (parameter values and doses) were sorted by ascending dose to facilitate analysis and identify outliers. Trials with physiologically unrealistic values, that occurred only in a few instances in simulations with normally distributed parameter values in spite of our efforts to truncate the distributions at 0 in advance (i.e., negative excretion rates and negative biotransformation rates), were eliminated from the final analysis. Averages, SDs, percentiles of interest, and contributions to variance were calculated based on the restricted data set. Contribution to variance was calculated using rank correlations between the input parameters and the dose as described in the Crystal Ball® user's manual (Decisioneering, 1996).

Impact of excursions and alternative work schedule. In addition to an exposure of 8 h/day, 5 days/week, 2 other exposure scenarios were considered. In one scenario, it was assumed that an individual is exposed to airborne EGME, EGEEA, or EGEE for only 15 minutes per day. In another, it assumed that people work 60 h/week, in 5 12-h shifts. Airborne concentrations under these alternative scenarios that produce internal doses equivalent to the 8-h TWA PELs proposed in this paper were determined through PBPK modeling.

RESULTS

Sensitivity and Uncertainty Analyses

The results of the sensitivity analyses are summarized in Tables 1 and 2. Based on these results, the rat models were modified slightly, as described in the Methods section under Uncertainty Analysis. Generally, the results of the sensitivity analysis were similar among the models, as would be expected given the similarities in the partitioning and metabolic characteristics of these compounds. The average daily blood AUCs were most sensitive to parameters that describe the amount of parent compound removed from inhaled air (inhalation rate, body weight, percent retention of inhaled compound, and exposure concentration) and the urinary excretion rate. It should be noted that the urinary excretion rates are fitted parameters, a source of uncertainty, while all other parameters were fixed, but exhibit known variability.

Frequency distributions for those parameters included in the uncertainty analysis are summarized in Tables 3–6. Mean values of the input parameter distributions were those reported for the deterministic models (Gargas *et al.*, 2000a,b), with the exception of the rat body weights. Variation in rat body mass and exposure concentration were obtained from the study that established the NOEL (Doe, 1984; Hanley *et al.*, 1984). Variations in alveolar ventilation rate were not reported for either critical study, so the degree of variability assumed was taken from the literature (Allen *et al.*, 1996; Cronin *et al.*, 1995). The

TABLE 2
Parameters Used in EGME Model Uncertainty Analyses for Rat and Human

Parameter	Model sensitivity × parameter coefficient of variation	
	Human	Rat
KEXC, KEX	−0.27	−0.27
QPC	0.16	0.15
BW0, BWC	0.12	0.021
CONCC	—	0.10
ALV	0.05	0.05
KMAAC	0.03	0.08
KEGC	−0.03	−0.10
PRA	—	−0.012

Note. Parameters listed above contribute >99% of the expected variation in the dose estimate. KEX, KEXC, urinary excretion rate of MAA; QPC, pulmonary ventilation rate; BW0, initial body weight; BWC, body weight (expressed in a table function); CONCC, exposure concentration of EGME (expressed in a table function); ALV, alveolar retention of EGME; KMAAC, rate of conversion of EGME to MAA; KEGC, rate of conversion of EGME to EG; PRA, richly perfused tissue:blood partition coefficient for MAA. Negatives indicate that an increase in the parameter value produced a decrease in the average daily blood AUC of MAA ("dose"). The dose was insensitive to other model parameters, such as cardiac output, tissue blood flow distribution, tissue volumes, and partition coefficients other than PRA.

TABLE 3
EGEEA/EGEE Parameters Used in PBPK Model for the Human

Parameter	Type of distribution	Mean value	CV	Source of CV
BW0	Lognormal	58 kg	0.15	Allen *et al.*, 1996
QPC	Normal	15.3 l/h/kg$^{0.74}$	0.16	Cronin *et al.*, 1995
ALV	Normal	0.65	0.07	Groeseneken *et al.*, 1986
KEX	Lognormal	0.4 l/h	0.3	Allen *et al.*, 1996[a]
KEAAC	Lognormal	57 l/h/kg liver	0.3	Green *et al.*, 1996
KEGC	Lognormal	30.4 l/h/kg liver	0.3	Green *et al.*, 1996

Note. CV, coefficient of variation; BW0, initial body weight; QPC, pulmonary ventilation rate; ALV, alveolar retention of EGEEA and EGEE; KEX, urinary excretion rate of EAA; KEAAC, rate of conversion of EGEE to EAA; KEGC, rate of conversion of EGEE to ethylene glycol.
[a]Parameter with unknown variability.

variability of the alveolar absorption fractions of EGEE and EGME in male human volunteers was reported by Groeseneken *et al.* (1986 and 1989 for EGEE and EGME, respectively) and assumed to be appropriate for pregnant female humans and rats. Because the urinary excretion rates of EAA and MAA were derived by model fitting, our incomplete knowledge of the "true" value of these parameters is perhaps better described as "uncertainty" rather than variability, although there is likely to be variability among individuals as well. The CVs of the parameter distribution for urinary excretion rates are taken from a published estimate of uncertainty for metabolic parameter values (Allen *et al.*, 1996). The variability for the biotransformation rates of EGEEA, EGEE, and EGME are derived from the amount of variation seen in the experiments with hepatocytes from which the rates were derived by Gargas *et al.* (2000a,b).

In Table 7, the point estimates generated using mean values of parameters are compared to the distributions generated by Monte Carlo simulation. Cumulative distributions of dose metric are provided in Figures 4–8. The 95th percentile individual (a pharmacokinetically-sensitive individual) is approximately equal to twice the value of the mean or median of the distribution. The point estimate used for the estimation of dose in the EGEE-exposed rat is less than the mean and approximately equal to median, and is thus an appropriate target for determining a safe human dose. The point estimate in the human (at the previously determined human equivalent concentration) is also close to the mean and median, indicating that it is appropriate for deriving a no-effect level.

Likewise, the point estimates used for the estimation of dose in EGME-exposed rats and humans are also similar in value to the medians and means determined by Monte Carlo simulation.

The contributions of the different model parameters to the overall variance in the dose are presented in Table 8. As expected from the sensitivity analysis, uncertainty regarding the urinary elimination rate of the alkoxyacetic acids was the main source of variability in predicted doses, with secondary contributions from pulmonary ventilation rate and rates of metabolism.

Calculation of OEL Recommendations

Uncertainty factors. Pharmacodynamics is the description of the qualitative and quantitative differences in the response or mechanism of action associated with the toxic action of a chemical (in animals or humans). Currently available data do

TABLE 4
EGEEA/EGEE Parameters Used in PBPK Model for the Rat

Parameter	Type of distribution	Mean value	CV	Source of CV
CONC	Normal	50.8 ppm	0.05	Doe, 1984
BW0	Uniform	0.2 to 0.28 kg	not applicable	Doe, 1984
QPC	Normal	14 l/h/kg$^{0.74}$	0.15	Allen *et al.*, 1996
ALV	Normal	0.65	0.07	Groeseneken *et al.*, 1986
KEX	Lognormal	0.3 l/h	0.3	Allen *et al.*, 1996[a]
KEAAC	Lognormal	223 l/h/kg liver	0.5	Green *et al.*, 1996
KEGC	Lognormal	66.9 l/h/kg liver	0.5	Green *et al.*, 1996

Note. CV, coefficient of variation; CONC, exposure concentration of EGEEA; BW0, initial body weight; QPC, pulmonary ventilation rate; ALV, alveolar retention of EGEEA and EGEE; KEX, urinary excretion rate of EAA; KEAAC, rate of conversion of EGEE to EAA; KEGC, rate of conversion of EGEE to ethylene glycol.
[a]Parameter with unknown variability.

TABLE 5
EGME Parameters Used in PBPK Model for the Human

Parameter	Type of distribution	Mean values	CV	Source of CV
BW0	Lognormal	58 kg	0.15	Allen *et al.*, 1996
QPC	Normal	15.3 l/h/kg$^{0.74}$	0.16	Cronin *et al.*, 1995
ALV	Normal	0.76	0.05	Groeseneken *et al.*, 1989
KEX	Lognormal	0.3 l/h	0.3	Allen *et al.*, 1996[a]
KMAAC	Lognormal	4.9 l/h/kg liver	0.5	Green *et al.*, 1996
KEGC	Lognormal	0.3 l/h/kg liver	0.5	Tyson *et al.*, 1989

Note. CV, coefficient of variation; BW0, initial body weight; QPC, pulmonary ventilation rate; ALV, alveolar retention of EGEEA and EGEE; KEX, urinary excretion rate of MAA; KMAAC, rate of conversion of EGME to MAA; KEGC, rate of conversion of EGME to ethylene glycol.
[a]Parameter with unknown variability.

not provide insight regarding these differences between rats and humans or among individual humans for this class of chemicals. In these situations, use of the default UF (of 2.5 or $10^{0.5} = 3.2$) for pharmacodynamic differences is recommended (Renwick and Lazarus, 1998; Renwick, 1993).

Another "unknown" in trying to extrapolate the animal data to humans is that there are pharmacokinetic differences among humans. The results of the uncertainty analyses also indicate that human intraspecies variability/uncertainty due to pharmacokinetic differences is limited. We have chosen to use the 95th percentile dose divided by the point estimate to calculate UFs of 1.8 for both EGEEA and EGEE, and 1.7 for EGME for intraspecies PK differences. The 95th percentile value of the simulation is reproducible with the number of iterations (1400–1600) used (data not shown). The 95th percentile value for a distribution is generally considered to be a reasonable surrogate for a worst-case or "sensitive" population, but a greater degree of conservatism could be incorporated by choosing the 99th percentile dose (increasing the intraspecies PK uncertainty factors to 2.2 for EGEEA, 2.4 for EGEE, and 2.1 for EGME). The default intraspecies UF of 3.2 is equivalent to the 99.9 percentile of the human EGEE doses, but exceeds all 1600 trials of the human EGEEA and EGME doses. The values for the 99th percentile doses and the percentile equivalents of the default UF should be considered approximations due to insufficient iterations to stabilize these values.

The model results were somewhat sensitive to the choice of lognormal or normal distributions. When all parameters were assumed to be normally distributed, the UF for intraspecies PK differences increased from 1.8 for EGEEA and EGEE and 1.7 for EGME to 2.0 for all 3 compounds. When all parameters were assumed to be lognormally distributed, the UFs decreased to 1.4 for EGEEA and EGEE and 1.5 for EGME.

Proposed occupational exposure limits. Applying UFs of 2.5 (for interspecies pharmacodynamic differences), $10^{0.5}$ (for intraspecies pharmacodynamic differences, i.e., differences among humans), and 1.8 (for intraspecies pharmacokinetic differences) results in a total uncertainty factor of about 14 being applied to the previously calculated human equivalent concentration of 25 ppm EGEEA or EGEE. This calculation yields a recommended exposure limit of 2 ppm (25/[2.5 $\times$

TABLE 6
EGME Parameters Used in PBPK Model for the Rat

Parameter	Type of distribution	Mean value	CV	Source of CV
CONC	Normal	10 ppm	0.1	Hanley *et al.*, 1984
BW0	Lognormal	0.175 kg[a]	0.03	Hanley *et al.*, 1984
QPC	Normal	14 l/h/kg$^{0.74}$	0.15	Allen *et al.*, 1996
ALV	Normal	0.76	0.05	Groeseneken *et al.*, 1989
KEX	Lognormal	0.004 l/h	0.3	Allen *et al.*, 1996[b]
KMAAC	Lognormal	31 l/h/kg liver	0.4	Green *et al.*, 1996
KEGC	Lognormal	4.03 l/h/kg liver	0.5	Tyson *et al.*, 1989
PRA	Lognormal	1.05	0.2	B. Elswick, CIIT, personal communication

Note. CV, coefficient of variation; CONC, exposure concentration of EGME; BW0, initial body weight; QPC, pulmonary ventilation rate; ALV, alveolar retention of EGME; KEX, urinary excretion rate of MAA; KMAAC, rate of conversion of EGME to MAA; KEGC, rate of conversion of EGME to ethylene glycol; PRA, richly perfused tissue:blood partition coefficient for MAA.
[a]Range of 0.159 to 0.2 kg (Hanley *et al.*, 1984).
[b]Parameter with unknown variability.

TABLE 7
Comparison of Distribution to Point Estimates of Blood EAA or MAA AUC (h-mg/L per day)

Model	Point estimate	Median of distribution	Mean of distribution ± SD
EGEEA (human)[a]	183	184	194 ± 71
EGEE (human)[a]	194	194	207 ± 80
EGEE (rat)[b]	216	230	242 ± 88
EGME (human)[a]	164	166	174 ± 60
EGME (rat)[c]	157	160	171 ± 62

[a]Human model values based on PBPK modeling of the first 8 weeks of pregnancy (8 h/day, 5 days/week) at the human equivalent NAEL estimate.
[b]Rat model values based PBPK modeling of EGEE study of Doe (1984).
[c]Rat model values based PBPK modeling of EGME study of Hanley *et al.* (1984).

$10^{0.5} \times 1.8$]), or 11 mg/m^3 EGEEA or 7 mg/m^3 EGEE. Similarly, for EGME, UFs of 2.5 (for interspecies pharmacodynamic differences), $10^{0.5}$ (for intraspecies pharmacodynamic differences), and 1.7 (for intraspecies pharmacokinetic differences) result in a total uncertainty factor of 13. Using a human equivalent concentration of 12 ppm to calculate the recommended exposure limit gives 0.9 ppm EGME (12 ppm/(2.5 $\times$ $10^{0.5}$ $\times$ 1.7)), or 3 mg/m^3. Uncertainty factors for interspecies pharmacokinetic differences are omitted (assumed equal to 1) because this extrapolation was performed using the PBPK models.

Impact of Excursions and Alternative Work Schedule

To assess the need for short-term exposure limits, scenarios involving short excursions (15 min) to elevated concentrations of EGEEA, EGEE, or EGME were simulated. Once-daily 15-min exposures (inhalation only) to 29 ppm EGME or 64 ppm EGEEA or EGEE produce the same dose (average daily blood AUC of acid metabolite) as the 8-h TWA exposure to 0.9 ppm EGME and 2 ppm EGEE (see Figure 9 for predicted EAA time courses in women exposed to EGEE). Similarly, 4 15-min

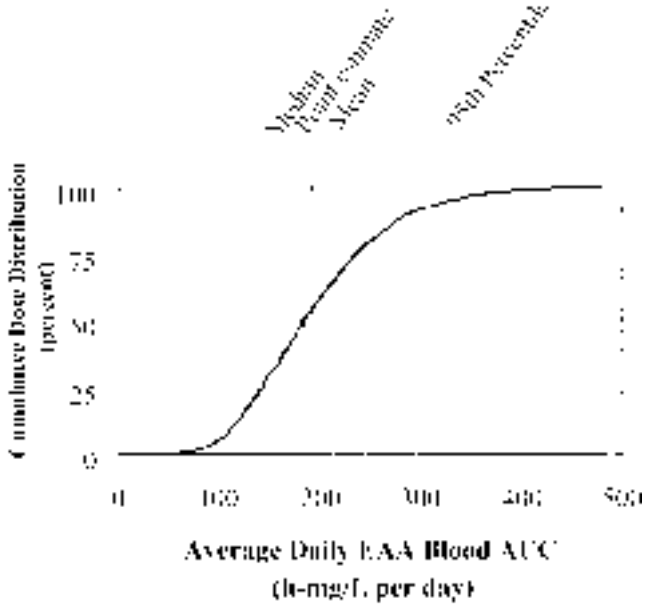

FIG. 4. Average daily AUC values for EAA in blood, calculated for pregnant women exposed to 25 ppm EGEEA for 8 h/day, 5 days/week for 8 weeks.

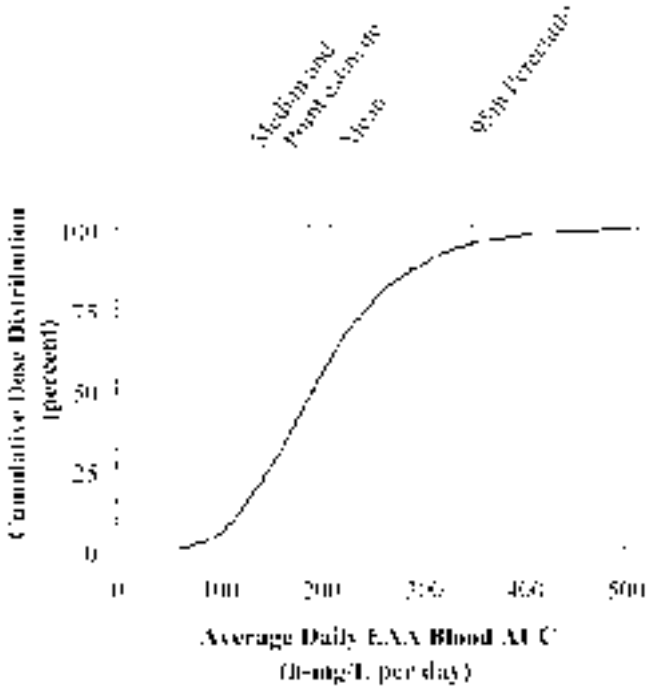

FIG. 5. Average daily AUC values for EAA in blood, calculated for pregnant women exposed to 25 ppm EGEE for 8 h/day, 5 days/week for 8 weeks.

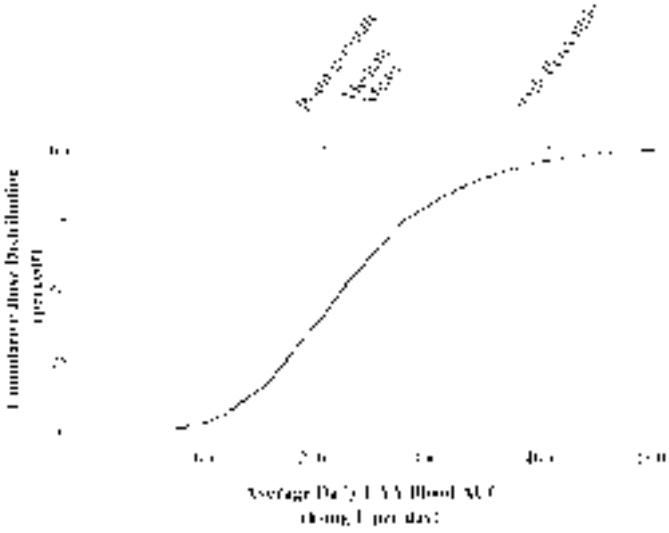

FIG. 6. Gestation day (GD) 13–15 average daily AUC values for EAA in blood, calculated for pregnant rats exposed to 50 ppm EGEE for 6 h/day on GD 6–15.

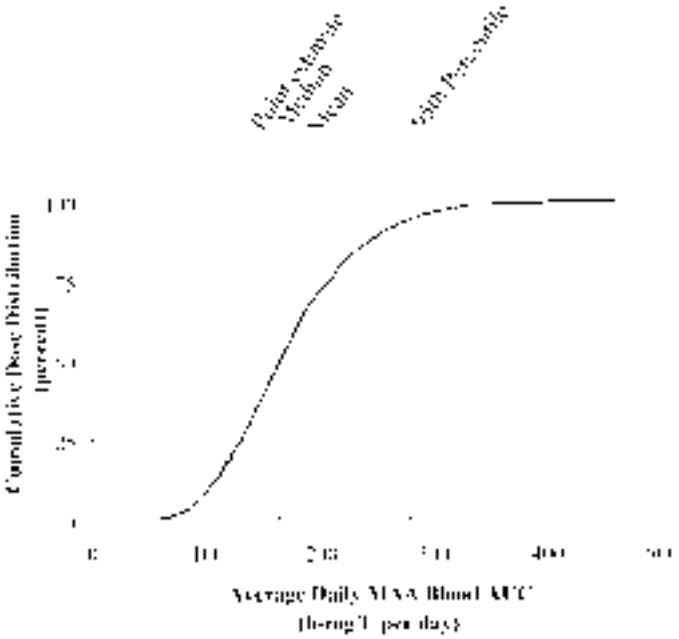

FIG. 7. AUC values for MAA in blood, calculated for pregnant women exposed to 12 ppm EGME for 8 h/day, 5 days/week for 8 weeks.

exposures to 16 ppm EGEEA or EGEE or 7 ppm EGME produce the same average daily blood AUCs of acid metabolite as the 8-h TWA exposure to 2 ppm EGEEA or EGEE or 0.9 ppm EGME, respectively.

Allowable time-weighted average concentrations of these chemicals for workers exposed up to 60 h/week (5 12-h shifts) were also computed. TWA exposures at 0.6 ppm EGME and 1.3 ppm EGEEA or EGEEE for 12-h shifts produce the same dose as the 8-h TWA exposure to 0.9 ppm EGME and 2 ppm EGEE.

In the traditional work week and both of the scenarios described above, equivalent internal doses (average daily blood AUC) were achieved for constant C × T (8 h at 2 ppm = 12 h at 1.3 ppm = 0.25 h at 64 ppm).

DISCUSSION

EGME and EGEE are known to be reproductive and developmental toxicants in laboratory animals. Therefore it is prudent to establish OELs that are protective against such effects occurring in humans. Our literature review concluded that the studies selected by OSHA (1993) (Doe, 1984 and Hanley *et al.*, 1984) remain the most relevant for this category of adverse effects. Unfortunately, exposure assessments in the various epidemiology and case studies evaluating these effects in humans have been too imprecise for establishing OELs. Our approach to establishing these limits based on these studies differs from the one used by OSHA since ours relied upon PBPK models to perform interspecies extrapolation. Additionally, PBPK modeling combined with Monte Carlo simulation to derive the uncertainty factors is used to account for interindividual variability.

The proposed OELs, 2 ppm for EGEEA or EGEE (11 mg/m^3 EGEEA or 7 mg/m^3 EGEE) and 0.9 ppm for EGME (3 mg/m^3) (TWA_8) are much lower than the current PELs, slightly lower than the current TLVs, but higher than OSHA's proposed PELs (Table 9). OSHA's current and proposed PELs for EGEEA and EGEE are 4- to 8-fold greater than their current and proposed EGME values. While the proposed OSHA PELs reflect the 5-fold difference in the rodent NOELs (10 ppm for EGME, 50 ppm for EGEEA and EGEE), incorporation of pharmacokinetics gives human equivalent concentrations that differ by only a factor of about 2 (12 ppm EGME, 25 ppm EGEEA and EGEE).

The human equivalent concentration for EGME is slightly greater than the rodent NOEL, due to greater efficiency in elimination of MAA. EAA, on the other hand, is less efficiently eliminated by humans, resulting in human equivalent concentrations for EGEEA and EGEE that are lower than the rodent NOEL (Gargas *et al.*, 2000a,b). This pharmacokinetic difference is the reason that the recommended OELs for EGME and EGEEA/EGEE differ by a factor of about 2 when the rodent NOELs differ by a factor of 5.

Our proposed OELs only address the risks posed by inhaled EGEEA, EGEE, and EGME. It is acknowledged that additional dermal uptake of EGME vapor may be worthy of special consideration; for example, Shih *et al.* (2000b) report that human whole-body dermal uptake of the vapors may be similar to the uptake rate by inhalation. However, since our approach was based on animal studies where the whole body of the animal was exposed, and rodent skin is nearly always more permeable to solvent vapor than human skin, the dermal uptake of vapor is inherent in the NOEL value. The Shih *et al.* (2000b)

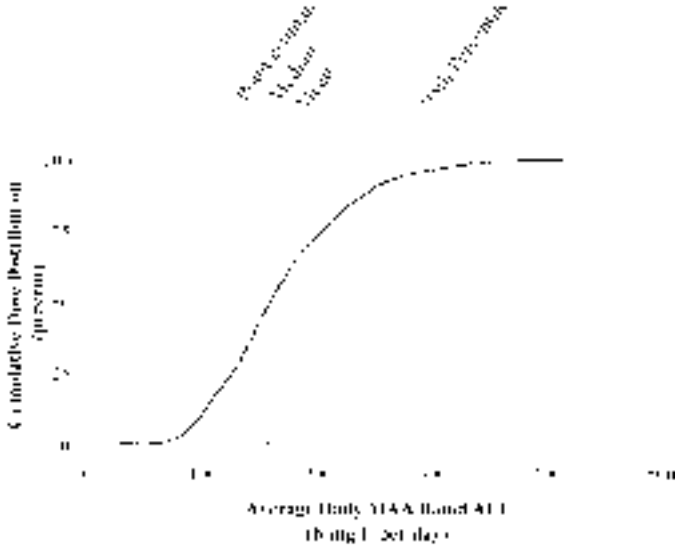

FIG. 8. Average daily gestation day (GD) 13–15 average daily AUC values for MAA in blood, calculated for pregnant rats exposed to 10 ppm. EGME for 6 h/day on GD 6–15.

TABLE 8
Contributions to Variance

Model	Parameter							
	KEX	QPC	KEAAC/KMAAC	KEGC	BW	ALV	CONC	PRA
EGEEA (human)	0.59	0.20	0.060	0.071	0.039	0.039	N/A	N/A
EGEE (human)	0.58	0.19	0.060	0.089	0.053	0.027	N/A	N/A
EGEE (rat)	0.67	0.22	0.033	0.025	0.036	0.0005	0.016	N/A
EGME (human)	0.47	0.048	0.13	0.17	0.066	0.11	N/A	N/A
EGME (rat)	0.63	0.19	0.034	0.034	0.001	0.019	0.094	0.00006

Note. KEX, urinary excretion rate of alkoxyacetic acid; QPC, pulmonary ventilation rate; KEAAC, rate of conversion of EGEE to EAA; KMAAC, rate of conversion of EGME to MAA; KEGC, rate of conversion of EGME or EGEE to EG; BW, initial body weight, ALV, alveolar retention of parent compound; CONC, exposure concentration; PRA, richly perfused tissue:blood partition coefficient for MAA.

results should, however, be considered an upper limit on possible dermal EGME absorption, as EGME "uptake" was calculated by difference, and they may not have accounted for all losses from the system. In addition, it is biologically implausible that absorption across an epithelial membrane would be the same for an organ with a large surface area specifically designed for uptake of gaseous materials (lung) and an organ with less surface area designed to protect from uptake of materials with which it comes in contact (skin).

The simulations of 15-min excursions to elevated levels of EGEEA and EGEE indicate that there is no need for a special short-term exposure limit (STEL) for these glycol ethers on the basis of reproductive hazards. Because adverse effects are mediated through slowly eliminated metabolites (alkoxyacetic acids), a short-term increase in the exposure concentration does not create a spike in blood and tissue concentrations of the toxicant. Thus, we conclude that maintaining airborne TWA_8s (for a 40-h work week) of 2 ppm EGEEA and EGEE and 0.9 ppm EGME will also provide protection against harmful effects potentially mediated by exposure to higher concentrations these ethylene glycol ethers for shorter time periods (e.g., 15 min).

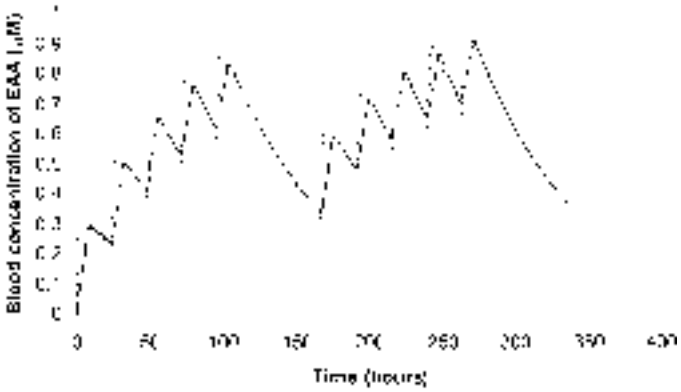

FIG. 9. Concentrations of EAA in the blood of women occupationally exposed to EGEE during a 2-week period (5 days of exposure followed by 2 days unexposed each week) predicted by the PBPK model of Gargas *et al.* (2000b). Solid line, exposure to 2 ppm EGEE 8 h/day, 5 days/week for 2 weeks. Dashed line, exposure to 64 ppm EGEE for 15 min at the beginning of each work day, 5 days/week for 2 weeks.

Overall, the degree of confidence that may be placed in the OEL calculation stems from: (1) the degree of confidence in the selection of NOELs from the critical studies, (2) confidence in the pharmacokinetic models used in interspecies extrapolation, and (3) confidence in the uncertainty factors applied in the OEL calculation. Each of these issues is addressed in turn.

Confidence in NOEL Selection

The selected critical studies are summarized in Table 10. For all 3 compounds the NOELs were based on the observation of developmental (anatomic) variants. When the 3 primary studies (Doe, 1984; Hanley *et al.*, 1984; Tyl *et al.*, 1988), were conducted, the prevailing scientific and regulatory philosophy considered these endpoints indicative of perturbed development. In keeping with that philosophy, the authors cautiously interpreted these observations of anatomical variants as significant, adverse effects.

However, the current view of the significance of these endpoints by teratologists has changed. Today, these effects are thought to lack toxicologic significance, particularly in the absence of frank malformation. It is also generally accepted that the interpretation of the significant developmental variants

TABLE 9
Current and Proposed Occupational Exposure Limits

	EGEEA	EGEE	EGME
OSHA PEL[a]	100 ppm	200 ppm	25 ppm
ACGIH TLV[b]	5 ppm	5 ppm	5 ppm
This study	2 ppm	2 ppm	0.9 ppm
OSHA PEL[c]	0.5 ppm	0.5 ppm	0.1 ppm

[a]Established 1971, OSHA, 1993.
[b]Established 1984, documentation updated in 1991, ACGIH, 1999.
[c]Proposed, OSHA, 1993.

TABLE 10
Developmental Effects Relative to Potencies of Selected Glycol Ethers

Author	Test compound	Maternal toxicity NOAEL	Endpoints and exposures (ppm) characterizing developmental toxicity in the rat					
			Structural malformations	IUGR	Prenatal mortality	LOEL	NOEL	Critical endpoint
Hanley *et al.,* 1984	EGME	10	None	None	None	50	10	Variations alone (lumbar spurs, delayed ossification: centra, ribs)
Driscoll *et al.,* 1998	EGME	25	None	None	None	25	ND	Variations alone (delayed skeletal ossification, rudimentary ribs)
Doe, 1984	EGEE	250	None	250	250	50	50 (10)[a]	Variations alone (unossified sternebrae, extra ribs)
Tyl *et al.,* 1988	EGEEA	100	300	200	300	100	50	Variations alone (unossified cervical centra, split centra, delayed ossification of a process of the atlas)

Note. IUGR, intrauterine growth retardation; ND = not determined (not designed as a dose-response study).
[a]Original author called 10 ppm NOEL but OSHA in 1993 (proposed rule in *Federal Register,* OSHA, 1993) changed to 50 ppm due to absence of statistically significant effect in high exposure group.

is confounded in the presence of intrauterine growth retardation. For example, based on the experience of one of the present authors (J. F. H.), these variants are rarely if ever considered to be of toxicological significance by the Food and Drug Administration. In general, the use of these endpoints by the U.S. EPA depends on the sector and individual responsible for reviewing the data, and no protocol has been established for classifying these developmental effects.

The minor anatomic variations are viewed with uncertainty because many occur at high frequencies in control animals, their incidences vary over time, their visual determination is highly subjective, they are frequently shown to be decreased by treatment, and whether they significantly affect normal growth, development, and salubrity of progeny is unknown. Additionally, some studies indicate that they may not persist into postnatal life (Hayasaka *et al.,* 1985; Kast, 1994; Wickramaratne, 1988) or they represent "normal" deviations in morphology (Woo and Hoar, 1972). Further inspection of Table 10 reveals that in the selected critical studies for this group of compounds there was no concordance between studies for the type of developmental variants reported. However, there was strong agreement among study outcomes that intrauterine growth retardation, prenatal mortality, and malformation were produced in the exposure range of 250–300 ppm. The Driscoll *et al.* (1998) study used EGME as a positive control agent; a single exposure level of 25 ppm was studied, limiting interpretation due to absence of dose-response design.

Additionally, studies demonstrating concordance between laboratory animal studies and adverse human developmental outcomes have not established whether developmental variants are valid signals for potential adverse effects to human development (Holson *et al.,* 1981; Kimmel *et al.,* 1984). In the most robust study of human concordance, that reported by Kimmel *et al.,* malformation, intrauterine growth retardation, and functional deficits were the only endpoints established as qualitatively and quantitatively valid signals of potential adverse effects to human development.

For the purpose of the present report, the NOELs as reported by the authors were used, with the exception of the Doe (1984) study, for which the original NOEL of 10 ppm was restated as 50 ppm by OSHA (1993). It should be recognized that these NOEL values are conservative estimates of the adverse effects of these compounds due to the nature of the endpoints used in deriving the NOELs and the substantial spacing between exposure levels (i.e., 10 vs. 50 ppm vs. 100 ppm, etc.). The spacing of exposure levels is based on practical considerations in conducting the studies, but is significant to OEL setting, given the obviously steep slope of the dose-response curves for these compounds. The salient adverse developmental effects of these compounds occur in the laboratory animal studies between 100–300 ppm, below frank maternal toxicity; hence the conservatism of using the originally reported NOELs. The consistent findings in several species (mice, rats, and rabbits) give a high level of confidence that OELs (and NOELs) based on these studies should be valid.

Confidence in Interspecies Extrapolation Conducted Using PBPK Models

The confidence in the interspecies extrapolation (converting an animal NOEL to an exposure concentration that results in equivalent internal human doses) derives from the confidence in the predictive ability of the rodent and human PBPK models (Gargas *et al.,* 2000a,b). The rodent models for EGME and EGEE disposition accurately predict blood concentrations of the alkoxyacetic acid metabolites in rats exposed to EGME and EGEE by inhalation at the NOEL and LOEL exposure concentrations in the critical studies (Gargas *et al.,* 2000a,b). Thus there is high confidence in the ability of these rodent models to predict what the internal doses of alkoxyacetic acid metabolites were in the critical studies.

The human models for EGEEA and EGEE are based on urinary excretion of EAA in humans exposed to 3 different concentrations of EGEEA and EGEE. The exposure concentrations in these studies (Groeseneken *et al.*, 1987a,b, 1988) are only slightly lower (factor of 2) than the calculated human equivalent concentrations, so the model does not have to be extrapolated very far outside the range of validation. The confidence in the model of EGEEA/EGEE disposition in humans would thus be assessed as relatively high. The human model for EGME pharmacokinetics is less well validated, as it is based on a single exposure concentration (Groeseneken *et al.*, 1989). As with EGEEA and EGEE, the human EGME inhalation study was conducted at a concentration that was lower than the calculated human equivalent NAEL by a factor of about 2. The confidence in this model is assessed to be moderate due to the single validation data set, but modest extrapolation requirement. In general, the confidence in the interspecies extrapolations is high.

Confidence in Uncertainty Factor Selection

The degree of pharmacokinetic variability among humans, as calculated by Monte Carlo simulation, is somewhat dependent on the shape chosen for the parameter distribution, for example, lognormal or normal distribution. We have followed the example of Clewell *et al.* (1999) in the selection of the distribution shapes. The distribution shapes selected by Clewell *et al.* are the same as those in Portier and Kaplan (1989) and Thomas *et al.* (1996) with the exception of alveolar ventilation rate (normal in Clewell *et al.*, lognormal in Thomas *et al.* and Portier and Kaplan). Justification for the selection of a particular shape for model parameters has generally been lacking in these studies and lends uncertainty to estimates produced by Monte Carlo simulation. The differences in intraspecies pharmacokinetic variability, as calculated using different distribution shapes, are small, so we are confident that the calculated $UF_{h,pk}$ value will lead to a reliable OEL.

We have retained the default uncertainty factor of 2.5 (interspecies) or 3.16 (interindividual) for pharmacodynamic variability/uncertainty in these calculations. It could be argued that the identification of a NOEL in a large group of animals accounts for variability in response. If the most pharmacodynamically-sensitive individuals have an adverse response to a compound, that dose is defined as a LOEL, not a NOEL. Furthermore, for EGME, EGEE, and EGEEA, the most sensitive endpoint is developmental toxicity. One could argue that the NOEL is based on an effect in the most sensitive subpopulation (embryonic/fetal animals), so concern about sensitivity may not require an adjustment factor, since selection of a sensitive subpopulation was incorporated in the study design.

In vitro experiments with cultured rat and human luteal cells have demonstrated effects (increased progesterone production) at, but not below, 1 mM MAA (Almekinder *et al.*, 1997). Interestingly, 1 mM MAA is the *in vivo* concentration at which developmental effects are observed to occur in animals (Welsch *et al.*, 1995). If these *in vitro* results could be linked to a mode of action for developmental effects in rats *in vivo*, an interspecies pharmacodynamic uncertainty factor of 1 could be supported. However, in the absence of sufficient mechanistic data on mode of action in rats, we retained the health-protective, default UF for pharmacodynamic differences between rats and humans.

Summary of Confidence in OEL Calculation Using the PBPK-Monte Carlo Approach

Our confidence that the NOELs selected from the animal studies are health protective is high. We deem the use of default UFs for pharmacodynamics to be necessarily health protective, as data from which to derive compound-specific UFs for pharmacodynamics are lacking. For interspecies extrapolation in pharmacokinetics and development of the intraspecies PK UF, we are confident that pharmacokinetics at the exposure concentrations of interest are properly described by the models.

As in traditional approaches, the PBPK-Monte Carlo approach relies on identification of the critical studies. However, instead of relying on default uncertainty factors to derive acceptable human exposure levels from animal data, we used PBPK models for rats and humans to conduct interspecies extrapolation. Monte Carlo simulation of intraspecies physiological and pharmacokinetic variability further allows us to replace uncertainty with knowledge of how variability affects internal dose estimates. We believe that this approach makes the maximum use of the data available and leads to OELs with a stronger basis in science than traditional approaches.

ACKNOWLEDGMENT

This project was supported by Union Carbide Corporation.

REFERENCES

ACGIH (1991). *Documentation of the Threshold Limit Values and Biological Exposure Indices.* American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

ACGIH (1999). *1999 TLVs and BEIs; Threshold Limit Values for Chemical Substances and Physical Agents Biological Exposure Indices.* American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Allen, B. C., Covington, T. R., and Clewell, H. J. (1996). Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. *Toxicology* **111,** 289–303.

Almekinder, J. L., Lennard, D. E., Walmer, D. K., and Davis, B. J. (1997). Toxicity of methoxyacetic acid in cultured human luteal cells. *Fundam. Appl. Toxicol.* **38,** 191–194

Andersen, M. E., Clewell, H., III, and Krishnan, K. (1995). Tissue dosimetry, pharmacokinetic modeling, and interspecies scaling factors. *Risk Anal.* **15,** 533–537.

Andrew, F. D., and Hardin, B. D. (1984). Developmental effects after inhalation exposure of gravid rabbits and rats to ethylene glycol monoethyl ether. *Environ. Health Perspect.* **57,** 13–23.

Baird, S. J. S., Cohen, J. T., Graham, J. D., Shlyakter, A. I., and Evans, J. S. (1996). Noncancer risk assessment: A probabilistic alternative to current practice. *Human Ecol. Risk Assess.* **2,** 79–102.

Barton, H. A., Andersen, M. E., and Clewell, H. J., III, (1998). Harmonization: Developing consistent guidelines for applying mode of action and dosimetry information to cancer and noncancer risk assessment. *Human Ecol. Risk Assess.* **4,** 75–115.

Chia, S., Foo, S., Khoo, N., and Jeyaratnam, J. (1997). Menstrual patterns of workers exposed to low levels of 2-ethoxyethylacetate (EGEEA). *Am. J. Ind. Med.* **31,** 148–152.

Clarke, D. O., Duignan, J. M., and Welsch, F. (1992). 2-Methoxyacetic acid dosimetry-teratogenicity relationships in CD-1 mice exposed to 2-methoxyethanol. *Toxicol. Appl. Pharmacol.* **114,** 77–87.

Clewell, H. J., Gearhart, J. M., Gentry, P. R., Covington, T. R., Van Landingham, C. B., Crump, K. S., and Shipp, A. M. (1999). Evaluation of the uncertainty in an oral Reference Dose for methylmercury due to interindividual variability in pharmacokinetics. *Risk Anal.* **19,** 547–558.

Cook, R. R., Bodner, K. M., Kolesar, R. C., Uhlmann, C. S., VanPeenen, P. F. D., Dickson, G. S., and Flanagan, K. (1982). A cross-sectional study of ethylene glycol monomethyl ether process employees. *Arch. Environ. Health* **37,** 346–351.

Cronin, W.J., IV, Oswald, E. J., Shelley, M. L., Fisher, J. W., and Flemming, C. D. (1995). A trichloroethylene risk assessment using a Monte Carlo analysis of parameter uncertainty in conjunction with physiologically-based pharmacokinetic modeling. *Risk Anal.* **15,** 555–565.

Davis, B. J., Almekinder, J. L., Flagler, N., Travlos, G., Wilson, R., and Maronpot, R. R. (1997). Ovarian luteal cell toxicity of ethylene glycol monomethyl ether and methoxy acetic acid *in vivo* and *in vitro*. *Toxicol. Appl. Pharmacol.* **142,** 328–337.

Decisioneering (1996). *Crystal Ball® Version 4.0. User Manual.* Decisioneering, Denver, CO.

Delic, J. I., Lilly, P. D., MacDonald, A. J., and Loizou, G. D., (2000). The utility of PBPK in the safety assessment of chloroform and carbon tetrachloride. *Regul. Toxicol. Pharmacol.* **32,** 144–155.

Doe, J. E. (1984). Ethylene glycol monoethyl ether and ethylene glycol monoethyl ether acetate teratology studies. *Environ. Health Perspect.* **57,** 33–42.

Dourson, M. L., Felter, S. P., and Robinson, D. (1996). Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol.* **24,** 108–120.

Dourson, M. L., and Stara, J. F. (1983). Regulatory history and experimental support of uncertainty (safety) factors. *Regul. Toxicol. Pharmacol.* **3,** 224–238.

Driscoll, C. D., Valentine, R., Staples, R. E., Chromey, N. C., and Kennedy, G. L., Jr. (1998). Developmental toxicity of diglyme by inhalation in the rat. *Drug Chem. Toxicol.* **21,** 199–136.

Foster, P. M. D., Creasy, D. M., Foster, J. R., Thomas, L. V., Cook, M. W., and Gangolli, S. D. (1983). Testicular toxicity of ethylene glycol monomethyl and monoethyl ethers in the rat. *Toxicol. Appl. Pharmacol.* **69,** 385–399.

Gargas, M. L., Tyler, T. R., Sweeney, L. M., Corley, R. A., Weitz, K. K., Mast, T. J., Paustenbach, D. J., and Hays, S. M. (2000a). A toxicokinetic study of inhaled ethylene glycol ethyl ether acetate and validation of a physiologically based pharmacokinetic model for rat and human. *Toxicol. Appl. Pharmacol.* **165,** 63–73.

Gargas, M. L., Tyler, T. R., Sweeney, L. M., Corley, R. A., Weitz, K. K., Mast, T. J., Paustenbach, D. J., and Hays, S. M. (2000b). A toxicokinetic study of inhaled ethylene glycol monomethyl ether (2-ME) and validation of a physiologically based pharmacokinetic model for the pregnant rat and human. *Toxicol. Appl. Pharmacol.* **165,** 53–62.

Green, C. E., Gordon, G. R., Cohen, P. M., Nolen, H. W., Peters, J. H., and Tyson, C. A. (1996). *In vitro* metabolism of glycol ethers by human and rat hepatocytes. *Occup. Hyg.* **2,** 67–75.

Groeseneken, D., Veulemans, H., and Masschelein, R. (1986). Respiratory uptake and elimination of ethylene glycol monoethyl ether after experimental human exposure. *Br. J. Ind. Med.* **43,** 544–549.

Groeseneken, D., Veulemans, H., Masschelein, R., and Van Vlem, E. (1987a). Ethoxyacetic acid: A metabolite of ethylene glycol monoethyl ether acetate in man. *Br. J. Ind. Med.* **44,** 488–493.

Groeseneken, D., Veulemans, H., Masschelein, R., and Van Vlem, E. (1987b). Pulmonary absorption and elimination of ethylene glycol monoethyl ether acetate in man. *Br. J. Ind. Med.* **44,** 309–316.

Groeseneken, D., Veulemans, H., Masschelein, R., and Van Vlem, E. (1989). Experimental human exposure to ethylene glycol monomethyl ether. *Int. Arch. Occup. Environ. Health* **61,** 243–247.

Hanley, T. R., Jr., Yano, B. L., Nitschke, K. D., and John, J. A. (1984). Comparison of the teratogenic potential of inhaled ethylene glycol monomethyl ether in rats, mice, and rabbits. *Toxicol. Appl. Pharmacol.* **75,** 409–422.

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* **35,** 321–328.

Hayasaka, I., Tamake, F., Uchiyana, K., *et al*. (1985). Azosemide-induced fetal wavy ribs and their disappearance after birth. *Cong. Anom.* **25,** 121–127.

Hays, S. M., Elswick, B. A., Blumenthal, G. M., Welsch, F., Conolly, R. B., and Gargas, M. L. (2000). Development of a physiologically based pharmacokinetic model of 2-methoxyethanol and 2-methoxyacetic acid disposition in pregnant rats. *Toxicol. Appl. Pharmacol.* **163,** 67–74.

Holson J. F., Kimmel, C. A., Hogue, C., and Carolo, G. (1981). Suitability of experimental studies for predicting hazards to human development. Presented at the 1981 annual winter meeting of the Toxicology Forum.

Johanson, G. (2000). Toxicity review of ethylene glycol monomethyl ether and its acetate ester. *Crit. Rev. Toxicol.* **30,** 307–345.

Kast, A. (1994). "Wavy ribs": A reversible pathologic effect in rat fetuses. *Exp. Toxicol. Pathol.* **46,** 203–210.

Kimmel, C. A., Holson, J. F., Hogue, C. J., and Carlo, G. L. (1984). Reliability of experimental studies for predicting hazards to human development. NCTR technical report for experiment 6015. National Center for Toxicological Research, Jefferson, AR.

Ku, W. W., Wine, R. N., Chae, B. Y., Ghanayem, B. I., and Chapin, R. E. (1995). Spermatocyte toxicity of 2-methoxyethanol (ME) in rats and guinea pigs: Evidence for the induction of apoptosis. *Toxicol. Appl. Pharmacol.* **134,** 100–110.

Miller, R. R., Ayres, J. A., Young, J. T., and McKenna, M. J. (1983). Ethylene glycol monomethyl ether. I. subchronic vapor inhalation study with rats and rabbits. *Fundam. Appl. Toxicol.* **3,** 49–54.

Nagano, K., Nakayama, E., Oobayashi, H., Yamada, T., Adachi, H., Nishizawa, T., Ozawa, H., Nakaichi, M., Okuda, H., Minami, K., and Yamazaki, K. (1981). Embryotoxic effects of ethylene glycol monomethyl ether in mice. *Toxicology* **20,** 335–343.

Nelson, B. K., Conover, D. L., Brightwell, W. S., Shaw, P. B., Werren, D., Edwards, R. M., and Lary, J. M. (1991). Marked increase in the teratogenicity of the combined administration of the industrial solvent 2-methoxyethanol and radiofrequency radiation in rats. *Teratology* **43,** 621–634.

Nelson, B. K., Conover, D. L., Shaw, P. B., Snyder, D. L., and Edwards, R. M. (1996). Interactions of radiofrequency radiation on 2-methoxyethanol teratogenicity in rats. *J. Appl. Toxicol.* **17,** 31–39.

Nelson, B. K., Vorhees, C. V., Scott, W. J., Jr., and Hastings, L. (1989). Effects of 2-methoxyethanol on fetal development, postnatal behavior, and embryonic intracellular pH of rats. *Neurotoxicol. Teratol.* **11,** 273–284.

OSHA (1997). Occupational exposure to methylene chloride. Occupational Safety and Health Administration. *Fed. Regist.* 62, 1494–1619.

OSHA (1993). Occupational exposure to 2-methoxyethanol, 2-ethoxyethanol and their acetates (glycol ethers): proposed rule. Occupational Safety and Health Administration. *Fed. Regist.* 58, 15526–15632.

Paustenbach, D. J. (2000). The history and biological basis of occupational exposure limits for chemical agents. In *Patty's Industrial Hygiene* (R. L. Harris, Ed.), 5th ed., pp. 1903–2000. John Wiley and Sons, New York.

Portier, C. J., and Kaplan, N. L. (1989). Variability of safe dose estimates when using complicated models of the carcinogenic process. A case study: Methylene chloride. *Fundam. Appl. Toxicol.* **13,** 533–544.

Ratcliffe, J. M., Schrader, S. M., Clapp, D. E., Halperine, W. E., Turner, T. W., and Hornung, R. W. (1989). Semen quality in workers exposed to 2-ethoxyethanol. *Br. J. Ind. Med.* **46,** 399–406.

Reitz, R. H., Gargas, M. L., Andersen, M. E., Provan, W. M., and Green, T. L. (1996). Predicting cancer risk from vinyl chloride exposure with a physiologically based pharmacokinetic model. *Toxicol. Appl. Pharmacol.* **137,** 253–267.

Renwick, A. G. (1993). Data-derived safety factors for the evaluation of food additives and environmental contaminants. *Food Addit. Contam.* **10,** 275–305.

Renwick, A. G., and Lazarus, N. R. (1998). Human variability and noncancer risk assessment—an analysis of the default uncertainty factor. *Regul. Toxicol. Pharmacol.* **27,** 3–20.

Ritter, E. J., Scott, W. J., Jr., Randall, J. L., and Ritter, J. M. (1985). Teratogenicity of dimethoxyethyl phthalate and its metabolites methoxyethanol and methoxyacetic acid in the rat. *Teratology* **32,** 25–31.

Samuels, D. M., Doe, J. E., and Tinston, D. J. (1984). The effects on the rat testis of single inhalation exposures to ethylene glycol monoalkyl ethers, in particular ethylene glycol monomethyl ether. *Arch. Toxicol. Suppl.* **7,** 167–170.

Shih, T.-S., Hsieh, A.-T., Liao, G.-D., Chen, Y.-H., and Liou, S.-H. (2000a). Haematological and spermatotoxic effects of ethylene glycol monomethyl ether in copper clad laminate factories. *Occup. Environ. Med.* **57,** 348–352.

Shih, T.-S., Wang, P. Y., Chen, C.-Y., Smith, T. J., and Hu, Y. -P. (2000b). Measurement of percutaneous uptake of 2-methoxyethanol vapor in humans. *J. Occup. Environ. Med.* **42,** 475–482.

Sleet, R. B., Welsch, F., Myers, C. B., and Marr, M. C. (1996). Developmental phase specificity and dose-response effects of 2-methoxyethanol in rats. *Fundam. Appl. Toxicol.* **29,** 131–139.

Slob, W., and Pieters, M. N. (1998). A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: general framework. *Risk Anal.* **18,** 787–798.

Smialowicz, R. J., Riddle, M. M., Luebke, R. W., Copeland, C. B., Andrews, D., Rogers, R. R., Gray, L. E., and Laskey, J. W. (1991). Immunotoxicity of 2-methoxyethanol following oral administration in Fischer 344 rats. *Toxicol. Appl. Pharmacol.* **109**(3), 494–506

Swartout, J. C., Price, P. S., Dourson, M. L., Carlson-Lynch, H. L., and Keenan, R. E. (1998). A probabilistic framework for the Reference Dose (probabilistic RfD). *Risk Anal.* **18,** 271–282.

Terry, K. K., Elswick, B. A., Stedman, D. B., and Welsch, F. (1994). Developmental phase alters dosimetry-teratogenicity relationship for 2-methoxyethanol in CD-1 mice. *Teratology* **49,** 218–227.

Thomas, R. S., Bigelow, P. L., Keefe, T. J., and Yang, R. S. H. (1996). Variability in Biological Exposure Indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. *Am. Ind. Hyg. Assoc. J.* **57,** 23–32.

Toraason, M., Niemeier, R. W., and Hardin, B. D. (1986). Calcium homeostasis in pregnant rats treated with ethylene glycol monomethyl ether (EGME). *Toxicol. Appl. Pharmacol.* **86,** 197–203.

Tyl, R. W., Pritts, I. M., France, K. A., Fisher, L. C., and Tyler, T. R. (1988). Developmental toxicity evaluation of inhaled 2-ethoxyethanol acetate in Fischer 344 rats and New Zealand white rabbits. *Fundam. Appl. Toxicol.* **10,** 20–39.

Tyson, C., Green, C., and Peters, J. (1989). Method development to assess human metabolism of chemical xenobiotics. Prepared for National Institute of Environmental Health Sciences under contract NIH-NOI-ES-55109. SRI International, Menlo Park, CA.

U.S. EPA (2000). IRIS substance file – vinyl chloride, CASRN 75–01–4. U. S. Environmental Protection Agency. Availabele at: http://www.epa.gov/iris/subst/1001.htm. Accessed April 18, 2001.

U.S EPA (1999). IRIS Substance File – Ethylene glycol monobutyl ether (EGBE) (2-Butoxyethanol), CASRN 111–76–2. U. S. Environmental Protection Agency. Available at: http://www.epa.gov/ngispgm3/iris/subst/0500.htm. Accessed April 18, 2001.

Veulemans, H., Steeno, O., Masschelein, R., and Groeseneken, D. (1993). Exposure to ethylene glycol ethers and spermatogenic disorders in man: A case-control study. *Br. J. Ind. Med.* **50,** 71–78.

Welch, L. S., Schrader, S. M., Turner, T. W., and Cullen, M. R. (1988). Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male reproduction. *Am. J. Ind. Med.* **14,** 509–526.

Welsch, F., Blumenthal. G. M., and Conolly, R. B. (1995). Physiologically based pharmacokinetic models applicable to organogenesis: Extrapolation between species and potential use in prenatal toxicity risk assessments. *Toxicol. Lett.* **82–83,** 539–547.

Wickramaratne, G. A. de S. (1988). The post-natal fate of supernumerary ribs in rat teratogenicity studies. *J. Appl. Toxicol.* **8,** 91–94.

Wier, P. J., Lewis, S. C., and Traut, 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 Carcinog. Mutagen.* **7,** 55–64.

Woo, D. C., and Hoar, R. M. (1972). Apparent hydronephrosis as a normal aspect of renal development in late gestation in rats: The effect of methyl salicylate. *Teratology* **6,** 191–196.