INTERIM: 09/2009

ACUTE EXPOSURE GUIDELINE LEVELS (AEGLs)

FOR

ETHYLBENZENE

(CAS Reg. No. 100-41-4)

C_8H_{10}

INTERIM

PREFACE

Under the authority of the Federal Advisory Committee Act (FACA) P. L. 92-463 of 1972, the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC/AEGL Committee) has been established to identify, review and interpret relevant toxicologic and other scientific data and develop AEGLs for high priority, acutely toxic chemicals.

AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Three levels — AEGL-1, AEGL-2 and AEGL-3 — are developed for each of five exposure periods (10 and 30 minutes, 1 hour, 4 hours, and 8 hours) and are distinguished by varying degrees of severity of toxic effects. The three AEGLs are defined as follows:

AEGL-1 is the airborne concentration (expressed as parts per million or milligrams per cubic meter [ppm or mg/m^3]) of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, non-sensory effects. However, the effects are not disabling and are transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration (expressed as ppm or mg/m^3) of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death.

Airborne concentrations below the AEGL-1 represent exposure levels that could produce mild and progressively increasing but transient and nondisabling odor, taste, and sensory irritation or certain asymptomatic, non-sensory effects. With increasing airborne concentrations above each AEGL, there is a progressive increase in the likelihood of occurrence and the severity of effects described for each corresponding AEGL. Although the AEGL values represent threshold levels for the general public, including susceptible subpopulations, such as infants, children, the elderly, persons with asthma, and those with other illnesses, it is recognized that individuals, subject to unique or idiosyncratic responses, could experience the effects described at concentrations below the corresponding AEGL.

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SUMMARY

Ethylbenzene is a flammable liquid that is insoluble in water and miscible with most organic solvents (O'Neil et al. 2001). The chemical is used mainly in the production of styrene with other uses less than 1% of the total ethylbenzene produced (ECETOC 1986). In 2001, world demand for ethylbenzene was about 23 million metric tons. Use of the chemical is projected to increase at an annual rate of 4.6% from 2001-2006 (Ring and Linak 2002). The most common manufacturing process is by reaction of benzene and ethylene with the ethylbenzene "mostly" produced and used at the same site (ECETOC 1986). Ethylbenzene is often present in mixed xylenes with the technical xylene product containing approximately 40% m-xylene and approximately 20% each of o-, and p-xylene and ethylbenzene (Fishbein, 1988).

Experimental data on the effects of ethylbenzene on humans showed irritation at high concentrations for short durations but possible CNS effects with lower concentrations for longer durations. Limited data suggest that the young animal is the most susceptible to effects of ethylbenzene and that this susceptibility is dependent on the body weight of the animal. Signs of irritation were observed in laboratory animals at concentrations >1000 ppm. Narcosis developed at ≥2000 ppm. The cochlear ducts in the inner ear may be a target organ following repeated exposure, but no data were found which indicate ototoxicity after a single exposure to ethylbenzene. Decreased body weight gain occurred in animals exposed repeatedly.

Experimental data on the effects of ethylbenzene on humans were available for the derivation of AEGL-1 values. No problems were reported by nine individuals exposed to 100 ppm for 8 hours. However, during exposure of eleven individuals to 180 ppm for 8 hours, some complained of irritation of the upper respiratory tract and eye and headache and sleepiness towards the end of the exposure; transient feelings of drunkenness were also reported (Bardodej and Bardodejova 1961). Motor activity in rats increased following exposures to 400-1500 ppm for four hours then decreased – perhaps indicative of CNS depression – at higher concentrations (Molnár et al. 1986). A number of experimental studies in adult animals indicate that clinical signs and systemic effects are not observed at concentrations less than 1000 ppm following single or repeated exposures. These concentrations are much greater than those causing effects in humans. Therefore, a concentration of 100 ppm for 8 hours was chosen as the point of departure for derivation of AEGL-1 values. This is the highest concentration in humans which did not produce clinical signs after a single exposure. A total uncertainty factor of 3 was used which includes 3 for intraspecies extrapolation because the point of departure was a no effect level for irritation and is below that which would cause CNS effects. An intraspecies UF of 3 is appropriate because direct acting irritant effects at the portal of entry are not expected to vary between individuals. The same UF is appropriate for mild CNS effects (see rationale below). Because the point of departure is below that causing systemic effects, time scaling was not performed.

The AEGL-2 is based upon the highest non-narcotic level in rats. Motor activity was monitored in male CFY rats during a 4-hour exposure to 400-2180 ppm ethylbenzene (Molnár et al. 1986). Exposure resulted in a biphasic response with increased activity between 400-1500 ppm followed by a decrease in activity at higher concentrations. A concentration of 2180 ppm was listed as the minimum narcotic concentration with 1500 ppm the highest non-narcotic

concentration. It is assumed that the central nervous system response observed following ethylbenzene exposure is directly related to the concentration of parent material reaching the brain, and that venous blood concentrations correlate with brain concentrations. Therefore, the venous blood concentration (Cv) of ethylbenzene following a 4-hour exposure to 1500 ppm would be expected to provide an internal dose measurement correlating with the no effect for a narcotic response. Using a physiologically-based pharmacokinetic (PBPK) model, the internal dose (Cv) producing the highest non-narcotic condition in rats was determined. Then, the human PBPK model was run for each defined AEGL time point to determine the equivalent exposure concentration producing the target Cv. It is acknowledged that the resulting AEGL 2 values may not be protective of ototoxicity which occurs after repeated exposures, however no data are available to assess this endpoint following a single exposure to ethylbenzene.

Human exposure data relevant to derivation of AEGL-3 values were not available. The most appropriate animal data relevant to derivation of AEGL-3 values are those of Andersson et al. (1981). The highest non-lethal exposure of adult rats to 2000 ppm, 6 hours/day for 3 days was used as the basis for deriving the 10-min, 30-min, 1-hour, 4-hour, and 8-hour AEGL-3 values. As for the AEGL-2, it is assumed that the central nervous system effects observed following ethylbenzene exposure are directly related to the concentration of parent material reaching the brain. Therefore, PBPK modeling was again used to calculate the internal dose (Cv) correlating with an exposure to 2000 ppm for 6 hours which was the highest non-lethal concentration. The human PBPK model was then run for each defined AEGL time point to determine the equivalent exposure concentration producing the target Cv.

A total uncertainty factor of 3 was applied to the AEGL-2 and -3 dose metrics. An interspecies uncertainty factor of 1 was applied because PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1 and the pharmacodynamic component is also reduced to 1 because it appears similar exposure effects (central nervous system effects) occur in humans and animals. An intraspecies uncertainty factor of 3 was applied because the mode of action of ethylbenzene is similar to anaesthetic chemicals. The minimum alveolar concentration (MAC - produces a lack of motor response in 50% of individuals exposed to that concentration) for different age groups from newborns to the elderly and pregnant women has been studied for a number of anaesthetic gases. It varies from 2-3 fold (NRC 2001).

The calculated values are listed in the table below.

Summary of AEGL Values for Ethylbenzene						
Classification	**10-minute**	**30-minute**	**1-hour**	**4-hour**	**8-hour**	**Endpoint (Reference)**
AEGL−1 (Nondisabling)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	Highest no effect level in humans (Bardodej and Bardodejova 1961)
AEGL−2 (Disabling)	2900 ppm (13,000 mg/m^3)	1600 ppm (7000 mg/m^3)	1100 ppm (4800 mg/m^3)	660 ppm (2900 mg/m^3)	580 ppm (2500 mg/m^3)	No effect level for narcosis in rats (Molnár et al. 1986)
AEGL−3 (Lethal)	4700 ppm (20,400 mg/m^3)	2600 ppm (11,000 mg/m^3)	1800 ppm (7800 mg/m^3)	1000 ppm (4400 mg/m^3)	910 ppm (4000 mg/m^3)	Highest non-lethality in rats (Andersson et al. 1981)

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1. INTRODUCTION

Ethylbenzene is a flammable liquid that is insoluble in water and miscible with most organic solvents (O'Neil et al. 2001). The principle hazards associated with ethylbenzene release are fire and explosion. The pure chemical is used mainly in the production of styrene with other uses less than 1% of the total ethylbenzene produced (ECETOC 1986, ATSDR 1999); these other uses include as a solvent, as a constituent of asphalt and of naphtha, and in fuels (ATSDR 1999). In 2001, world demand for ethylbenzene was about 23 million metric tons. Use of the chemical is projected to increase at an annual rate of 4.6% from 2001-2006 (Ring and Linak 2002). The most common manufacturing process is by reaction of benzene and ethylene with the ethylbenzene "mostly" produced and used at the same site (ECETOC 1986). Ethylbenzene is often present in mixed xylenes with the technical xylene product containing approximately 40% m-xylene and approximately 20% each of o-, and p-xylene and ethylbenzene (Fishbein, 1988).

Selected chemical and physical properties of ethylbenzene are listed in Table 1.

TABLE 1: Chemical and Physical Properties of Ethylbenzene

Parameter	Value	Reference
Synonyms	phenyl ethane	ECETOC 1986
Chemical formula	C_8H_{10}	O'Neil et al. 2001
Molecular weight	106.16	O'Neil et al. 2001
CAS Reg. No.	100-41-4	
Physical state	liquid	O'Neil et al. 2001
Solubility in water	practically insoluble	O'Neil et al. 2001
Vapor pressure	9.31 mmHg at 20°C	ECETOC 1986
Vapor density (air =1)	3.7	ECETOC 1986
Liquid density (water =1)	0.866	O'Neil et al. 2001
Melting point	-95.01°C	O'Neil et al. 2001
Boiling point	136.25°C	O'Neil et al. 2001
Auto-ignition	432.0°C	ATSDR 1999
Flammability limits (% in air)	0.99-6.70	ECETOC 1986
Lower Explosive Limit	0.8%	NIOSH 1996, ATSDR 1999
Conversion factors	1 ppm = 4.35 mg/m^3 1 mg/m^3 = 0.233 ppm	ECETOC 1986

2. HUMAN TOXICITY DATA

2.1. Acute Lethality

No reports of human fatalities from ethylbenzene exposure were found.

2.2. Nonlethal Toxicity

2.2.1. Odor Threshold/Odor Awareness

AIHA (1995) listed an odor detection of 0.092 ppm from an unreviewed source and a range of reported odor thresholds as 0.46-0.60 ppm; however, all values were rejected by AIHA after a critique of the data.

The thresholds for eye irritation and odor were compared for a series of alcohols, ketones, and alkylbenzenes (Cometto-Muñiz and Cain 1995). For ethylbenzene, the ratio for eye irritation threshold/odor threshold was approximately 1133; threshold data were presented graphically and the odor threshold appeared to be slightly less than 10 ppm.

2.2.2. Case Reports

Bone marrow toxicity and pancytopenia were reported in a 47-year old male following topical and subcutaneous contact with a solution of lead chromate, xylene, and ethylbenzene (Erickson et al. 1994). The patient sustained a severe degloving injury (avulsion of the skin and subcutaneous layer with disruption of the underlaying deep fascia and muscle layers) to the lower left leg; at the time of the injury a large volume of xylene/ethylbenzene solvent and paint flooded the wound. Pancytopenia developed within 48 hours and was progressive over the next several weeks; the patient was septic and died on the 57^{th} day after the accident.

2.2.3. Epidemiologic Studies/Occupational Exposures

Concentrations of ethylbenzene at four job sites in a styrene production plant were 0.08-0.53 ppm as measured by area sampling of the workplaces (Holz et al. 1995). Samples were collected in charcoal tubes every 10 minutes over a period of one hour and quantitated with a gas chromatograph. Personal monitoring by passive sampling over the entire eight hour workshift, showed that workers were actually exposed to 3.42 ppm of ethylbenzene. At the end of the workshift, ethylbenzene was measured in the expired air of workers (0.022 ± 0.018 ppm) and metabolites were measured in the urine. No information on the health status of the workers (age range 20-58 years) was given.

Health status was monitored for 20 years in approximately 200 workers (mean age 36.6 years) at an ethylbenzene production facility (Bardodêj and Círek 1988). Exposure was assessed as mandelic acid and mercapturate excretion in urine; air concentrations were not measured. Average mandelic acid concentrations were 0.2-0.3 mmol/L with postshift mercapturate levels 2.3x preshift levels. None of the exposed workers showed any adverse effects on hematology or liver function tests and no increased incidence in any tumor type was found.

2.2.4. Clinical Studies

Thirty-five male workers involved in spraying vehicles with varnishes dissolved in mixed xylenes and ethylbenzene were examined for hematopoetic changes (Angerer and Wulf 1985). The age of the workers was 24-52 years and the average length of employment was 8.2 years. Overall average concentrations of the solvents, monitored by personal air samplers during the work shift, were 2.1-7.9 ppm for the xylenes and 4.0 ppm for ethylbenzene. Solvent concentration in blood and metabolite concentrations in urine were directly correlated with exposure levels. Compared to age- and sex-matched unexposed controls, the workers had slightly increased numbers of lymphocytes and decreased numbers of segmented granulocytes; RBC counts and hemoglobin levels were at the lower range of normal. Similar results were found with repeated sampling four and nine months after the initial blood cell counts. No adverse health effects or other confounding factors were found to correlate with the changes in blood cell counts.

2.2.5. Experimental Studies

Six men were exposed to various concentrations of ethylbenzene; details of the chemical purity, exposure chamber and subjects' health status were not given (Yant et al. 1930). Concentration in the chamber atmosphere was determined by calculation of the quantity of material used during the study. A concentration of 1000 ppm caused eye irritation with profuse lacrimation that decreased with continued exposure to the point of being hardly noticed after a minute or two. At 2000 ppm the eye irritation was almost intolerable on first entering the chamber and was accompanied by throat irritation and a feeling of constriction of the chest; however, symptoms decreased with continued exposure. One individual remained in the chamber at 2000 ppm for five minutes and noted that irritation gradually disappeared but vertigo developed. The concentration of 5000 ppm was intolerable.

The results of the previous study support the statement by Thienes and Haley (1972) that 1000 ppm ethylbenzene is momentarily irritating and 2000 ppm was intolerably irritating to the eyes, nose, and throat. A primary reference was not given.

No problems were reported by nine individuals exposed to 100 ppm for 8 hours. However, during exposure of eleven individuals to 180 ppm for 8 hours, some complained of irritation of the upper respiratory tract and eye and headache and sleepiness towards the end of the exposure; transient feelings of drunkenness were also reported (Bardodej and Bardodejova 1961). Exposures were interrupted in the middle for a one-hour lunch break outside the chamber. Atmospheres were monitored spectrophotometrically. No additional experimental details were given.

In pharmacokinetic studies with ethylbenzene, no adverse effects were reported in volunteers exposed to up to 46 ppm for 8 hours (Gromiec and Piotrowski 1984), up to 85-100 ppm for 8 hours (Bardodej and Bardodejova 1961, 1970), or to 150 ppm for 4 hours (Engström et al. 1984).

Gamberale et al. (1978) conducted two series of experiments assessing the effects of xylene exposure in healthy male volunteers age 21 to 33 years old. The xylene mixture contained

20.7% ethylbenzene. In the first experiment, groups of 5 males were exposed to 0, 100, or 300 ppm xylene for 70 minutes on day 1, 2, or 3, with the sequence of the exposure balanced among the 3 groups (i.e., on day 1, groups 1, 2, and 3 were exposed to 0, 300, or 100 ppm xylene, respectively). In the second experiment, a group of 8 volunteers (who had also participated in the first series) was exposed to 300 ppm xylene for 70 minutes; the volunteers exercised on a bicycle ergometer (100 W) the first 30 minutes of the exposure, and sat in a chair the last 40 minutes of the exposure. In both experiments, a breathing valve with low resistance was used to supply the air or xylene, and menthol crystals were placed in the tube of the mouthpiece to mask the odor of solvent. A total hydrocarbon analyzer was used to continuously measure the inspired xylene concentration during exposure, and a gas chromatographic technique was used to measure the alveolar air concentration of xylene. Heart rate was checked regularly. Five performance tests were administered to volunteers during exposure: one administered at the beginning of the exposure period and all five during the last 35 minutes of exposure. The performance tests included: critical flicker fusion, reaction time addition, simple reaction time, short term memory, and choice reaction time. All of the tests utilized visual stimulation with electronic recording of responses. Lastly, after each exposure trial, subjects were requested to fill out a questionnaire addressing subjective symptoms observed by the subjects during exposures.

No exposure-related changes in heart rate were observed. Although a slight increase in the frequency of headache, sickness, and intoxication were noted, the number of subjects affected was not provided. However, the authors stated that most of the subjects reported no or only negligible subjective symptoms. Xylene exposure at rest did not significantly affect the results of the performance tests of subjects exposed to 100 or 300 ppm xylene. When xylene exposure was combined with 100W of work, impaired performance was observed on all tests, with statistical significance ($p<0.05$) attained in the reaction time addition test and the short term memory test.

No skin sensitization was produced in 25 volunteers following application of 10% ethylbenzene (Fishbein 1985).

2.3. Neurotoxicity

In the study by Yant et al. (1930), vertigo was reported after exposure to 2000 ppm for about 5 minutes. Gamberale et al. (1978) reported significant reductions in the reaction time addition test and the short term memory test following exposure to 100 and 300 ppm of a xylene mixture containing 20.7% ethylbenzene.

2.4. Developmental/Reproductive Toxicity

No information was found regarding the reproductive or developmental toxicity of ethylbenzene in humans.

2.5. Genotoxicity

Genotoxic effects were measured in workers exposed to aromatic hydrocarbons at a styrene plant (Holz et al. 1995). Ethylbenzene concentrations in the workplace air ranged from 0.08 ppm to 0.53 ppm; styrene, benzene, toluene, and xylenes were also measured. For the exposed workers, no increases in DNA adducts, DNA single strand breaks, sister chromatid exchange, or the frequency of total micronuclei were found in peripheral lymphocytes.

2.6. Carcinogenicity

IARC (2000) lists ethylbenzene as *possibly carcinogenic to humans* based on inadequate evidence in humans but sufficient evidence in experimental animals. US EPA (2004) lists ethylbenzene as not classifiable as to human carcinogenicity due to lack of animal bioassays and human studies. It should be noted that the US EPA assessment has not been revised since long-term studies in rats and mice were completed by NTP (1999).

2.7. Summary

Very little information is available concerning human exposure to ethylbenzene despite the large quantities of the chemical that are produced each year. However, the mainly industrial use of the chemical as an intermediate limits potential exposure to the general population. No deaths have been reported from exposure to the ethylbenzene. Concentrations ≥1000 ppm are irritating to the eyes and mucous membranes on initial exposure. Although symptoms may diminish after several minutes of exposure, sufficiently high ethylbenzene exposures can elicit vertigo. Exposure for several hours to 180 ppm caused narcosis.

3. ANIMAL TOXICITY DATA

3.1. Acute Lethality

3.1.1. Guinea pigs

Female albino guinea pigs (n = 8) were exposed in whole-body chambers to 0 or 2500 ppm of ethylbenzene (purity 99%) for 5 days; exposures were for 8 hours on day 1 and 6 hours on days 2-5 (Cappaert et al. 2002). Chamber atmospheres were generated by injecting saturated gas into the air supply and were monitored by a dual gas monitor. At the end of the first 8-hour exposure, two animals were motionless and did not respond to painful stimuli while the others were lethargic. One of the motionless animals died. Subsequent exposures were reduced to 6 hours; no adverse clinical signs were observed and all animals survived. Body weight was not affected by exposure over the 5 days. The remaining examinations focused on the potential ototoxicity of ethylbenzene. Four to eight weeks after the last exposure, animals were anesthetized and the right and left cochlea were exposed. Auditory evoked responses to a series of stimuli were recorded at the apex of each cochlea. After electrocochleography, each cochlea was fixed and processed for histological examination. No threshold shift was measured and no loss of outer hair cells was observed.

Guinea pigs (strain and sex not specified; n = 6) were exposed whole-body in flow through chambers to nominal concentrations of ethylbenzene of 1000, 2000, 5000, or 10,000 ppm for up to 480 minutes (Yant et al. 1930). The test article (purity not given) was evaporated from gauze placed in the chamber and mixed by a fan. Concentration in the chamber atmosphere was determined by calculation of the quantity of material used during the study. The concentration of 10,000 ppm resulted in death of two animals after approximately 2 hours of exposure. Clinical signs of irritation were observed at 1000 ppm after 3-8 minutes, but these disappeared after 30 minutes and no further adverse effects of exposure were seen at this concentration. At 2000, 5000 and 10,000 ppm signs of immediate irritation included squinting of the eyes, lacrimation, and rubbing and scratching at the nose the with severity increased with concentration. Unsteadiness and ataxia were observed after 390 and 480 minutes, respectively, at 2000 ppm, after 26-30 minutes at 5000 ppm, and after only 4-10 minutes at 10,000 ppm. The two highest concentrations also caused tremors, unconsciousness, and abnormal respiration. Gross pathology findings in animals that died included cerebral congestion, congestion and edema of the lungs, and congestion throughout the abdominal viscera. The surviving animals were killed immediately after exposure or 4-8 days later. Necropsy findings in survivors were similar to those of decedents, but the severity was less and most lesions were no longer evident by 8 days post-exposure (Yant et al. 1930).

3.1.2. Rats

Groups of male Fischer 344 rats (n = 5) were exposed in whole body inhalation chambers to 0, 400, 1200, or 2400 ppm of ethylbenzene (purity 99.7%) for 6 hours/day for 4 days (Bio/dynamics Inc. 1986). Atmospheres were generated by feeding the test material directly into an air atomizing nozzle and delivering air through a pressure gauge to generate a vapor. Chamber concentrations were monitored using a Miran® 1A Ambient Air analyzer and a strip chart recorder. Mean analytical concentrations were within 3% of target. All animals exposed to 2400 ppm died, one on day 1 and two each on days 2 and 3. All remaining animals survived the 4-day exposure. Clinical signs at 2400 ppm included lacrimation, shallow breathing, prostration, and yellow anogenital staining. At 1200 ppm, lacrimation was observed on two animals after the first day of exposure and on three animals after the second day of exposure. Lacrimation was also observed on 5/5 animals in the 400 and 1200 ppm groups after days 3 and 4 and on 4/5 controls after day 4. Body weight gain by the 1200-ppm group was 53% of the controls during the 4-day period. At termination, absolute liver weight was significantly increased ($p \leq 0.01$) in the low- and mid-concentration groups compared with that of the control group. No treatment-related gross or microscopic lesions were observed in animals surviving to scheduled termination. In the 2400-ppm animals, congestion of visceral organs was considered normal for animals not exsanguinated prior to examination.

An older report noted lethality in rats exposed to various concentrations of ethylbenzene for 4 hours (Mellon Institute 1949) but no information was included on strain of animals or testing methods. Mortality was 6/6 at 8000 ppm, 3/6 at 4000 ppm, and 0/6 at 2000 ppm. Exposure to saturated vapor resulted in death of 0/6 after 1 hour, 2/6 after 2 hours, and 6/6 after 4 hours. These data were likely the basis for a 4-hour LC_{50} of 4000 ppm reported by Smyth et al. (1962).

Ivanov (1962) reported mortality in rats exposed to 6897-17,241 ppm for 2 hours and, from these data, calculated an LC_{50} of 13,343 ppm. However, these were nominal, not analytical, concentrations, and details of test atmosphere generation and exposure apparatus were not given.

3.1.3. Mice

Groups of male B6C3F$_1$ mice (n = 5) were exposed in whole body inhalation chambers to 0, 400, 1200, or 2400 ppm of ethylbenzene (purity 99.7%) for 6 hours/day for 4 days (Bio/dynamics Inc. 1986). Atmospheres were generated by feeding the test material directly into an air atomizing nozzle and delivering air through a pressure gauge to generate a vapor. Chamber concentrations were monitored using a Miran® 1A Ambient Air analyzer and a strip chart recorder. Mean analytical concentrations were within 3% of target. All animals exposed to 2400 ppm died on day 2 and four animals exposed to 1200 ppm died on day 3. All remaining animals survived the 4-day exposure. Clinical signs in the 1200- and 2400-ppm animals included lacrimation, shallow breathing, prostration, and eyes closed. At 400 ppm, lacrimation was observed on all animals after the third day of exposure and on four animals after the last day of exposure. Body weight gain by the 400-ppm group and by the surviving 1200-ppm animal was similar to that of the controls during the 4-day period. No treatment-related organ weight changes or gross or microscopic lesions were observed in animals surviving to scheduled termination. In the 1200- and 2400-ppm animals that died, congestion of visceral organs was considered normal for animals not exsanguinated prior to examination.

Gerarde (1960) listed prostration in mice exposed to 3500 ppm and death at 10,382-10,400 ppm for 2 hours. Further details were not given.

3.2. Nonlethal Toxicity

3.2.1. Rabbits

Groups of male New Zealand white rabbits (n = 4) were exposed in whole body inhalation chambers to 0, 400, 1200, or 2400 ppm of ethylbenzene (purity 99.7%) for 6 hours/day for 4 days (Bio/dynamics Inc. 1986). Atmospheres were generated by feeding the test material directly into an air atomizing nozzle and delivering air through a pressure gauge to generate a vapor. Chamber concentrations were monitored using a Miran® 1A Ambient Air analyzer and a strip chart recorder. Mean analytical concentrations were within 3% of target. All animals survived the four-day exposure. Lacrimation was also observed on 2/4 high-concentration animals on day 1, on one animal in each of the mid- and high-concentration groups after day 2, and on 2-3 animals of all exposed groups and one control animal on day 3. A concentration-related decrease in body weight gain by all exposed groups was seen during the 4-day period (68, 65, and 6%, respectively, of control level). No treatment-related organ weight differences or gross or microscopic lesions were observed.

Groups of eight male New Zealand white rabbits were exposed to 750 ppm ethylbenzene (analytical grade) for 12 hrs/day for 7 days (Romanelli et al. 1986, Mutti et al. 1988). Vapors were generated by direct injection into the chamber airflow and the atmosphere was monitored by gas chromatography. Clinical signs were not reported. Exposure resulted in significant

depletion of dopamine in the striatum and tuberoinfundibular regions of the brain. Norepinephrine levels were not affected. No other endpoints of toxicity were measured.

Groups of New Zealand white rabbits (n = 5/sex) were exposed whole body to analytically measured concentrations of 0, 382, 782, or 1610 ppm ethylbenzene (purity 99.7%) for 6 hr/day, 5 days/week, for 4 weeks (Cragg et al. 1989). A fluid metering pump was used to meter the chemical into an air atomizing nozzle; air was introduced into the atomizer to generate an aerosol that immediately volatilized. Particle size was measured to assure that ethylbenzene was in the vapor phase. Chamber atmospheres were monitored with an infrared gas analyzer. No mortality, clinical signs, changes in hematology or clinical chemistry, ophthalmoscopic findings, or gross lesions were seen in any animal. At 1610 ppm, males and females lost weight during the first week (13 and 62 g, respectively) and body weight gain by females was slightly decreased during weeks 3 and 4.

3.2.2. Rats

Female Wag/Rij rats (n = 8) were exposed in whole-body chambers to 0 or 550 ppm of ethylbenzene (purity 99%) 8 hours/day for 5 days (Cappaert et al. 2002). Chamber atmospheres were generated by injecting saturated gas into the air supply and were monitored by a dual gas monitor. No adverse clinical signs were observed and all animals survived. Body weight was not affected by exposure over the five days. The remaining examinations focused on the potential ototoxicity of ethylbenzene. Four to eight weeks after the last exposure, animals were anesthetized and the right and left cochlea were exposed. Auditory evoked responses to a series of stimuli were recorded at the apex of each cochlea. After electrocochleography, each cochlea was fixed and processed for histological examination. A large threshold shift was measured in the mid-frequency range with the maximum shift >30 dB (presented graphically). Pronounced outer hair cell loss was observed in the 11- 21-kHz region of the cochlea.

Motor activity was monitored in male CFY rats (n = 8) during a 4-hour exposure to 400-2180 ppm ethylbenzene (purity >99%) (Molnár et al. 1986). Atmospheres were generated by mixing saturated vapor into the air flow of each whole-body, dynamic chamber. Chamber concentrations were monitored with an ultraviolet spectrophotometer. Activity data were presented graphically and clinical signs were not reported. Exposure resulted in a biphasic response with increased activity between 400-1500 ppm followed by a decrease in activity at higher concentrations. A concentration of 2180 ppm was listed as the minimum narcotic concentration.

Groups of 6 male Sprague-Dawley rats were exposed by whole body to a mean analytically determined concentration of 2000 ppm ethylbenzene (purity >99%) for 6 hours/day for 3 days (Andersson et al. 1981). No deaths or clinical signs of toxicity were observed. Dopamine and noradrenaline levels and turnover in various parts of the brain were evaluated 16-18 hours after the last exposure. With one exception, exposure did not produce any effect on the level of either dopamine or catecholamine fluorescence in various regions of the forebrain. The exception was a decrease in catecholamine in the magnocellular part of the paraventricular hypothalamic nucleus. However, turnover of both neurotransmitters was increased in several specific nerve terminals. Prolactin levels were also greatly reduced in exposed animals.

Rats in the study described above, were also used for evaluation of metabolic enzyme activities (Toftgård and Nilsen 1982). Exposure resulted in a phenobarbital-type of enzyme induction in the liver but not in the kidney or lung.

Groups of Fischer 344 rats (n = 5/sex) were exposed whole body to analytically measured concentrations of 0, 99, 382, or 782 ppm ethylbenzene (purity 99.7%) for 6 hr/day, 5 days/week, for 4 weeks (Cragg et al. 1989). A fluid metering pump was used to meter the chemical into an air atomizing nozzle; air was introduced into the atomizer to generate an aerosol that immediately volatilized. Particle size was measured to assure that ethylbenzene was in the vapor phase. Chamber atmospheres were monitored with an infrared gas analyzer. No mortality, effects on body weight, changes in clinical chemistry or urinalysis, ophthalmoscopic findings, or gross lesions were seen in any animal. Sporadic incidences of salivation and lacrimation were observed in animals exposed to 382 and 782 ppm, but the incidence was too low to determine an exposure-response relationship (timing of clinical signs was not given). At 782 ppm, liver weight was increased in both sexes, platelet count was increased in males, and leukocyte count was increased in males and females.

3.2.3. Mice

The RD_{50} for ethylbenzene (analytical grade) in male Swiss-Webster mice was 4060 ppm (Nielsen and Alarie 1982). Groups of animals (n = 4) were exposed head-only to 410-9640 ppm for 30 minutes followed by a 20-minute recovery period. Each animal was placed in a plethysmograph for measurement of tidal volume and respiratory rate. Chamber atmospheres were generated by evaporating the chemical in a glass nebulizer; concentration was monitored with an infrared analyzer. After the initiation of exposure, the decrease in respiratory rate occurred rapidly followed by a fade in the response (i.e., rate began to increase to pre-exposure levels). After about 10 minutes, a second depression in the respiratory rate occurred following the fade of the first response. It was noted that at 7800 and 9640 ppm, the animals were sedated and anesthetized toward the end of exposure. The next lower concentration that did not cause sedation was not stated.

In contrast to the study above, de Ceaurriz et al. (1981) reported the RD_{50} for male Swiss OF_1 mice (n = 6) as 1432 ppm. Groups of mice were exposed nose-only for about 5 minutes to one of at least four concentrations of ethylbenzene (purity stated as high) and respiratory rate monitored with a plethysmograph. Chamber atmospheres were monitored by gas chromatography.

Groups of $B6C3F_1$ mice (n = 5/sex) were exposed whole body to analytically measured concentrations of 0, 99, 382, or 782 ppm ethylbenzene (purity 99.7%) for 6 hr/day, 5 days/week, for 4 weeks (Cragg et al. 1989). A fluid metering pump was used to meter the chemical into an air atomizing nozzle; air was introduced into the atomizer to generate an aerosol that immediately volatilized. Particle size was measured to assure that ethylbenzene was in the vapor phase. Chamber atmospheres were monitored with an infrared gas analyzer. No mortality, clinical signs, effects on body weight, changes in hematology, ophthalmoscopic findings, or gross lesions were seen in any animal. At 782 ppm, liver weight was increased in both sexes.

3.3. Neurotoxicity

Neurotoxicity was evaluated in mice using a functional observational battery (FOB) (Tegeris and Balster 1994). Male CFW albino mice (n = 8) were exposed to 0, 2000, 4000, or 8000 ppm ethylbenzene (purity 99%) for 20 minutes in a static exposure chamber. Concentrations were monitored by infrared spectrometry. Immediately following exposure, the animals were subjected to a complete FOB adapted for mice; open field duration was 2 minutes. During the final two minutes of exposure, decreased rearing and increased palpebral closure were observed at all concentrations. In the FOB, CNS effects observed at all concentrations included decreased arousal, increased ease of removal from the chamber, lacrimation, gait abnormalities, decreased righting reflex and forelimb grip strength, and lower sensory reactivity. The severity of all effects was concentration-related.

In guinea pigs, unsteadiness and ataxia were observed after 390 and 480 minutes, respectively, at 2000 ppm, after 26-30 minutes at 5000 ppm, and after only 4-10 minutes at 10,000 ppm (Yant et al. 1930). Mice appeared sedated and anesthetized toward the end of a 30-minute exposure to 7800 and 9640 ppm; a no effect level was not given (Nielsen and Alarie 1982). Exposure of rats to a range of concentrations resulted in a biphasic motor activity response with a minimal narcotic concentration listed as 2180 ppm (Molnár et al. 1986).

3.4. Developmental/Reproductive Toxicity

Groups of 29-33 female Sprague-Dawley rats were exposed whole body to ethylbenzene at concentrations of 0, 100, or 1000 ppm for 7 hrs/day, 5 days/week for three weeks prior to mating followed by exposure for 7 hrs/day on gestation days 1-19 (Andrew et al. 1981, Hardin et al. 1981). The chemical used was described as “pure grade” and the reported measured impurities were <0.3%. Test atmospheres were generated by heating the chemical in a vapor generation system and mixing the vapor with fresh air to attain the desired chamber concentration. Atmospheres were monitored by gas chromatography. Mean analytically determined concentrations during the study were 96-97 and 958-985 ppm, respectively. No treatment-related mortality or clinical signs of toxicity were observed in the dams. Maternal body weight and food consumption were similar between the treated and control groups. At necropsy, absolute liver, kidney, and spleen weights were significantly increased in high-concentration animals. No differences between the treated and control groups were found in the numbers of corpora lutea, implantations, live fetuses, or resorptions, or fetal and placental weights. No treatment-related external, visceral, or skeletal major malformations were observed in any fetus. The incidence of the variation of extra ribs was significantly increased in litters from the high concentration group (7/31 litters compared with 1/33 control litters).

Groups of 29-30 female New Zealand white rabbits were exposed whole body to ethylbenzene at concentrations of 0, 100, or 1000 ppm for 7 hrs/day on gestation days 1-24 (Andrew et al. 1981, Hardin et al. 1981). Test atmosphere generation and monitoring are described above. Mean analytically determined concentrations during the study were 99 and 962 ppm, respectively. No treatment-related mortality or clinical signs of toxicity were observed in the does. Maternal body weight and food consumption were similar between the treated and

control groups. At necropsy, liver weight relative to body weight was significantly increased in high-concentration animals. No differences between the treated and control groups were found for numbers of corpora lutea, implantations, live fetuses, or resorptions, or fetal and placental weights. No treatment-related external, visceral, or skeletal malformations or variations were observed in any fetus.

Female Sprague-Dawley rats (n = 21-25) were exposed to 100-2000 ppm ethylbenzene (purity >99%) by whole body inhalation for 6 hours/day on gestation days 6-20 (Saillenfait et al. 2003). Test atmospheres were generated by passing air flow through the fritted disk of a heated bubbler containing the test chemical. The vaporized compound was carried into the main air inlet pipe and concentration was adjusted by varying the airflow passing through the bubbler. Atmospheres were monitored by a gas chromatograph equipped with a flame ionization detector. Mean measured concentrations differed by less than 1% of nominal. Maternal toxicity was evident as decreased body weight gain and reduced food consumption at concentrations of 1000 ppm and higher throughout the exposure interval. All dams survived to scheduled sacrifice. Clinical signs of toxicity, including ataxia and decreased motor activity, were observed at 2000 ppm (incidence not given). Three dams in the high-concentration group had complete litter resorption. Fetal body weight was decreased at 1000 and 2000 ppm. No treatment-related external, visceral, or skeletal malformations were observed.

Ungváry and Tátrai (1985) tested ethylbenzene for developmental toxicity in mice, rats, and rabbits exposed by whole-body inhalation. Mice were exposed to 500 mg/m^3 (117 ppm) for for 3×4 h/day intermittently from gestation days 6-15; rats were exposed to 600, 1200, or 2400 mg/m^3 (140, 280, or 560 ppm) for 24 hr/day on gestation days 7-15; and rabbits were exposed to 500 or 1000 mg/m^3 (117 or 233 ppm) for 24 h/day on gestation days 7-20. Purity of the test material, methods of atmosphere generation, and analytical data from chamber monitoring were not included. Maternal toxicity was noted as moderate and concentration-dependent in rats, but specific effects were not described. Maternal weight gain was reduced in rabbits at 233 ppm and all does in this group aborted. Fetal body weight was decreased in rats at 560 ppm and in rabbits at 117 ppm. Reduced ossification (listed only as percent of fetuses with skeletal retardation) was found in rat fetuses at all concentrations. The only fetal malformation given was of the "uropoetic apparatus" (assumed to be kidney) in rats at 560 ppm and in mice at 117 ppm.

In a two-generation reproductive toxicity study, groups of 25-30 Crl:CD rats/sex were exposed by whole-body inhalation to 0, 25, 100, or 500 ppm of ethylbenzene (>99% purity) (Faber et al. 2006). Exposures of parental animals were for 6 h/day for at least 70 consecutive days prior to mating. For the F_0 and F_1 females, exposures continued throughout mating, during gestation days 0-20, and during lactation days 5-21. On lactation days 1-4, females received ethylbenzene in corn oil by gavage at doses of 0, 26, 90, or 342 mg/kg/day; these doses were calculated from a physiologically-based pharmacokinetic model to provide equivalent maternal blood area-under-concentration as provided by a 6-hour inhalation exposure (Tardif et al. 1997). F_1 generation animals were weaned on lactation day 21 and began exposures on post-natal day 22. To generate the test atmospheres, the chemical was metered from an amber glass reservoir, vaporized, and mixed with nitrogen gas. The vaporization nitrogen carried the ethylbenzene vapor to the chamber inlet where the concentration was reduced to the desired level with chamber ventilation air (Stump 2003). Mean daily analytical concentrations, as determined by

gas chromatography equipped with a flame ionization detector, were 0, 25, 100-101, and 500-501 ppm (Faber et al. 2006).

No treatment-related clinical findings were noted for any animal of either generation during the daily observations before, during, and one hour after exposure. Parental systemic toxicity was limited to decreased body weight gain by the 500-ppm F_0 and F_1 males resulting in mean body weight 4.5-5.6% lower than that of controls. Absolute and/or relative (to body weight) liver weights were slightly increased in males and females of both generations exposed to 500 ppm, but no corresponding microscopic pathology was observed. Reproductive performance and offspring growth and survival were not affected in either generation.

A range-finding one-generation study was conducted prior to the Faber et al. (2006) study. Groups of 20 Crl:CD rats/sex were exposed by whole-body inhalation to 0, 100, 500, or 1000 ppm of ethylbenzene (>99% purity) (Stump 2003). Exposures of F_0 animals prior to mating were for 6 h/day for at least 4 weeks for males and two weeks for females. For the F_0 females, exposures continued throughout mating, during gestation days 0-20, and during lactation days 5-21. On lactation days 1-4, one-half of the females received ethylbenzene in corn oil by gavage at doses of 0, 90, 342, or 621 mg/kg/day; these doses were calculated from a physiologically-based pharmacokinetic model to provide equivalent maternal blood area-under-concentration as provided by a 6-hour inhalation exposure (Tardif et al. 1997). Offspring were weaned on either lactation day 21 or 28 and were treated beginning on post-natal day 22 or 29, respectively, through post-natal day 33. To generate the test atmospheres, the chemical was metered from an amber glass reservoir, vaporized, and mixed with nitrogen gas. The vaporization nitrogen carried the ethylbenzene vapor to the chamber inlet where the concentration was reduced to the desired level with chamber ventilation air. Mean daily analytical concentrations, as determined by gas chromatography equipped with a flame ionization detector, were 0, 99-101, 500, and 1000-1008 ppm (Stump 2003).

All F_0 parental animals survived to scheduled sacrifice and no clinical signs of toxicity were observed during the study. Body weight gain by the mid- and high-concentration males (38 and 13%, respectively of controls) and females (45% of controls for both groups) was significantly reduced during the first week of treatment. Reduced weight gain in the 1000-ppm males resulted in significantly decreased absolute body weight at weeks 2 and 3 compared to the controls. In the mid- and high-concentration groups, food consumption was reduced in males and females (83-89% of controls for all groups) and food efficiency was reduced in males (39 and 15%, respectively, of controls). For females, body weight, body weight gain, and food consumption were similar between the treated and control groups during gestation and lactation. Reproductive performance was not affected by treatment. Gross necropsy of parental animals was unremarkable. In the mid- and high-concentration groups, absolute and relative liver weights were increased in males and females and kidney weight was increased in males (Stump 2003).

Body weight of male and female offspring from high-concentration dams was significantly less than controls at birth. Body weight and body weight gain of pups from dams treated with 1000 ppm/621 mg/kg/day were reduced throughout lactation compared with those of controls. On post-natal days 0-4, offspring survival was reduced in dams treated with 1000 ppm and 1000

ppm/621 mg/kg/day due to one dam in each group with close to complete litter loss. Offspring survival was not affected after culling on day 4 (Stump 2003).

Exposure for the F_1 animals was initiated on post-natal day 22 or 29, and continued through post-natal day 33. Mid- and high-concentration animals in both exposure regimens had slightly or significantly decreased mean body weight during the exposure interval with the most pronounced effect a reduced weight gain after the first day of exposure. After one day of exposure beginning on day 22, weight gain by the mid- and high-concentration animals was decreased by 37-53% and 71-94%, respectively, in males and 14-35% and 71-79%, respectively, in females compared to that of controls. Likewise, after one day of exposure beginning on day 29, weight gain by the mid- and high-concentration animals was decreased by 29-33% and 47-50%, respectively, in males and 20-50% and 45-54%, respectively, in females compared to controls (Stump 2003).

No deaths or treatment-related clinical signs were observed in F_1 animals that began treatment on day 29. In contrast, deaths and treatment-related clinical signs were observed in mid- and high-concentration animals that began exposure on post-natal day 22 (Table 2). These findings were generally noted after the first one to four days of treatment. In the high-concentration group, clinical signs observed one hour post-exposure included labored respiration, eyelids half-closed, prostration, animal unable to right itself, and rocking, lurching and swaying while ambulating. Two of the deaths in the 1000-ppm group occurred on day 22. In the mid-concentration group, one animal was observed with labored respiration after two exposures and was found dead the next day, post-natal day 24. Gross pathology of the animals found dead was unremarkable.

TABLE 2: Clinical Findings in F_1 Animals One Hour Post-exposure to Ethylbenzene (no. affected/no. animals)

Observation	0 ppm	100 ppm	500 ppm	1000 ppm
Found dead (days 22-26)	0/30	2[a] /31	1/38	6/35
Labored respiration	0/30	0/31	1/38	10/35
Eyelids half-closed	0/30	0/31	0/38	10/35
Prostration	0/30	0/31	0/38	7/35
Animal unable to right itself	0/30	0/31	0/38	2/35
Rocking, lurching, swaying	0/30	0/31	0/38	6/35

Data from Stump (2003); includes offspring from dams treated by inhalation only and dams treated by inhalation/gavage, males and females combined.
[a]Littermates that were small at weaning; deaths are not considered treatment-related and no clinical signs were observed prior to death.

At 500 ppm the exposure-related adverse effects in the F_1 weanlings seen immediately after the initiation of exposure in the range-finding study, (Stump 2003) were not observed in the

main study (Faber et al. 2006). The difference in apparent sensitivity at this concentration may have been due to a slight variation in protocol in the main study. Because offspring body weight is generally reduced in inhalation studies due to removal of the dams for exposure beginning on post-natal day 5, weanlings in the main study were housed with a littermate during the first week of exposure in an attempt to reduce stress to the animals. This transition period for the F_1 animals allowed them to gain additional weight before individual housing was implemented for the premating phase. The supposition that weanlings with lower body weight are more sensitive to ethylbenzene exposure is supported by the fact that no deaths or clinical signs were seen in the range-finding study at 500 and 1000 ppm for animals that were weaned on post-natal day 28 (Stump 2003).

3.5. Genotoxicity

Results of bacterial assays with ethylbenzene found it was not mutagenic in *Salmonella typhimurium* with or without metabolic activation at concentrations up to 1000 µg/plate (TA100, TA1535, TA97, TA98) (NTP 1992) or at concentrations up to 2000 µg/plate (TA100, TA1535, TA1537, TA1538, TA98) (Dean et al. 1985) or 0.4 mg/plate (Nestmann et al. 1980). The chemical also failed to induce mutation in *Escherichia coli* (Dean et al. 1985) or gene conversion in *Saccharomyces cerevisiae* (Dean et al. 1985, Nestmann and Lee 1983).

In cultured Chinese hamster ovary cells, ethylbenzene was negative for induction of sister chromatid exchange and chromosomal aberrations. An increase in trifluorothymidine-resistant colonies of L5178Y/TK$^{\pm}$ mouse lymphoma cell was observed at 80 µg/mL (highest nonlethal concentration) without metabolic activation (NTP 1992). Chromosome damage was not induced in cultured rat liver cells (Dean et al. 1985). No induction of micronucleus formation was found in peripherial blood erythrocytes of male and female mice after 13 weeks of inhalation exposure of up to 1000 ppm (NTP 1992).

A very slight, marginal increase in sister chromatid exchange was found in human lymphocytes cultured for 48 hours with 10 mM ethylbenzene (Norppa and Vainio 1983).

Ethylbenzene exposure failed to induce recessive lethal mutations in Drosophilia (Donner et al. 1980).

3.6. Subchronic and Chronic Toxicity/Carcinogenicity

Male Wistar rats (n = 5) were exposed to 0, 50, 300, or 600 ppm ethylbenzene (purity 99%) for 6 hours/day, 5 days/week, for up to 16 weeks (Elovaara et al. 1985). Atmospheres were generated by mixing saturated vapor into the air flow of each whole-body, dynamic chamber. Chamber concentrations were monitored with an infrared spectrophotometer. Clinical signs were not reported. Body weight gain was reduced in the 300- and 600-ppm groups after two weeks. Electron microscopy showed proliferation of the smooth endoplasmic reticulum in hepatocytes from animals of all exposure groups after two weeks, but only in the 600-ppm group after 16 weeks. In the liver, concentration- and time-related increases were noted for microsomal protein content and several enzyme activity levels.

Groups of male and female F344/N rats and B6C3F$_1$ mice (n = 10) were exposed to 0, 100, 250, 500, 750, or 1000 ppm ethylbenzene (purity 99%) for 6 hours/day, 5 days/week, for 13 weeks (NTP 1992). Atmospheres were generated using a dispersion-type system in which zero-grade nitrogen was passed though liquid ethylbenzene. Concentrations in the chambers were monitored by an automatic sampling system coupled to a gas chromatograph. At the highest concentration, rats had slightly (not significant) lower body weight gain. Absolute liver weight was increased in male and female rats at ≥500 ppm and in male and female mice at ≥750 ppm. In rats, absolute lung weight was increased at ≥250 ppm and inflammation was observed in 9/10 males and 10/10 females in all groups at ≥250 ppm. No other treatment-related changes were observed in males or females of either species (NTP 1992).

Groups of male and female F344/N rats and B6C3F$_1$ mice (n = 50) were exposed to 0, 75, 250, or 750 ppm ethylbenzene (purity >99%) for 6 hours/day, 5 days/week, for 103 weeks (NTP 1999). Atmospheres were generated by a flash evaporator unit and nitrogen gas carried the ethylbenzene vapor to the exposure chambers. Concentrations were created by varying the flow rate. Concentrations in the chambers were monitored by an automatic sampling system coupled to a gas chromatograph. Survival of male rats was decreased at the highest concentration. No biologically significant effects on body weight were observed in males or females of either species. In rats, the incidences of renal tubule neoplasms (adenoma and carcinoma) and of renal tubule hyperplasia were increased in males and females at 750 ppm. The severity of nephropathy was also increased in male rats at 750 ppm and in all groups of treated female rats. Male rats also had an increased incidence of interstitial cell adenoma in the testis at 750 ppm. In male mice, the incidence of alveolar/bronchiolar neoplasms and of alveolar epithelial hyperplasia were increased at 750 ppm. In female mice, the incidence of hepatocellular neoplasms was increased at 750 ppm. Nonneoplastic liver changes (hepatocyte syncytial alteration, hypertrophy, and necrosis) were also increased in high-concentration male mice. The incidence of hyperplasia of the pituitary gland in female mice at 250 and 750 ppm and the incidence of thyroid gland follicular cell hyperplasia in male and female mice at 750 ppm were increased.

Similar results to those described above were found in an older study (Wolf et al. 1956). Male and/or female rats (n = 10-25; 400-2200 ppm), guinea pigs (n = 5-10; 400-1250 ppm), rabbits (n = 1-2; 400-1250 ppm), and rhesus monkeys (n = 1-2; 400-600 ppm) were exposed to ethylbenzene for 7 hr/day, 5 days/week, for up to six months. Decreased body weight gain occurred in rats and guinea pigs at ≥1250 ppm. Increased liver weight was found in guinea pigs and monkeys at 600 ppm and in rats at all concentrations.

3.7. Summary

Non-lethal, developmental, and reproductive toxicity experimental animal exposures are summarized in Table 3. Signs of irritation were observed in laboratory animals at concentrations >1000 ppm. Narcosis developed at ≥2000 ppm. The cochlear ducts in the inner ear may be a target organ. Decreased body weight gain occurred in animals exposed repeatedly. Evidence for hepatic enzyme induction has been observed in several species following long-term exposure.

Developmental toxicity studies in the rat and rabbit did not indicate an increased sensitivity of the developing fetus. However, in reproductive toxicity studies weanling rats were more sensitive than adult rats.

Lethality data in animals are summarized in Table 4. Data were insufficient to assess the concentration-response curve.

TABLE 3: Summary of Nonlethal Animal Data Following Ethylbenzene Exposure

Species/sex	Conc. (ppm)	Duration	Effects	Reference
Guinea pig/not stated	1000-10,000	up to 480 min	1000: irritation after 3-8 min disappeared after 30 min 2000: immediate irritation, unsteadiness after 390 min, ataxia after 480 min 5000: immediate irritation, unsteadiness and ataxia after 26-30 min, tremors, abnormal respiration	Yant et al. 1930
Rabbit/m	400-2400	6 hours/day for 4 days	lacrimation in 2/4 at 2400 ppm on day 1, 2-3/4 in all groups on day 3	Bio/dynamics Inc. 1986
Rabbit/m,f	382-1610	6 hours/day, 5 days/week, 4 weeks	no clinical signs, decr wt gain at 1610 ppm	Cragg et al. 1989
Rat/f	550	8 hours/day, 5 days	no clinical signs, hair cell loss and threshold shift in cochlea	Cappaert et al. 2002
Rat/m	400, 1200	6 hours/day for 4 days	400: lacrimation after 3 days 1200: lacrimation on 2/5 after 1 day	Bio/dynamics Inc. 1986
Rat/m	400-2180	4 hours	400-1500: increased activity >1500: decreased activity 2180: minimum narcotic	Molnár et al. 1986
Rat/m	2000	6 hours/day for 3 days	no death or clinical signs	Andersson et al. 1981
Rat/f	100 or 1000	7 hr/d, 5 d/wk, 3 wks plus 7 hr/d on GDs 1-19	Maternal: 1000: increased liver, kidney, and spleen weights Developmental: 1000: slight increase in extra ribs	Andrew et al. 1981, Hardin et al. 1981

TABLE 3: Summary of Nonlethal Animal Data Following Ethylbenzene Exposure				
Rabbit/f	100 or 1000	7 hr/d on GDs 1-24	Maternal: 1000: increased liver weight relative to body weight Developmental: no effects	Andrew et al. 1981, Hardin et al. 1981
Rat/f	100-2000	6 hr/d on GDs 6-20	Maternal: ≥1000: decreased weight gain and food consumption Developmental: ≥1000: decreased body weight	Saillenfait et al. 2003
Rat/m,f	25-500	6 hr/d; 70 d prior to mating; two generations	500: parental: incr liver wt (F_0, F_1:m,f) ; incr kidney wt (F_0, F_1:m); decr body wt gain (F_0, F_1:m); offspring: no effects (F_1, F_2)	Faber et al. 2006
Rat/m,f	100-1000	6 hr/d; 2 or 4 wks prior to mating; one generation with F_1 exposed post-natal days 22 or 29 through 33	500: parental: incr liver wt (m,f) ; incr kidney wt (m); decr body wt gain (m,f); offspring: clinical signs, decr wt gain, death after two exposures 1000: parental: as for 500; offspring: decr wt at birth; decr survival; decr wt gain, clinical signs and death after day 22	Stump 2003
Rat/m,f Mice/m,f	99-782	6 hours/day, 5 days/week, 4 weeks	no clinical signs, incr liver wt at 782 ppm	Cragg et al. 1989
Mice/m	400	6 hours/day for 4 days	lacrimation after 3 days	Bio/dynamics Inc. 1986

TABLE 4: Summary of Animal Lethality Data Following Ethylbenzene Exposure				
Species/sex	Conc. (ppm)	Duration	Effects	Reference
Guinea pig/f	2500	8 hours 6 hours	1/8 died no effects	Cappaert et al. 2002
Guinea pig/not stated	10,000	2 hours	2/6	Yant et al. 1930
Rat/m	2400 1200	6 hours/day; 4 days	5/5; one on day 1 lacrimation	Bio/dynamics Inc. 1986
Rat/not stated	4000	4 hours	LC_{50}	Smyth et al. 1962; Mellon Institute 1949
Mouse/m	2400 1200	6 hours/day; 4 days	5/5; all on day 2 4/5; on day 3	Bio/dynamics Inc. 1986

4. SPECIAL CONSIDERATIONS

4.1. Metabolism and Disposition

Ethylbenzene is rapidly absorbed and excreted in both humans and rats. Six healthy men (27-32 years old) exposed to up to 46 ppm for 8 hours had an average of 49% pulmonary retention; exposures were via a "breathing valve" but it was not specified whether this was a mouthpiece or nose tube (Gromiec and Piotrowski 1984). A slightly higher retention of 64% was measured in volunteers (ages not given) exposed to up to 85 ppm for 8 hours; following the chamber exposure, only trace amounts of unchanged chemical were found in expired air (Bardodej and Bardodejova 1970). Pulmonary absorption by male rats was calculated as 44% during a 6-hour whole-body exposure to 233 ppm of radio-labeled chemical (Chin et al. 1980). Circulating ethylbenzene concentrations in workers were 0.69-0.80 mg/L at a mean workplace atmosphere of 41 ppm (Angerer and Lehnert 1979) and 61.4 µg/L at a mean workplace atmosphere of 4 ppm (Angerer and Wulf 1985).

Cappaert et al. (2002) measured circulating concentrations in rats and guinea pigs exposed to 500 ppm ethylbenzene 8 hours/day for three days. After day 1, the concentration of the chemical was approximately 8.3× higher in rat blood than in guinea pig blood. After day 3, the concentration decreased in both species with respect to day 1 but remained about 4.3× higher in rats compared with guinea pigs.

Once absorbed, ethylbenzene is metabolized by liver microsomal enzymes mainly to mandelic acid and excreted in the urine. Of the total retained ethylbenzene in humans, 55-64% was excreted as mandelic acid (Gromiec and Piotrowski 1984, Bardodej and Bardodejova 1970)

and 25% was excreted as phenylglyoxylic acid (Bardodej and Bardodejova 1970). Excretion of mandelic acid was biphasic with half-life values of 3.1 and 24.5 hours (Gromiec and Piotrowski 1984). Following chamber exposure of male volunteers (ages 33-40 years) to 150 ppm for 4 hours, urinary mandelic and phenylglyoxylic acids accounted for 90% of the metabolites with excretion nearly complete by 8 hours after the initiation of exposure (Engström et al. 1984). In rats, greater than 80% of the radioactivity was recovered in the urine with about 8% in expired air and excretion was nearly complete within 24 hours after the start of a 6-hour exposure (Chin et al. 1980).

Some qualitative species differences in metabolism of ethylbenzene may occur. For example, the main metabolite in rabbits after an oral dose is hippuric acid formed probably by oxidative decarboxylation of phenylglyoxylic acid (ATSDR 1999). However, the R-enantiomer was the main form of mandelic acid found to be excreted by both humans (two volunteers ages 25 and 39 years) and rats (Drummond et al. 1989, Korn et al. 1992).

Urinary mandelic acid levels have been used as biological exposure indices of ethylbenzene (Angerer and Wulf 1985, Holz et al. 1995, Korn et al. 1992) and correlations to workplace exposures were shown as higher levels postshift compared with preshift (Holz et al. 1995).

Very small amounts of ethylbenzene are retained in tissues after exposure. In rats, less than 0.2% of the total radioactivity was found in the carcass 42 hours after exposure to 233 ppm for 6 hours (Chin et al. 1980). Subcutaneous fat samples from workers in a styrene polymerization plant contained only 0.1-0.7 ppm of ethylbenzene (Wolff 1976, Wolff et al. 1977).

4.2. Mechanism of Toxicity

Little is known about the mechanism of ethylbenzene toxicity. At higher concentrations, direct irritation of mucous membranes was apparent in both human and animal studies (Yant et al. 1930). Alterations in neurotransmitter levels may be involved in mediation of central nervous system effects (Andersson et al. 1981, Romanelli et al. 1986, Mutti et al. 1988).

4.3. Structure Activity Relationships

Ethylbenzene is often present in mixed xylenes with the technical grade xylene product containing approximately 40% m-xylene and approximately 20% each of o-, and p-xylene and ethylbenzene (Fishbein, 1988). If exposure to ethylbenzene as a component of mixed xylenes is of concern, AEGL values for xylenes should be consulted (NAC 2005).

Ototoxicity has been shown in rats repeatedly exposed to styrene (Campo et al., 2001), toluene (Pryor et al., 1984), and mixed xylenes (Pryor et al., 1987). Thus, the cochlear ducts in the inner ear may be a target organ following repeated exposure to these aromatic hydrocarbons, but no data were found which indicate ototoxicity after a single exposure to any of these chemicals, including ethylbenzene. As discussed below in section 4.4.3, the rapid onset and transient nature of central nervous system effects combined with the transient nature of the ethylbenzene-induced nervous system disturbances are likely due to direct interaction of the chemical with molecular receptors in the central nervous system followed by rapid elimination.

Therefore, the venous blood concentration (Cv) of ethylbenzene following a single exposure would be expected to provide an internal dose measurement correlating with clinical signs. In contrast, the repeated exposures required for ototoxicity suggest that the cumulative measure of area under the curve (AUC; and not the Cmax) is likely responsible for ototoxicity.

Among the alkyl benzene derivatives, both potency of irritation to the mucous membranes and narcotic potency decrease with increasing substituent chain length (Gerarde 1960).

4.4. Other Relevant Information

4.4.1. Species Variability

Little evidence for species variability in nonlethal endpoints was found but qualitative and quantitative metabolism differences may exist. Clinical signs of ethylbenzene intoxication were similar between guinea pigs, rabbits, rats, and mice following a single exposure. Repeated exposures resulted in decreased body weight and increased liver weight in rabbits, rats, and mice. In regard to lethality, mice were more sensitive in a four-day repeat exposure study than rats and rabbits. Much higher concentrations were required to cause death in guinea pigs compared to the other species.

Data in the mouse suggest an extremely steep concentration-response curve. Although deaths were seen after three days of exposure to 1200 ppm for 6 hours/day (Bio/dynamics Inc. 1986), no adverse effects were found after exposure to 1000 ppm, 6 hours/day for 13 weeks (NTP 1992).

4.4.2. Susceptible Populations

Limited data suggest that the young animal is the most susceptible to effects of ethylbenzene and that this susceptibility is dependent on the body weight of the animal. At 500 ppm the exposure-related adverse effects in the F_1 weanlings seen immediately after the initiation of exposure in a range-finding study, (Stump 2003) were not observed in another study (Faber et al. 2006). The difference in apparent sensitivity at this concentration may have been in a slight variation in protocol for the Faber et al. study. Because offspring body weight is generally reduced in inhalation studies due to removal of the dams for exposure beginning on post-natal day 5, weanlings in the Faber et al. study were housed with a littermate during the first week of exposure in an attempt to reduce stress on the animals. This transition period for the F_1 animals allowed them to gain additional weight before individual housing was implemented for the premating phase. The supposition that weanlings with lower body weight are more sensitive to ethylbenzene exposure is supported by the fact that no deaths or clinical signs were seen at 500 and 1000 ppm for animals that were weaned on post-natal day 28 (Stump 2003).

4.4.3. Concentration-Exposure Duration Relationship

The two primary effects of ethylbenzene exposure are those of irritation and central nervous system effects. Irritation is considered a threshold effect and therefore should not vary

over time. An AEGL value based on irritation is therefore not scaled across time, but rather the same value is applied across all times.

The central nervous system effects of ethylbenzene are attributed to the low molecular weight and lipophilic nature of the chemical which allow it to readily cross the blood:brain barrier. The rapid onset and transient nature of central nervous system effects combined with the transient nature of the ethylbenzene-induced nervous system disturbances are likely due to direct interaction of the chemical with molecular receptors in the central nervous system followed by rapid elimination. The arterial or venous blood concentration of ethylbenzene is a reliable index of the brain level, and in turn, the magnitude of the CNS depression that is due to the parent compound. Thus, the blood concentration is a key determinant of impaired central nervous system activity. Therefore, the venous blood concentration (Cv) of ethylbenzene following exposure would be expected to provide an internal dose measurement correlating with clinical signs. Using physiologically-based pharmacokinetic (PBPK) modeling (see Appendices C and D), the internal dose (Cv) producing the clinical sign of interest (no effect level for narcosis for the AEGL-2; and highest non-lethal effect for the AEGL-3) in rats was determined. The human PBPK model of ethylbenzene was then run for each defined AEGL time point to determine the equivalent atmospheric exposure concentration producing the target Cv.

5. DATA ANALYSIS FOR AEGL-1

5.1. Summary of Human Data Relevant to AEGL-1

Experimental data on the effects of ethylbenzene on humans were available for the derivation of AEGL-1 values. No problems were reported by nine individuals exposed to 100 ppm for 8 hours. However, during exposure of eleven individuals to 180 ppm for 8 hours, some complained of irritation of the upper respiratory tract and eye and headache and sleepiness towards the end of the exposure; transient feelings of drunkenness were also reported (Bardodej and Bardodejova 1961).

5.2. Summary of Animal Data Relevant to AEGL-1

Motor activity in rats increased following exposures to 400-1500 ppm for four hours then decreased – perhaps indicative of CNS depression – at higher concentrations (Molnár et al. 1986). Lacrimation was observed in male rats, mice and rabbits exposed to 400 ppm for 6 hours/day for four days (Bio/dynamics Inc. 1986).

A number of experimental studies in adult animals indicate that clinical signs and systemic effects are not observed at concentrations less than 1000 ppm following single or repeated exposures. This concentration is much greater than that causing effects in humans.

5.3. Derivation of AEGL-1 values

A concentration of 100 ppm for 8 hours was chosen as the point of departure for derivation of AEGL-1 values. This is the highest concentration in humans which did not produce clinical signs after a single exposure. A total uncertainty factor of 3 was used which includes 3 for

intraspecies extrapolation because the point of departure was a no effect level for irritation and is below that which would cause CNS effects. An intraspecies UF of 3 is appropriate because direct acting irritant effects at the portal of entry are not expected to vary between individuals. The same UF is appropriate for mild CNS effects (see rationale below). Because the point of departure is below that causing systemic effect, time scaling was not performed. AEGL-1 values are shown in Table 5.

TABLE 5: AEGL-1 Values for Ethylbenzene

10-minute	30-minute	1-hour	4-hour	8-hour
33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)

6. DATA ANALYSIS FOR AEGL-2

6.1. Summary of Human Data Relevant to AEGL-2

Limited experimental data on the effects of ethylbenzene on humans were available for the derivation of AEGL-2 values. During exposure of eleven individuals to 180 ppm for 8 hours, some complained of irritation of the upper respiratory tract and eye and headache and sleepiness towards the end of the exposure; transient feelings of drunkenness were also reported (Bardodej and Bardodejova 1961). Severity of effects and the number of individuals affected were not reported.

6.2. Summary of Animal Data Relevant to AEGL-2

Animal data are available for derivation of AEGL-2 values. Motor activity was monitored in male CFY rats during a 4-hour exposure to 400-2180 ppm ethylbenzene (Molnár et al. 1986). Exposure resulted in a biphasic response with increased activity between 400-1500 ppm followed by a decrease in activity at higher concentrations. A concentration of 2180 ppm was listed as the minimum narcotic concentration with 1500 ppm as the highest non-narcotic concentration.

Female Wag/Rij rats exposed to 550 ppm of ethylbenzene for 8 hours/day for 5 days had changes in the inner ear (Cappaert et al. 2002). No adverse clinical signs were observed and all animals survived. Body weight was not affected by exposure over the five days. A large threshold shift was measured in the mid-frequency hearing range and pronounced outer hair cell loss was observed in the 11- 21-kHz region of the cochlea.

In a range-finding reproductive toxicity study, exposure to 500 or 1000 ppm for 6 hours resulted in decreased body weight gain in F_1 animals that began treatment on post-natal day 22 or 29 (Stump 2003). Concentration-related clinical signs were observed in the 500- and 1000-ppm animals that began exposure on post-natal day 22. These findings were generally noted after the first one to four days of treatment. In the high-concentration group, clinical signs

observed one hour post-exposure included death, labored respiration, eyelids half-closed, prostration, animal unable to right itself, and rocking, lurching and swaying while ambulating. In the 500-ppm group, one animal was observed with labored respiration after two exposures and was found dead the next day, post-natal day 24.

6.3. Derivation of AEGL-2 values

Animal data were used for derivation of AEGL-2 values. A concentration of 1500 ppm for 4 hours which was the no effect level for narcosis was chosen as the point of departure. It is assumed that the central nervous system response observed following ethylbenzene exposure is directly related to the concentration of parent material reaching the brain, and that venous blood concentrations correlate with brain concentrations. Therefore, the venous blood concentration (Cv) of ethylbenzene following a 4-hour exposure to 1500 ppm would be expected to provide an internal dose measurement correlating with the minimum narcotic response. Using a physiologically-based pharmacokinetic (PBPK) model, the internal dose (Cv) producing minimum narcotic condition in rats was determined. Then, the human PBPK model was run for each defined AEGL time point to determine the equivalent exposure concentration producing the target Cv (Appendix C).

A total uncertainty factor of 3 was applied to the AEGL-2 dose metric. An interspecies uncertainty factor of 1 was applied because PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1 and the pharmacodynamic component is also reduced to 1 because it appears similar exposure effects (central nervous system effects) occur in humans and animals. An intraspecies uncertainty factor of 3 was applied because the mode of action of ethylbenzene is similar to anaesthetic chemicals. The minimum alveolar concentration (MAC - produces a lack of motor response in 50% of individuals exposed to that concentration) for different age groups from newborns to the elderly and pregnant women has been studied for a number of anaesthetic gases. It varies from 2-3 fold (NRC 2001). It is acknowledged that the resulting AEGL 2 values may not be protective of ototoxicity which occurs after repeated exposures, however no data are available to assess this endpoint following a single exposure to ethylbenzene. AEGL-2 values are shown in Table 6.

TABLE 6: AEGL-2 Values for Ethylbenzene

10-minute	30-minute	1-hour	4-hour	8-hour
2900 ppm (13,000 mg/m^3)	1600 ppm (7000 mg/m^3)	1100 ppm (4800 mg/m^3)	660 ppm (2900 mg/m^3)	580 ppm (2500 mg/m^3)

7. DATA ANALYSIS FOR AEGL-3

7.1. Summary of Human Data Relevant to AEGL-3

Human exposure data relevant to derivation of AEGL-3 values were not available. No reports of human lethality from exposure to ethylbenzene were found in the literature. A concentration of 5000 ppm was intolerable (Yant et al. 1930).

7.2. Summary of Animal Data Relevant to AEGL-3

The most appropriate animal data relevant to derivation of AEGL-3 values are those of Andersson et al. (1981). The highest non-lethal scenario was exposure of adult rats to 2000 ppm for 6 hours/day for 3 days. Deaths in mice at a lower concentration occurred after multiple exposures (Bio/dynamics Inc. 1986). In a range-finding reproductive toxicity study (Stump 2003), clinical signs and decreased body weight gain were seen in F_1 animals exposed to 1000 ppm for 6 hours/day beginning on post-natal day 22 or 29. Two deaths occurred at 1000 ppm after a single exposure, but this was not repeated in the main reproductive toxicity study.

7.3. Derivation of AEGL-3 values

The highest non-lethal exposure of rats to 2000 ppm for 6 hours was used to derive the 10-min, 30-min, 1-hour, 4-hour, and 8-hour AEGL-3 values. As for the AEGL-2, it is assumed that the central nervous system effects observed following ethylbenzene exposure are directly related to the concentration of parent material reaching the brain. Therefore, PBPK modeling was again used to calculate the internal dose (Cv) correlating with an exposure to 2000 ppm for 6 hours which was the highest non-lethal concentration. The human PBPK model was then run for each defined AEGL time point to determine the equivalent exposure concentration producing the target Cv (Appendix D).

A total uncertainty factor of 3 was applied to the AEGL-3 dose metric. An interspecies uncertainty factor of 1 was applied because PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1 and the pharmacodynamic component is also reduced to 1 because it appears similar exposure effects (central nervous system effects) occur in humans and animals. An intraspecies uncertainty factor of 3 was applied because the mode of action of ethylbenzene is similar to anaesthetic chemicals. The minimum alveolar concentration (MAC - produces a lack of motor response in 50% of individuals exposed to that concentration) for different age groups from newborns to the elderly and pregnant women has been studied for a number of anaesthetic gases. It varies from 2-3 fold (NRC 2001). AEGL-3 values are shown in Table 7.

TABLE 7: AEGL-3 Values for Ethylbenzene

10-minute	30-minute	1-hour	4-hour	8-hour
4700 ppm (20,400 mg/m^3)	2600 ppm (11,000 mg/m^3)	1800 ppm (7800 mg/m^3)	1000 ppm (4400 mg/m^3)	910 ppm (4000 mg/m^3)

8. SUMMARY OF AEGLS

8.1. AEGL Values and Toxicity Endpoints

The derived AEGL values for various levels of effects and durations of exposure are summarized in Table 8. AEGL-1 was based on a no-effect level in humans. AEGL-2 values were based on the no effect level for narcosis in the adult rat. The basis for AEGL-3 was the highest non-lethal level in the rat.

TABLE 8: Summary of AEGL Values

Classification	Exposure Duration				
	10-minute	30-minute	1-hour	4-hour	8-hour
AEGL-1 (Nondisabling)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)	33 ppm (144 mg/m^3)
AEGL-2 (Disabling)	2900 ppm (13,000 mg/m^3)	1600 ppm (7000 mg/m^3)	1100 ppm (4800 mg/m^3)	660 ppm (2900 mg/m^3)	580 ppm (2500 mg/m^3)
AEGL-3 (Lethal)	4700 ppm (20,400 mg/m^3)	2600 ppm (11,000 mg/m^3)	1800 ppm (7800 mg/m^3)	1000 ppm (4400 mg/m^3)	910 ppm (4000 mg/m^3)

8.2. Comparison with Other Standards and Guidelines

Standards and guidance levels for workplace and community exposures are listed in Table 9. The time-weighted average exposure concentration for workers is 100 ppm (ACGIH 2006, NIOSH 1996, 2005, OSHA 1999). A NIOSH IDLH has been established at 800 ppm based only on 10% of the lower explosive limit of 0.8%. The occupational exposure limit from The Netherlands and Sweden is 50 ppm. Germany has designated ethyl benzene as a substance for which observance of the established MAK value on its own does not guarantee the prevention of adverse effects on health, that is, dermal exposure increases the body burden.

TABLE 9: Extant Standards and Guidelines for Ethylbenzene

Guideline	Exposure Duration				
	10 minute	**30 minute**	**1 hour**	**4 hour**	**8 hour**
AEGL-1	33 ppm	33 ppm	33 ppm	33 ppm	33 ppm
AEGL-2	2900 ppm	1600 ppm	1100 ppm	660 ppm	580 ppm
AEGL-3	4700 ppm	2600 ppm	1800 ppm	1000 ppm	910 ppm
SMACs[a]			180 ppm		
REL-TWA (NIOSH)[b]					100 ppm
REL-STEL (NIOSH)[c]	125 ppm				
IDLH (NIOSH)[d]		800 ppm			
TLV-TWA (ACGIH)[e]					100 ppm
TLV-STEL (ACGIH)[f]	125 ppm				
PEL-TWA (OSHA)[g]					100 ppm
MAK (Germany)[h]					H
MAC (The Netherlands)[i]					50 ppm
OEL-TWA (Sweden)[j]					50 ppm
OEL-STEL (Sweden)[k]	100 ppm				

[a] **SMACs (Spacecraft Maximum Allowable Concentrations)** (NRC 1997) provide guidance on chemical exposures during normal operations of spacecraft as well as emergency situations. The one-hour SMAC is a concentration of airborne substance that will not compromise the performance of specific tasks by astronauts during emergency conditions or cause serious or permanent toxic effects. Such exposure may cause reversible effects such as skin or eye irritation, but they are not expected to impair judgment or interfere with proper responses to emergencies.

[b] **NIOSH REL-TWA (National Institute of Occupational Safety and Health, Recommended Exposure Limits - Time Weighted Average)** (NIOSH 1996, 2005) is defined analogous to the ACGIH-TLV-TWA.

[c] **NIOSH REL-STEL (Recommended Exposure Limits - Short Term Exposure Limit)** (NIOSH 1996, 2005) is defined analogous to the ACGIH TLV-STEL.

[d] **IDLH (Immediately Dangerous to Life and Health, National Institute of Occupational Safety and Health)** (NIOSH 1996, 2005) represents the maximum concentration from which one could escape within 30 minutes without any escape-impairing symptoms, or any irreversible health effects.

[e] **ACGIH TLV-TWA (American Conference of Governmental Industrial Hygienists, Threshold Limit Value - Time Weighted Average)** (ACGIH 2002, 2006) is the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which nearly all workers may be repeatedly exposed, day after day, without adverse effect. SEN:sensitizer

[f]**ACGIH TLV-STEL (Threshold Limit Value - Short Term Exposure Limit)** (ACGIH 2002, 2006) is defined as a 15-minute TWA exposure which should not be exceeded at any time during the workday even if the 8-hour TWA is within the TLV-TWA. Exposures above the TLV-TWA up to the STEL should not be longer than 15 minutes and should not occur more than 4 times per day. There should be at least 60 minutes between successive exposures in this range.

[g]**OSHA PEL-TWA (Occupational Safety and Health Administration, Permissible Exposure Limits - Time Weighted Average)** (OSHA 1999) is defined analogous to the ACGIH-TLV-TWA, but is for exposures of no more than 10 hours/day, 40 hours/week.

[h]**MAK (Maximale Arbeitsplatzkonzentration [Maximum Workplace Concentration])** (Deutsche Forschungsgemeinschaft [German Research Association] 2007) is defined analogous to the ACGIH-TLV-TWA. "H" designates substances for which observance of the established MAK value on its own does not guarantee the prevention of adverse effects on health, that is, when dermal exposure increases the body burden.

[i]**MAC (Maximaal Aanvaarde Concentratie [Maximal Accepted Concentration])** (SDU Uitgevers 2000 [under the auspices of the Ministry of Social Affairs and Employment], The Hague, The Netherlands) is defined analogous to the ACGIH-TLV-TWA.

[j]**OEL-TWA (Occupational Exposure Limits - Time-weighted-average)** (Swedish National Board of Occupational Safety and Health 2005) is an occupational exposure limit value for exposure during one working day.

[k]**OEL-STEL (Occupational Exposure Limits - Short-term exposure limit)** (Swedish National Board of Occupational Safety and Health 2000) is an occupational exposure limit value for exposure during a reference period of fifteen minutes.

8.3. Data Adequacy and Research Needs

Limited human and animal data were available despite the widespread use of the chemical. Because of lack of data, a clear concentration-response was difficult to assess for both non-lethal and lethal concentrations. Data regarding potential ototoxicity following a single exposure were not found.

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APPENDIX A: Derivation of AEGL Values

Derivation of AEGL-1

Key Study:	Bardodej and Bardodejova 1961
Toxicity endpoint:	No effects in human volunteers exposed to 100 ppm for 8 hours
Time scaling:	none
Uncertainty factors:	3 (3 for intraspecies extrapolation because the point of departure was a no effect level for irritation and is below that which would cause CNS effects. An intraspecies UF of 3 is appropriate because direct acting irritant effects at the portal of entry are not expected to vary between individuals.)
Modifying factor:	None
Calculations:	(C/UFs) (100 ppm/3) = 33 ppm

Derivation of AEGL-2

Key Study:	Molnár et al. 1986
Toxicity endpoint:	No effect level for narcosis in rats after exposure to 1500 ppm for 4 hours
Time scaling	It is assumed that the central nervous system response observed following ethylbenzene exposure is directly related to the concentration of parent material reaching the brain, and that venous blood concentrations correlate with brain concentrations. Therefore, the venous blood concentration (Cv) of ethylbenzene following a 4-hour exposure to 1500 ppm would be expected to provide an internal dose measurement correlating with the minimum narcotic response. Using a physiologically-based pharmacokinetic (PBPK) model, the internal dose (Cv) producing minimum narcotic condition in rats was determined. Then, the human PBPK model was run for each defined AEGL time point to determine the equivalent exposure concentration producing the target Cv (Appendix C).
Uncertainty factors:	3 (3 for intraspecies variability and 1 for interspecies variability)
Modifying factor:	None
10-minute AEGL-2:	Application of PBPK model: 2900 ppm
30-minute AEGL-2:	Application of PBPK model: 1600 ppm
1-hour AEGL-2:	Application of PBPK model: 1100 ppm
4-hour AEGL-2:	Application of PBPK model: 660 ppm
8-hour AEGL-2:	Application of PBPK model: 580 ppm

Derivation of AEGL-3

Key Study:	Andersson et al. 1981
Toxicity endpoint:	Highest non-lethal exposure in rats of 2000 ppm for 6 hours/day for 3 days
Time scaling	It is assumed that the central nervous system response observed following ethylbenzene exposure is directly related to the concentration of parent material reaching the brain, and that venous blood concentrations correlate with brain concentrations. Therefore, the venous blood concentration (Cv) of ethylbenzene following a 6-hour exposure to 2000 ppm would be expected to provide an internal dose measurement correlating with the non-lethal response. Using a physiologically-based pharmacokinetic (PBPK) model, the internal dose (Cv) producing a non-lethal condition in rats was determined. Then, the human PBPK model was run for each defined AEGL time point to determine the equivalent exposure concentration producing the target Cv (Appendix D).
Uncertainty factors:	3 (3 for intraspecies variability and 1 for interspecies variability)
Modifying factor:	None
10-minute AEGL-3:	Application of PBPK model: 4700 ppm
30-minute AEGL-3:	Application of PBPK model: 2600 ppm
1-hour AEGL-3:	Application of PBPK model: 1800 ppm
4-hour AEGL-3:	Application of PBPK model: 1000 ppm
8-hour AEGL-3:	Application of PBPK model: 910 ppm

APPENDIX B: Derivation Summary for Ethylbenzene AEGLs

ACUTE EXPOSURE GUIDELINE LEVELS FOR ETHYLBENZENE (CAS Reg. No. 100-41-4) DERIVATION SUMMARY

AEGL-1 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
33 ppm	**33 ppm**	**33 ppm**	**33 ppm**	**33 ppm**

Key Reference: Bardodêj, Z. and E. Bardodêjova. 1961. [Usefulness and application of exposure tests.] Cesk. Hyg. 6:537-545. (Czech)

Test Species/Strain/Number: human/9-11

Exposure Route/Concentrations/Durations: Inhalation/ 100 and 180 ppm/ 8 hours

Effects:
100 ppm: no effects

180 ppm: upper respiratory tract and eye irritation; CNS effects

Endpoint/Concentration/Rationale: The highest no effect level in humans.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, human data
Intraspecies: 3, because the point of departure was a no effect level for irritation and is below that which would cause CNS effects; an intraspecies UF of 3 is appropriate because direct acting irritant effects at the portal of entry are not expected to vary between individuals.

Modifying Factor: None

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: none; point of departure was below the level causing effects

Data Adequacy: Study details were limited.

AEGL-2 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
2900 ppm	**1600 ppm**	**1100 ppm**	**660 ppm**	**580 ppm**

Key Reference: Molnár, J., K.Á. Paksy, and M. Náray. 1986. Changes in the rat's motor behaviour during 4-hr inhalation exposure to prenarcotic concentrations of benzene and its derivatives. Acta Physiol. Hung. 67:349-354.

Test Species/Strain/Number: rat/ CFY / 8 males

Exposure Route/Concentrations/Durations: Inhalation/ 400-2180 ppm/ 4 hours

Effects: biphasic response with increased motor activity between 400-1500 ppm followed by a decrease in activity at higher concentrations; 2180 ppm was the minimum narcotic concentration with 1500 ppm the highest non-narcotic concentration

Endpoint/Concentration/Rationale: No effect level for narcosis of 1500 ppm for 4 hours.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, because PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1, the pharmacodynamic component is also reduced to 1 because it appears similar exposure effects (central nervous system effects) occur in humans and animals.
Intraspecies: 3, because the mode of action of ethylbenzene is similar to anaesthetic chemicals. The minimum alveolar concentration (MAC - produces a lack of motor response in 50% of individuals exposed to that concentration) for different age groups from newborns to the elderly and pregnant women has been studied for a number of anaesthetic gases. It varies from 2-3 fold (NRC 2001).

Modifying Factor: None

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: It is assumed that the central nervous system response observed following ethylbenzene exposure is directly related to the concentration of parent material reaching the brain, and that venous blood concentrations correlate with brain concentrations. Therefore, the venous blood concentration (Cv) of ethylbenzene following a 4-hour exposure to 2180 ppm would be expected to provide an internal dose measurement correlating with the minimum narcotic response. Using a physiologically-based pharmacokinetic (PBPK) model, the internal dose (Cv) producing minimum narcotic condition in rats was determined. Then, the human PBPK model was run for each defined AEGL time point to determine the equivalent exposure concentration producing the target Cv (Appendix C).

Data Adequacy: Supporting data were available in both humans and animals. It is acknowledged that the resulting AEGL 2 values may not be protective of ototoxicity which occurs after repeated exposures, however no data are available to assess this endpoint following a single exposure to ethylbenzene.

AEGL-3 VALUES				
10-minute	**30-minute**	**1-hour**	**4-hour**	**8-hour**
4700 ppm	**2600 ppm**	**1800 ppm**	**1000 ppm**	**910 ppm**

Key Reference: Andersson, K., K. Fuxe, O.G. Nilsen, R. Toftgård, P. Eneroth, and J.-Å. Gustafsson. 1981. Production of discrete changes in dopamine and noradrenaline levels and turnover in various parts of the rat brain following exposure to xylene, *ortho*-, *meta*-, and *para*-xylene, and ethylbenzene. Toxicol. Appl. Pharmacol. 60:535-548.

Test Species/Strain/Number: rat/Sprague-Dawley/6 males

Exposure Route/Concentrations/Durations: Inhalation/ 2000 ppm/ 6 hours/day, 3 days

Effects: no deaths or clinical signs; highest non-lethal concentration and duration

Endpoint/Concentration/Rationale: Exposure to a concentration of 2000 ppm, 6 hours/day for three days was the highest non-lethal exposure level in the rat.

Uncertainty Factors/Rationale:
Total uncertainty factor: 3
Interspecies: 1, because PBPK modeling reduced the toxicokinetic component of the uncertainty factor to 1, the pharmacodynamic component is also reduced to 1 because it appears similar exposure effects (central nervous system effects) occur in humans and animals.
Intraspecies: 3, because the mode of action of ethylbenzene is similar to anaesthetic chemicals. The minimum alveolar concentration (MAC - produces a lack of motor response in 50% of individuals exposed to that concentration) for different age groups from newborns to the elderly and pregnant women has been studied for a number of anaesthetic gases. It varies from 2-3 fold (NRC 2001).

Modifying Factor: None

Animal to Human Dosimetric Adjustment: Not applicable

Time Scaling: It is assumed that the central nervous system response observed following ethylbenzene exposure is directly related to the concentration of parent material reaching the brain, and that venous blood concentrations correlate with brain concentrations. Therefore, the venous blood concentration (Cv) of ethylbenzene following a 6-hour exposure to 2000 ppm would be expected to provide an internal dose measurement correlating with the non-lethal response. Using a physiologically-based pharmacokinetic (PBPK) model, the internal dose (Cv) producing a non-lethal condition in rats was determined. Then, the human PBPK model was run for each defined AEGL time point to determine the equivalent exposure concentration producing the target Cv (Appendix D).

Data Adequacy: Limited data for concentration-response evaluation.

1

APPENDIX C: Physiologically-Based Pharmacokinetic Modeling of Ethylbenzene – AEGL 2

PBPK-Modeling Based Derivation of AEGL 2 Values for Ethylbenzene

To be submitted to the National Advisory Committee for AEGLs (NAC) as an Appendix to the Ethylbenzene AEGL document

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FINAL

May 13, 2008

Summary

Physiologically based pharmacokinetic (PBPK) modeling was applied to the extrapolation of the identified Acute Exposure Guideline Level—severity 2 (AEGL 2) effects of ethylbenzene in rats to human exposure guidelines for various durations, as specified in the AEGL guidance (NRC, 1993, 2001). The resulting AEGLs are summarized below:

Assuming a total uncertainty factor (UF) of 3:

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr
AEGL 2	2900 ppm	1600 ppm	1100 ppm	660 ppm	580 ppm

Introduction

Physiologically based pharmacokinetic (PBPK) modeling has been identified as a tool that can improve the scientific basis of various extrapolations (e.g., interspecies, dose route, duration) common in human health risk assessment. Guidance has recently been developed for the use of PBPK modeling to support the derivation of Acute Exposure Guideline Levels (AEGLs) (Dennison and Troxel, 2006). The derivations presented in this document are consistent with the guidance and a previous application of that guidance (Oak Ridge National Laboratory [ORNL], 2007), and apply PBPK modeling to the derivation of AEGLs for ethylbenzene.

The AEGL 1 endpoint for ethylbenzene would most logically be associated with dose metrics for which internal dosimetry modeling does not currently provide an improved theoretical basis for human risk assessment as compared to reliance on external dose. The previously derived AEGL 3 values (Sweeney, 2008) were well received by the National Advisory Committee on AEGLs, but a different endpoint was selected for the AEGL 2. Therefore, the focus for this document is on dosimetry modeling relevant to the AEGL 2. The assessment involved the following steps.

Step 1) Identify the appropriate dose metric.

Step 2) Identify PBPK models for the test species (rat) and humans that adequately describe the pharmacokinetics of ethylbenzene with respect to the above dose metrics.

Step 3) Calculate the dose metric under the conditions corresponding to the critical study using the rat PBPK model.

Step 4) Apply the uncertainty factor (UF) to the dose metric.

Step 5) Determine the human equivalent concentrations (HECs) using the human PBPK model for the target dose metric for each duration of interest.

For the ethylbenzene AEGL derivations, key studies and UFs were identified as described in the Technical Support Document (TSD—in preparation).

When PBPK modeling is being considered for potential use in AEGL derivation, it is customary to review the PBPK modeling literature for that chemical, identify appropriate models, and validate the model by comparing the model predictions to the available experimental data (Dennison and Troxel, 2006). This review has previously been completed (American Chemistry Council [ACC], 2007, Appendix P; Sweeney, 2008).

Results and Discussion

Key Study and Point of Departure

The critical studies were identified as discussed in the TSD.

The key study for the AEGL 2 was Molnar et al. (1986); a 4-hr exposure of male CFY (Sprague-Dawley derived) rats to 2180 ppm elicited narcotic effects, while exposures of shorter duration or lesser intensity (1500 ppm or less) produced moderate activation. The most appropriate dose metric for narcotic (neurotoxic) effects is the peak concentration of ethylbenzene in the brain (richly perfused tissue), and the most appropriate model is the model developed by Kannan Krishnan and co-workers (Haddad et al., 2000), as modified by Sweeney et al. (2007) for higher exposure concentrations (ACC, 2007). Animals were reported to weigh 0.2 kg at the time of exposure. The point-of-departure of 4 hrs exposure to 1500 ppm ethylbenzene is equivalent to a brain concentration of 177 mg/L

Potential AEGL Values

A potential uncertainty factor of 3 was applied to the point-of departure to arrive at a target human brain concentration of 59 mg/L ethylbenzene. The results for the different durations are reported below in Table 1. Because the AEGL 2 values were based on peak blood concentration, at longer AEGL durations the AEGL values tend to plateau because the blood concentrations approach steady state.

Table 1. AEGL 2 results with UF = 3 applied before extrapolation

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr
AEGL 2	2900 ppm	1600 ppm	1100 ppm	660 ppm	580 ppm

References

American Chemistry Council (2007). Voluntary Children's Chemical Evaluation Program (VCCEP) Tier 1 Pilot Submission for Ethylbenzene (CAS No. 100-41-4). August 10, 2007. http://www.tera.org/peer/VCCEP/Ethylbenzene/VCCEP%20Ethylbenzene%20Revised%20Doc%20-august%2010%202007.pdf

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APPENDIX D: Physiologically-Based Pharmacokinetic Modeling of Ethylbenzene – AEGL 3

PBPK-Modeling Based Derivation of AEGL Values for Ethylbenzene

To be submitted to the National Advisory Committee for AEGLs (NAC) as an Appendix to the Ethylbenzene AEGL document

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January 18, 2008

Summary

Physiologically based pharmacokinetic (PBPK) modeling was applied to the extrapolation of the identified Acute Exposure Guideline Level—severity 2 (AEGL 2) and AEGL—severity 3 (AEGL 3) effects of ethylbenzene in rats to human exposure guidelines for various durations, as specified in the AEGL guidance (NRC, 1993, 2001). Ethylbenzene PBPK models for rats and humans were recently extensively reviewed for an assessment under U.S. EPA's Voluntary Children's Chemical Evaluation Program (VCCEP) (American Chemistry Council [ACC], 2007, Appendix P). In this current document, the same PBPK models used in the VCCEP assessment were applied to the AEGL derivation, and the results are reported herein. The model documentation available in ACC (2007) is supplemented in the present document by additional analyses (sensitivity analyses conducted at the higher concentrations relevant to AEGL exposure scenarios) and the model code is provided. The resulting AEGLs are summarized below:

Assuming a total uncertainty factor (UF) of 3:

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr
AEGL 2	11,000[a] ppm	3800[a] ppm	1900[a] ppm	510 ppm	280 ppm
AEGL 3	4700 ppm	2600 ppm	1800 ppm	1000 ppm	910 ppm

[a]Superceded by AEGL 3

Assuming a UF of 10:

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr
AEGL 2	4000[a] ppm	1400[a] ppm	710[a] ppm	200 ppm	120 ppm
AEGL 3	1400 ppm	810 ppm	580 ppm	360 ppm	320 ppm

[a]Superceded by AEGL 3

It should be noted that the AEGLs noted above were derived with the UF applied to the rat internal dose before the extrapolations to the human. If the order were reversed (interspecies extrapolation and duration adjustment, followed by UF application), slightly lower AEGLs would be derived. Also, these extrapolations do not take into account the possibility of an increased level of exertion during the AEGL exposure scenario. If an exertion of 50 W is assumed, the AEGLs decrease by as much as a factor of 2 (10-minute AEGL 3), with smaller differences for the AEGL 2 and exposure scenarios of longer duration.

Introduction

Physiologically based pharmacokinetic (PBPK) modeling has been identified as a tool that can improve the scientific basis of various extrapolations (e.g., interspecies, dose route, duration) common in human health risk assessment. Guidance has recently been developed for the use of PBPK modeling to support the derivation of Acute Exposure Guideline Levels (AEGLs) (Dennison and Troxel, 2006). The derivations presented in this appendix are consistent with the guidance and a previous application of that guidance (Oak Ridge National Laboratory [ORNL], 2007), and apply PBPK modeling to the derivation of AEGLs for ethylbenzene.

The AEGL 1 endpoint for ethylbenzene would most logically be associated with dose metrics for which internal dosimetry modeling does not currently provide an improved theoretical basis for human risk assessment as compared to reliance on external dose. Therefore, the focus for this appendix is on dosimetry modeling relevant to the AEGL 2 and AEGL 3 endpoints. The assessment involved the following steps

Step 1) Identify the appropriate dose metrics.

Step 2) Identify PBPK models for the test species (rat) and humans that adequately describe the pharmacokinetics of ethylbenzene with respect to the above dose metrics.

Step 3) Calculate the dose metrics under the conditions corresponding to the critical study using the rat PBPK model.

Step 4) Apply the uncertainty factors (UFs) to the dose metrics.

Step 5) Determine the human equivalent concentrations (HECs) using the human PBPK model for each target dose metric for each duration of interest.

Alternatively, the extrapolations for interspecies kinetic differences and for the various durations may be done prior to applying the UF.

For the ethylbenzene AEGL derivations, key studies and UFs were identified as described in the Technical Support Document (TSD—in preparation).

When PBPK modeling is being considered for potential use in AEGL derivation, it is customary to review the PBPK modeling literature for that chemical, identify appropriate models, and validate the model by comparing the model predictions to the available experimental data (Dennison and Troxel, 2006). A review with similar coverage has previously been conducted for an assessment under U.S. EPA's Voluntary Children's Chemical Evaluation Program (VCCEP) (American Chemistry Council [ACC], 2007, Appendix P). This review is provided as an attachment. The model documentation available in ACC (2007) is supplemented in the present document by additional analyses (sensitivity analyses conducted at the higher concentrations relevant to AEGL exposure scenarios) and the model code is provided.

Results and Discussion

Key Studies and Points of Departure

The critical studies were identified as discussed in the TSD.

The key study for the AEGL 2 was Cappaert et al. (2002); in the ototoxicity portion of the study, rats were exposed to 550 ppm EB for 8 hrs. As discussed in ACC (2007), the most appropriate dose metric for ethylbenzene-induced ototoxicity is cumulative exposure of the cochlea (area under the concentration vs. time curve for richly perfused tissue--AUCR) to ethylbenzene, and the most appropriate model is the model developed by Kannan Krishnan and co-workers (Haddad et al., 2000), as modified by Sweeney et al. (2007) for higher exposure concentrations. (ACC, 2007) For the purpose of these AEGL derivations, it was assumed that a one day-exposure to ethylbenzene had the potential to produce hearing impairment. Animals were reported to weigh 0.2 kg upon receipt; a BW = 0.25 kg was assumed for the time of exposure. The 24 hr AUCR for 8 hrs exposure was 573.8 mg-hr/L. **(note: this was subsequently changed, see Appendix C)**

The key study for the AEGL 3 was Andersson et al. (1981) were no lethality was observed in rats exposed to 2000 ppm ethylbenzene for six hours. The lethal effects of high concentrations of ethylbenzene and other solvents are generally understood to be related to central nervous system depression. Thus an appropriate dose metric for the human extrapolations is assumed to be the peak concentration in the richly perfused tissues (peak CR). The animal body weight was not stated in the Andersson et al. (1981) paper, so a value of 0.25 kg was assumed. The estimated peak CR for this study was 290.3 mg/L.

Potential AEGL Values

Enhanced transparency regarding the impact of selected approaches to the derivation of AEGLs is provided by presenting the results of different assumptions and procedures. In the effort documented here, two different potential uncertainty factors were considered (3 or 10), two sequences for the steps for deriving the AEGLs were considered (i.e., uncertainty factor application followed by extrapolation or extrapolation followed by uncertainty factor application), and the influence of assumptions about the level of exertion was explored. The results of the different cases are reported below in Tables 1-4. Because the AEGL 3 values were based on peak blood concentration, at longer AEGL durations, the AEGL values tend to plateau because the blood concentrations approach steady state. In contrast, the AEGL 2 values are based on cumulative exposure (AUC), so as the AEGL duration increases, the acceptable external concentration decreases. As a result, the AEGL 2 values for shorter durations (10 minutes to 1 hr) were frequently superceded by the AEGL 3 values.

Case 1: Apply the uncertainty factor, then extrapolate to human exposure of varying durations

Case 1A. Assume a UF of 3:

AEGL 2 target: 191.3 mg-hr/L
AEGL 3 target: 96.8 mg/L

Table 1. AEGL results with UF = 3 applied before extrapolation

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr
AEGL 2	11,000[a] ppm	3800[a] ppm	1900[a] ppm	510 ppm	280 ppm
AEGL 3	4700 ppm	2600 ppm	1800 ppm	1000 ppm	910 ppm

[a]Superceded by AEGL 3

Case 1B: Assume a UF of 10:

AEGL 2 target: 57.38 mg-hr/L
AEGL 3 target: 29.03 mg/L

Table 2: AEGL results with UF = 10 applied before extrapolation

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr
AEGL 2	4000[a] ppm	1400[a] ppm	710[a] ppm	200 ppm	120 ppm
AEGL 3	1400 ppm	810 ppm	580 ppm	360 ppm	320 ppm

[a]Superceded by AEGL 3

Case 2: Extrapolate to human exposure of varying durations, then apply uncertainty factor

Case 2A. Assume a UF of 3:

Table 3. AEGL results with UF = 3 applied after extrapolation

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr
AEGL 2	10,000[a] ppm	3300[a] ppm	1700 ppm	430 ppm	230 ppm
AEGL 3	4700 ppm	2500 ppm	1800 ppm	970 ppm	870 ppm

[a]Superceded by AEGL 3

Case 2B. Assume a UF of 10:

Table 4. AEGL results with UF = 10 applied after extrapolation

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr

AEGL 2	3000[a] ppm	1000[a] ppm	500 ppm	130 ppm	68 ppm
AEGL 3	1400 ppm	750 ppm	530 ppm	290 ppm	260 ppm

[a]Superceded by AEGL 3

For simulation of exertion at the level of 50 W, the alveolar ventilation rate, cardiac output, and blood flow to the tissue groups were adjusted in the same manner described for toluene (ORNL, 2007). The results are summarized in Table 5.

Table 5. AEGL results for 50 W with UF=10 applied after extrapolation

Severity	Duration				
	10 min.	30 min.	1 hr	4 hr	8 hr
AEGL 2	2000[a] ppm	650[a] ppm	330[a] ppm	84 ppm	43 ppm
AEGL 3	710 ppm	370 ppm	270 ppm	190 ppm	180 ppm

[a]Superceded by AEGL 3

Sensitivity Analyses

Sensitivity analyses were conducted to determine the influence of the parameter values on the points of departure for the AEGL derivations. The results are summarized in Table 6 below. The results indicate that the points of departure were most sensitive to the exposure concentration, the richly perfused tissues partition coefficient, and the alveolar ventilation rate. Metabolic parameters had no significant impact on the AEGL 3 POD and a moderate influence on the AEGL 2 POD.

Table 6. Sensitivity Analyses for Points of Departure in the Rat

Parameter[a]	Normalized sensitivity coefficients	
	AEGL 2 POD (AUCR estimate)	AEGL 3POD (Peak CR estimate)
Body weight (BW)	--[b]	-0.12
Alveolar ventilation rate (KQP)	0.76	0.61
Fractional volume of adipose tissues (KVF)	-0.18	-0.38
Blood:air partition coefficient (PB)	-0.16	--
Fat:air partition coefficient (KPF)	-0.18	-0.38
Richly perfused tissues:air partition coefficient (KPR)	1.0	0.99
Maximum metabolic capacity, high affinity pathway (KVMAX)	-0.32	--
Maximum metabolic capacity, low affinity pathway (KVMAX2)	-0.23	--
Michaelis constant for low affinity pathway (KM2)	0.13	--
Exposure concentration (CONC)	1.4	1.1

[a]Parameter abbreviations in parenthesis are the terms used in the model code
[b]Parameter sensitivity coefficients were not listed in the table if |SC|<0.1

A time course sensitivity analysis was conducted for the predicted concentration of ethylbenzene in richly perfused tissues (CR) of humans exposed to 910 ppm ethylbenzene for up to eight hours (the potential AEGL 3, if a UF = 3 is applied). Results are shown in Figures 1 and 2 for those parameters for which |SC| was >0.1 at some point between 0.25 and 8 hours. The values of the metabolic parameters had no significant impact on CR at any point during the simulation. CR was most sensitive to the exposure concentration, the richly perfused tissues partition coefficient, and the alveolar ventilation rate. The blood flow rate, volume, and partition coefficient for the slowly perfused tissues have some influence on CR at early time points, but the effect decreases toward the end of the exposure period.

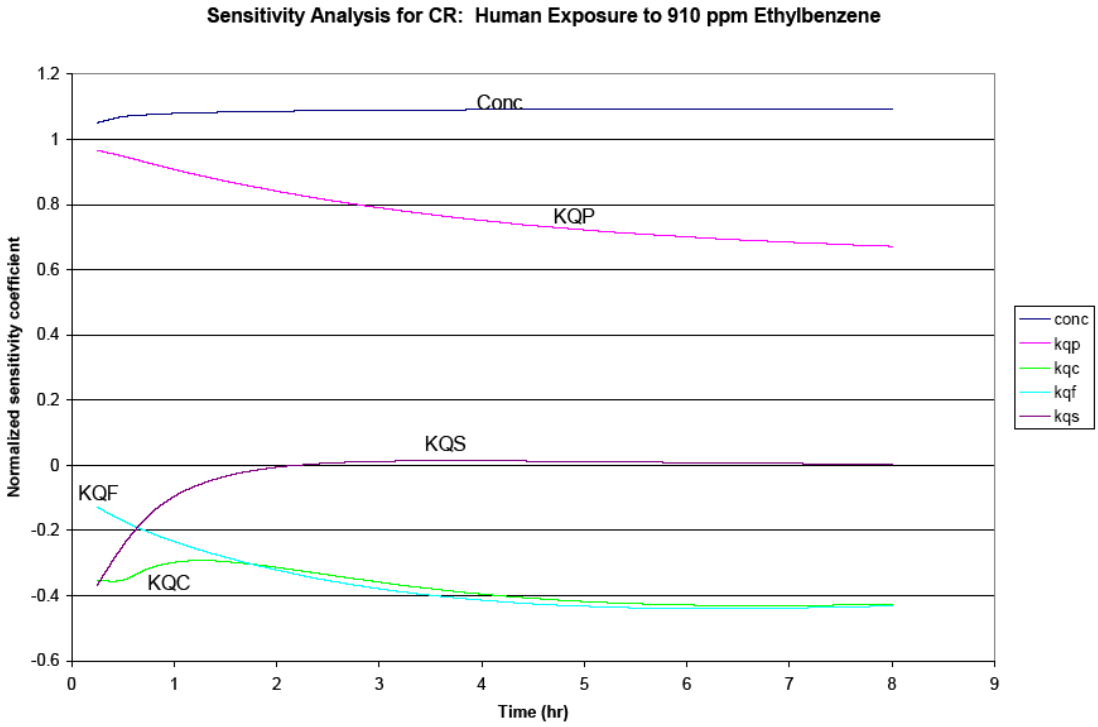

Figure 1. Sensitivity time course for the predicted concentration of ethylbenzene in human richly perfused tissues for up to eight hours exposure to 910 ppm ethylbenzene.

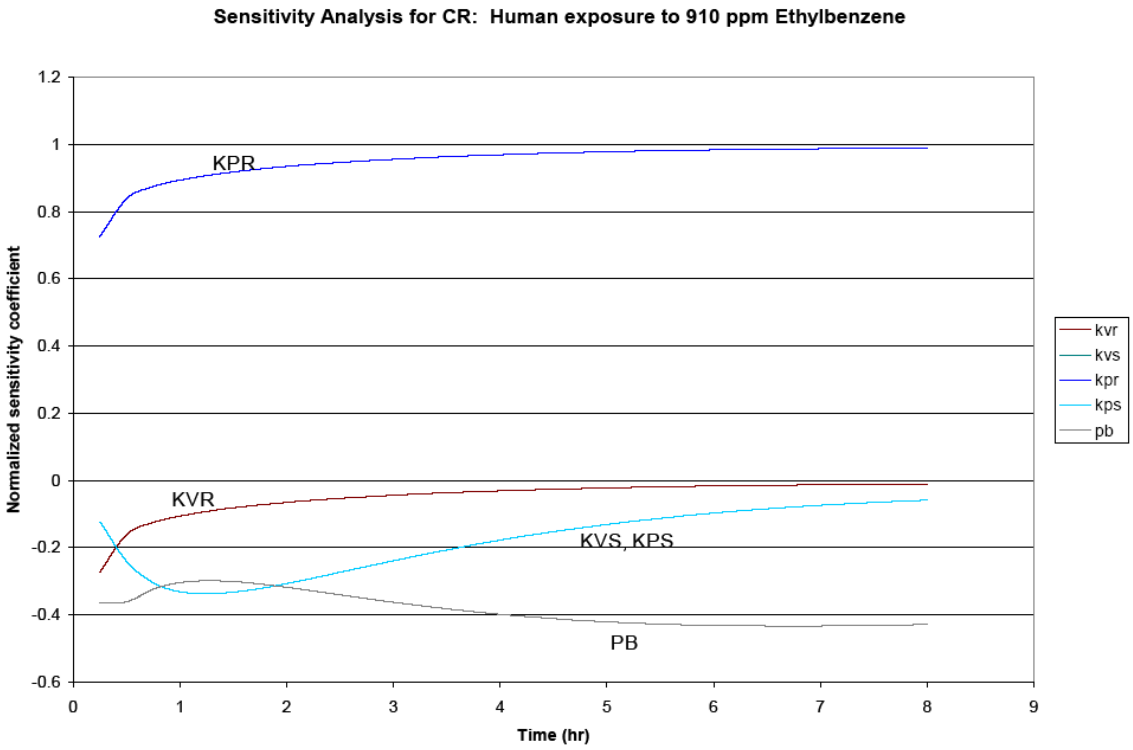

Figure 2. Sensitivity time course for the predicted concentration of ethylbenzene in human richly perfused tissues for up to eight hours exposure to 910 ppm ethylbenzene.

The potential impact of human model parameter values on potential AEGL 2 values was assessed by conducting a sensitivity analysis of AUCR predictions for humans exposed to 120 ppm ethylbenzene for eight hours. The analysis was limited to one time point because at shorter durations (10 minutes to 1 hr), the calculated AEGL 2 values were typically superceded by the lower AEGL 3 values (Tables 1-5) and sensitivity of CR predictions generally did not change appreciably between 4 and 8 hrs at a higher exposure concentration (Figures 1 and 2). The results are summarized in Table 7. The AUCR prediction was most sensitive to the exposure concentration, alveolar ventilation rate, and the richly perfused tissues:air partition coefficient. The prediction was also moderately sensitive to the blood:air partition coefficient and maximum metabolic capacity.

Table 7. Sensitivity Analysis for Human AUCR

Parameter[a]	Normalized Sensitivity Coefficient
Alveolar ventilation rate (KQP)	1.1
Cardiac output (KQC)	-0.33
Fractional blood flow to adipose tissues (KQF)	-0.22
Fractional blood flow to the liver (KQL)	-0.12
Blood:air partition coefficient (PB)	-0.55
Richly perfused tissues:air partition coefficient (KPR)	1.0
Maximum metabolic capacity (KVMAX)	-0.58
Michaelis constant (KM)	0.21
Exposure concentration (CONC)	1.3

[a]Parameter abbreviations in parenthesis are the terms used in the model code. Parameter sensitivity coefficients were not listed in the table if |SC|<0.1

AEGL Confidence with Respect to PBPK Modeling

The AEGL PODs in rats were most sensitive to parameter values that are generally considered to be well characterized—the exposure concentration, physiological parameters, and the partition coefficients. There is slightly less confidence in the AEGL 2 POD than the AEGL 3 POD because the AEGL 2 POD was somewhat sensitive to the metabolic parameters, which are frequently less confidently determined than other parameters. Likewise, the AEGL 3 HECs also lack sensitivity to metabolic parameters, while the AEGL 2 has some sensitivity to the values determined for the metabolic capacity. However, since the human model lacks a "low affinity" metabolism pathway for ethylbenzene, any error in the model parameters is most likely to produce an overestimate of blood and tissue ethylbenzene models, and hence is conservative with respect to the AEGL endpoints. Overall, confidence is high with respect to the AEGL recommendations derived using PBPK modeling.

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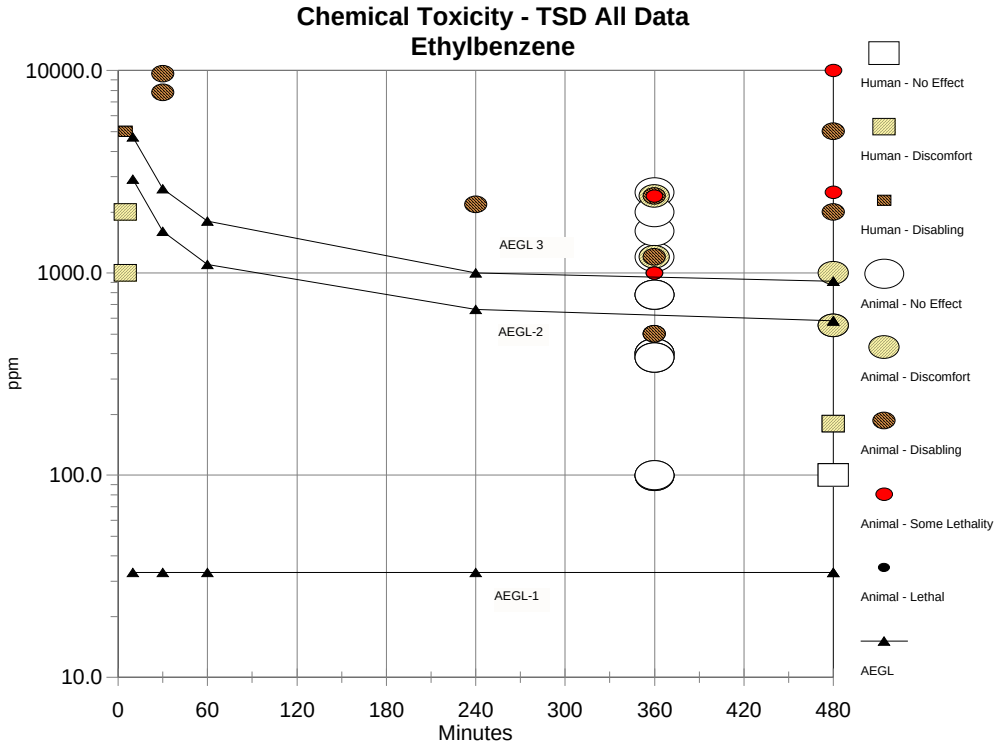
APPENDIX E: Time-scaling Category Plot for Ethylbenzene



Source	Species	Sex	# Exposures	ppm	Minutes	Category	Comments
NAC/AEGL-1				33	10	AEGL	
NAC/AEGL-1				33	30	AEGL	
NAC/AEGL-1				33	60	AEGL	
NAC/AEGL-1				33	240	AEGL	
NAC/AEGL-1				33	480	AEGL	
NAC/AEGL-2				2900	10	AEGL	
NAC/AEGL-2				1600	30	AEGL	
NAC/AEGL-2				1100	60	AEGL	
NAC/AEGL-2				660	240	AEGL	
NAC/AEGL-2				580	480	AEGL	
NAC/AEGL-3				4700	10	AEGL	
NAC/AEGL-3				2600	30	AEGL	
NAC/AEGL-3				1800	60	AEGL	
NAC/AEGL-3				1000	240	AEGL	
NAC/AEGL-3				910	480	AEGL	
Yant et al. 1930	human	m		1000	5	1	Eye irritation, lacrimation; decreased severity after a

							minute or two
Yant et al. 1930	human	m		2000	5	1	Eye and throat irritation; decreased severity with duration
Yant et al. 1930	human	m		5000	5	2	Intolerable
Bardodej and Bardodejova 1961	human	?		100	480	0	No effects
Bardodej and Bardodejova 1961	human	?		180	480	1	Irritation, headache, sleepiness
Cappaert et al. 2002	guinea pig	f	4	2500	360	0	No adverse effects
Cappaert et al. 2002	guinea pig	f	1	2500	480	SL	One death; animals motionless or lethargic
Yant et al. 1930	guinea pig	?		1000	480	1	Initial clinical signs of irritation disappeared after 30 minutes
Yant et al. 1930	guinea pig	?		2000	480	2	Signs of irritation, unsteadiness, ataxia
Yant et al. 1930	guinea pig	?		5000	480	2	Signs of irritation, unsteadiness, ataxia; tremors, unconciousness
Yant et al. 1930	guinea pig	?		10000	480	SL	Two deaths after 2 hours
Bio/dynamics 1986	rat	m	4	400	360	0	Lacrimation after 3 days
Bio/dynamics 1986	rat	m	4	1200	360	1	Lacrimation; dcr wt gain
Bio/dynamics 1986	rat	m	4	2400	360	SL	One death after first exposure; two each on days 2 and 3
Bio/dynamics 1986	mice	m	4	400	360	0	Lacrimation after day 3
Bio/dynamics 1986	mice	m	4	1200	360	2	Lacrimation, prostration; death after day 3
Bio/dynamics 1986	mice	m	4	2400	360	2	Lacrimation, prostration; death after day 2
Bio/dynamics 1986	rabbit	m	4	400	360	0	Lacrimation after day 3
Bio/dynamics 1986	rabbit	m	4	1200	360	0	Lacrimation after day 2
Bio/dynamics 1986	rabbit	m	4	2400	360	1	Lacrimation
Cragg et al. 1989	rabbit	m/f	5 d/wk; 4 wk	382	360	0	No clinical signs
Cragg et al. 1989	rabbit	m/f	5 d/wk; 4 wk	782	360	0	No clinical signs
Cragg et al. 1989	rabbit	m/f	5 d/wk; 4 wk	1610	360	0	No clinical signs; weight loss during first week
Cappaert et al. 2002	rat	f	5	550	480	1	No clinical effects; ototoxicity
Molnar et al. 1986	rat	m		2180	240	2	Minimum narcotic concentration

Andersson et al. 1981	rat	m	3	2000	360	0	No clinical effects
Cragg et al. 1989	rat	m/f	5 d/wk; 4 wk	99	360	0	No clinical effects
Cragg et al. 1989	rat	m/f	5 d/wk; 4 wk	382	360	0	No clinical effects
Cragg et al. 1989	rat	m/f	5 d/wk; 4 wk	782	360	0	No clinical effects; increased liver wt
Nielsen and Alarie 1982	mice	m		7800	30	2	Sedation
Nielsen and Alarie 1982	mice	m		9640	30	2	Sedation
Cragg et al. 1989	mice	m/f	5 d/wk; 4 wk	99	360	0	No clinical effects
Cragg et al. 1989	mice	m/f	5 d/wk; 4 wk	382	360	0	No clinical effects
Cragg et al. 1989	mice	m/f	5 d/wk; 4 wk	782	360	0	No clinical effects; increased liver wt
Stump 2003	rat	m/f	At least 4 wk	100	360	0	No clinical effects in either generation
Stump 2003	rat	m/f	At least 4 wk	500	360	2	Dcr wt gain and food consumption
Stump 2003	rat	m/f	At least 4 wk	1000	360	SL	Reduced offspring survival

For Category: 0 = No effect, 1 = Discomfort, 2 = Disabling, SL = Some Lethality, 3 = Lethal