ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR CHLOROBENZENE

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CONTENTS

ADDENDUM for Chlorobenzene
Supplement to the 1990 Toxicological Profile for Chlorobenzene

Background Statement

This addendum to the Toxicological Profile for Chlorobenzene supplements the profile that was released in 1990.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the CERCLA Priority List of Hazardous Substances and that the profiles be revised "no less often than once every three years." CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide to the public and to federal, state, and local agencies a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1990.

Chapter numbers in this addendum coincide with the Toxicological Profile for Chlorobenzene (1990). This document should be used in conjunction with the profile. It does not replace it.

2. HEALTH EFFECTS

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

2.2.1 Inhalation Exposure

2.2.1.2 Systemic Effects

Respiratory Effects: Although no studies were located in the literature that implicate chlorobenzene, specifically, as a cause of lung inflammation *in vivo*, a number of human studies have reported a possible link between low-level exposure to various mixtures of volatile organic compounds (VOCs), including those containing chlorobenzene, and inflammation of the lung (Harving et al., 1991; Koren et al., 1992; Wieslander et al., 1997; Deitz et al., 2000; Lehman et al., 2001). In addition, chlorobenzene has been used as a model VOC in several *in vitro* studies to investigate possible mechanisms of lung inflammation. The authors of these studies suggested that low levels of chlorobenzene exposure might initiate inflammatory reactions in the lung (Fischäder et al., 2008; Röder-Stolinski et al., 2008; Lehman et al., 2008; Feltens et al. 2010).

Harving et al. (1991) evaluated forced expiratory volumes (FEVs) in 11 bronchial asthmatics exposed for 90 minutes to a mixture of 22 VOCs at levels of 0.0, 2.5, and 25 mg/m^3 (0.0, 0.543, and 5.43 ppm). Forced expiratory volumes (FEV_1) were decreased, although not significantly, following exposure to the highest dose of organic solvents. Although chlorobenzene was not a component of the VOCs evaluated in this study, the reported effects served as the impetus for a number of follow-up VOC investigations, many of which did include chlorobenzene.

Koren et al. (1992) exposed 14 human subjects to a mixture of VOCs (not including chlorobenzene) at a concentration of 25 mg/m^3 total hydrocarbon. These air levels were reported to be representative of those found in new homes and office buildings at the time of the study. Significant increases in polymorphonucleated neutrophils (PMNs) were observed in nasal lavage fluid immediately following a 4-hour exposure and at 18 hours post-exposure. The authors concluded that similar VOCs mixtures may also have the potential to induce bronchial irritation (Koren et al. 1992).

Wieslander et al. (1997) evaluated the incidence of self-reported asthma and airway symptoms in male house painters, aged 20 - 65, in relation to their use of solvent-based paint (SBP) versus water-based paint (WBP). The study design did not include any unexposed external reference group. The subjects of the study were employed in 1989 at the five largest firms in mid-Sweden. They were followed up through April 1991 or 1992. The cohort was made up of those considered to have an increased susceptibility to possible solvent-induced airway symptoms, and included smokers (21%), and individuals who reported: a history of atopy (18%); a family history of allergy (14%); asthma as an adult (5%); and hay fever (9%). Mean total VOC exposures associated with indoor painting with SBP and WPB were assumed to be 600 mg/m^3 and 4 mg/m^3, respectively, as suggested by previous studies. By multiplying the appropriate mean exposure by the relative degree of use of SBP and WBP reported by the subjects in self-administered questionnaires, the authors estimated each individual subject's total VOC exposure for a 40-hr workweek. No significant increase of self-reported asthma, hay fever, or atopy was observed during the study period. However, the incidence of self-reported asthma and airway symptoms was found to be greater in those who used more SBP than WBP. In addition, painters who had the highest estimated average solvent exposure (100 - 380 mg/m^3) also reported the most pronounced increase of airway symptoms. The investigators concluded that "VOCs found in both the work and home environments may influence lung function and are probably of importance as bronchial irritants." One of the known uses of chlorobenzene is as a solvent in paints. However, the authors did not identify any of the individual VOCs contained in the paints used in their study.

In a study known as the Leipzig Allergy Risk Children Study (LARS), researchers investigated the influence of indoor VOC exposure on the health of premature atopy-risk infants and children during the first years of life (Diez et al., 2000; Lehmann et al., 2001). The "atopy-risk children" in this study were premature neonates both of whose parents had positive family histories of atopy. (Atopy is the genetic tendency to develop allergic diseases characterized by the excess production of immunoglobulin E (IgE) in response to common environmental antigens). The infants' bedrooms were monitored for 25 different VOCs in air, including chlorobenzene, during the first four weeks after birth. The parents answered questionnaires, and the babies received medical examinations at six weeks and one year of age. The results of LARS suggested that early exposure to higher concentrations of certain VOCs (including chlorobenzene) associated with renovation activities during pregnancy (e.g., solvents emitted from adhesives in floor covering and wall-to-wall carpet) were also associated with the later development of allergies and chronic lung diseases in atopy-risk children (Lehmann et al., 2001). The LARS study is discussed in more detail in Section 2.2.1.3 Immunological and Lymphoreticular Effects.

In Vitro Studies: Lehmann and co-workers developed a unique *in vitro* system described by Lehmann et al. (2008) and Feltens et al. (2010). This system allowed the researchers to take cultivated human lung carcinoma cells (A549 cell line) that had been pre-stimulated with tumor necrosis factor-alpha (TNF-α) and expose them directly in the gas phase, without medium coverage, thereby more closely approximating *in situ* exposure.

Using this system, Fischäder et al. (2008) exposed TNF-α-stimulated A549 *in vitro* via the gas phase for 20 hours to chlorobenzene at doses that ranged from 1 ng/m^3 (0.001 µg/m^3 or 0.00022 ppbv) to 100 g/m^3 (100,000,000 µg/m^3 or about 22,000,000 ppbv). They saw statistically significant increases (roughly 20-40% higher than controls) in the release of the pro- inflammatory cytokine, monocyte chemoattractant protein 1 (MCP-1) at non-cytotoxic chlorobenzene concentrations of 10 and 100 µg/m^3 (approximately 2 and 22 ppbv). These concentrations of chlorobenzene were comparable to the levels of single VOCs (up to 20 µg/m^3 or 4.3 ppbv) that Fischäder et al. (2008) cited as being found in normal houses. MCP-1-release was significantly decreased at the highest chlorobenzene concentration (100,000,000 µg/m^3 or roughly 22,000,000 ppbv). Interleukin 8 (IL-8) release was significantly increased, compared to controls, at 1,000,000 and 10,000,000 µg/m^3. Increased release of MCP-1 was stimulated by two other aromatic VOCs besides chlorobenzene (styrene and m-xylene), but no stimulation was detected following exposure to aliphatic VOCs, either singly or in combination. The authors concluded that these *in vitro* results "could be relevant for *in vivo* exposure" and planned additional *in vitro* studies based on macrophages and lymphocytes, as well as lung epithelial cells, "to verify this hypothesis" (Fischäder et al., 2008).

Lehmann et al. (2008) performed an *in vitro* experiment very similar to that of Fischäder et al. (2008). Their experiment was designed to determine the effects of chlorobenzene on directly exposed human peripheral blood mononuclear cells (PBMC), as well as on lung carcinoma (A549) cells. Neither the vitality nor the proliferation of PBMC or A549 cells was impaired by highest concentrations of chlorobenzene employed in this *in vitro* study. Following 20 hours of exposure, no direct effect of chlorobenzene was found on the cytokine secretion of PBMC. However, 100 g/m^3 (i.e., 100,000,000 µg/m^3) chlorobenzene did induce IL-8 production, and 10–10,000,000 µg/m^3 increased (by approximately 40% over controls) the release of MCP-1 from A549 lung alveolar epithelial cells that were pre-stimulated with rh-TNFα (recombinant human tumor necrosis factor-alpha). The authors concluded that "chlorobenzene induces the production of inflammatory mediators in lung cells" (Lehmann et al., 2008).

Röder-Stolinski et al. (2008) studied the mechanism by which chlorobenzene enhances the TNF-α-stimulated release of MCP-1 in A549 cells exposed directly in the gas phase to concentrations of

chlorobenzene ranging from 0.1 μg/m^3 to 100 grams/m^3 (approximately 0.022 ppbv to 22,000,000 ppbv). To simulate cell-cooperation with alveolar macrophages, TNF-α, an obligatory component of the cytokine cascade which itself activates both the p38 MAPK (mitogen-activated protein kinases) and NF-κB (nuclear factor kappa-light-light-chain enhancer of activated B cells) pathways, was added at a concentration of 1 ng/ml to all samples; without it, the investigators were unable to detect any effect of VOC exposure in A549 cell cultures (Fischäder et al., 2008). The results of these *in vitro* studies suggested that the chlorobenzene-enhancement of TNF-α-induced release of MCP-1 was dependent on the NF-κB and MAPK pathways (Röder-Stolinski et al., 2008). Most of the levels of exposure produced significant increases (approximately 1.4-fold) in MCP-1 release, relative to controls (Röder-Stolinski et al., 2008). (The highest concentration of chlorobenzene (100 g/m^3 or 100,000,000 μg/m^3), which reportedly had no significant effect on the viability of the cells *in vitro*, caused a significant *decrease* in MCP-1 and IL-6 release, compared to controls.) Chlorobenzene concentrations ranging from 10 to 100,000,000 μg/m^3 produced similar, significant increases in the relative activity of p38 MAPK. In the presence of a specific inhibitor of NF-κB, the chlorobenzene-induced release of MCP-1 was suppressed, suggesting that chlorobenzene produced the latter effect by activating the NF-κB pathway.

Because the range of concentrations they used in these *in vitro* experiments embraced concentrations relevant to both indoor exposures and workplace exposures, the authors concluded that "… it can be assumed that chlorobenzene induces inflammatory responses in lung tissue *in vivo* in exposed individuals" (Röder-Stolinski and Fischäder, 2008).

MAPKs (mitogen-activated protein kinases), NF-κB (nuclear factor kappa-light-light-chain enhancer of activated B cells), MCP-1 (monocyte chemotactic protein-1), and TNF-α (tumor necrosis factor-alpha) are all involved in signaling cascades that control various cellular responses (including inflammation) to cytokines, environmental toxicants, and pathological stressors (Weinberg 2007). P_{38} MAPKs are involved in cell differentiation and apoptosis (programmed cell death). TNF-α is a potent cytokine that is produced mainly by macrophages. It attracts immune cells, triggers localized inflammation in many epithelial tissues, and can cause apoptosis. By promoting the "suicide" of initiated cells, TNF-α can inhibit carcinogenesis and the growth of tumors. Once activated in the cytoplasm by any of a number of diverse signals (including TNF-α), the NF-κB family of transcription factors enter the cell nucleus where they, in turn, activate the expression of at least 150 different target genes, including several that code for important anti-apoptotic (and, hence, tumor - promoting) proteins, and the gene for TNF-α that can produce the opposite effect (i.e., apoptosis). In many cancer cell types, the NF-κB pathway is highly active (i.e., deregulated or constitutively expressed). Finally, MCP-1 is an inducible factor involved in

attracting monocytes to sites of injury and infection. It is expressed in significant quantities in epithelial cancer cell types, and the macrophages it attracts play an important role in stimulating angiogenesis (i.e., the development of new blood vessels), thereby increasing the blood supply for growing tumors (Weinberg 2007). Cell signaling cascades are extraordinarily complicated *in vivo*. It is the complex interplay of all the components, rather than the concentration of any single component, that determines the net effect.

Feltens et al. (2010) used a revised version of the air-liquid cell culture apparatus previously developed in their laboratory (see Lehmann et al., 2008) to monitor cellular markers for oxidative stress (including heme oxidase-1, glutathione-S-transferase π1, superoxide dismutase, prostaglandin-endoperoxidase synthase 2, and dual specificity phosphatase 1) and reactive oxygen species in TNF-α-stimulated A549 cells (a human lung carcinoma cell line) treated for 24 hrs. with 100, 1000, or 10,000 µg chlorobenzene/m^3 (22, 220 or 2200 ppbv). *In vivo*, the MCP-1 released during pulmonary inflammation recruits and activates alveolar macrophages that then release TNF- α and other cytokines that enhance the inflammatory response. However, this *in vitro* system contained no macrophages. Therefore, to at least partially compensate for the absence of macrophages and to pre-stimulate MCP-1 release, all samples of A549 cells were cultured in TNF-α–conditioned medium prior to chlorobenzene exposure (Feltens et al., 2010). Without this pre-stimulation, no effect of VOC exposure in A549 cell cultures was detectable (Fischäder et al., 2008).

The expression of all monitored cellular markers for oxidative stress was elevated by treatment with chlorobenzene. In addition, intracellular levels of reduced glutathione (GSH) were reduced by chlorobenzene treatment in a concentration-dependent manner. After incubation of A549 cells for 24-hours at 100, 1000, and 10,000 µg chlorobenzene/m^3, intracellular GSH levels were reduced by about 29%, 41%, and 63%, respectively. Lung cells are particularly well equipped with respect to their antioxidant resources and their ROS [reactive oxygen species]-scavenging enzymes. *In vivo*, epithelial lung cells are afforded additional protection by the relatively high levels of GSH and antioxidant enzymes (e.g., glutathione peroxidase and superoxide dismutase) that are naturally contained in the lung's epithelial lining fluid (Feltons et al., 2010).

Feltons et al. concluded that their "findings show that seemingly innocuous, aromatic compounds such as chlorobenzenes and styrene, in their capacity as generators of oxidative stress in cultured lung epithelial cells, definitely show the potential to initiate an inflammatory response. It seems plausible that chronic chlorobenzenes exposition (not possible to investigate with only cellular system) will cause prolonged

oxidative stress and persistent inflammation, which even at a low level may be enough to set the stage for disease manifestation." As noted by these authors, this plausible potential cannot be confirmed in isolated cellular systems, alone. Appropriate follow-up studies in vivo would be required (Feltons et al., 2010).

2.2.1.3 Immunological and Lymphoreticular Effects

In the previously mentioned LARS study (see Section 2.2.1.2., Systemic Effects of Inhalation Exposure), early exposure to higher concentrations of certain VOCs (including chlorobenzene) was associated with the later development of allergies and chronic lung diseases in atopy-risk children (Diez et al., 2000; Lehmann et al., 2001). Diez et al. (2000) found no association between similar exposures and IgE secretion in the first year of life. However, Lehmann et al. (2001) did find such an association in three-year-olds previously exposed to indoor VOCs during the LARS.

A significantly higher prevalence of sensitization to food allergens (as measured by the prevalence of IgE to eggs and cow's milk) was observed in three-year-old children with prior exposure to a few of the 26 VOCs monitored during the LARS, including chlorobenzene. However, the concentration difference between each quartile of exposure for all but a few of the 26 VOCs monitored was only 1 or 2 ppbv or less. (Exceptions were limonene, α-pinene, and toluene.) For chlorobenzene, specifically, the difference between the first (lowest) and fourth (highest) quartiles of indoor levels (0.12 and 0.56 $\mu g/m^3$, respectively) was 0.44 $\mu g/m^3$ or 96 parts per trillion (Lehmann et al., 2001). In addition to an association with increased risk of allergic sensitization to milk and egg white antigens, low-level chlorobenzene exposure was associated with higher percentages of IL-4-producing CD3+T cells (Lehmann et al. 2001). The authors noted that "the result of cytokine measurement must be interpreted carefully" and that their results needed to be confirmed on a larger population. Because the association between VOC exposure and allergic sensitization or modified T-cell responsiveness had been demonstrated only in atopy-risk children, the authors planned future studies to determine whether children not at risk of atopy are similarly affected by VOC exposure (Lehmann et al., 2001).

2.2.1.4 Neurological Effects

When four volunteers were exposed via inhalation to 60.2 ppm (276.9 mg/m3) chlorobenzene for 7 hrs during a study of urinary metabolites in exposed workers, all complained of disagreeable odor and drowsiness, three complained of headache, two of throbbing pain in eyes, and one of sore throat (Ogata et al., 1991). In addition, mean flicker-fusion value declined 3.1 cycles/s in exposed subjects, compared to controls (Ogata et al., 1991).

Acute neurological impairment is particularly relevant in the development of IDLH values (i.e., concentrations in air that are immediately dangerous to life and health) because the latter are specifically designed to identify inhalation concentrations that could impair the ability of exposed workers to escape potentially life-threatening situations. Such effects were observed in a Shell Oil Company sponsored an acute inhalation toxicology study conducted in 1977 by the Utah Biomedical Test Laboratory (1978). Results of this study were submitted to NIOSH by Shell Oil Company in 1991 (Shell Oil Company 1991). In that study, ten male and ten female rats and guinea pigs were exposed for 30 minutes to 2,990, 5,850, or 7,970 ppm (13,756, 26,932, or 36,691 mg/m3) chlorobenzene in air. They were observed both during exposure and for 14 days after exposure. Necropsies were performed to identify irreversible pathological lesions (Shell Oil Company 1991). At the lowest dose administered (2,990 ppm or 13,756 mg/m3), reversible eye and nasal irritation was observed in up to half of the exposed rats and guinea pigs. No irreversible lesions were found on necropsy 14 days later. Ataxia and narcosis were observed at 5,850 ppm (or 26,932 mg/m3) progressing to “twitching movements” at 7,970 ppm (or 36,691 mg/m3). Guinea pigs were more sensitive than rats. At the 5,850 ppm dose of chlorobenzene most rats were narcotic by 25 minutes, but they recovered rapidly after removal from the test chamber. All guinea pigs were narcotic at 30 minutes (Shell Oil Company 1991). On the basis of these data in rats and guinea pigs, NIOSH revised its IDLH for chlorobenzene downward from 2,400 ppm to 1,000 ppm (NIOSH 2005).

2.2.1.7 Genotoxic Effects

High pressure liquid chromatography (HPLC) analysis of urine from chlorobenzene-exposed rats suggested the presence of a guanine adduct similar (but not identical) to N7-phenylguanine. This adduct was excreted on days 1 and 2 and between days 4 and 6 post-administration (Krewet et al., 1989). The

authors concluded that dehalogenated phenolic metabolites may be capable of binding to DNA, but the data were not conclusive.

In a mouse bone marrow micronucleus study, chlorobenzene showed negative results at intraperitoneal (ip) exposures of 128.8, 257.5 and 515 mg/kg (Shelby et al., 1993). There was no significant difference between controls and exposed mice at any dose administered. In a further comparison of 65 chemicals in a National Institute of Environmental Health Sciences (NIEHS) database, there was 80% concordance of results between tests for induction of chromosomal aberrations (CA) and micronuclei (MN). Chlorobenzene was among the discrepancies (Shelby and Witt 1995). Chlorobenzene tested negative for MN, and it gave only equivocal results for CA. In the first trial, CA was elevated, insignificantly, at the lowest administered dose of chlorobenzene (312.5 mg/kg), compared to controls and all higher doses. In a second trial, CA was significantly elevated at the highest dose (1,000 mg/kg) only, but it exhibited a statistically significant trend at all three doses. The authors concluded that the evidence for the clastogenicity of chlorobenzene was weak and less than definitive (Shelby and Witt 1995).

Vaghef and Hellman (1994) treated female C57BL/6 mice with either one or three daily intraperitoneal (ip) injections of 750 mg/kg chlorobenzene diluted in olive oil. The animals were sacrificed 16 hours after the last injection and their peripheral blood lymphocytes and bone marrow cells were evaluated for DNA damage using the “Comet Assay” under alkaline conditions. The ip LD50 of chlorobenzene in mice has been reported to be as low as 515 mg/kg and as high as 1355.2 mg/kg (HSDB 2011). Vaghef and Hellman noted that 750 mg/kg severely affected the condition of the animals. Nevertheless, single doses had no effect on DNA of either lymphocytes or bone marrow cells. Repeated doses (3 X 750 mg/kg) had no effect on bone marrow cells, but did increase the number of lymphocytes with DNA damage.

Siddiqui et al. (2006) administered i.p. doses of 750, 1000, or 1250 mg chlorobenzene/kg body weight to rats and evaluated the bone marrow cells at 12, 24, and 48 hours post-administration for MN and CA. While MN and CA frequencies were both elevated, compared to tap water and solvent (0.2% ethanol) controls, at all doses and times of observation, they reached statistical significance only at the highest dose and only 24 hours post-administration, after which MN and CA frequencies declined. The actual numbers of MN and CA were small. The mean frequency of MN per 1000 PCEs (polychromatic erythrocytes of bone marrow) were only 2.74 ± 0.8, 5.42 ± 0.3, and 3.07 ± 0.3 at 12, 24, and 48 hours, respectively, in the high-dose (1250 mg/kg) animals. The corresponding numbers of CA were even smaller (Siddiqui et al., 2006). The intraperitoneal doses of chlorobenzene employed by Siddiqui et al. in this animal study were 45 - 76% of the acute rat ip LD50 of 1655 mg/kg (HSDB 2011).

2.2.2 Oral Exposure

2.2.2.1 Death

There have been no documented human deaths from chlorobenzene exposure. Ingestion of 140 mL of 90% chlorobenzene by a suicidal 40-year-old, 58-kg, male alcoholic resulted in severe liver necrosis (Babany et al., 1991; Reygagne et al., 1992). Daily alcohol consumption was estimated at approximately 200 grams, but the patient had no history of chronic liver disease. While consciousness was normal and the liver was not enlarged, aspartate amino transferase (AST) and amino alanine transferase (ALT) levels on the 3rd day after chlorobenzene ingestion were 345 and 201 times, respectively, the upper limit of normal. Furthermore, liver biopsy showed centrilobular and mediolobular necrosis. The concentration of chlorobenzene in serum was 500 ppb on day 3. By day 15, serum levels had declined to 2 ppb. The authors speculated that chronic alcohol consumption may have potentiated chlorobenzene hepatotoxicity by inducing cytochrome P-450 IIE1 in their patient who eventually recovered (Babany et al., 1991).

2.2.2.2 Systemic Effects

Chlorobenzene (0.04 mL per 180 gram rat, or approximately 246 mg/kg b.w., assuming the chlorobenzene was pure) caused extensive liver necrosis in rats pre-treated with phenobarbital, but little or none in rats that were that were not pretreated with phenobarbitol (Brodie et al., 1971). In another study, the severity of chlorobenzene-induced necrosis was decreased by pre-treatment with the microsomal enzyme inhibitor SKF 525A. The authors concluded that reactive metabolites of chlorobenzene which were formed in the liver may have subsequently reacted with tissue macromolecules (Brodie et al., 1971).

During a 13-week study conducted by Hazelton Laboratories for Monsanto Company, 4 male and 4 female beagle dogs in each dose group were orally administered gelatin capsules containing either 0, 0.025, 0.050 or 0.250 milliliters of pure chlorobenzene/kg/day (Monsanto Company, 1967; Knapp et al. 1971). (Assuming a specific gravity of 1.1058 for pure chlorobenzene, these doses would correspond to: 0, 28, 55 and 277 mg/kg/day.) Clinical observations for the low- and intermediate-dose dogs did not differ significantly from those for controls. However, four of the high-dose animals died or became comatose and were sacrificed between the third and fifth weeks of the study. The surviving high-dose animals were sacrificed after 13 weeks of dosing. They exhibited elevated liver enzymes, low hemogram, increased

immature WBCs, cachexia (a wasting syndrome), icterus (jaundice), grayish-yellow discoloration of the hepatic parenchyma, distended gallbladder, and red discoloration of the renal medulla.
Acute liver, kidney and thyroid toxicity was evaluated after equi-molar doses (1, 2, or 4 mMol/kg) of a series of chlorinated benzenes (ranging from chlorobenzene to penta-chlorobenzene) were administered to groups of male Wister rats by single i.p. injections (Den Besten et al., 1991). For chlorobenzene, doses of 1, 2, and 4 mMol/kg corresponded to 112.5, 225, and 450 mg/kg, respectively. Chlorobenzene was the least toxic of the chlorinated benzenes evaluated in the study. It was found to be moderately hepatotoxic, as indicated by elevated ALT levels at the two highest doses, but it produced no statistically significantly adverse effects on the kidney or thyroid, at any dose tested. The degree of hepatotoxicity did not correlate with the extent of GSH depletion. Similar degrees of GSH depletion were produced by moderately hepatotoxic homologues like chlorobenzene and severely hepatotoxic homologues like 1, 2, 4- tri-chlorobenzene. Accumulation of proteinaceous droplets in renal proximal tubular epithelium cells, a common observation with higher-chlorinated benzenes, was not seen with chlorobenzene exposure even at the highest dose (450 mg/kg) tested.

In an in vitro study, chlorobenzene was found to be toxic to male Sprague Dawley or Fischer 344 rat liver slices exposed at 5 mM (563 ppm) for 6 hours, but not at 1 or 2 mM (113 or 226 ppm) (Fisher et al., 1990).

2.2.2.7 Genotoxic Effects

HPLC analysis of urine samples from chlorobenzene-exposed rats suggested the presence of a guanine adduct similar (but not identical) to N7-phenylguanine. This adduct was excreted on days 1 and 2 and between days 4 and 6 post-administration (Krewet et al., 1989). The authors concluded that dehalogenated phenolic metabolites may be capable of binding to DNA (Krewet et al., 1989).

In a mouse bone marrow micronucleus study of 49 different chemicals at three different doses, i.e., 128.8, 257.5 and 515 mg/kg, chlorobenzene tested negative at all doses (Shelby et al., 1993). No significant difference was exhibited between controls and animals in the highest dose group. Only 4 of the 24 non-carcinogens and 5 of the 25 carcinogens did test positive in this assay.

In a further comparison of 65 chemicals in an NIEHS database, there was 80% concordance of results between tests for induction of MN and CA, but chlorobenzene was among the discrepancies (Shelby and Witt, 1995). Chlorobenzene tested negative for MN, and gave only equivocal results for CA. In the first

trial, chlorobenzene exposure resulted in elevated CAs at the lowest dose (312.5 mg/kg) although the elevation was not significant. However, in a second trial, chlorobenzene exposure resulted in significantly elevated results (5.25 ± 2.33% cells with aberrations, compared to 1.00 ± 0.53% for controls) at the highest dose (1,000 mg/kg) only**.** The authors concluded that the evidence provided by this assay for the clastogenicity of chlorobenzene was weak and less than definitive (Shelby and Witt, 1995).

Chlorobenzene produced no increase in the number of sex-linked recessive lethal mutations in *Drosophila* (Valencia 1982).

2.3 TOXICOKINETICS

2.3.1.1 Inhalation Exposure

Knecht and Woitowitz (2000) exposed 8 volunteers to Germany's maximum workplace concentration (MAK) of 10 ppm chlorobenzene 8-hr/day for 5 days. There was no apparent tendency for chlorobenzene or its metabolites to accumulate in blood or urine with prolonged exposure. Blood levels reached a steady state (mean, 197.0 ± 9.7 µg/L) after the first hour of exposure. The mean concentration of chlorobenzene in blood in 5 subjects exposed during physical exercise (75 W, 10 min/hr on a bicycle) was 217 µg/L. In 2 subjects exposed during mild exercise (50 W, 10 min/hour on a bicycle) and one subject exposed while at rest, mean blood levels of chlorobenzene were approximately 133 and 78 µg/L, respectively. Half-lives of elimination of chlorobenzene from blood were 53 min in the first hour after cessation of exposure, and 150 min thereafter. The major urinary metabolite of chlorobenzene was 4-chlorocatechol (74%), with the remainder comprised of chlorophenol isomers of which para-chlorophenol (13%) was the most abundant. Urinary para-chlorophenol was still useful as a biomarker of exposure due to its longer half-life (approx. 12 hr). The elimination half-life of urinary 4-chlorocatechol was half that (i.e., 6.4 hr) (Knecht and Woitowitz 2000).

Thrall et al. (2004) developed a rat physiologically based pharmacokinetic (PBPK) model for chlorobenzene in air, using metabolic data derived from groups of F-344 male rats exposed to chlorobenzene levels ranging from 82 to 6750 ppm in air. Physiological values (e.g., breathing rate, organ volumes, etc.) were taken from the literature, and partition coefficients were determined from in vitro experiments with rat tissues and blood samples. The finished model was evaluated by using it to predict the chlorobenzene levels in exhaled breath of rats exposed by corn oil gavage (127 mg/kg) or intraperitoneal injection (131 mg/kg).

2.3.1.2 Oral Exposure

A physiologically-based pharmacokinetic (PBPK) model was developed to estimate the amount of 19 different VOCs that a nursing infant would receive from its occupationally exposed mother (Fischer et al., 1997). In a simulation of a lactating woman exposed to the threshold limit value (TLV) concentration of chlorobenzene in air at the workplace, the amount of chlorobenzene tranfered to a nursing infant from mother's milk was calculated to be 0.229 mg for a 10 kg infant.

2.3.3 Metabolism

Cytochrome P-450 2E1 is the main enzyme involved in the oxidation of chlorobenzene in mice, rats and humans. Cytochrome P-450 3A also appears to play a role in the generation of reactive metabolites in mice, rats and humans. It is important to note, however, that, compared to mice and rats, the rate of metabolism of chlorobenzene to soluble metabolites is higher in humans, and the formation of covalently bound products is lower (Nedelcheva et al., 1998). In addition, there is up to a 10-fold difference in the rate of metabolism of chlorobenzene in different human livers. There are also significant species and sex differences in the metabolism of chlorobenzene with markedly higher rates of oxidation in male mice than in male rats and female mice (Nedelcheva et al., 1998).

Linear correlations between urinary 4-chlorocatechol excretion and airborne exposure of workers to chlorobenzene were established by Yoshida et al. (1986), after monitoring end-of-shift urinary metabolites in healthy male workers in two chemical factories where chlorobenzene was used as a solvent. The primary urinary metabolites were 4-chlorocatechol (mean 76.9±1.6%, n=11) and 4-chlorophenol (mean 12.4±1.1%, n-11). In factories A and B, average chlorobenzene concentrations in air were 3.16 ppm (range 1.72 to 5.78 ppm) and 3.14 ppm (range 2.68 to 3.68 ppm), respectively. These levels of exposure in factories A and B corresponded, respectively, to mean 4-chlorocatechol levels of 0.362 μmoles/mg creatinine (range 0.166 to 0.787 μmoles/mg creatinine) and 0.482 μmoles/mg creatinine (range 0.354 to 0.655 μmoles/mg creatinine) in urine (Yoshida et al., 1986).

Assessing forty-four maintenance workers in a diphenylmethane 4, 4'-diisocyanate plant for chlorobenzene exposure, Kusters and Lauwerys (1990) also found that the main urinary metabolites at the end of shift were 4-chlorophenol and 4-chlorocatechol, with the latter being 3 times more abundant than the former. The time-weighted average exposure to chlorobenzene in air (mean 1.2 ppm, range 0.05 to 106 ppm) was less than the German MAK value of 50 ppm that was established in 1989, as well as the

current MAK value of 10 ppm established in 1995. More than 80% of the metabolites were eliminated within 16 hours after the end of exposure, and there was no tendency for an increase in concentration during the working week (Kusters and Lauwerys1990).

Ogata et al. (1991) reported that, in order of abundance, the main urinary metabolites of chlorobenzene in exposed workers were 4-chlorocatechol, and p-chlorophenylmercapturic acid. The concentrations of chlorobenzene in blood and metabolites in urine (e.g., 4-chlorocatechol, approx 26% of exposure) were both proportional to the concentration of chlorobenzene in air. The molar ratio of urinary chlorocatechol to inhaled chlorobenzene was estimated to be approximately 26%, and the mean slope of regression line for chlorobenzene in air versus blood was 4.6 ± 1.15 µg/L for 1 ppm chlorobenzene. The measured biological half-time of 4-chlorocatechol was reported to be 2.9 hrs (Ogata et al. 1991).

2.4 RELEVANCE TO PUBLIC HEALTH

According to the results of CDC's Fourth National Health and Nutrition Survey (NHANES IV), blood levels in the general public were undetectable at a detection limit (LOD) of 0.011 ng/L or nanograms per milliliter (CDC 2009). The same LOD may also be expressed as 0.011 µg/L (parts per billion or ppb) or 11 parts per trillion (ppt).

Air: In 1982, the highest chlorobenzene concentrations reported in urban air averaged 3 $\mu g/m^3$ or 0.66 ppbv (ATSDR 1990). In a study of urban VOC concentrations in the United States between 1996 and 1997, the highest levels of chlorobenzene were < 1 ppbv (< 4.6 $\mu g/m^3$) at all 13 monitoring stations (Mahmoud et al. 2002). The lowest known less serious inhalation LOAEL (Lowest Observed Adverse Effect Level) for chlorobenzene is 75,000 ppbv for decreased levels of the liver enzymes lactate dehydrogenase (LDH) and serum glutamic-oxaloacetic transaminase (SGOT) in rats and rabbits exposed 7 hr/day, 5 days/week, for 120 days (ATSDR 1990).

Water: The potential for toxic exposure to chlorobenzene via the water supply may be somewhat limited by the relatively low solubility of chlorobenzene in water, as evidenced by the fact that environmental levels of chlorobenzene in groundwater and surface water are generally in the low parts per billion range (HSDB 2011). Even in three Florida counties where surface water and groundwater were contaminated by municipal landfill leachate, chlorobenzene concentrations ranged from <0.20 to 302 µg/L (Hallbourg et al., 1992). The current Maximum Contaminant Level (EPA MCL) for chlorobenzene is 100 µg/L (EPA 2009); MCLs are set "…using the best available analytical and treatment technologies and taking cost into consideration."

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Yoshida et al. (1986) demonstrated linear correlations between urinary 4-chlorocatechol excretion and airborne exposure of workers to chlorobenzene. These authors suggested that the former might be an effective biomarker of exposure in humans. As a consequence of that work ACGIH recommends measurement of 4-chlorocatechol in urine at the end of the last shift of the work week as an effective biomarker of exposure to chlorobenzene in humans. The corresponding BEI is 100 mg 4-ClCat/gram creatinine (ACGIH 2011).

Kumagi and Matsunaga (1994; 1995) also found that the major urinary metabolites of chlorobenzene in humans, including 4-chlorocatechol (especially) and ρ-chlorophenol, are good biomarkers of recent exposure in workers. The slopes of the regression line for urinary metabolite concentration versus inhalation exposure concentration do appear to vary somewhat between studies, probably because of differences in workloads (active vs. at rest) and patterns of exposure (acute vs. chronic). Nevertheless, controlled chamber studies with workers have demonstrated that the concentrations of both major urinary metabolites of chlorobenzene correlate well with workers' eight hour time weighted average exposure to chlorobenzene, and reflect variations in workplace exposure to chlorobenzene (Kumagai and Matsunaga, 1995). Accordingly, ACGIH has since developed biological exposure indices (BEIs) based on both of these urinary metabolites for use in the workplace (ACGIH 1996-2011).

In an occupational study by Knecht and Woitowitz, (2000), the major urinary metabolite of chlorobenzene was 4-chlorocatechol (74%). The remainder consisted of chlorophenol isomers of which para-chlorophenol (13%) was the most abundant. In spite of its being less than 20% as abundant as 4-chlorocatechol, urinary para-chlorophenol was still considered to be potentiallyuseful as a biomarker of exposure due to its longer halflife (approx. 12 hr) (Knecht and Woitowitz, 2000). The elimination half-life of urinary 4-chlorocatechol was half that (i.e., 6.4 hr) (Knecht and Woitowitz, 2000).

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

On the basis of limited data, asthmatics may represent a population that is unusually susceptible to the inhalation effects of chlorobenzene. Regarding the potential for infants to be an unusually susceptible population, a PBPK model developed by Fischer et al. (1997) calculated that a 10 kg nursing

infant would be exposed to 0.229 mg chlorobenzene over a 24 hour period from breast milk from an occupationally exposed lactating woman.

3. CHEMICAL AND PHYSICAL INFORMATION

3.2 CHEMICAL AND PHYSICAL PROPERTIES

The odor threshold for chlorobenzene in humans has been reported to be as low as 0.21 ppm or 0.97 mg/m^3 (Leonardos et al., 1969; ACGIH 2001). However, others have reported its "almond-like odor" to be "barely perceptible" at 60 ppm (Von Burg 1981; Willhite and Book 1990; ACGIH 2001).

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

4.1 PRODUCTION

The Hazardous Substance Data Bank (HSDB) lists the following figures for U.S. production capacity (in lbs/yr): 368 million in1990; 371 million in1993; 370 million in 1996; 358 million in 1999; and 205 million in 2004. Assuming a constant annual rate of decline (-3%), production in 2010 was at least 60% less than in 1990 (HSDB 2011). Current usage data were not available. However, a trend report generated for the available years, 1988-2009, from the Toxic Release Inventory database (TRI 2011) indicates that total releases of chlorobenzene have declined by 95% in the last 20 years. Assuming that total releases of chlorobenzene by U.S. industries should be roughly proportional to total production/use of chlorobenzene by those industries, this trend report implies that use of chlorobenzene by U.S. industry has continued its historical decline from its peak in the 1960s to its current minimum.

4.2 IMPORT/EXPORT

From 2002 to 2003, US exports of chlorobenzene declined from 3.5 million pounds to 1.5 million pounds, annually. Imports remain negligible (HSDB 2011; Kirschner, 2004).

4.3 USE

Currently, chlorobenzene is used primarily as a chemical intermediate in the production of nitrochlorobenzenes and diphenyl oxide (OEHHA 2000). To a lesser extent, it is still used as a solvent in degreasing processes, paints, adhesives, waxes, and polishes.

4.4 DISPOSAL

A trend report generated for the available years, 1988 to 2009, from the Toxic Release Inventory database (TRI 2011) suggests that total releases of chlorobenzene have declined 95% in the 20 years since ATSDR last prepared a toxicological profile for chlorobenzene (1990).

5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

According to the results of NHANES 2003-2004, the levels of chlorobenzene in all 1,366 tested blood samples from blacks, whites, and Mexican-Americans were below the applicable detection limit of 0.011 ng/ml (µg/L or ppb) or 11 parts per trillion (ppt) (CDC 2009).

5.2 RELEASES TO THE ENVIRONMENT

As previously stated, a trend report generated for the available years 1988 to 2009, from the U.S. EPA's Toxics Release Inventory database (TRI, 2011) indicates that total releases of chlorobenzene have declined 95% in the 20 years since ATSDR last prepared a toxicological profile for chlorobenzene (1990). While TRI data are only rough estimates, this trend report suggests that use of chlorobenzene by U.S. industry has continued its historical decline from its peak in the 1960s to its current minimum.

5.3 ENVIRONMENTAL FATE

5.3.2 Transformation and Degradation

Under hypoxic conditions in groundwater, shifts in the bacterial community may occur as a result of syntrophic rather than competitive interactions, facilitating the degradation of chlorobenzene (Kiesel et al., 2007). (Syntrophy occurs when one organism lives off the product of another organism, rather than the organism itself.)

5.3.2.2 Water

Few data are available from the field, but evaporation, hydrolysis, and microbial degradation, in that order, are likely to be the major fates of chlorobenzene discharged to water.

Bioconcentration of chlorobenzene does not appear to be a significant process in aquatic environments. However, bio-concentration factors for chlorobenzene do increase somewhat in phytoplankton as temperature increases between 4.5 and 27.6 °C (Koelmans and Sanchez, 1994).

Oxygen appears to be required for the initial activation of chlorobenzene and the fission of the aromatic ring, although it can be partially replaced by nitrate (Nestler et al., 2007).
Adrian and Görisch, (2002) reviewed reductive dechlorination of chlorinated benzenes in laboratory systems. Metabolic dechlorination of chlorobenzenes seems to proceed fastest under methanogenic conditions (Adrian and Görisch, 2002; Ramanand et al., 1993). While the negative changes in Gibbs free energy associated with all 20 possible dechlorination reactions of chlorobenzenes are large enough to be coupled to adenine triphosphate (ATP) generation, not all of those reactions have been observed in laboratory systems, and the extent to which any of them occurs in nature remains unknown (Adrian and Görisch, 2002).

The potential for anaerobic degradation has also been studied in contaminated groundwater plumes, where oxygen levels are generally lower than they are outside the plume. In a study of three North Central Florida landfills, Hallbourg et al. (1992) found that due to the high water table, anaerobic degradation predominated. However, Kaschl et al. (2005), were among the first to demonstrate the slow anaerobic microbial transformation of chlorobenzene in the field. They showed that, in a contaminated aquifer in Bitterfeld, Germany, the decreases of chlorobenzene concentrations at the horizontal fringes of the plume and at shallower depths were accompanied by changes in isotopic composition (i.e., enrichment in ^{13}C)

that suggested the *in situ* anaerobic degradation of chlorobenzene was occurring, albeit slowly. Since the known aerobic pathway initiated by dioxygenases in chlorobenzene-degrading strains (Ralstonia sp. DSM 8910, Acidovorax facilis UFZ B517, Rhodococcus erythropolis UFZ B528, and Pseudomonas verinii UFZ B547) did not result in isotopic fractionation, it was concluded that a novel anaerobic pathway resulting in isotopic fractionation was the predominant process of chlorobenzene degradation in this aquifer. This aquifer, which was near a site where lindane had been formerly produced since the early 1900s, was contaminated with up to 30 ppm chlorobenzene. The anaerobic microbial degradation of [$^{13}C_6$]-chlorobenzene was confirmed by Nijenhuis et al. (2007). In a constructed wetland designed to treat contaminated groundwater, Braeckevelt et al. (2007a) observed an isotope shift that was higher than expected for aerobic chlorobenzene degradation and concluded that an anaerobic degradation pathway must be making a significant contribution to the overall degradation (Braeckevelt et al., 2007a). Natural attenuation of 13C-labeled chlorobenzene in this constructed wetland was indicated by (1) detection of 13C-labeled (i.e., reductively dechlorinated) benzene, (2) incorporation of 13C-labeled carbon derived from chlorobenzene into bacterial fatty acids, and (3) a systematic correlation between decreasing chlorobenzene concentration and significant enrichment in $\delta^{13}C$ with increasing distance from the source of contamination (Braeckevelt et al., 2007b).

5.3.2.3 Soil

Evaporation and microbial degradation, in that order, are likely to be the major fates of chlorobenzene in soils. However, very few data are available from the field. Most relevant information comes from laboratory studies on amended soils and strains of soil bacteria isolated from contaminated water, soil, or sediments.

Starting with soil samples from a site in Niagra Falls, NY, Ramanand et al., 1993 prepared anoxic soil slurries amended with a mixture of higher chlorobenzenes. The authors inoculated the latter with microbial cultures able to dechlorinate higher chlorobezenes. They then monitored the sequential reductive dechlorination of the latter to chlorobenzene. While inoculation of the soil slurries greatly facilitated dechlorination activity (as evidenced by the rapid formation of chlorobenzene coincident with the decay of the mixture of higher chlorobenzenes), it was found that endogenous soil microbes alone also dechlorinated higher chlorobenzenes to chlorobenzene, though at a much slower rate.

Under aerobic conditions, all 15 volatile and semivolatile organic compounds (including chlorobenzene) in a soil-applied mixture disappeared rapidly due to abiotic factors during a 7-day observation period

(Anderson et al., 1991). Feidieker et al., 1995 documented the aerobic degradation of chlorobenzene with mixed bacterial cultures. Complete metabolism of chlorinated benzenes is not a feature that is generally found in aerobic bacteria. However, at chlorobenzene-contaminated sites, indigenous bacteria populations appear able to evolve the capacity for natural attenuation of chlorobenzene (Van der Meer et al., 1998). Pseudomonas putida MST that was previously isolated in the presence of α-methylstyrene was shown to regioselectively hydroxylate chlorobenzene to 3-chlorocatechol, and 2- and 4-chlorophenol to 3- and 4-chlorocatechol, respectively (Bestetti et al 1992). Inoculation of a soil slurry with Pseudomonas aeruginosa (105 microbes/gram of soil) led to rapid and complete degradation of the 0.8 mM chlorobenzene within 30 hours (Brunsbach and Reineke 1994). Indigenous soil microbes also degraded chlorobenzene, but the higher chlorobenzenes persisted.

Chlorobenzene contamination of soil stimulates the growth of indigenous, chlorobenzene metabolizing bacteria. The latter may even out-compete inoculated strains of Pseudomonas (Nishino et al., 1994).

In an in vitro study, Nowak et al. (1996) demonstrated the total reductive dechlorination of chlorobenzenes by a methanogenic culture enriched from Saale River sediment. Dechlorination of chlorobenzene to benzene was also observed in these cultures. However, the amount of benzene formed was extremely low and the reaction occurred only in the presence of higher chlorinated benzenes (Nowak et al., 1996). Presumably, this was a co-metabolic process, i.e., one in which the metabolism of chlorobenzene occurred without benefit to the organism, but was co-incident with the metabolism of the substrate on which the microbe actually depended for energy production. Such reactions are useful in bioremediation, because they can proceed at concentrations far below those required to support the organism (Hazen 2009).

As previously documented in the field for pesticides and other contaminants , the residue of chlorobenzene in soil that is not volatilized or metabolized tends to bind more tightly to soil with time, a phenomenon known as “aging” (Sharer et al., 2003). As a result, degradation occurs at lower rates and to a lesser extent, even though chlorobenzene-degrading bacteria still have access to sorbed chlorobenzene in aged wetland soils (Lee et al., 2008).

The reductive dechlorination of chlorobenzenes in an anaerobic estuarine sediment followed first-order reaction kinetics with rate constants ranging from 0.0016 to 0.0389 day-1 or half-lives between 17 and 433 days (Kochany and Boltob 1992; Masunaga et al., 1996). From the detected intermediates, it was apparent that the removal of chlorine atoms occurred at all possible positions on the aromatic ring, but

removal followed a thermodynamically favored order, i.e., a chlorine atom flanked on both sides by another > one of two adjacent chlorine atoms > a chlorine with no adjacent chlorine atoms (e.g., the dechlorination of chlorobenzene) (Masunaga et al., 1996).

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.1 Air

At the University of Gadańsk, Poland, investigators measured the quality of indoor air using solid-phase micro-extraction (SPME) and gas chromatography-mass spectro-graphy (GC-MC) in two different laboratories and in 16 different flats in relatively prosperous districts located far from industrial areas and roads with high traffic (Gorlo et al., 1999). The level of chlorobenzene in none of the 16 flats was detectable at the detection limit of 0.002 mg/m3 (0.44 ppbv). In the 2 laboratories, average concentrations of chlorobenzene in air were 2.15 mg/m3 and 18.65 mg/m3 (0.47 ppmv and 4.1 ppmv), respectively (Gorlo et al., 1999).

Meek et al., 1994 measured mean concentrations of chlorobenzene that ranged from 0.10 to 0.21 µg/m3 in ambient air from 18 Canadian sites in five provinces; corresponding estimated intakes in the general population ranged from 0.03 to 0.09 µg/kg/day.

5.4.2 Water

Chlorobenzene was generally detected in the low ppb range, when found at all, in three North Central Florida landfills (Hallbourg et al., 1992). Using headspace gas chromatographic analysis with ECD to avoid the necessity of special cleanup procedures, Först et al. (1993) detected an average of 868 ppb chlorobenzene in leachate samples from a highly contaminated landfill. In the early 1990s, chlorobenzene in drinking water was below the limits of detection (1.0 ppb) in 30 different locations within Canada (Meek et al., 1994). Of the 2,401 groundwater samples from domestic wells and the 1,096 samples from public wells, over 90% of the chlorobenzene concentrations were less than 1 ppb and none were as high as 5 ppb (Zogorski et al., 2006).

A risk assessment on chlorobenzene for the marine environment (the North Sea area) was conducted in which "risk" was indicated by the ratio of predicted environmental concentration (PEC) to the predicted no-effect concentration (PNEC) (set to 32 µg/l) for the marine aquatic environment (Van Wijk et al., 2004). Since monitoring data indicated that chlorobenzene in surface waters was below detection limits of 0.1, 0.2, and 0.5 µg/l, the worst-case PEC was assumed to be 0.5 µg/L, yielding a PEC/PNEC of at least 60, without even taking into account dilution of chlorobenzene-containing surface waters in the sea. The authors concluded that chlorobenzene is not a toxic, persistent, or bioaccumulating substance, and that current use of the compound posed no unacceptable risk to the aquatic environment (Van Wijk et al., 2004).

5.4.4 Other Environmental Media

A national survey of the United States indicated that chlorobenzene was below detection limits in milk supplies (Schaum et al., 2003). Carrots, potatoes, cabbage, cauliflower, lettuce, onions, beans, peas, and tomatoes have been analyzed for mono- through hexa-chlorobenzenes (Wang and Jones 1994). Chlorobenzene was found only in cabbage, but at levels (207 ppb, dry wt. basis) almost seven times higher than the level of all the other chlorobenzenes detected in all nine vegetables combined. Assuming consumption of raw vegetables only, and using United Kingdom default consumption rates, chlorobenzene exposure via consumption of vegetables (i.e., almost entirely from cabbage) was estimated to be 850 µg/person/year, or approximately 0.33 µg/kg/day for a 70-kg adult (Wang and Jones 1994).

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

According to the results of NHANES IV, chlorobenzene was undetectable in blood samples of every age group, gender, race, and ethnicity studied (CDC 2009). The detection limit was 0.011 ng/ml (ppb) or 11 parts per trillion (ppt).

6. ANALYTICAL METHODS

6.2 ENVIRONMENTAL SAMPLES

Using headspace gas chromatographic analysis with electron capture detection to avoid the necessity of special clean-up procedures, Först et al., (1993) detected an average of 868 ppb chlorobenzene in leachate samples from a highly contaminated landfill. Lehotay and Hromul'akova (1997) were able to detect as little as 185 parts per trillion in 100 ml of tap water, using an HPLC method they developed with spectrophotometric detection at 220 nm UV. This assayed procedure has also been applied to river water. Roy and Mielczarski 2002 devised a method whereby chlorobenzene concentrations of 13 ppb or more in water could be quickly and simply detected in the field using an infrared sensor with an attenuated total reflection ZnSe trapezoidal element covered with a very thin (1 μm) film of decahydronaphthalene. Using a drop-based liquid phase micro-extraction and gas chromatographic-electron capture detection (GC-ECD) method, Tor (2006) was able to detect as little as 0.008 μg/L chlorobenzene in a 5-ml sample of water.

Page and Lacroix (1995) described a steam distillation purge and trap gas chromatographic procedure for the determination of halogenated analytes in foods and beverages. Recoveries from spiked samples were generally >60% for a 1-gram food sample, and the method detection limits ranged from 0.02 to 0.2 ppb for the 32 analytes that were tested.

7. REGULATIONS AND ADVISORIES

The ACGIH time-weighted average threshold limit value (TLV-TWA) for chlorobenzene is currently 10 ppm (46 mg /m3). The corresponding occupational biological exposure Indices (BEIs) currently adopted by ACGIH for chlorobenzene are 100 mg 4-chlorocatechol/g creatinine and 20 mg ρ-chlorophenol/g creatinine in urine (after hydrolysis of the conjugates) at the end of shift (ACGIH 2011).

ACGIH currently classifies chlorobenzene as an A3 carcinogen, i.e., a confirmed animal carcinogen with unknown relevance to humans (ACGIH 2011). The U.S. EPA designates chlorobenzene a class D carcinogen, i.e., unclassifiable as to its potential carcinogenicity in humans (IRIS 1991). Both of these

classifications (ACGIH and EPA) are based on the same data in rodents (NTP 1985) and on the absence of any evidence of chlorobenzene-induced carcinogenicity in humans.

On the basis of the results of a NIOSH-sponsored animal study submitted in 1991 by Shell Oil Company, NIOSH's 1985 IDLH (Immediately Dangerous to Life and Health) value for chlorobenzene was lowered from the previous 2400 ppm (as listed in ATSDR's 1990 Toxicological Profile) to 1000 ppm (NIOSH 1993; 2005).

ATSDR's 1990 Toxicological Profile for Chlorobenzene reported that the state drinking water standards for chlorobenzene for California, Maine, Minnesota, New Jersey and Wisconsin were 30, 47, 60, 2, and 600 ppb, respectively . Those values have been changed to 70, 140, 100, 50, and 100 ppb, respectively (HSDB 2011). In addition, the state drinking water standards for chlorobenzene for Massachusetts, Ohio, Kansas, and Colorado have all been set 100 ppb (Massachusetts, 2012; Ohio, 2010; Wisconsin, 2013; Kansas, 1989).

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