Benzene, ethyl-: Human health tier II assessment

30 June 2020

CAS Number: 100-41-4

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using he Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted

to address specific concerns that could not be resolved during the Tier II assessment.

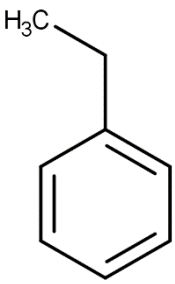
These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit: **www.nicnas.gov.au**

Disclaimer

Chemical Identity

Synonyms	Ethylbenzene Phenylethane Ethylbenzol
Structural Formula	
Molecular Formula	C8H10
Molecular Weight (g/mol)	106.167
Appearance and Odour (where available)	Liquid.
SMILES	c1(CC)ccccc1

Import, Manufacture and Use

Australian

The National Pollutant Inventory (NPI) holds data for all sources of emission of the chemical in Australia. The following domestic uses have been identified through: the NPI (2019) and the NHMRC (2011).

The chemical has reported domestic uses in consumer products including:

- household paints, paint thinners, paint and varnish removers;
- automotive paints, primers and polishes;
- floor and furniture polishes; and
- oven, tile and upholstery cleaning and sanitation agents.

The chemical has reported commercial uses including:

- as a component of gasoline (2 % w/w);
- production of plastic foam insulation in building and construction; and
- a constituent of asphalt and naphtha.

The chemical has reported site-limited use including as a precursor/intermediate to produce styrene and synthetic polymers.

The chemical has non-industrial uses in herbicides and insecticides.

International

The following international uses have been identified through: the European Union (EU) Registration, Evaluation and Authorisation of Chemicals (REACH) dossiers; the European Chemicals Agency (ECHA) risk assessment report (RAR); Environmental Health Criteria (EHC); Galleria Chemica; eChemPortal; the United Stated Environmental Protection Agency (US EPA) Consumer Product Information Database (CPID) and Chemical Products and Categories (CPCat) database; and the European Commission Cosmetic Ingredients and Substances (CosIng) database.

The chemical is not listed on the International Fragrance Association (IFRA) transparency list (IFRA, 2017). The IFRA Transparency List is an ordered register of all fragrance ingredients used in consumer goods by the fragrance industry's customers worldwide. It represents a snapshot of all the ingredients used in active formulas at the time of publication.

The chemical has reported domestic uses including:

- as a component of sealants, fillers and adhesives;
- as a component of paints and drawing supplies;
- in cleaning products (detergents and surfactants); and
- as an aerosol propellant.

The US EPA’s Consumer Product Information Database (CPID) lists the majority of domestic products containing ethylbenzene as having a concentration of ≤5 % of the chemical. Few listed products, including mixed xylenes (for thinning paints, epoxy, adhesives and varnishes), sealants, fuel additives and some spray paints, have concentrations of ethylbenzene in the range of 10-30 %.

The chemical has reported commercial uses including:

- as a thinning agent in paints, varnishes, lacquers, adhesives and glues;
- in the maintenance and repair of motor vehicles, ships and boats;
- as insulation and fillers, plastics, and building materials in the building and construction industry;
- as a bleaching agent;
- as a colouring agent;
- as a welding and soldering agent;
- as a wood preserving agent;
- as a component of fuel for heating or for use in motorised vehicles; and

- in lubricant products such as greases and oils.

The chemical has site-limited use including:

- primarily as a precursor/intermediate to produce styrene;
- as a chemical intermediate to produce acetophenone, cellulose acetate, diethylbenzene and propylene oxide;
- as a constituent in commercial xylenes (known as mixed xylenes) used as a component of solvents, and
- can be blended into gasoline as an anti-knock agent;
- as a solvent in the rubber and chemical manufacturing industries, and as a process solvent/reactant in
- unsaturated polyesters;
- in the manufacture of electronic equipment including computers and optical products;
- in the manufacture of machinery, metals, paints, paper products and plastics;
- as a fracking additive in hydraulic fracturing processes;
- as a negative photoresist solvent in the semiconductor industry; and
- in coating agents, furniture manufacture, inks and toners.

The chemical has non-industrial use in agriculture as a component of pesticides.

Restrictions

Australian

The chemical is listed in the National Health and Medical Research Council (NHMRC) National Water and Quality Management Strategy, Australian Drinking Water Guidelines 6, with a guideline value for health effects of 0.3 mg/L (NHMRC, 2011).

The NSW Government Department of Planning, Industry and Environment has banned the use of volatile organic compounds benzene, toluene, ethylbenzene and xylene (BTEX) in all coal seam gas (CSG) drilling and hydraulic fracturing activities under *the Petroleum (Onshore) Act 1991*, effective from 2012.

International

The World Health Organisation (WHO) Guidelines for Drinking Water Quality (Guideline values for chemicals that are of health significance in drinking water) recommends a limit of 0.3 mg/L of the chemical (Galleria Chemica).

The chemical is subject to restrictions within the EU (ECHA, 2020):

Directive 94/33/EC for the Protection of Young People at Work, whereby persons under 18 years of age may not be exposed to the chemical in the workplace.

Directive 2010/75/EU – Annex VII – Organic Solvents, which defines emission limit values for polluting substances released as industrial emissions under the Integrated Pollution Prevention and Control (IPPC) Directive. Volatile organic compounds are limited to an emission value of ≤ 150 mg/m³ under all listed industrial activities (e.g. use as a coating agent, varnish or cleaning agent).

Directive 98/24/EC, which lays down the minimum requirements for the protection of the health and safety of workers from the risks related to chemical agents at work.

Existing Work Health and Safety Controls

Hazard Classification

The chemical is classified as hazardous, with the following hazard categories and hazard statements for human health in the Hazardous Chemical Information System (HCIS; Safe Work Australia):

Acute toxicity – Category 4; H332 (Harmful if inhaled)

Eye irritation – Category 2A; H319 (Causes serious eye irritation)

Skin irritation – Category 2; H315 (Causes skin irritation)

Exposure Standards

Australian

The chemical has an exposure standard of 100 ppm (434 mg/m³) time-weighted average (TWA) and 125 ppm (543 mg/m³) short-term exposure limit (STEL) (Safe Work Australia). In January 2020 Safe Work Australia reviewed and recommended a change to this workplace exposure standard (Safe Work Australia, 2020).

International

The following exposure standards are identified (Galleria Chemica):

- a TWA of 434 – 442 mg/m³ (100 ppm) in different countries such as New Zealand, the USA (NIOSH), Canada (Yukon), throughout EU member countries and the UK;
- a TWA of 88 mg/m³ (20 ppm) in Canada (British Columbia), which is equivalent to the threshold limit value (TLV) in the USA (US ACGIH), and the maximum concentration value at the workplace (MAK) in Germany;
- a STEL of 543 – 552 mg/m³ (125 ppm) in New Zealand, the USA (NIOSH) and the UK; and
- a STEL of 884 mg/m³ (200 ppm) in EU member countries (EU occupational exposure value).

The US Occupational Safety and Health Administration (OSHA) has set a legal limit of 100 ppm ethylbenzene in workplace air averaged of an 8-hour work day (ATSDR, 2010).

Health Hazard Information

Toxicokinetics

Ethylbenzene is readily absorbed following inhalation, oral, and dermal exposures; distributed throughout the body; and excreted primarily through urine. There are two different metabolic pathways for ethylbenzene with the primary pathway being the α-oxidation of ethylbenzene to 1-phenylethanol, mostly as the R-enantiomer. The pattern of urinary metabolite excretion varies with different mammalian species. In humans, ethylbenzene is excreted in the urine as mandelic acid and phenylgloxylic acids; whereas rats and rabbits excrete hippuric acid and phenaceturic acid as the main metabolites (OECD, 2005).

Acute Toxicity

Oral

Based on the reported median lethal doses (LD50) in experimental animals, the chemical has low acute oral toxicity (LD50 = 3500 mg/kg bodyweight (bw) in rats) (ChemIDPlus, REACH).

The chemical or mixtures containing the chemical could have the potential to cause chemical pneumonitis if aspirated. This would be dependent on the viscosity of the chemical as introduced. The threshold viscosity value for classification as an aspiration hazard is 20.5 mm^2/s at 40 °C.

Dermal

The chemical has low acute dermal toxicity based on results from animal tests following dermal exposure (LD50 = 17,800 mg/kg bw in rabbits) (REACH).

Inhalation

The chemical is classified as hazardous in the HCIS (Safe Work Australia) as ‘Acute toxicity – Category 4; H332 (Harmful if inhaled)’. The reported LD50 in rats (≤ 20 mg/L/4 hours) supports this classification.

Exposure to ethylbenzene ≥ 1432 ppm (6.2 mg/L) for 5-30 min caused 50 % respiratory depression in mice (ATSDR, 2010).

The following LC50 values have been obtained for ethylbenzene inhalation in different strains of rat (ATSDR, 2010; OECD, 2005):

- 58.1 mg/L (13367 ppm) over 2 h in rats
- 17.4 mg/L (4000 ppm) over 4 h in Carworth Wistar rats
- 5.2 mg/L (1200 ppm) for 6 h/d for 4 days in Fischer 344 rats

Observation in humans

The following values are reported as the lowest published toxic concentration in humans in various studies (Galleria Chemica):

- 4.35 mg/L;
- 8.7 mg/L/6-minutes;
- 10 ppm (0.044 mg/L)/4-hours; and
- 21.7 mg/L

Corrosion / Irritation

Respiratory Irritation

Based on the available data ethylbenzene may cause sensory and respiratory tract irritation (see **Observations in humans**), but limited data and confounding factors in many instances precludes classification.

The primary source of epidemiological information on human exposure to ethylbenzene is from factory workers exposed to mixed solvents, including xylenes, toluene, benzene and styrene. As such, the precise role of ethylbenzene cannot be ascertained in the onset of toxic effects in many cases (ATSDR, 2010).

Reduction in breathing rate of 50 % was observed in mice exposed to 1432 ppm ethylbenzene for 5 min, which was attributed to sensory irritation. Nasal irritation was observed in guinea pigs (expressed as excessive rubbing of the nose) exposed to 1000 ppm of the chemical for ≥ 3 min.

Guinea pigs exposed to sublethal concentration of ethylbenzene (≤ 10000 ppm for < 100 min) showed moderate pulmonary oedema and congestion, which resolved following a 4–8 day recovery period, suggesting that the treatment-related effects were transient (ATSDR, 2010).

Skin Irritation

The chemical is classified as hazardous in the HCIS (Safe Work Australia) as ‘Skin irritation – category 2; H315 (Causes skin irritation)’. The available data are support this classification.

The chemical is reported to cause moderate skin irritation in rabbit studies, particularly following occlusive application of the undiluted chemical (Galleria Chemica):

- Ethylbenzene was evaluated for dermal irritation in rabbits and, under the conditions of the study, was reported as a moderate (grade 4) skin irritant;
- The chemical was moderately irritating to the skin of rabbits under occlusive conditions and caused moderate necrosis;
- The undiluted substance was applied to the intact and abraded skin of the rabbit belly and kept under occlusion for 24 hours. The reaction was classed as moderately irritating.

Eye Irritation

The chemical is classified as hazardous with the hazard category and hazard statement for human health in the HCIS (Safe Work Australia) as follows: Eye irritation – Category 2A; H319 (Causes serious eye irritation). The available data support this classification.

The chemical is reported to cause moderate eye irritation in animal studies (Galleria Chemica):

- Ethylbenzene caused slight conjunctival irritation and no corneal injury. The results of this study suggest that ethylbenzene is slightly irritating to the eye of rabbit.
- In another study, ethylbenzene gave grade 3 results (instillation of 0.5 mL undiluted gives injury up to 5 points while 0.1

mL gives a score of less than 5 points), indicating moderate eye irritation in rabbits.

- In guinea pigs, 5000 and 10000 ppm of ethylbenzene vapour produced immediate and intense irritation of the conjunctiva, while 2000 ppm caused moderate eye and nose irritation within one minute.

Observation in humans

Ethylbenzene caused reversible conjunctival and respiratory tract irritation in 9 volunteers exposed to 25 ppm for 7.5 h. Mucosal irritation was reported by 3 subjects (MAK, 2012).

Volunteers (n = 24) exposed to 10 or 98 ppm ethylbenzene reported limited respiratory irritation. Investigations of nasal respiratory flow and changes in heart rate and respiration rate provided no evidence of exposure-related changes in the chemosensory (rhinological) or autonomous functions (MAK, 2012).

Sensitisation

Skin Sensitisation

Based on the limited information available and quantitative structural activity relationship [(Q)SAR] predictions, the chemical is not considered to be a skin sensitiser.

In a human repeat insult patch test (HPRIT), human subjects (n = 25) exposed to ethylbenzene (10 % in petrolatum) reported negative results (details not available) (OECD, 2005).

QSAR modelling for skin sensitisation using the OECD QSAR Toolbox (version 4.2) indicated that there were no alerts for skin sensitisation.

Modelling using the knowledge based expert system Deductive Estimation of Risk from Existing Knowledge (DEREK) Nexus version 6.2.2 indicated that the chemical was a non-sensitiser. The chemical did not match any structural alerts or examples for skin sensitisation or contained any unclassified or misclassified features. Therefore, the chemical were predicted to be a non-sensitiser.

Repeated Dose Toxicity

Oral

Based on available data, the chemical is not expected to cause severe health effects following repeated oral exposure, apart from hearing damage at high doses of the chemical (900 mg/kg bw/day).

In a sub-chronic study, Wistar rats (10/sex/dose) were given repeated oral gavage doses of the chemical in corn oil at 0, 75, 250 and 750 mg/kg bw/day twice daily, with 8-hour interval for 3 months (OECD TG 408). There were no mortalities. Clinical signs of toxicity were post dose salivation at 250 and 750 mg/kg bw/day and discolouration of urine at 750 mg/kg bw/day. Salivation could be due to local irritation to the upper digestive tract. The urine finding was unexplained because no urine discoloration was seen during the urinalysis. There were no treatment related findings in home cage observations, open field observations, or sensorimotor tests or reflexes. Body weight in males at 750 mg/kg bw/day was significantly decreased from day 28, being 13.8 % below control values on day 91. There were no significant treatment related changes in body weight or body weight change in males at lower dose levels, or in females at any dose level. There were no treatment related changes in ophthalmological examination and in gross pathology. The only histopathologic finding attributed to treatment was an increase of hyaline droplet storage in the tubular epithelium of male rat kidneys. The increase in hyaline droplets was considered to result from an increase in production of the male specific protein a-2u-globulin.

Morphologic signs of chronic progressive nephropathy were similar in the control and treatment groups. The no observed adverse effect level (NOAEL) was considered to be 75 mg/kg bw/day with a lowest observed adverse effect level (LOAEL) of 250 mg/kg bw/day based on changes in haematology, indicative of a mild regenerative anaemia together with changes in clinical chemistry parameters, increased liver weights and centrilobular hepatocellular hypertrophy indicative of hepatic microsomal enzyme induction (REACH).

The chemical was administered to Sprague Dawley rats by gavage at 900 mg/kg bw/day, 5 days/week for 2 weeks. No clinical effects or body weight changes were reported. Complete loss of outer hair cells (OHCs) and minute loss of inner hair cells in the cochlear, indicating hearing loss was observed. The data suggest that the chemical is specifically toxic to hearing organs.

Ethylbenzene quantitatively demonstrated the highest ototoxic potency compared to other aromatic solvents, such as toluene, styrene or xylenes (ECHA, 2010).

Dermal

No data are available.

Inhalation

Based on the available animal and human data (see **Observations in humans**), the chemical causes damage to hearing organs following repeated inhalation exposure. Other treatment-related systemic effects were not considered severe. Hazard classification for ototoxicity is warranted given that the concentrations used within key studies align with the GHS guidance value range for classification (0.2-1.0 mg/L) (ECHA, 2012; GHS, 2009).

In a repeat dose toxicity study, male Sprague Dawley rats (14/dose) were exposed to ethylbenzene (0, 200, 400, 600 and 800 ppm; equal to 0, 0.87, 1.74, 2.60 and 3.47 mg/L) by inhalation for 6 h/day, 6 days/week for 13 weeks. Electrophysiological measurements were recorded at the end of the 4th, 8th and 13th weeks of exposure, and following 8 weeks of recovery (week 21). No significant weight difference between exposed and control groups, and no severe systemic toxicity, were observed. Audiometric thresholds were measured from the 4th week onward and were higher in animals exposed to ≥400 ppm ethylbenzene. The highest degree of hearing loss was observed in groups exposed to 600 and 800 ppm. The magnitude of auditory damage persisted unchanged at weeks 4 and 13 of exposure and 8 weeks following final exposure, demonstrating that the damage resulting in hearing loss was permanent. Shifts in audiometric thresholds were less in animals exposed to 400 ppm, and no shift was observed in control animals or in the 200 ppm exposed group (CLH, 2010; Gagnaire et al., 2007).

Exposure to 600 and 800 ppm ethylbenzene caused near complete loss in the three rows of outer hair cells (OHCs) in the organ of Corti in the mid- to low-frequency region of the cochlear; this is a more sensitive endpoint than auditory threshold and the most commonly reported cause of hearing loss. There were also losses of inner hair cells (IHCs) in the basal (high-frequency) part of the organ of Corti, reaching 32 and 14 % in the 800- and 600-ppm exposed groups, respectively. Exposure to 400 ppm ethylbenzene caused considerable OHC losses, with highest loss in the third (outer) and lowest in the first (inner) row, with some IHC losses in the basal region. Exposure to 200 ppm caused significant losses in OHC in the third row (up to 30 %) of the OHCs in four out of eight animals. The average losses in the third row of OHCs of the animals exposed to 200 ppm ethylbenzene were 4 %. A lowest observed adverse effect concentration (LOAEC) of 200 ppm (0.87 mg/L) was reported (Gagnaire et al., 2007).

Significant and permanent adverse auditory effects were reported in male rats (Wistar and Wag/Rij strains) following inhalation exposures to ethylbenzene (8 hours/day, 5 days/week) following both acute (1 week) and subchronic (13 weeks) durations. Acute exposure to ethylbenzene (300 – 800 ppm) resulted in a dose-dependent loss of OHCs between 25 % to 66 % (Cappaert et al, 2000; CLH, 2010).

At doses of 300 and 400 ppm ethylbenzene, the simultaneous application of significant auditory stimulation (noise at 105 dB) produced a synergistic effect, resulting in greater OHC loss than the sum of those due to ethylbenzene or noise exposures alone (Cappaert et al, 2001; CLH, 2010).

In a sub-chronic study, New Zealand White rabbits (5/sex/dose) were exposed to 0, 400, 800 or 1600 ppm (1.7, 3.4, 7.1 mg/L) of the chemical 6 hr/day, 5 days/week for four weeks. The lowest observed effect concentration (LOEC) was 1600 ppm (no adverse effects, but reduced body weight at this dose level) in rabbits after four weeks' exposure to the chemical vapour (REACH). The 28-day NOAEC was 728 ppm (3390 mg/m^3) in rabbits (OECD, 2005).

In 4-week repeat dose inhalation (whole body exposure) studies with ethylbenzene vapour, rats and mice (M/F) had increased liver weights at ≥ 382 ppm. Haematology changes in rats were reported at 782 ppm (small increases in leukocyte counts); male rats had increased platelet counts at 782 ppm. There were no effects on mortality, clinical chemistry observations, urinalysis and gross/microscopic (including ophthalmologic) observations. The reported LOEC was 382 ppm (1656 mg/m^3) in rats and mice (REACH).

Susceptibility to ethylbenzene is species dependent. No adverse auditory effects were observed in female guinea pigs exposed to 2500 ppm ethylbenzene for 5 days (Cappaert et al., 2002; CLH, 2010).

Observation in humans

Epidemiological studies provided evidence of systemic effects in workers occupationally exposed by inhalation to organic solvent

mixtures, including ethylbenzene. However, the systemic effects of ethylbenzene alone remains unclear due to the presence of other solvents, most of which are typically present in mixtures at higher concentrations than ethylbenzene (ATSDR, 2010).

A positive correlation between occupational exposure to mixed solvents and the risk of developing hearing loss, with odds ratios (OR) estimated to be 1.37, 3.25 and 4.5 for low, moderate and high exposure levels, respectively was reported (Hormozi et al., 2017). Exposure indices (EI) were calculated by dividing the sum of the mean time-weighted exposure to each solvent by the occupational exposure limit (threshold limit value, TLV) recommended by the ACGIH (20 ppm TWA). Low, moderate and high level exposures were defined as having EI <0.5, EI = 0.5-1 and EI >1, respectively, where EI >1 indicates that the organic solvent mixture concentration exceeded the TLV. Analysis suggested that increasing concentrations of mixed solvents are causally associated with developing hearing loss, even when the concentrations of individual solvents are within exposure limits. Further, increasing the number of solvents present within a mixture increases the risk of developing hearing loss, as does the duration of exposure (≥ 5 years). Simultaneous exposures to noise and organic solvent mixtures significantly increases the risk of hearing loss relative to solvent exposure or noise alone (2 to 11-fold), suggestive of an additive or synergistic effects. It was also identified that the latency period for developing hearing loss from solvent exposures varies from 2 to 3 or even ≥ 5 years following exposure. The mode of action (MOA) was not identified by this study, nor was the precise role of each individual solvent. (Hormozi et al., 2017).

A cross-sectional epidemiological study of house painters (n = 105) exposed to workplace ethylbenzene concentrations up to 12.9 mg/m³ (3 ppm) revealed significant neurobehavioural changes including change in personality and short-term memory capacity compared to non-exposed controls (n = 53). In a subgroup of exposed house painters with pre-narcotic symptoms, the differences were found to be more pronounced. No definitive conclusions on the causative agent for these effects can be drawn from these data due to concomitant exposure to other organic solvents (ethyl acetate, toluene, butyl acetate, methyl isobutyl ketone and xylene) (US EPA, 2007).

Workers (n = 35) occupationally exposed to an average concentration of 1.64 ppm ethylbenzene over an average of 8.2 years employment demonstrated increased levels of lymphocytes (41.5–68.8 %) and lower haemoglobin values (5.2–7.1 %) relative to an unexposed control group. Workers were concurrently exposed to other chemicals (xylenes, lead, toluene); therefore, the role of ethylbenzene in causing these adverse haematological effects cannot be established (ATSDR, 2010).

Genotoxicity

Based on the available genotoxicity data, the chemical is not considered genotoxic in the absence of pronounced toxicity.

In vitro assays: There are several in vitro genotoxicity tests reporting negative results (OECD, 2005), including:

- six Ames tests using Salmonella typhimurium strains (and one test also with an Escherichia coli strain) with or without metabolic activation (highest doses tested up to ~3200 µg/plate);
- three gene mutation assays in Saccharomyces cerevisiae (one according to OECD TG 481);
- production of p53 tumour suppressor protein in the mouse fibroblast cell line NCTC 929;
- a cell transformation assay using embryonic cells of a Syrian Golden Hamster (62-1000 µg/mL);
- sister chromatid exchanges in Chinese hamster ovary (CHO) cells (~99.5 µg/mL) with or without metabolic activation (dose range limited by toxicity);
- mouse lymphoma (L5178Y TK+/-) forward mutation assay with or without metabolic activation;
- chromosomal aberration test using the CHO cell lines with or without metabolic activation up to 125 µg/mL (dose range restricted by cytotoxicity at 150 µg/mL) and;
- a chromosomal aberration test with rat liver RL1 cells up to 100 µg/mL (cytotoxic above this dose).

Ethylbenzene gave positive results in the Syrian Hamster Embryo (SHE) cell transformation assay following seven day exposure at 150 and 200 µg/mL, but gave negative results at 100 and 125 µg/mL. Results were negative after 24 hour exposure at 100-500 µg/mL. According to the authors, a possible explanation for the negative 24 hour and the positive 7 day exposure result is that the test substance must be continuously present in the culture medium for the induction of morphological transformations (OECD, 2005).

A micronucleus test with SHE cells produced a statistically significant dose-related increase in numbers of micronucleated SHE cells compared to controls at dose range 25-200 µg/mL. The cell line used was reported to have some metabolic competence

(OECD, 2005).

Ethylbenzene was evaluated in a mouse lymphoma assay (mouse lymphoma cells L5178Y at doses 10 to 160 µg/mL) in the absence of metabolic activation. A positive response was observed at a single cytotoxic dose level (80 µg/mL) in each of two independent assays. Positive results, accompanied by substantial increases in cytotoxicity, complicate interpretation of results. In the absence of a statistically significant dose response trend or peak response, this study was considered negative in NTP TR466, but has been reported as positive by the US EPA gene toxicology program (Mitchell et al., 1997) (OECD, 2005).

Ethylbenzene was evaluated for gene mutation in mouse lymphoma L5178Y cells (soft agar method) (OECD TG 476). The assay was conducted both with and without metabolic activation. Six independent experiments were carried out using a treatment period of 4 hours and duplicate cultures. The second study did not confirm the findings of the first experiment. The third study in the absence of metabolic activation corroborated the lack of mutagenicity observed in the second study. However, with metabolic activation (with S9) the increased cytotoxicity experienced at the same dose levels used in the earlier studies resulted in a total lack of viable cultures. When the study was eventually repeated in the presence of S9 using a much lower dose range there was no evidence of an increased mutation rate even at cytotoxic dose levels. In view of the variability in cytotoxicity and lack of reproducibility of the positive response the results of this study are considered equivocal. Ethylbenzene is considered to be non-mutagenic in this mouse lymphoma assay, since the effects observed in the first experiment were not reproduced in two additional experiments carried out independently of each other. The author concluded that the findings of the first experiment were caused by toxicity-related secondary effects and did not indicate a true mutagenic potential of the test substance. However, the results of the experiments have to be regarded as ambiguous (OECD, 2005).

In a sister chromatid exchange assay with human lymphocytes without metabolic activation, ethylbenzene produced a marginal, though significant, positive effect ($p<0.01$) at the highest toxic dose (about 30 % reduction in differentially stained cells vs. controls). The coefficient of linear regression was significant indicating a dose relationship in spite of the extremely weak overall effect. The marginal positive response, reported at only one cytotoxic dose level, was not confirmed using an independent experiment. The lack of documentation available for assessment and non-standard methodology calls into question the validity of this observation (OECD, 2005).

QSAR predicted ethylbenzene as a non-mutagen based on weight-of-evidence (OECD, 2005).

Ethylhydroquinone (EHQ) and 4-ethylcatechol (EC) were identified as minor metabolites in the metabolism of ethylbenzene. These metabolites were shown to cause DNA damage in the presence of Cu(I), they also induced the formation of the DNA adduct 8-oxo-7,8-dihydro-2'-deoxyguanosine in calf thymus DNA. The enhancing effect of nicotinamide adenine dinucleotide (NADH) on oxidative damage by EC could suggest that reactive species are generated in the redox cycle. These active dihydroxylated ethylbenzene metabolites (EHQ and EC) would be involved in the mechanism of ethylbenzene carcinogenesis (OECD, 2005).

In vivo assays: Ethylbenzene did not induce micronucleus formation in peripheral blood erythrocytes of mice following treatment up to the maximum tolerated concentration of 4.74 mg/L (1000 ppm). In another micronucleus assay in mice, there was no increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of NMRI mice following intraperitoneal administration of ethylbenzene up to 645 mg/kg bw (twice with an interval of 24 h). In an autoradiographic technique, ethylbenzene gave negative results for unscheduled DNA synthesis in the liver of B6C3F mice (OECD, 2005).

A metabolite of ethylbenzene (1-phenyl ethanol) administered to mice at dose levels up to 750 mg/kg/day did not increase the rate of development of micronuclei in polychromatic erythrocytes. At this dose level there were overt clinical signs of toxicity (OECD, 2005).

Henderson et al. (2007) concluded that both in vitro and in vivo tests have been predominantly negative in the absence of excessive toxicity. Mouse lymphoma gene mutation studies produced a mixed series of responses that have proved difficult to interpret. An increase in morphological transformation of SHE cells was also found. Results from a more relevant series of in vivo genotoxicity studies, including acute and sub-chronic micronucleus tests and the mouse liver UDS assay, indicate a lack of in vivo genotoxic activity.

Carcinogenicity

The chemical is not classified as carcinogenic based on lack of conclusive data.

The International Agency for Research on Cancer (IARC) has classified ethylbenzene as 'Possibly carcinogenic to humans

(Group 2B)' based on inadequate evidence in humans but sufficient evidence in experimental animals for the carcinogenicity (IARC, 2000).

The chemical is considered to be an animal carcinogen, but the relevance of these findings to humans is reported as unknown (OECD, 2005). In the US EPA list of carcinogens, ethylbenzene is listed under category D - not classifiable as to human carcinogenicity (Galleria Chemica).

Some evidence of carcinogenic activity of ethylbenzene was reported (increased incidences of alveolar/bronchiolar neoplasms in male mice and increased incidences of hepatocellular neoplasms in female mice at 1084 and 325 mg/m³, respectively) (MAK, 2012). The increased tumour incidences in rats and mice in the high dose range were considered the result of chronic damage to organ functions, as ethylbenzene causes not only increased cell proliferation but also enzyme induction in those organs in which tumours develop (MAK, 2012).

The US Department of Health and Human Services (2010) reported that no association has been found between the occurrence of cancer in humans and occupational exposure to ethylbenzene.

In a combined chronic toxicity/carcinogenicity study (OECD TG 453, GLP), B6C3F1 mice (50/sex/dose) were exposed (whole-body) to ethylbenzene vapour (99 %) at 0, 75, 250 or 750 ppm for 6 hours/day, 5 days/week for 103 weeks. Mortality or survival was not affected by the treatment. The following were reported during the histopathological examination: At 750 ppm, males had effects in lungs (increased incidence of alveolar epithelial metaplasia); and non-neoplastic effects in liver (syncytial alteration of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis). At 750 ppm females had liver effects (increased incidence of eosinophilic foci). At ≥250 ppm females showed increased incidence of hyperplasia of the pituitary gland pars distalis. At the highest dose, increased incidences of thyroid follicular cell hyperplasia were reported in both males and females. The NOAEL (F/M) was 75/250 ppm (325/1084 mg/m³). The study reported some evidence of carcinogenic activity of ethylbenzene in male mice based on increased incidences of alveolar/bronchiolar neoplasms and in female mice based on increased incidences of hepatocellular neoplasms (REACH; OECD, 2005).

In a 104-week inhalation (whole body) study in F344/N rats exposed to 0, 75, 250, or 750 ppm ethylbenzene vapour, survival rate in males decreased at 750 ppm and mean body weight of males decreased at ≥250 ppm. At 750 ppm, males had increased incidences of renal tubule adenoma and adenoma or carcinoma (combined), and increased incidence of interstitial cell adenoma in the testis. Both sexes had increased incidence of renal tubule adenoma and hyperplasia at 750 ppm. The NOAEL for both sexes was 250 ppm (1084 mg/m³), based on increased incidences of renal lesions seen in experimental animals at 750 ppm. The study reported clear evidence of carcinogenic activity of ethylbenzene in male rats based on increased incidences of renal tubule neoplasms and increased incidence of testicular adenoma. There was some evidence of carcinogenic activity in female rats based on increased incidences of renal tubule adenomas (REACH). The apparent increase in renal tumours was strongly associated with chronic progressive nephropathy, a spontaneous age-related disease in rodents with no identical counterpart in humans (OECD, 2005).

Reproductive and Developmental Toxicity

Based on the available data, the chemical is not expected to cause adverse effects on fertility or development following inhalation exposure.

In a two generation study (OECD TG 416), 30 male and 30 female Sprague Dawley rats (P/F0 generation) were exposed via inhalation to vapours of the chemical at 25, 100 and 500 ppm (6 hours/day); males exposed for 70 days (pre-mating period F0 and F1 generation) and females exposed 70 days prior to mating, through gestation day 20 and from lactation days 5–21 and via oral by gavage at 26, 90 and 342 mg/kg bw/day (divided into 3 equal doses, approximately two hours apart) from lactation days 1–4. There were no adverse effects on reproductive or developmental endpoints at dose levels up to 500 ppm (REACH; MAK, 2012).

Parental toxicity was minimal with treatment related effects confined to transient decreases in bodyweight gain in the highest dose males and increased liver weights in males and females of both generations. In the absence of histopathological change, the organ weight changes were considered adaptive. The NOAEL was 500 ppm for parental systemic toxicity, reproductive toxicity and developmental effects. The no observed effect level (NOEL) is 500 ppm for reproductive toxicity and developmental effects, and 100 ppm for parental systemic toxicity (REACH, MAK, 2012).

In 13 week inhalation studies in rats and mice expsoed to 100, 500, or 1000 ppm (0, 434 or 4335 mg/m³) ethylbenzene vapour, there were no treatment related effects on reproductive parameters, such as testicular morphology, sperm motility, spermatid

counts or the length of oestrus cycle (see **Repeat dose toxicity**) (OECD, 2005).

Inhalation of the chemical at 0, 100 or 1000 ppm (0, 434 or 4335 mg/m³) during gestation days one to 19 in rats resulted in an increased incidence (14 %) of skeletal variations (supernumerary ribs) at the maternal toxic dose of 1000 ppm. There were increased liver, kidney, and spleen weight changes (approximate change of 22 %, 10 %, and 10 %, respectively), without histopathological effects. NOAEL for maternal and developmental toxicity is 100 ppm (434 mg/m³) (OECD, 2005).

In a study designed to investigate the neurotoxic effects on postnatal development in SD rats (described above), the F2 offspring of the 2-generation study (Faber et al. 2006) were subjected to a functional observational battery (postnatal days 4, 11, 22, 45 and 60), a motor activity test (postnatal days 13, 17, 21 and 61), an acoustic startle reaction test (postnatal days 20 and 60), a learning and memory test in the Biel water maze (initiated on postnatal days 26 or 62) and morphometric and histological investigations of the brain and the nervous system (postnatal days 21 and 72). No exposure-related changes were found up to the highest concentration tested of 500 ppm (MAK, 2012).

Rabbits exposed to the chemical at 0, 100 or 1000 ppm during gestation days one to 24 showed no developmental effects. The NOAEL for maternal toxicity was 100 ppm (434 mg/m³) based on increased liver weights. The NOAEL for developmental toxicity is 1000 ppm (4335 mg/m³) (OECD, 2005).

Other Health Effects

Neurotoxicity

Limited human data are available.

Dizziness and vertigo was reported after exposure to high concentrations (2000-5000 ppm) of ethylbenzene (Yant et al., 1930 cited in ATSDR, 2010).

Neurotoxic effects including depressive and narcotic effects have been observed after exposure to other structurally similar aromatic solvents including styrene and toluene. Therefore, ethylbenzene may cause similar effects.

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic long-term effects of ototoxicity following oral or inhalation exposure. The chemical can also cause eye and skin irritation, and is an inhalation hazard.

Public Risk Characterisation

The chemical has reported use in domestic, commercial and site-limited products in Australia (refer **to Import, Manufacture and Use** section). The chemical has reported use as a fuel additive. The majority of products are expected to contain less than 5 % of the chemical; use of higher concentration products would likely be infrequent. At a concentration below 5 %, the chemical in commercial/domestic products will not cause irritation and inhalation hazards to the public.

The public risk from this chemical is not considered to be unreasonable and further risk management is not considered necessary for public safety.

Should additional information to better characterise exposure become available, further assessment may be required.

Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemical at lower concentrations could also occur while using formulated products containing the chemical. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical health effects, the chemical could pose an unreasonable risk to workers if adequate control measures to minimise ocular, oral and inhalation exposure to the chemical are not implemented. The chemical should be appropriately

classified and labelled to ensure that a person conducting a business or undertaking (PCBU) or an employee at a workplace, has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the Hazardous Chemical Information System (HCIS) (Safe Work Australia) (see **Recommendation** section).

NICNAS Recommendation

Assessment of the chemical is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Further risk management is required if this chemical is used in widely used cosmetic and domestic products at high concentrations. The chemical may be recommended for further assessment to evaluate the concentrations and uses in domestic products manufactured or imported into Australia, to identify if an unacceptable risk of exposure exists from the chemical.

Regulatory Control

Work Health and Safety

The chemical is recommended for classification and labelling under the current approved criteria and adopted Globally Harmonized System of Classification and Labelling of Chemicals (GHS) as below.

For mixtures containing the chemical, the aspiration hazard classification should only be applied if the kinematic viscosity criteria for aspiration classification in the GHS is met.

From 1 January 2017, under the model Work Health and Safety Regulations, chemicals are no longer to be classified under the Approved Criteria for Classifying Hazardous Substances system.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Not Applicable	May be fatal if swallowed and enters airways - Aspi. Cat. 1 (H304) Harmful if inhaled - Cat. 4 (H332)*
Irritation / Corrosivity	Not Applicable	Causes serious eye irritation - Cat. 2A (H319)* Causes skin irritation - Cat. 2 (H315)*
Repeat Dose Toxicity	Not Applicable	May cause damage to the hearing organs through prolonged or repeated exposure - Cat. 2 (H373)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labellling of Chemicals (GHS) United Nations, 2009. Third Edition

[*] Existing Hazard Classification. No change recommended to this classification

Advice for consumers

Products containing the chemical should be used according to instructions on the label.

Advice for industry

Control measures

Control measures to minimise the risk from oral/ocular/inhalation exposure to the chemical should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate or minimise risk arising from storage, handling and use of a hazardous chemical are dependent on the physical form and the manner in which the chemical is used. Examples of control measures which may minimise the risk include but are not limited to:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemical from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemical, if valid techniques are available to monitor the effect on the worker's health;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- minimisation of manual processes and work tasks through automation of processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that, the worker does not come into contact with the chemical.

Guidance on managing risks from hazardous chemicals are provided in the Managing Risks of Hazardous Chemicals in the Workplace—Code of Practice available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

References

Agency for Toxic Substances & Disease Registry (ATSDR) 2010. Toxicological Profile for Ethylbenzene. Accessed April 2020 at https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=383&tid=66

Approved Criteria for Classifying Hazardous Substances [NOHSC: 1008(2004)] Third edition. Accessed May 2020 at https://www.safeworkaustralia.gov.au/doc/approved-criteria-classifying-hazardous-substances-nohsc-1008-2004

Cappaert, N. L. M., Klis, S. F. L., Baretta, A.B., Muijser, H. and Smoorenburg, G. F. (2000). Ethyl benzene-induced ototoxicity in rats: a dose-dependent mid-frequency hearing loss. J Assoc. Res. Otolaryngol. 1(4): 292-299

Cappaert, N. L. M., Klis, S. F. L., Muijser, H. and Kulig, B. M. (2002). Differential susceptibility of rats and guinea pigs to the ototoxic effects of ethyl benzene. Neurotoxicol Teratol 24(4): 503-510

Cappaert, N. L. M., Klis, S. F. L., Muijser, H., Kulig, B. M. and Smoorenburg, G. F. (2001). Simultaneous exposure to ethyl benzene and noise: synergistic effects on outer hair cells. Hearing Res 162(1-2): 67-79

ECHA (2007). European Union Risk Assessment Report (EU RAR): Ethylbenzene (CAS No. 100-41-4). Office for Official Publications of the European Communities, Luxembourg. Accessed April 2020 at https://echa.europa.eu/documents/10162/f9b4577b-c57f-439b-b15a-9e268b1d0a58

ECHA (2010). CLH Report (Annex XV dossier) Proposal for Harmonised Classification and Labelling of Ethylbenzene. Submitted November 2010. Accessed April 2020 at https://echa.europa.eu/documents/10162/3e2f9bdd-4ab0-dd90-eb01-982e63ce0d9a

ECHA (2012) Opinion: Proposing harmonised classification and labelling at EU level of ethylbenzene (ECHA/RAC/CLH-O-0000001542-81-03/F). Committee for Risk Assessment (RAC). Accessed April 2020 at https://echa.europa.eu/documents/10162/d01123d0-a4d5-3bd3-6c33-9cdcc1cdf8ce

ECHA (2019). Summary of Classification and Labelling – Ethylbenzene. Last updated April 2019. Accessed April 2020 at https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/274

ECHA (2020). Brief Profile of Ethylbenzene. World Health Organisation, International Programme on Chemical Safety (WHO IPCS). Last updated March 2020. Accessed April 2020 at https://echa.europa.eu/brief-profile/-/briefprofile/100.002.591.

ECHA (2020). REACH Dossier for Ethylbenzene (CAS No. 100-41-4). Last updated March 2020. Accessed April 2020 at https://echa.europa.eu/registration-dossier/-/registered-dossier/15377

European Commission (EC) Cosmetic Ingredients Database (CosIng). Accessed April 2020 at https://ec.europa.eu/growth/sectors/cosmetics/cosing_en

Gagnaire, F., Langlais, C., Grossmann, S. and Wild, P. (2007). Ototoxicity in rats exposed to ethylbenzene and to two technical xylene vapours for 13 weeks. Arch Toxicol 81: 127-143.

Globally Harmonised System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third edition. Accessed May 2020 at http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html

Henderson L, Brusick D, Ratpan F, Veenstra G 2007. A review of the genotoxicity ofethylbenzene. Mutat Res 635:81-89.

Hormozi, M, Ansari-Moghaddam, A, Mirzaei, R, Dehghan Haghighi, J& Eftekharian, F (2017). The risk of hearing loss associated with occupational exposure to organic solvents mixture with and without concurrent noise exposure: A systematic review and meta-analysis. Int J Occup Environ Health, 30, 521-535.

International Agency for Research on Cancer (IARC) (2000). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 77. IARC Working Group on the Evaluation of Carcinoenic Risks to Humans. Lyon, France. Accessed April 2020 at https://www.ncbi.nlm.nih.gov/books/NBK390937/

Kim, JK, Jung, KH, Noh, JH, Eun, JW, Bae, HJ, Xie, HJ, Jang, J-J, Ryu, JC, Park, WS, Lee, JY& Nam, SW (2011). Identification of characteristic molecular signature for volatile organic compounds in peripheral blood of rat. Toxicol Appl Pharm, 250, 162-169.

National Health and Medical Research Council (NHMRC) (2011). Australian Drinking Water Guidelines 2011. Australian Government Department of Health. Last updated May 2019. Accessed April 2020 at https://www.nhmrc.gov.au/about-us/publications/australian-drinking-water-guidelines#block-views-block-file-attachments-content-block-1

National Pollutant Inventory (NPI) 2018/2019 data within Australia – Ethylbenzene from All Sources. Australian Government Department of the Environment and Energy. Last updated 2019. Accessed April 2020 at http://www.npi.gov.au/npidata/action/load/summary-result/criteria/substance/42/destination/ALL/source-type/ALL/subthreshold-data/Yes/substance-name/Ethylbenzene/year/2019

National Pollutant Inventory (NPI) Substance Fact Sheet on Ethylbenzene. Australian Government Department of the Environment and Energy. Last updated November 2018. Accessed April 2020 at http://www.npi.gov.au/resource/ethylbenzene

NSW Government Department of Planning, Industry and Environment (2012). Ban on use of BTEX compounds in CSG activities. Accessed April 2020 at https://www.industry.nsw.gov.au/policies/items/ban-on-use-of-btex-compounds-in-csg-activities

OECD (2005), SIDS Initial Assessment Report (SIAR) Ethylbenzene (CAS No. 100-41-4). Accessed May 2020 at https://hpvchemicals.oecd.org/UI/handler.axd?id=%2032c3b40f-2ebc-41a4-ac06-5097a4b5a71e

Safe Work Australia (SWA) Hazardous Chemical Information System (HCIS). Exposure Standards. Accessed April 2020 at http://hcis.safeworkaustralia.gov.au/ExposureStandards/

Safe Work Australia (SWA) Hazardous Chemical Information System (HCIS). Hazardous Chemical Details and History – Ethylbenzene. Last updated June 2018. Accessed April 2020 at http://hcis.safeworkaustralia.gov.au/HazardousChemical/Details?chemicalID=370

Safe Work Australia Workplace Exposure Standards Review. Accessed June 2020 at https://engage.swa.gov.au/

Sweeney, L. M., Kester, J. E., Kirman, C. R., Gentry, P. R., Banton, M. I., Bus, J. S.& Gargas, M. L. (2015). Risk assessments for chronic exposure of children and prospective parents to ethylbenzene (CAS No. 100-41-4). Crit Rev Toxicol, 45, 662-726

The International Programme on Chemical Safety (IPCS) (1996). Environmental Health Criteria (EHC) 186 – Ethylbenzene. Accessed March 2020 at http://www.inchem.org/documents/ehc/ehc/ehc186.htm

The MAK Collection for Occupational Health and Safety (2012). Ethylbenzene [MAK Value Documentation, 2012]. Accessed June 2020 at https://onlinelibrary.wiley.com/doi/full/10.1002/3527600418.mb10041e5214

United Stated Environmental Protection Agency (US EPA) Chemical and Product Categories (CPCat) Database. Accessed June 2020 at https://actor.epa.gov/cpcat/faces/chemicalUse.xhtml?casrn=100-41-4

United Stated Environmental Protection Agency (US EPA) Consumer Product Information Database (CPID). Accessed April 2020 at https://www.whatsinproducts.com/chemicals/view/1/180/000100-41-4/Ethylbenzene

United States Environmental Protection Agency (US EPA) Voluntary Children's Chemical Evaluation Program (VCCEP) (2007). Tier 1 Assessment for Ethylbenzene (CAS No. 100-41-4). Accessed May 2020 at https://www.tera.org/Peer/VCCEP/Ethylbenzene/VCCEP%20Ethylbenzene%20Revised%20Doc%20-august%2010%202007.pdf

US Department of Health and Human Services (US DHHS) 2010. Toxicological Profile for Ethylbenzene. Public Health Service, Agency for Toxic Substances and Disease Registry. 1600 Clifton Road NE, Mailstop F-62, Atlanta, Georgia 30333.