HAP Task Force
P.O. Box 331, Millwood, VA 22646 (540) 837-1602

September 4, 2015

BY CDX

Document Control Office
Office of Pollution Prevention and Toxics
Room 6428
U.S. Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Re: Testing Consent Order for Ethylene Dichloride;
Final PBPK Modeling Report (Docket No. OPPT-2003-0010)

Dear Sirs:

Enclosed is the final report required under the Enforceable Consent Agreement for Ethylene Dichloride, 68 Fed. Reg. 33125 (June 3, 2003) (the "ECA"), as amended, entitled "Route-to-Route Extrapolation of 1,2-Dichloroethane Studies from the Oral Route to Inhalation Using Physiologically Based Pharmacokinetic Models: Extended One Genera tion Toxicity Study and Reproductive Toxicity Studies in the Rat."

Please do not hesitate to contact me if there is any question about this report.

Sincerely,

Peter E. Voytek /wcn

Peter E. Voytek, Ph.D.
Manager

Enclosures

cc: John E. Schaeffer (w/out enclosure)
W. Caffey Norman, Esq.

Route-to-Route Extrapolation of 1,2-Dichloroethane Studies from the Oral Route to Inhalation Using Physiologically Based Pharmacokinetic Models: Extended One Generation Toxicity Study and Reproductive Toxicity Studies in the Rat

Prepared for
HAP Task Force, Millwood, Virginia

Prepared by

Lisa M. Sweeney, Ph.D., DABT
Michael L. Gargas, Ph.D.
Independent Consultants, Dayton, Ohio

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EXECUTIVE SUMMARY

A need has been identified for a quantitative understanding of the hazards of 1,2-dichloroethane (ethylene dichloride, EDC, CAS No. 107-06-2) exposure by the inhalation route. Under Section 4 of the Toxic Substances Control Act (TSCA), the U.S. EPA issued a testing consent order (Enforceable Consent Agreement, ECA) to perform toxicity testing and mechanistic and pharmacokinetic studies to satisfy EPA's data needs for EDC (U.S. EPA, 2003, 2009). As part of Tier I testing, pharmacokinetic studies were conducted (Saghir et al., 2006) and a physiologically based pharmacokinetic (PBPK) model was developed that describes the disposition of EDC in rats by the oral and inhalation routes (Sweeney et al., 2008).

This report serves to fulfill the identified data need for route-to-route extrapolation to the inhalation route of results of an extended one-generation reproductive toxicity (EOGRT) study recently conducted via drinking water ingestion and previously identified tests of the reproductive toxicity of EDC. The EOGRT study serves as a substitute for the oral subchronic neurotoxicity and reproductive toxicity tests called for in the amended ECA (U.S. EPA, 2003, 2009). The route-to-route extrapolation of results of the EOGRT study of EDC using the PBPK model was based on selection of the no adverse effects levels for the critical endpoints of neurotoxicity and reproductive/developmental toxicity, the most appropriate internal dose measures, and an appropriate point of departure. The specified points of departure were the NOAEL, the highest tested doses, of 169 and 155 mg/kg-day, respectively. Based on PBPK modeling results presented here, the NOAELs in rats exposed by drinking water in the neurotoxicity and reproductive/developmental are equivalent to continuous exposure of rats to minimums of 76 ppm and 62 ppm EDC, respectively, using total metabolism of EDC (normalized to body weight) as the dose metric that is equivalent in the drinking water and inhalation scenarios. Sensitivity analyses of the key internal dose metric at the oral and inhalation-equivalent NOAEL indicated that in both scenarios, the total amount of metabolized was sensitive to the ingested dose/exposure concentration and the maximum rate of oxidative metabolism of EDC. Ingested dose was well-characterized in the study, as drinking water consumption was measured. The maximum rate of oxidative metabolism in adult rats was well-characterized in the model development stage (Sweeney et al., 2008), where sensitivity analysis indicated high sensitivity of blood EDC concentrations to this optimized parameter. Metabolic rates have not, however, been characterized in lifestages other than adult rats, introducing some uncertainty in internal dosimetry for neonatal rats.

INTRODUCTION

1,2-Dichloroethane (ethylene dichloride, EDC, CAS No. 107-06-2) is used as primarily as an intermediate in the production of vinyl chloride; use of EDC as a solvent has been reduced and largely phased out. A need has been identified for a quantitative understanding of the hazards of EDC exposure by the inhalation route. Under Section 4 of the Toxic Substances Control Act (TSCA), the U.S. EPA issued a testing consent order (Enforceable Consent Agreement, ECA) to perform toxicity testing and mechanistic and pharmacokinetic studies to satisfy EPA's data needs for EDC (U.S. EPA, 2003); this consent order was subsequently modified with respect to timing (U.S. EPA, 2009). This report describes the PBPK-based extrapolation of NOAEL doses identified for neurotoxicity and reproduction in an extended one-generation reproductive toxicity (EOGRT) study conducted by the drinking water route (Charlap, 2015) to equivalent continuous inhalation exposures. The EOGRT replaces the separate subchronic oral neurotoxicity and reproductive toxicity studies specified in the amended ECA (U.S. EPA, 2003, 2009).

Summary of Physiologically Based Pharmacokinetic (PBPK) Model

A combination of experimental studies (D'Souza et al., 1987, 1988; Gargas et al. 1986; Spreafico et al., 1980; Payan et al., 1995; Reitz et al., 1982; Withey and Karpinksi, 1985; Saghir et al., 2006) and modeling (Sweeney et al., 2008) was used to develop a physiologically based pharmacokinetic (PBPK) model that would allow route-to-route extrapolation from existing and future data describing hazards of oral EDC exposure to potential inhalation hazards.

The Sweeney et al. (2008) model is an expansion and refinement of previous PBPK models (D'Souza et al., 1987, 1988; Staats et al., 1991; ENVIRON 2004) for disposition of EDC in male F344 rats based on EDC kinetics data following oral gavage exposure. Sprague-Dawley rats receiving intravenous (iv) doses of EDC (Spreafico et al., 1980) rapidly cleared EDC from the blood. However, the D'Souza et al. (1987, 1988) model structure was not able to replicate the clearance (even when applying a large rate of metabolism in the liver), suggesting that EDC also undergoes extrahepatic metabolism. EDC studies (Spreafico et al., 1980; Saghir et al., 2006) indicate that pulmonary metabolism is not significant as evidenced by the lack of GSH depletion in the lung; thus lung extrahepatic metabolism is not needed, although it was included in the D'Souza et al. (1987, 1988) model. Oral absorption of EDC was initially described using a simple first-order description (D'Souza et al., 1987, 1988). However, when uptake from the GI tract was measured in rats dosed with radiolabeled EDC, it was found that approximately 50% of the administered radiolabeled EDC dose remained in the GI tract at 1 hr post dosing, but only an additional 15% was absorbed in the following 3 hrs, indicating bi-phasic absorption (Payan et al., 1995). The expanded model (Sweeney et al., 2008) included a two compartment GI tract where

EDC was rapidly absorbed from the first compartment then sent to either the portal blood or to a second GI compartment where additional absorption occurs, and the extrahepatic metabolism was modeled as occurring in a venous blood "pool" fed by the slowly perfused tissues, kidney and other richly perfused tissues. Updated model parameters determined from previous models and literature were tested against the available data. An iterative approach was used to optimize the model parameters (hepatic metabolism, extrahepatic metabolism, exhalation rates, and absorption rates); the modified model was able to provide adequate estimates of GSH-dependent metabolism. The revised model provided an adequate fit to all the rat data except fetal tissue (a 3.3-fold difference was found between modeled and predicted fetal tissue concentrations). The model can be used to calculate specified dose metrics of interest (blood concentrations, tissue concentrations, or amounts metabolized) after inhalation or gavage (corn oil or water). Model code is provided in Appendix A.

SUMMARY OF KEY STUDY

Study Design

An EOGRT study of EDC administered in drinking water (Charlap, 2015) was conducted using 3 EDC-dosed groups and a control group (27/sex/group) of sexually mature male and virgin female Crl:CD (Sprague-Dawley, SD) rats (F_0 parental generation). The study design was intended to conform with the Organisation for Economic Development (OECD) Test Guideline (TG) 443 (OECD, 2011). The intent of the EOGRT test is to identify the potential for test-chemical induced effects arising from prenatal, postnatal, and juvenile/young adult exposures. A particular emphasis is placed upon reproductive effects. The basic design in TG 443 includes three possible F_1 (first generation offspring) cohorts for testing for reproductive/developmental effects (Cohort 1), neurodevelopmental effects (Cohort 2), and developmental immune effects (Cohort 3). Cohort 1 is considered essential to the conduct of the study, and may be extended to a second generation (F_2) if "triggered" by reproductive findings in the F_1 generation. Cohorts 2 and 3 are optional, and their inclusion or exclusion is to be based on existing knowledge of the chemical and needs of the regulating authorities. Consistent with requirement for a subchronic neurotoxicity test and a reproduction study examining fertility index, gestation index, gross necropsy, organ weight, histopathology, estrous cycle, sperm evaluation, vaginal opening, and preputial separation as endpoints of interest in the ECA (U.S. EPA, 2003), Charlap (2015) included Cohorts 1 and 2, but not Cohort 3.

Based on the lack of findings in previous reproductive toxicity studies (Almuot et al., 1976, Rao et al., 1980), Sweeney and Parker (2011) recommended that doses intended to potentially yield reproductive effects in rats should exceed 50 mg/kg-day in diet or drinking water. However, in

consideration of maternal toxicity identified in previous studies and possible toxicokinetic saturation (a situation to be avoided, if possible, per OECD TG 443 [OECD, 2011]), they recommend that the highest dose should not exceed 300 mg/kg-day. Charlap (2015) therefore selected 50, 150, and 300 mg/kg/day as target exposure levels.

Study Results

Actual Doses

The actual EDC doses in the Charlap (2015) EOGRT study were less than the targeted doses with the exception of lactating F_0 females. For the F1 generation, intake is averaged over the entire study period. For the F0 generation, the lowest intake among the various study phases (pre- or post-mating for males; premating, gestation, or lactation for females) was selected as a possible point of departure for route-to-route extrapolation. The applicable average daily intakes for the different generations, sexes, and doses are summarized in Table 1.

Table 1. Applicable daily intakes for route-to-route extrapolation

Generation/Sex	Low dose (Target: 50 mg/kg/day)	Middle dose (Target: 150 mg/kg/day)	High dose (Target: 300 mg/kg/day)
F_0 male	31 (after mating)	79 (after mating)	155 (after mating)
F_0 female	40 (prior to mating)	95 (prior to mating)	182 (prior to mating)
F_1 male	37	97	184
F_1 female	39	93	169

Charlap (2015) evaluated many endpoints in the F_0 animals and the two cohorts of F_1 offspring. The many negative findings will not be enumerated here; notably, the potential breeding and evaluation of an F_2 generation was not triggered. Charlap (2015) identified key findings and their associated no-adverse effect levels (NOAELs) in the "Conclusions" section (Section 1.4) of their report. In the summary table in this current report, the corresponding lowest-observed adverse effect levels (LOAELs) are also noted (Table 2). The key findings of Charlap (2015) consisted of decreases in body weight, body weight gain, and food consumptions. Charlap (2015) suggested that rats may have found EDC in drinking water to have poor palatability; drinking water consumption was generally decreased, relative to controls, in a dose-related manner. Charlap (2015) further suggested that food consumption thus may have been decreased to

parallel the reduced water intake. While this explanation is one possibility, it should be noted that decreased body weight gains have been noted in studies where EDC was delivered by other means. In a subchronic, 90-day study of EDC conducted by gavage in male and female Sprague-Dawley rats, significant decreases in body weight gain and food consumption were identified in males from the 150 mg/kg/day dose group (Daniel et al., 1994). Likewise, exposures of 300 ppm EDC for 6-7 hrs/day were found to produce adverse effects on maternal body weight or body weight gain in teratogenicity/developmental toxicity studies (Rao et al., 1980; Payan et al., 1995). As a health protective measure, we have chosen to include decreased body weight gain as a potential basis for route-to-route extrapolation.

In the body of the report, additional findings (statistically significant or otherwise) beyond food consumption and body weight-related effects were described by Charlap (2015). These other statistically-significant findings were dismissed as reflecting variation within the laboratory's historical ranges, or for lack of a consistent, monotonic dose-response relationship. We agree that these findings are not of concern with respect to identifying appropriate points of departure and key endpoints to serve as bases for route-to-route extrapolation of the study results. We have, however, noted one finding where the changes were outside of the WIL historical ranges—relative liver weight in F_0 females. As no significant histological findings were identified in the liver (or any other tissue), the organ weight finding may be considered non-adverse. Charlap (2015) reports other organ weight changes (within WIL historical ranges) that were consistent with the Daniel et al. (1994) findings of increased relative kidney, liver, and brain weights in the previously-mentioned 90-day study.

Table 2. NOAELs, LOAELs, and key findings from Charlap (2015) EOGRT study

Endpoint	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Responses at the LOAEL per Charlap (2015) conclusions, unless otherwise noted.
F_0 male (systemic)	31	79	Lower mean body weights, body weight gains, and/or food consumption
F_0 female (systemic)	40	95	Lower mean body weights, body weight gains, and/or food consumption Higher relative liver weights at 95 and 182 mg/kg/day, exceeding historical ranges (not highlighted by Charlap, 2015)

Endpoint	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Responses at the LOAEL per Charlap (2015) conclusions, unless otherwise noted.
F_0 male (reproductive)	155	None identified	None identified
F_0 female (reproductive)	182	None identified	None identified
F_1 adult male (systemic)	37	97	Lower mean body weights, body weight gains, and/or food consumption
F_1 adult female (systemic)	93	169	Lower mean body weights, body weight gains, and/or food consumption
F_1 neonatal male	97	184	Lower body weights and body weight gains throughout postnatal period
F_1 neonatal female	93	169	Lower body weights and body weight gains throughout postnatal period
F_1 male (reproductive)	184	None identified	None identified
F_1 female (reproductive)	169	None identified	None identified
F_1 male (neurotoxicity)	184	None identified	None identified
F_1 female (neurotoxicity)	169	None identified	None identified

OTHER REPRODUCTIVE TOXICITY STUDIES IDENTIFIED IN THE ECA

The ECA (U.S. EPA, 2003) specified that quantitative route-to-route extrapolation (oral to inhalation) would be conducted for the studies conducted under Tier II (neurotoxicity and reproductive toxicity) and the extant reproductive toxicity studies Alumot et al. (1976), Rao et al. (1980), and Lane et al. (1982). These existing studies did not identify any effects of EDC on

reproduction (Almuot et al., 1976, Rao et al., 1980, Lane et al. 1982) nor was any developmental toxicity for EDC observed (Payan et al., 1995, Lane et al. 1982). While the request for extrapolation of the findings of Alumot et al. (1976), a study conducted via dietary exposure, makes some sense, the other requests are somewhat puzzling. Rao et al. (1980) was a developmental toxicity study conducted by the inhalation route, obviating the need for extrapolation. The Lane et al. (1982) study was conducted in mice; but no PBPK model development and/or approval for mice was specified in the ECA. The Appendices of the ECA state that merely that the results of the newly-conducted study will be "compared" to those of the extant studies.

Alumot et al. (1976)

Alumot et al. (1976) describe studies in which groups of 18 rats per sex per group were fed EDC-fumigated mash or a control diet. In the reproduction test, the rats consumed the fumigated diet for 6 weeks prior to mating. The feeding procedure, designed to minimize loss of fumigant, consisted of feeding the animals two portions of feed daily. In the first daily feeding, 20 percent of the daily allotment was provided between 11 a.m and noon, and it was quickly consumed. The remaining 80 percent of daily intake was made available to the rats between 7 and 9 p.m.; of this portion, roughly 66 percent was consumed in the first hour, with the remainder consumed during the second hour. The authors computed that 250 ppm EDC in diet was equivalent to 25 mg/kg/day, so they assumed that their dose of 500 ppm EDC in diet was equivalent to 50 mg/kg/day. The lack of findings in the Alumot et al. (1976) study is consistent with the lack of findings in the more extensive Charlap (2015) EOGRT study, which was conducted at higher ingested doses.

Rao et al. (1980)

Rao et al. (1980) evaluated the effects on reproduction of inhaled EDC in Sprague-Dawley rats. During the pre-breeding period, groups of rats (30/sex in the control group and 20/sex in each treated group) F_0 rats were exposed to 25, 75, or 150 ppm EDC for 6 hours/day, 5 days per week. For the rest of the study, exposure was 6 hours/day, 7 days per week, with the exception that maternal animals were not exposed from gestation day 21 through the 4^{th} day postpartum. No significant findings were identified in this study.

Lane et al. (1982)

Mice in a multigeneration study (Lane et al., 1982) were exposed to EDC in drinking water at concentrations intended to yield doses of 0, 5, 15, or 50 mg/kg/day. The study design included dominant lethal and teratology investigations. No reproductive/developmental effects were

identified in this study, consistent with the lack of findings in rats exposed to higher doses of EDC in drinking water in the Charlap (2015) EOGRT study.

SELECTION OF CRITICAL ENDPOINTS AND DOSE MEASURES

Critical Endpoints

The critical endpoints for systemic effects in F_0 adults and adult and neonatal F_1 rats in Charlap (2015) were decreased body weights and body weight gains. No neurotoxic, reproductive, or developmental effects were identified in the study.

Critical Dose Measures

Consistent with U.S. EPA's *Guidelines for Carcinogen Risk Assessment* and PBPK modeling guidance (U.S. EPA, 2005, 2006), the selection of an appropriate dose measure for toxicological endpoints of interest (both cancer and non-cancer endpoints) requires a careful consideration of the mode of action by which a chemical exerts an effect. Parent compound (peak, average, or area under the concentration vs. time curve [AUC]) and amount metabolized were suggested as dose metrics in the ECA (U.S. EPA, 2003). The dose metrics to be considered were expanded beyond those suggested in the ECA. The following additional dose metrics were considered: total amount of EDC metabolized in the liver (normalized to liver weight) (AMLVL), the minimum concentration of glutathione in the liver (GSHLmin) (per Sweeney and Parker, 2010), the peak concentration of EDC in arterial blood (CAmax) and the average concentration of EDC in arterial blood (CAavg, computed as AUCB/duration) were also evaluated, in order to provide a comprehensive assessment.

The data indicate that the most appropriate dose metric for route to route extrapolation of hepatic effects (i.e., increased liver weights) is the minimum post exposure concentration of GSH in the liver (GSHLmin). Igwe et al. (1986) evaluated the potential interaction of dietary disulfiram (Antabuse) and EDC exposure in male Sprague-Dawley rats treated with the compounds for 30 days. Disulfiram acts to block the metabolism of aldehydes by aldehyde dehydrogenase. One pathway for EDC metabolism, which competes with GSH conjugation, is oxidative metabolism to chloroacetaldehyde (Figure 1); co-exposure to disulfiram would block the metabolism of chloroacetaldehyde by aldehyde dehydrogenase (ALDH), resulting in potentially greater accumulation of chloroacetaldehyde and subsequent diversion of the metabolism of this intermediated from chloroacetic acid to the chloroacetaldehyde-GSH conjugate, with the consequence of more extensive depletion of GSH. Igwe et al. (1986) found that the combined exposure to EDC and disulfiram increased hepatotoxicity (mid-zonal necrosis), decreased spleen

weight, and resulted in testicular atrophy. These findings support the use of dose metrics related to EDC metabolism rather than parent compound as indicators of hepatotoxicity.

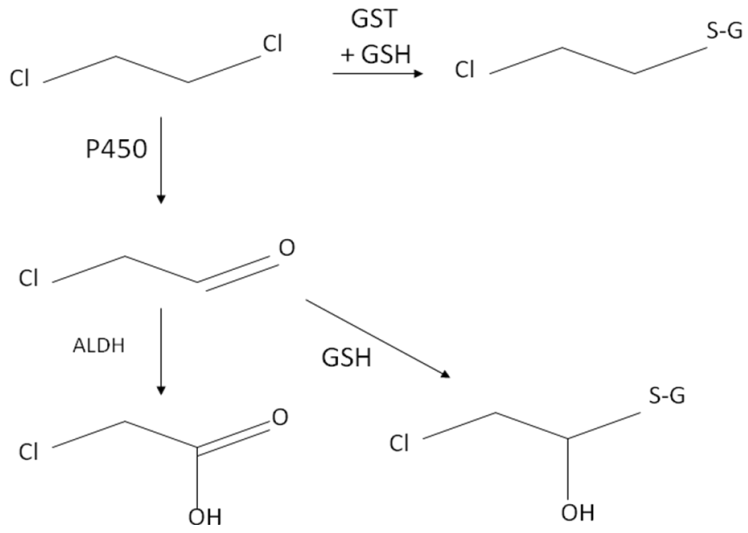

Figure 1. Metabolic scheme for EDC

Chloroacetaldehyde has been demonstrated to be hepatotoxic when administered directly to mice in drinking water (Daniel et al., 1992) and chloroacetaldehyde is cytotoxic to isolated rat hepatocytes (Sood and O'Brien, 1993). The studies by Sood and O'Brien (1993) included incubations with added antioxidants or GSH-depletion prior to the addition of chloroacetaldehyde. The greater susceptibility of hepatocytes when GSH was depleted indicates that the toxicity is not likely to be due to the generation of the GSH-chloroacetaldehyde conjugate. Incubations of hepatocytes with chloroacetaldehyde and added antioxidants produced delays in cytotoxicity, indicating that the overall antioxidant status is the best indicator of potential hepatotoxicity in chloroacetaldehyde-exposed liver cells. Thus, GSHLmin (lowest GSH concentration in the liver after EDC exposure) is supported as the most appropriate dose metric for identifying an EDC inhalation concentration that would be expected to produce hepatotoxicity.

Because reproductive and developmental effects were not observed in the toxicity studies of EDC conducted thus far, our evaluation of potential dose measures will be based on evaluation of evidence regarding potential modes of action for structurally related compounds, specifically low-molecular-weight halogenated hydrocarbons, as was done for developmental toxicity of 1,1,2-trichloroethane (The Sapphire Group, 2005). It was concluded that while developmental toxicity related to the parent compound is a plausible mode of action for halogenated

hydrocarbons, for 1,1,2-tricholoroethane, toxicity due to metabolic activation was considered a more likely mode of action for potential developmental effects (The Sapphire Group, 2005). By analogy, we also assume that EDC metabolites, rather than parent compound, were more likely to be reproductive toxicants in the rat, had such effects been identified.

ROUTE TO ROUTE EXTRAPOLATION METHODS

Route-to-route extrapolation of the NOAELs and LOAELs for various key endpoints in the Charlap (2015) EOGRT study were performed using the PBPK model (Sweeney et al., 2008). Minor changes were made to the model code by Sweeney and Parker (2011) to convert it from ACSL 11.8.4 to acslX (Version 2.5.0.6) and to facilitate computation of the dose metrics to be considered in the subchronic route to route extrapolation, interpretation of previously-conducted reproductive and developmental toxicology studies (Alumot et al., 1976; Rao et al., 1980), and design of the extended one-generation reproductive toxicity study (Sweeney and Parker, 2011, Appendix A). Internal dose metrics associated with the NOAELs and LOAELs were determined from PBPK model simulations (Appendix B, Tables B-1, B-2, and B-3) using group-specific body weights averaged over the full study or, in the case of neonates, relevant life stage. Equivalent inhalation exposure concentrations were derived using a body weight of 250 g for male and female rats (with the exception of neonates, assumed to weight 25 g) and assuming 24 hr/day exposure. Additional assumptions regarding the physiology and anatomy for neonates were (i.e., blood flow distribution, tissue volumes, ventilation rate, and cardiac output) were the same as those used by Sweeney et al. (2015) for simulation of PND 22 rats; the values used by Sweeney et al. (2015) were based on a data compilation prepared by Gentry et al. (2006). Inhalation exposure concentrations producing NOAEL- and LOAEL-equivalent internal dose metrics were derived by iteratively simulating various trial concentrations until a trial concentration approximately matching the simulated internal dose from the drinking water study was derived (Appendix B, Table B-5, B-6, and B-7). The level of precision of the equivalent exposure concentrations is two significant figures.

ROUTE TO ROUTE EXTRAPOLATION RESULTS

The consideration of an array of dose metrics yielded ranges of potential NOAEL and LOAEL equivalents for F_0 and F_1 rats, reported in Appendix B for systemic, reproductive, neurotoxic, and neonatal effects. Only those for the reproductive/developmental and neurotoxic endpoints are reported in this section, because those were the endpoints specified for study in the ECA (U.S. EPA, 2003).

For reproductive/developmental effects, the highest tested oral doses were designated NOAELs by Charlap (2015) (155 and 184 mg/kg/d for F_0 and F_1 males, respectively; 182 and 169 mg/kg/d

for F_0 and F_1 females, respectively). The internal dose metric which yielded the lowest equivalent inhalation concentrations was the total amount of EDC metabolized, normalized to body weight. This continuous exposure equivalent 62 ppm for F_0 males, the lowest inhalation equivalent for the 2 generations and sexes.

Neurotoxicity testing in the Charlap (2015) EOGRT study was conducted on an F_1 cohort. The highest doses were identified as NOAELs—184 mg/kg/d for male rats, 169 mg/kg/d for female rats. As with the extrapolation of the reproductive/developmental toxicity testing results, use of amount of EDC metabolized (normalized by body weight), yields the lowest equivalent continuous inhalation concentrations, 76 and 78 ppm for males and females, respectively. More commonly, however, neurotoxic effects of solvents are attributed to peak concentrations of the parent compound. Use of CAmax as the basis of the extrapolation would yield continuous inhalation concentrations of 160 ppm for males, 128 ppm for females.

SENSITIVITY ANALYSIS

The route-to-route extrapolation of the EOGRT of EDC in rats involved the consideration of multiple endpoints and internal dose metrics consistent with supportable modes of action, as computed using a PBPK model (Sweeney et al., 2008).

Reproductive/Developmental Toxicity Extrapolation

The sensitivity of the critical dose metric for reproductive/developmental toxicity, the simulated total amount EDC metabolized (AMETBW), to changes in model parameter values was evaluated for scenarios involving drinking water ingestion or continuous inhalation. In the drinking water scenario, a NOAEL of 155 mg/kg-day to male rats, was used as the basis of the analysis. The continuous inhalation equivalent for male rats, 62 ppm for 24 hrs/day, was used as the basis of the inhalation sensitivity analysis.

The sensitivity analysis was conducted by changing the value of an input parameter and noting the change in the model prediction of interest (AMETBW), normalized to the baseline prediction of that dose metric. The fractional increase in the prediction divided by the fractional increase in the parameter was defined as the normalized sensitivity coefficient (NSC). A negative value indicates that when the value of the input parameter increases, the output decreases in value. A value of 1 indicates a positive linear relationship between the input and output (e.g., a 1% increase in the input parameter produces a 1% increase in the output of interest). The results are summarized in Table 3. For the drinking water scenario, AMETBW was moderately sensitive to the dose, the time over which the dose was assumed to be ingested, and the rate of oxidative metabolism in the liver (VMAXLC). Ingested dose was well-characterized in the study, as

drinking water consumption was measured. The maximum rate of oxidative metabolism in adult rats was well-characterized in the model development stage (Sweeney et al., 2008), where sensitivity analysis indicated high sensitivity of blood EDC concentrations to this optimized parameter. Metabolic rates have not, however, been characterized in lifestages other than adult rats, introducing some uncertainty in internal dosimetry for neonatal rats. Under the inhalation scenario, AMETBW was most sensitive to the exposure concentration and ventilation rate.

Table 3. Sensitivity coefficients for total amount of EDC metabolized (AMETBW): reproductive/developmental toxicity extrapolation

Parameter[a]	**Drinking water dose (155 mg/kg-day)**	**Inhalation exposure (62 ppm)**
Drinking water dose (CDOSEC)	0.48	Not applicable
Dose ingestion duration (TING)	0.38	Not applicable
Inhaled concentration (CONC)	Not applicable	0.91
Alveolar ventilation rate (QPC)	-0.12	0.78
Body weight (BW)	-0.14	-0.27
Blood:air partition coefficient (PB)	|NSC| < 0.1	0.14
Maximum rate of oxidative metabolism of EDC in the liver (VMAXLC)	0.50	0.13
Maximal rate of extrahepatic EDC metabolism, as a fraction of maximum rate of oxidative metabolism of EDC in the liver (XLRATIO)	0.12	|NSC| < 0.1

[a] Parameters not listed had |NSC|<0.1 for both scenarios.

Neurotoxicity Extrapolation

The sensitivity of two relevant dose metrics for neurotoxicity, the more mechanistically realistic metric peak concentration of EDC (CAmax) and the most conservative metric, total EDC metabolism (AMETBW), to changes in model parameter values was evaluated for scenarios involving drinking water ingestion or continuous inhalation. In the drinking water scenario, a NOAEL of 155 mg/kg-day to F_1 male rats, was used as the basis of the analysis. The continuous inhalation equivalents for male rats, continuous exposure to76 ppm for AMETBW and 160 ppm for CAmax, were used as the basis of the inhalation sensitivity analyses.

The sensitivity analyses were conducted as described for reproductive/developmental toxicity. The results are summarized in Tables 4 and 5. For AMETBW, the neurotoxicity sensitivity analyses were very similar to the reproductive/developmental toxicity sensitivity analyses, and occurrence that was not surprising given that the dose metric and NOAELs were very similar. In

contrast, the maximum arterial blood concentration of EDC (CAmax) was sensitive to more parameters than AMETBW, and was sensitive to a much greater degree. It appears that EDC metabolism is saturated in these scenarios, since increases in dose or exposure concentration produce much greater than linear increases in CAmax (NSC >1), and increases in the capacity for oxidative metabolism likewise produce greater than linear decreases in the peak concentration. An increase in the blood:air partition coefficient (PB) also increases CAmax because of a decreased tendency for EDC clearance via exhalation. Under a drinking water scenario, ventilation functions only as a clearance mechanism, so the sensitivity coefficient is negative. Under an inhalation exposure, increase QPC yields a greater than linear increase in CAmax, but not as much of an increase as is produced by a similar increase in inhaled exposure concentration (CONC).

Table 4. Sensitivity coefficients for total amount of EDC metabolized (AMETBW): neurotoxicity extrapolation

Parameter[a]	Drinking water dose (184 mg/kg-day)	Inhalation exposure (76 ppm)
Drinking water dose (CDOSEC)	0.50	Not applicable
Dose ingestion duration (TING)	0.41	Not applicable
Inhaled concentration (CONC)	Not applicable	0.87
Alveolar ventilation rate (QPC)	-0.11	0.73
Body weight (BW)	-0.15	-0.27
Blood:air partition coefficient (PB)	|NSC| < 0.1	0.15
Maximum rate of oxidative metabolism of EDC in the liver (VMAXLC)	0.51	0.18
Maximal rate of extrahepatic EDC metabolism, as a fraction of maximum rate of oxidative metabolism of EDC in the liver (XLRATIO)	0.12	|NSC| < 0.1

[a]Parameters not listed had |NSC|<0.1 for both scenarios.

Table 5. Sensitivity coefficients for peak arterial blood concentration of EDC (CAmax): neurotoxicity extrapolation

Parameter[a]	Drinking water dose (169 mg/kg-day)	Inhalation exposure (128 ppm)
Drinking water dose (CDOSEC)	2.72	Not applicable
Dose ingestion duration (TING)	-2.67	Not applicable
Rate of absorption of EDC from the intestines (KAI)	0.13	Not applicable

Parameter[a]	Drinking water dose (169 mg/kg-day)	Inhalation exposure (128 ppm)
Inhaled concentration (CONC)	Not applicable	2.28
Alveolar ventilation rate (QPC)	-0.75	1.63
Body weight (BW)	0.78	|NSC| < 0.1
Blood:air partition coefficient (PB)	0.77	0.65
Maximum rate of oxidative metabolism of EDC in the liver (VMAXLC)	-1.72	-1.36
Michaelis constant for EDC oxidation (KM)	|NSC| < 0.1	0.11
Maximal rate of extrahepatic EDC metabolism, as a fraction of maximum rate of oxidative metabolism of EDC in the liver (XLRATIO)	-0.41	-0.35
Maximum rate conjugation of EDC with GSH in the liver (VMAXLGC)	-0.27	-0.12
Michaelis constant for EDC for GSH conjugation (KMG)	0.21	0.11

[a]Parameters not listed had |NSC|<0.1 for both scenarios.

CONCLUSIONS

The route-to-route extrapolation of results of the EDC EOGRT study using the PBPK model was based on selection of critical endpoints, an array of internal dose measures, and appropriate points of departure. For reproductive/developmental toxicity, a dose metric related to the most credible mode of action for chemically similar compounds was chosen. For neurotoxic effects, a conservative dose measure and the most plausible metric (on a mechanistic basis) were selected from among the options. The rat reproductive/developmental toxicity NOAEL of 155 mg/kg-day by drinking water was determined to be equivalent to continuous (24 hr/day) exposure of rats to 62 ppm EDC, when using the conservative dose metric of total EDC metabolism normalized to body weight. The male rat neurotoxicity NOAEL of 184 mg/kg-day by drinking water was determined to be equivalent to continuous (24 hr/day) exposure of rats to 76 ppm EDC, when using the conservative dose metric of total EDC metabolism normalized to body weight, and 160 ppm when using peak arterial blood EDC concentration, which is more reflective of a likely EDC neurotoxic mode of action.

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APPENDIX A: PBPK MODEL CODE

edc4.csl

```
PROGRAM:  GLUTATHIONE DEPLETION MODEL FOR EDC (EDC4.CSL)
!Initial parameter values for the rat (DSouza et al. 1987, 1988)
!Retyped, documentation modified by LMS 7/19/01
!revised February 23, 2005
!Add kidney, update tissue volumes and flows per Brown et al., 1997
!Adjust flow and volume calcs to ensure mass balance
!Add chamber for closed chamber simulations, add iv administration
!implement GSH delay per Environ report
!revise GSH parameter calculations to ensure ss in unexposed
!gsh turnover rates from Potter and Tran, tissue specific values
!Input pcs as tissue:air and calculate tissue:blood
!revised February 23, 2006
!add two compartment GI, repeat gavage dosing, GST conj of parent in liver
!turn off lung metab for now
!revised June 2006 extrahepatic metabolism, reformulate reactions
!for oxidative metabolite!
!2010-06-11 edited by Lisa M. Sweeney for acslX
!2010-06-17 added feeding scenario to accommodate simulation of Alumot et al.
1976
!and extended one gen ingestion scenario

INITIAL

!SPECIAL FLOW RATES!
CONSTANT QPC=15 $!alveolar ventilation rate L/hr/kg^0.74!
CONSTANT QCC=15 $!Cardiac output L/hr/kg^0.74!

!FRACTIONAL BLOOD FLOW TO TISSUES!
CONSTANT QLC = 0.18 $!Fractional blood flow to liver!
CONSTANT QFC = 0.07 $!Fractional blood flow to fat!
CONSTANT QSC = 0.34 $!Fractional blood flow to slow!
CONSTANT QKC = 0.14 !Fractional blood flow to kidney
   QRC = 1.0-(QLC + QFC + QSC + QKC)  $!Fractional blood flow to rapid!

!BODY WEIGHT!
CONSTANT BW = 0.22 $!Body weight (kg)!

!FRACTIONAL TISSUE VOLUMES!
CONSTANT VPC = 0.005 $!Fraction lung tissue!
CONSTANT VLC = 0.037 $!Fraction liver tissue!
CONSTANT VFC = 0.07  $!Fraction fat tissue!
CONSTANT VSC = 0.59  $!Fraction slow tissue (skin and muscle)!
CONSTANT VKC = 0.0073 !Fraction kidney
constant vvc = 0.05
VRC = 0.95- (vpc + vlc + vfc + vsc + vkc + vvc) $!Fraction rapid tissue!

!PARTITION COEFFICIENTS!
CONSTANT PPA = 33  $!Lung/air partition coefficient!
CONSTANT PLA = 33  $!Liver/air partition coefficient!
CONSTANT PFA = 340 $!Fat/air partition coefficient!
```

```
CONSTANT PSA = 23  $!Slowly perfused tissue/air partition coefficient!
CONSTANT PRA = 33  $!Richly perfused tissue/air  partition coefficient!
CONSTANT PKA = 33  $!Kidney/air  partition coefficient!
CONSTANT PB = 29   $!Blood/air partition coefficient!
         pp=ppa/pb
         pl=pla/pb
         pf=pfa/pb
         ps=psa/pb
         pr=pra/pb
         pk=pka/pb

CONSTANT MW = 98.96  $!Molecular weight (g/mol)!

!KINETIC CONSTANTS!
CONSTANT VMAXlC = 3.15 !!Maximum velocity of oxidative metabolism (mg/hr-
kg^0.7)!
CONSTANT KM = 0.25     $!Michaelis-Menten constant (mg/L)!

CONSTANT vmaxlgc=480.0 $!enzy conj rate const gsh w parent(mg/(hr-kg^-0.7))!
constant kmg = 2000 !Km with respect to EDC mg/L (not rate limiting at relev
conc?)
constant kmgg = 125 !Km with respect to liver GSH in uM (rate limiting when
gsh depleted)
CONSTANT KGSM =2.0 $!gsh conj rate const w metab (1/(uM-hr))!
CONSTANT KFEE=4600  $!conj rate const w non gsh (1/hr)!
CONSTANT Kdgsyn=0.32  $!GSH synthase breakdown (1/hr)!
CONSTANT Kdgshl=0.14   $!GSH turnover rate--liver!
CONSTANT KDGSHP =0.011  !Gsh turnover rate--lung
CONSTANT KSL = 200   $!Maximum GSH production control (liver)!
CONSTANT KSp = 200   $!Maximum GSH production control (lung)!
CONSTANT Tau = 2    $!Time delay constant (hr)!
CONSTANT GSOl = 5500 $!Initial GSH concentration (uM)!
constant gslmit = 1200 !mitochondrial GSH, does not conjugate or turnover
CONSTANT GSOp = 1200 $!Initial GSH concentration (uM)!
CONSTANT PLRATIO=0.0 $!MFO ratio lung/liver!
constant racpgp=0
CONSTANT xLRATIO=0.0  !MFO ratio extrahepatic/liver
constant kdet = 0.025 !P450 turnover constant (1/hr)
constant kdi = 0.0   !second order reaction rate for liver P450 inactivation
by oxid metab
                     ! 1/((mg-equiv/l)-hr)

!DOSING INFO!
CONSTANT CONC = 0 $!Inhaled concentration (ppm)!
CONSTANT VCHC = 9.1 !Total chamber volume (L)
CONSTANT NRATS = 0. !number of rats in closed chamber
    Vch = vchc-nrats*bw !net chamber volume
CONSTANT KLC = 0.0  !loss rate to chamber
    AI0 = conc*vch*mw/24450.0  !initial mass of chemical in chamber

constant odosec = 0  !gavage dose at t=0 in mg/kg
constant kas=1  !oral absorption rate from stomach
constant kt = 1 !transport rate from stom to intestines
constant kai = 1 ! oral absorption rate from intestines
constant ivdosec = 0. !iv dose at t=0  in mg/kg
constant tinf=0.01 !duration of iv injection (hr)
riv=ivdosec*bw/tinf
```

```
constant FDOSEC =0. ! daily dose in feed (Alumot study) in mg/kg
constant CDOSEC = 0. ! daily dose for continuous ingestion (water or feed)
mg/kg/d
constant TING = 12. ! ingestion duration for continuous ingestion scenario
(hrs)

!TIMING PARAMETERS!
CONSTANT TSTART =0. $!Start of exposure (hrs)!
CONSTANT TPER=24
CONSTANT TSTOP=120
CONSTANT POINTS=1200 $!Number of points in plot!
   CINT = TSTOP/POINTS $!Communication interval!
constant TCHNG=6

!SCALED PARAMETERS!
   QC = QCC*BW**0.74
   QP = QPC*BW**0.74
   QL = QLC*QC
   QF = QFC*QC
   QS = QSC*QC
   QK = QKC*QC
   qr = qc-(ql+qf+qs+qk)

   VP = VPC*BW
   VL = VLC*BW
   VF = VFC*BW
   VS = VSC*BW
   VR = VRC*BW
   vk = vkc*bw
   vv = vvc*bw

!Liver metabolism!
   VMAXl0=VMAXlC*BW**0.7
   vmaxlg = vmaxlgc*bw**0.7
   vmaxp= vmaxl0*plratio
   vmaxx=vmaxl0*xlratio
   AGSOl=GSOl*VL
!Lung metabolism!
   AGSOp=GSOp*VP

   P1=0
   P1R=0
   P2=0
   P2R=0
   P3=100
   P3R=100

KgshprodL0=agsol*kdgshl   !baseline liver gsh synthesis rate umol/hr
    r10=kgshprodl0
    r20=kgshprodl0
    kgshprodtdl0=kgshprodl0
kgshprodp0=agsop*kdgshp   !baseline lung gsh synthesis rate umol/hr
    rp10=kgshprodp0
    rp20=kgshprodp0
    kgshprodtdp0=kgshprodp0
kgsynprodl0=kgshprodl0*kdgsyn !baseline prod gsh synthetase umol/hr/hr
kgsynprodp0=kgshprodp0*kdgsyn !base prdxn of gsh synthetase in lung
```

```
END   $!End of initial!

DYNAMIC

ALGORITHM IALG=2  $!Gear method for stiff systems!

DERIVATIVE
    RAI = nrats*qp*(CA/pb - ci) -klc*ai
     AI = INTEG(RAI, AI0)
     cch= ai/vch
     cp = cch*24450/mw


!CI=Concentration in inhaled air (mg/l)!
      CI=cch*pulse(tstart, tper, tchng)
     RAinh=qp*ci
      Ainh=INTEG(RAINH,0.)

  !Algebraic solution for CA1 after gas exchange!
  !CA1 is arterial blood concentration after gas exchange!
 CA1= (QC*CV +QP*CI)/(QC + QP/PB)
 CX = CA1/PB
 raexh=qp*cx
aexh=integ(raexh,0.)

  !Mass balance for the lung tissue compartment!
   RAP = QC*(CA1-CA) - RAMpu
    AP = INTEG(RAP,0.0)
    Clung = AP/VP
    AUCP=INTEG(CP,0.0)
    CA=Clung/PP
    AUCB=INTEG(CA,0.0)

    !UPTAKE BY ORAL ROUTE!
       RFEEDA =
FDOSEC*BW*(0.528*(PULSE(0.0,24.,1.))+0.272*(PULSE(1.,24.,1.))+0.2*PULSE(16.,24
.,1.)) !Alumot study
       RCDOSE= CDOSEC*BW/TING*(PULSE(0.0, 24.,TING))
       RING=RFEEDA+RCDOSE
       AING = INTEG(RING, 0.)
       RSTOM = -(KAs+kt)*STOM+RING          $!dSTOM/dT!
      days=t/24
       ODOSE =odosec*bw*(1.0 + (int(days)))  !repeat dosing
       STOM =INTEG(RSTOM,0.0) +ODOSE   $!amount in stomach (mg)!
       rintest = kt*stom - kai*intest
      intest = integ(rintest, 0.)

    !AS = Amount in slowly perfused tissues (mg)!
    RAS = QS*(CA-CVS)
     AS = INTEG(RAS,0.0)
    CVS = AS/(VS*PS)
    CS  = AS/VS

    !AR = Amount in richly perfused tissues (mg)!
    RAR = QR*(CA-CVR)
     AR = INTEG(RAR,0.0)
    CVR = AR/(VR*PR)
```

```
    CR  = AR/VR

    !AK = Amount in kidney (mg)!
    RAk = Qk*(CA-CVk)
     Ak = INTEG(RAk,0.0)
    CVk = Ak/(Vk*Pk)
    Ck  = Ak/Vk

    !AF = Amount in fat tissues (mg)!
    RAF = QF*(CA-CVF)
     AF = INTEG(RAF,0.0)
    CVF = AF/(VF*PF)
    CF  = AF/VF

    !CV = Mixed venous blood concentration (mg/l)!

      rinf=riv*pulse(0., 2000., tinf)
      ivdose=integ(rinf,0.)
     RAVx = rinf + (QS*CVS + QR*CVR+ qk*CvK)-(qs+qr+qk)*CVx - ramx



     AV = integ(ravx, 0.)
     cvx=Av/vv


  cv = QFc*CVF + QLc*CVL + cvx*(qsc + qrc + qkc)

      !AL = Amount in liver tissue (mg)!
      RAL = QL*(CA-CVL)-RAMl + KAs*STOM + kai*intest
       AL = INTEG(RAL,0.0)
      CVL = AL/(VL*PL)
       CL = AL/VL
     AUCL = INTEG(CL,0.)

  !LIVER METABOLISM!
    !AM = Amount metabolized liver (mg)!
     RAMl=  RAMox + RAMCon
     RAMox =(VMAXl*CVL)/(KM+CVL)
     ramcon = vmaxlg*(GSl/(gsl+kmgg))*(CVL/(cvl + kmg))
      AMl=INTEG(RAMl,0.)
     AMPl=AMl*1000/MW
    RAMPl=RAML*1000/MW

!time-dependence of hepatic P450 oxidation due to inactivation by oxid
metabolite
    rvmaxl = (vmaxl0-vmaxl)*kdet - vmaxl*kdi*CML
     vmaxl = INTEG(rvmaxl, vmaxl0)

    !CMl=OXIDATIVE METABOLITE LIVER mg/L)!
     RAMMl= ramox -RACMGl*MW/1000-RACMEEl*MW/1000
        AMMl=INTEG(RAMMl,0.)
         CML=AMMl/VL
     AUCML=INTEG(CML,0.)

     !ACPGl = AMT PARENT CONJUGATED WITH GSH LIVER (uMOLES)!
      RACPGl=ramcon*1000/MW
```

```
       ACPGl=INTEG(RACPGl,0.)

     !ACMGl=AMT METABOLITE CONJUGATED WITH GSH LIVER (uMOLES)!
      RACMGl=KGSM*GSl*CMl*1000/MW*VL
       ACMGl=INTEG(RACMGl,0.0)

     !ACMEEl=AMT METABOLITE CONJUGATED WITH EVERYTHING ELSE LIVER!
      !uMOLES!
        RACMEEl=KFEE*VL*CMl*1000/MW
         ACMEEl=INTEG(RACMEEl,0.)

!LUNG METABOLISM!
  !AMp=Amount metabolized lung (mg)!
   RAMpu=RAMpox + rampcon
   rampox=(VMAXp*CA)/(KM+CA)
   rampcon=RACPGp*MW/1000
    AMp=INTEG(rampu,0.)
   AMPp=AMp*1000./MW
  RAMPp=RAMpu*1000./MW

!CMp=OXIDATIVE METABOLITE (mg/L)!
    RAMMp= rampox -RACMGp*MW/1000-RACMEEp*MW/1000
     AMMp=INTEG(RAMMp,0.)
     CMp=AMMp/VP

!ACMGp= AMT METABOLITE CONJUGATED WITH GSH LUNG (uMOLES)!
  RACMGp=KGSM*GSp*CMp*VP*1000/MW
ACMGp=INTEG(RACMGp,0.)

!ACMEEp=AMT METABOLITE CONJUGATED WITH EVERYTHING ELSE LUNG (uMOLES)!
  RACMEEp=KFEE*VP*CMp*1000/MW
   ACMEEp=INTEG(RACMEEp,0.)

!ACPGp=AMT PARENT CONJUGATED WITH GSH LUNG (uMOLES)!
  ! RACPGp=KGS*GSp*Ca*VP*1000/MW
 ACPGPp=INTEG(RACPGp,0.)

!extrahepatic METABOLISM!
   !AMx = Amount metabolized extrahepatic (mg)!
     RAMx=  RAMxox + RAMxCon
     amx=integ(ramx, 0.)
     RAMxox =(VMAXx*CVx)/(KM+CVx)
     amxox = integ(ramxox, 0.)
     ramxcon=0
     amxcon=integ(ramxcon, 0.)

!gsh synthesis rate--time dependent
  kgshprodl=integ(kgsynprodl0*(ksl+gsol)/(ksl+gsl) &
     -kdgsyn*kgshprodl,kgshprodl0)
  kgshprodp=integ(kgsynprodp0*(ksp+gsop)/(ksp+gsp) &
     -kdgsyn*kgshprodp,kgshprodp0)

!time delay (Bishchoff et al. 1971)
      r0=kgshprodl
     rr1=(r0-r1)/tau
      r1=integ(rr1,r10)
     rr2=(r1-r2)/tau
```

```
      r2=integ(rr2,r20)
     rr3=(r2-kgshprodtdl)/tau
   kgshprodtdl=integ(rr3, kgshprodtdl0)

      rp0=kgshprodp
     rrp1=(rp0-rp1)/tau
      rp1=integ(rrp1,rp10)
     rrp2=(rp1-rp2)/tau
      rp2=integ(rrp2,rp20)
     rrp3=(rp2-kgshprodtdp)/tau
   kgshprodtdp=integ(rrp3, kgshprodtdp0)

!GSl = GlUTAHIONE LIVER (uM)!
       RAMGSl=kgshprodtdl-Kdgshl*GSl*VL-RACMGl-RACPGl
        AMGSl=INTEG(RAMGSl,AGSOl)
          GSl=AMGSl/VL
       gshl = gsl + gslmit

!GSp=GLUTAHIONE LUNG (uM)!
    RAMGSp=Kgshprodtdp-Kdgshp*GSp*VP-RACMGp-RACPGp
     AMGSp=INTEG(RAMGSp,AGSOp)
       GSp=AMGSp/VP

!PCTGSH-PERCENT GSH COMPARED TO CONTROL!
  PCTGSHP=GSp/GSOp*100

!mass balance
anet = AING+ odose + ivdose + ainh - aexh
atissue=ap + as+ ar + al+ ak + af +av
agi = stom + intest
amet = aml + amp + amx
AMETBW = AMET/BW !total amount metabolized, normalized to BW
AMXBW = AMX/BW !extrahepatic metabolism normalized to BW
AMLVL = AML/VL !liver metabolism normalized to BW

termt(t.ge.tstop)
END  $!End of derivative!
END  $!End of dynamic!
END  $!End of program
```

Rat.m

```
% rat.m
% 2010-06-11 created from edc4.cmd proced rat by Lisa M. Sweeney
% physiol parameters from Brown et al. 1997
QPC=15; QCC=15;
QLC=0.18;QFC=0.07; QSC=0.34; QKC=0.14;
PB=29;
PLA=33; PPA=10; PFA=340;PRA=33; PSA=23; PKA=33;
VMAXLC=4.0; KM=0.25; PLRATIO=0.0; XLRATIO=0.3;
%PLRATIO WA0.026
VMAXLGC=4.80; KMGG=530; KMG=20.0;
KGSM=2.0;KFEE=4600.0;
KDGSYN=0.324;KDGSHL=0.14; KDGSHP=0.011;
GSOL=5500;GSOP=1200; TAU=0.89;
```

```
KSL=1200; KSP=240;
NRATS=0; KLC=0; CONC=0; IVDOSEC=0; ODOSEC=0;FDOSEC=0.;CDOSEC=0;
VCHC=9.1; KAS=8; KT=2; KAI=0.15; % Default absorption parameters are for corn
oil gavage
TSTART=0; TPER=24; TSTOP=24; TCHNG=24; TINF=0.01;
```

Sdrat.m

```
% sdrat.m
% updated 5/19/2006 by Lisa M. Sweeney
% updated 6/9/2006 LMS
% 2010-06-10 sdrat m file created from edc4.cmd files by Lisa M. Sweeney

%proced sdrat
%fat from schoeffner et al. 1999
%pcs from Dsouza et al., 1987
use rat
VFC=0.065;
PB=28; PLA=30; PSA=22;
BW=0.267; %default weight for a male SD rat in a subchronic study (EPA, 1988)
```

APPENDIX B

PBPK-Derived Internal Dosimetry and Route-to-Route Extrapolations for EDC-Exposed Rats

Values of the dose metrics of arterial blood EDC (peak and average concentrations), liver GSH concentration and daily metabolism occurring the liver (normalized to liver weight or body weight) were predicted using the PBPK models. Simulations were conducted at the relevant NOAELs and LOAELs for both sexes, using study specific body weights. The outputs are provided for F_0 rats (Table B-1), F_1 rats from birth to PND 123 (Table B-2), and F_1 rats from birth to PND 21 (Table B-3). The values of these same metrics in rats exposed to an array of inhaled concentrations of EDC (0-1000 ppm) 24 hrs/day were computed for rats of standard weight for a subchronic study (U.S. EPA, 1988) by Sweeney and Parker (2010); those values are reproduced here for the convenience of the reader.

Table B-1. Internal dose metrics corresponding to the NOAELs and LOAELs for F_0 rats in the Charlap (2015) rat EOGRT study, as computed using a PBPK model (Sweeney et al., 2008; Sweeney and Parker, 2011)

	F_0 male systemic toxicity		F_0 female systemic toxicity		F_0 male reproductive toxicity	F_0 female reproductive toxicity
Dose metric	NOAEL 31 mg/kg/d (554 g)	LOAEL 79 mg/kg/d (524 g)	NOAEL 40 mg/kg/d (306 g)	LOAEL 95 mg/kg/d (295 g)	NOAEL[a] 155 mg/kg/d (500 g)	NOAEL[a] 182 mg/kg/d (288 g)
Total amount of EDC metabolized daily, normalized by body weight (AMETBW) (mg/kg body weight/day)	30.3	72.6	39.1	87.2	109	128
Amount of EDC metabolized in liver each day, normalized by liver weight (AMLVL) (mg/kg liver/day)	726	1590	934	1907	2396	2802
Maximum concentration of EDC in arterial blood (CAmax) (mg/L)	0.0870	1.02	0.0994	1.09	7.27	7.40
Time-weighted average EDC in arterial blood (CAavg) (mg EDC/L blood)	0.0415	0.409	0.0470	0.434	2.95	3.04
Minimum concentration of glutathione in the liver (GSHLmin) (μM)	5089	3723	4713	3348	2805	2473

[a] Highest tested dose

Table B-2. Internal dose metrics corresponding to the NOAELs and LOAELs for F_1 rats (birth to PND 123) in the Charlap (2015) rat EOGRT study, as computed using a PBPK model (Sweeney et al., 2008; Sweeney and Parker, 2011)

	F_1 male systemic toxicity		F_1 female systemic toxicity		F_1 male reproductive toxicity and neurotoxicity	F_1 female reproductive toxicity and neurotoxicity
Dose metric	NOAEL 37 mg/kg/d (270 g)	LOAEL 97 mg/kg/d (261 g)	NOAEL 93 mg/kg/d (173 g)	LOAEL 169 mg/kg/d (162 g)	NOAEL[a] 184 mg/kg/d (240 g)	NOAEL[a] 169 mg/kg/d (162 g)
AMETBW[b]	36.2	89.3	88.2	134	132	134
AMLVL	895	1957	1965	2913	2889	2913
CAmax	0.0809	1.03	0.554	4.20	6.79	4.20
CAavg	0.0387	0.411	0.231	1.70	2.79	1.70
GSHLmin	4779	3297	3303	2434	2417	3303

[a] Highest tested dose

[b] For definitions and units, see Table B-1.

Table B-3. Internal dose metrics corresponding to the NOAELs and LOAELs for neonatal F_1 rats (birth to PND 21), 25 g, in the Charlap (2015) rat EOGRT study, as computed using a PBPK model (Sweeney et al., 2008; Sweeney and Parker, 2011)

	F_1 male neonatal toxicity		F_1 female neonatal toxicity	
Dose metric	NOAEL 97 mg/kg/d (27.4 g)	LOAEL 184 mg/kg/d (24.9 g)	NOAEL 93 mg/kg/d (26.0 g)	LOAEL 169 mg/kg/d (23.8 g)
AMETBW[a]	94.5	171	90.7	160
AMLVL	2304	3882	2223	3667
CAmax	0.1386	0.804	0.124	0.5594
CAavg	0.0646	0.333	0.0578	0.237
GSHLmin	3011	2121	3081	2211

[a]For definitions and units, see Table B-1.

Table B-4. Internal dose metrics corresponding to 24-hr exposure of adult rats to a range of inhalation concentrations (ppm), as computed using a PBPK model (Sweeney et al., 2008); reproduced from Sweeney and Parker (2010)

	Males				
Concentration	0	1	10	100	1000
AMETBW[a]	0	1.88	18.6	162	288
AMLVL	0	20.6	225	3323	6619
CAmax	0	0.00965	0.106	2.42	97.4
CAavg	0	0.00965	0.106	2.41	97.5
GSHLmin	6700	6673	6403	3491	1721

	Females				
Concentration	0	1	10	100	1000
AMETBW[a]	0	2.02	20.0	174	308
AMLVL	0	22.0	240	3572	7113
CAmax	0	0.00960	0.106	2.39	97.5
CAavg	0	0.00960	0.106	2.38	97.3
GSHLmin	6700	6671	6383	3339	1660

[a]For definitions and units, see Table B-1.

Table B-5. Inhaled concentrations (ppm) for 24-hr/day exposures to EDC that yield simulated internal doses (Sweeney et al., 2008; Sweeney and Parker, 2011) equivalent to NOAEL and LOAEL doses from the EOGRT study (Charlap, 2015) in 250 g F_0 rats

	F_0 male systemic toxicity		F_0 female systemic toxicity		F_0 male reproductive toxicity	F_0 female reproductive toxicity
Dose metric	NOAEL 31 mg/kg/d	LOAEL 79 mg/kg/d	NOAEL 40 mg/kg/d	LOAEL 95 mg/kg/d	NOAEL[a] 155 mg/kg/d	NOAEL[a] 182 mg/kg/d
AMETBW[a]	16	40	21	48	62	74
AMLVL	26	50	32	58	70	80
CAmax	8.4	60	9.4	63	160	166
CAavg	4.2	31	4.7	32	110	111
GSHLmin	44	85	54	102	142	184

[a]For definitions and units, see Table B-1.

Table B-6. Inhaled concentrations (ppm) for 24-hr/day exposures to EDC that yield simulated internal doses (Sweeney et al., 2008; Sweeney and Parker, 2011) equivalent to NOAEL and LOAEL doses from the EOGRT study (Charlap, 2015) in 250 g F_1 rats (birth to PND 123)

	F_1 male systemic toxicity		F_1 female systemic toxicity		F_1 male reproductive toxicity and neurotoxicity	F_1 female reproductive toxicity and neurotoxicity
Dose metric	NOAEL 37 mg/kg/d	LOAEL 97 mg/kg/d	NOAEL 93 mg/kg/d	LOAEL 169 mg/kg/d	NOAEL[a] 184 mg/kg/d	NOAEL[a] 169 mg/kg/d
AMETBW[b]	19	50	49	78	76	78
AMLVL	31	59	59	83	83	83
CAmax	7.8	61	39	128	160	128
CAavg	3.9	31	20	83	107	83
GSHLmin	52	105	105	190	193	190

[a] Highest tested dose

[b] For definitions and units, see Table B-1.

Table B-7. Inhaled concentrations (ppm) for 24-hr/day exposures to EDC that yield simulated internal doses (Sweeney et al., 2008; Sweeney and Parker, 2011) equivalent to NOAEL and LOAEL doses from the EOGRT study (Charlap, 2015) in neonatal F_1 rats (birth to PND 21)

	F_1 male neonatal toxicity		F_1 female neonatal toxicity	
Dose metric	NOAEL 97 mg/kg/d (27.4 g)	LOAEL 184 mg/kg/d (24.9 g)	NOAEL 93 mg/kg/d (26.0 g)	LOAEL 169 mg/kg/d (23.8 g)
AMETBW[a]	25	47	24	43
AMLVL	38	57	36	55
CAmax	12	49	11	37
CAavg	5.9	25	5.3	19
GSHLmin	63	101	61	95

[a]For definitions and units, see Table B-1.

The values of the "equivalent" inhaled concentrations were determined for all generations, endpoints, and life stages, and are reported in Tables B-5, B-6, and B-7. The "equivalent" values were highly dependent on the choice of internal dose metric. For example, the inhalation equivalent of 31 mg/kg-day via drinking water ingestion by male rats ranged from 4.2 ppm, based on the average concentration of EDC in arterial blood to 44 ppm, based on the minimum level of GSH in the liver. The endpoints of primary interest for this study, reproductive toxicity and neurotoxicity, had higher NOAELs that the example previously noted. At these higher NOAELs, the ranges of equivalent values span ~3-fold, rather than the >10-fold range identified at lower ingested doses. The most conservative doses metrics also shift from average arterial blood concentration (at lower doses) to amount metabolized in the liver (normalized to body weight) at higher doses.