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Establishing a total allowable concentration of *o*-toluidine in drinking water incorporating early lifestage exposure and susceptibility

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ABSTRACT

o-Toluidine is a monocyclic aromatic amine present in the formulation of some materials that contact drinking water. NSF/ANSI 61 Annex A (2011) and US EPA (2005a) risk assessment guidelines were used to determine an acceptable drinking water level. Occupational exposure to *o*-toluidine is associated with an increased risk of bladder cancer but human disease rates could not be used to establish risk values due to inadequate exposure data and coexposures in the epidemiology cohorts. Chronic dietary exposure to *o*-toluidine hydrochloride was associated with benign and malignant tumors in both sexes of F344 rats and B6C3F1 mice. *o*-Toluidine is genotoxic *in vitro* and *in vivo*. A 10^{-5} cancer risk level was extrapolated from the human equivalent $BMDL_{10}$ of 13 mg/kg-day for the combined incidence of papillomas and carcinomas of the bladder transitional epithelium in female rats. Considering varying susceptibility to tumor development at different life stages, the unit risk was modified to incorporate potency adjustments for early-life exposures. A framework for lifestage adjustment is presented that makes assumptions evident. For this assessment, the lifetime unit risk derived was ~2-fold greater than the unadjusted adult lifetime unit risk, and the resulting Total Allowable Concentration in drinking water is 20 μg/L.

1. Introduction

o-Toluidine is a monocyclic aromatic amine (CAS No. 95-53-4) with synonyms including 2-toluidine, 2-aminotoluene, 1-amino-2-methylbenzene, 2-amino-1-methylbenzene, 1-methyl-2-amino-benzene, 2-methyl-1-aminobenzene, 2-methylaniline, 2-methyl-benzamine, and 2-methylbenzeneamine (ChemIDplus, 2011). It is listed as a High Production Volume chemical in the United States (US EPA, 2010) and the European Union (OECD, 2004). The 2001 annual production volume of *o*-toluidine was estimated to be 59,000 metric tonnes (130 million pounds) worldwide (OECD, 2006). The 2006 aggregate production volume for manufactured and imported *o*-toluidine was between 10 and 50 million pounds (US EPA, 2007).

The principle use of *o*-toluidine is in the preparation of methyl ethyl aniline, an intermediate in the manufacture of certain chlorinated herbicides, such as acetochlor, metolachlor, and propisochlor (OECD, 2006). *o*-Toluidine is also an intermediate used for the synthesis of rubber chemicals, dyes and pigments, fungicides, pharmaceuticals, and curing agents for epoxy resin systems. It has been used as a corrosion inhibitor in paint formulations (WHO/IPCS, 1998), and a minor use is as a clinical laboratory reagent for the photometric determination of glucose in blood. No direct consumer use is known for *o*-toluidine (OECD, 2006). Occupational exposures occurred during *o*-toluidine production and/or during its use in dye and pigment manufacture (NTP, 2011a) and during rubber production (Weiss et al., 2005). Since *o*-toluidine is a minor metabolite of another High Production Volume chemical, *o*-nitrotoluene (NTP, 1996), occupational exposure to *o*-nitrotoluene is another potential source of *o*-toluidine exposure.

The International Agency for Research on Cancer (IARC) evaluated *o*-toluidine and its hydrochloride for carcinogenic potential (IARC, 1982, 1987, 2000, 2010; Baan et al., 2008). Most recently, *o*-toluidine was classified by IARC (Baan et al., 2008; IARC, 2010) as Group 1: *The agent is carcinogenic to humans*. The Working Group also reaffirmed magenta production (where *o*-toluidine is used as an intermediate) as "carcinogenic to humans," and as a known cause of bladder cancer. Based on a similar toxicological profiles between 4,4′-methylenebis(2-chloroaniline) and *o*-toluidine, these compounds were concluded to operate via a common mode of action, involving the interaction with DNA to form adducts in urothelial cells (IARC, 2009).*o*-Toluidine is present in certain elastomers, coatings, and sealants used in products that contact potable water. Its presence is occasionally detected in drinking water contact materials tested for compliance with health effects standards established by NSF/ANSI 61 (2011). There is no US EPA Maximum Contaminant Level (MCL) or Health Advisory for *o*-toluidine in drinking water. This risk assessment is the outcome of a

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comprehensive toxicological evaluation of *o*-toluidine, as an extractant from drinking water system components evaluated under NSF/ANSI 61 (2011).

2. Methods

We first reviewed the scientific literature related to the human health effects of *o*-toluidine, including metabolism, kinetic, toxicology, and epidemiology data. The literature search strategy employed was based on the Chemical Abstract Service Registry Number (CASRN) and the common name. As a minimum, the following data banks were searched:

- ChemID Plus.
- Registry of Toxic Effects of Chemical Substances (RTECS).
- Hazardous Substances Data Bank (HSDB).
- GENE-TOX.
- Environmental Mutagen Information Center (EMIC).
- Developmental and Reproductive Toxicology (DART).
- TOXLINE – Core and Special.
- TRI (Toxics Release Inventory).
- Chemical Carcinogenesis Research Information System (CCRIS).
- Medline (via PubMed).
- Integrated Risk Information System (IRIS).
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS).

Much of the pertinent literature had previously been reviewed by WHO (1998), IARC (2000), OECD (2006), and these secondary sources were used for some literature that pre-dated those publications. For the literature published subsequent to these reviews and all studies that were determined to be key to this risk assessment, the original publications were reviewed in their entirety and relevant information was included.

Although both non-cancer and cancer endpoints were considered, we focus here on endpoints related to cancer because they were determined to be pivotal to the current assessment. US EPA guidelines for cancer risk assessment (US EPA, 2005a) explicitly call for consideration of possible sensitive subpopulations and/or life stages, and the Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (US EPA, 2005b) focuses on assessing the effects of exposure to potential carcinogens during childhood for agents that act specifically through a mutagenic mode of action. Following US EPA guidelines, we evaluated the mode of action in determining the approach for dose–response assessment from positive human and experimental animal tumor data. Absent a biologically based dose–response model, which is the preferred method for cross-species and low-dose extrapolation, we used default procedures to derive human equivalent doses from experimental animal doses, and benchmark dose modeling to find the best-fit curve to the appropriate dose–response data (US EPA, 2000a, 2011a). Assuming a mutagenic mode of action for *o*-toluidine, we derived a drinking water lifetime unit risk that takes into account the impact of early lifestage susceptibility and exposure. We present a framework for lifestage adjustment that illustrates the assumptions made to facilitate future refinements to this analysis as new information becomes available.

3. Hazard identification

3.1. Toxicokinetics

Much of the available data on the metabolism and kinetics of *o*-toluidine has been reviewed previously (IARC 2000; OECD 2006). *o*-Toluidine is well absorbed by oral, dermal and inhalation routes of intake in animals and humans. Radiotracer studies in rats show the oral bioavailability is high, and tissue distribution is widespread. Approximately 92% of the dose was recovered in the urine within 24 h of a 50 mg/kg gavage dose (Cheever et al., 1980; Brock et al., 1990).

In both rats and humans, *o*-toluidine undergoes *N*-oxidation to a reactive metabolite that binds covalently to hemoglobin and DNA. *o*-Toluidine hemoglobin adducts have been used to monitor exposure to *o*-toluidine and have been found in exposed humans and rats, as well as in control human and rat groups with no known source of exposure. The potential for persistence of *o*-toluidine or its metabolites in humans was shown by comparison of the urinary concentrations determined in exposed and unexposed workers prior to the work shift (Teass et al., 1993; Brown et al., 1995).*o*-Toluidine is excreted primarily via the urine of humans and rats as *N*-acetylated metabolites (Son et al., 1980; El-Bayoumy et al., 1986; Teass et al., 1993; Brown et al., 1995; Williamson et al., 1995; Ward et al., 1996). The main metabolite is 4-amino-3-methylphenol (synonym 4-amino-m-cresol) and small amounts of the *N*-hydroxy-derivative are also formed (Son et al., 1980; Kulkarni et al., 1983). Unmetabolized *o*-toluidine is also excreted in the urine of rats at concentrations that are high (21–36%) in comparison to the noncarcinogenic *p*- and *m*-toluidine isomers (2.5% each), and contact of the parent compound with the urinary bladder was proposed as a basis for *o*-toluidine tumorigenesis in this organ (Cheever et al., 1980). A physiologically based pharmacokinetic model to permit quantitative comparisons between rats and humans, however, is not available. A partial metabolic scheme is depicted in Fig. 1.

The data available regarding the specific forms of cytochrome P450 (CYP) involved in the metabolism of *o*-toluidine are limited. Many aromatic amines are preferentially bioactivated by CYP1A2 in rats (Hammons et al., 1991) and humans (Kim and Guengerich, 2005). Smokers have significantly higher CYP1A2 activity compared to nonsmokers, yet smoking status did not influence the increase of *o*-toluidine hemoglobin adducts formed after treatment with the *o*-toluidine precursor, prilocaine (Gaber et al., 2007). On the other hand, *o*-toluidine itself is an inducer of hepatic CYP content and activities, and was especially effective in inducing caffeine metabolism, predominantly catalyzed by CYP1A2 (Jodynis-Liebert and Matuszewska, 1999), aryl hydrocarbon hydroxylase activity, predominantly catalyzed by CYP1A (Gnojkowski et al., 1984), ethoxyresorufin-*O*-deethylase (CYP1A1 and CYP1A2), and other CYP activities (ethoxycoumarin-*O*-deethylase and aldrin epoxidase) (Leslie et al., 1988). Additionally, induction of hepatic CYP1A2 among other isoforms by Phenobarbital treatment caused a 1.5-fold increase in the binding of *o*-toluidine to hemoglobin in the presence of rat microsomes *in vitro* (Teass et al., 1993) and a similar but variable response in rats *in vivo* (DeBord et al., 1992).

The production of ring-hydroxylated metabolites of *o*-toluidine was enhanced approximately 8-fold in rats given an ethanol liquid diet for 28 days as compared with rats given the control diet (Diaz Gomez et al., 2006), suggesting that ring-hydroxylation pathways may be catalyzed by CYP2E1.

3.2. Health effects in humans

Oral exposure information in humans was not identified. Cases of non-oral human poisoning by *o*-toluidine were reviewed by OECD (2006). The principle acute effect induced in humans following inhalation exposure is methemoglobinemia (OECD, 2006; ChemIDPlus, 2011), with clinical signs of central nervous system depression. Chronic effects in workers exposed to *o*-toluidine include anemia, anorexia, weight loss, skin lesions, central nervous system depression, cyanosis, methemoglobinemia, and bladder cancer (US EPA, 2000b; IARC, 2010; HSDB, 2011).