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.

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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*-nitro-toluene (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).
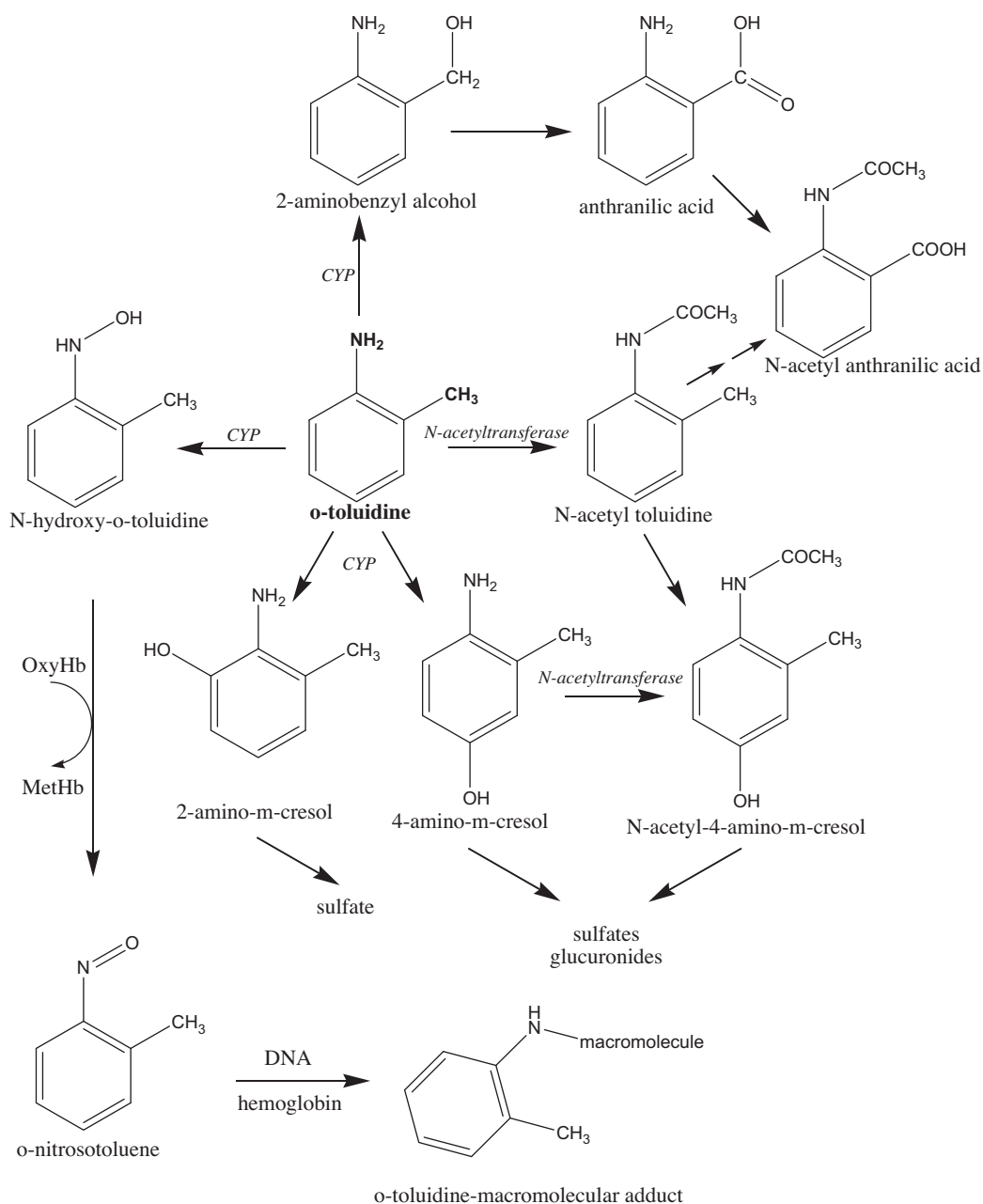
Fig. 1. Proposed metabolic pathway for *o*-toluidine, adapted from Son et al. (1980) and Riedel et al. (2006), with modifications.

Several human case studies and epidemiology studies involving *o*-toluidine have been reported and reviewed previously by IARC (2000, 2010), OECD (2006). A number of these studies suggested that occupational exposure to *o*-toluidine is associated with an increased risk of bladder cancer (see Table 1). It was not possible to definitively ascribe the bladder cancer to *o*-toluidine per se, in light of coexposure to other potential bladder carcinogens (including *p*-toluidine, aniline, *o*-nitrotoluene, rosaniline, safranine T, 4,4′-methylene-bis-(2-methylaniline), 2-naphthylamine and toluene), although co-exposures differed between studies (IARC, 2000).

An IARC Working Group (Baan et al., 2008) reassessed the carcinogenicity of certain aromatic amines, including *o*-toluidine, suspected of being carcinogenic. Following the re-evaluation of *o*-toluidine carcinogenicity data, it was noted that the occupational cohort studies showed greatly increased risks of bladder cancer, which could not be explained by smoking. Moreover, other known

bladder carcinogens were present only in trace amounts in two of the studies (Ward et al., 1991; Sorahan et al., 2000), and the highest risk of bladder cancer was associated with the longest duration of *o*-toluidine exposure (Sorahan, 2008; Baan et al., 2008). Based on the epidemiology studies in concert with several positive rodent carcinogenicity studies and the detection of *o*-toluidine hemoglobin adducts in humans, the IARC working group concluded that *o*-toluidine is carcinogenic to humans, and assigned it to evidence Group 1 (Baan et al., 2008; IARC, 2010).

3.3. Animal studies

Exposure to *o*-toluidine produces methemoglobinemia in laboratory animals as with humans. In short-term repeated oral dosing studies in rats, the earliest effects associated with *o*-toluidine are cyanosis, morphological changes associated with the spleen and

Table 1
Summary of selected studies of workers exposed to o-toluidine.

Population	Bladder cancer	Potential coexposures	Reference
NY State rubber chemical plant workers (1749 men)	SIR = 27.2 Latency 23/34 years	Aniline, 4-aminobiphenyl 2-aminofluorene, hydroquinone, toluene, carbon disulfide, sulfur, benzothiazole, 2-mercaptobenzothiazole, nitrobenzene	Ward et al. (1991), Prince et al. (2000), IARC (2010)
Italian dyestuff factory workers (906 men)	SMR = 62.5 Latency 24.9 years	4,4′-Methylene bis(2-methylaniline), magenta, safranine T, o-nitrotoluene, 2,5-diaminotoluene, aniline, o-aminoazotoluene, toluene, smoking	Rubino et al. (1982), IARC (2010)
German 4-chloro-o-toluidine plant workers (116 men)	SIR = 72.7 Latency 27.5 years	N-acetyl-o-toluidine, 6-chloro-o-toluidine, 4-chloro-o-toluidine	Stasik (1988), IARC, (2010)
Wales rubber chemical plant workers (2160 men)	SMR = 277 Latency >20 years	Aniline, 2-mercaptobenzothiazole, phenyl-β-naphthylamine	Sorahan et al. (2000), Sorahan (2008)

the urinary bladder, bone marrow hypercellularity, and in the liver, lipid peroxidation and the induction of antioxidant defenses. After 13- or 26-week exposures, male rats developed splenic capsule fibrosis, diffuse hyperplasia of the urinary bladder epithelium, and an increase in placental-GST positive foci in the liver. Hyperplasia of the epididymis was also observed after 26 weeks of exposure. After two-years of exposure to o-toluidine hydrochloride in the diet, non-neoplastic dose-related lesions were found in the spleen of rats and mice, and in the urinary bladder of rats. Decrements in body weight or body weight gain were seen consistently in animals exposed via the feed for durations of two weeks to two years.

No data were located on the reproductive effects or developmental toxicity of o-toluidine in the published literature. Chronic administration led to proliferative lesions and neoplasms of mesenchymal tissue in male rats including the peritoneal lining of the scrotum. In chronically treated female mice, ovarian cysts were observed. Standard reproductive parameters were not evaluated in these studies, and the data are not sufficient to determine whether exposure to o-toluidine can alter reproductive function. Repeated-dose studies along with the respective LOAEL and NOAEL values for non-cancer effects are summarized in Table 2.

Supporting the human epidemiological evidence of carcinogenicity are chronic studies in several species of animals. Table 3 summarizes the findings of carcinogenicity studies with the hydrochloride of o-toluidine given via the diet to rats and mice of different strains. In addition to these studies, o-toluidine induced urinary bladder papillomas in dogs after lifetime oral exposure (Pliss, 2004) and in rabbits and guinea pigs when administered by the subcutaneous route (US EPA, 1988b). The urinary bladder is the only suspected target in humans for o-toluidine carcinogenicity.

3.4. Genotoxicity

Studies of the genotoxic potential of o-toluidine were previously reviewed by Danford (1991), WHO/IPCS (1998), IARC (2000, 2010), OECD (2006). o-Toluidine was included in two international collaborative studies evaluating short-term tests for carcinogens, which contributed to a large database of studies available for this compound. Bacterial tests conducted under standard conditions in Salmonella typhimurium and Escherichia coli are largely negative, consistent with what is generally observed with monocyclic aromatic amines (IARC, 2010). However, o-toluidine is a weak bacterial, fungal and mammalian mutagen under nonstandard conditions when protocol variations included use of the fluctuation test (Gatehouse, 1981; Hubbard et al., 1981), addition of norharman (Nagao and Takahashi, 1981) or lithocholic acid

(Kawalek et al., 1983), and the use of hamster rather than rat liver S9 (Zeiger and Haworth, 1985) or high concentrations of S9 mix.

Results from several cytogenetic studies have demonstrated clastogenic activity of o-toluidine in mammalian cells in vitro, usually requiring prolonged exposure. Exogenous metabolic activation is not always a requirement, and incubation in serum-free media is necessary to evoke a positive response when hepatic S9 mix is added. In certain fungal and mammalian systems, o-toluidine induces aneuploidy or polyploidy. o-Toluidine was positive in an in vitro micronucleus test using human lymphocytes (Vian et al., 1993), without requirement for hepatic S9. Species differences may account for variable results observed in vivo. In mice, in vivo tests for bone marrow chromosomal aberrations and micronuclei were negative after intraperitoneal administration, and a mouse bone marrow micronucleus test by the oral route was similarly negative (OECD, 2006). When the micronucleus test was performed with Fischer 344 or Sprague–Dawley male rats by the oral or intraperitoneal routes, the results were reproducibly positive in peripheral blood reticulocytes and negative in hepatocytes (Suzuki et al., 2005). o-Toluidine causes single-strand DNA breaks in vitro and in vivo (Cesarone et al., 1982). It induces unscheduled DNA synthesis in vitro, with a requirement for S9 or intrinsic metabolic capability. Unscheduled DNA synthesis was also induced in vivo in the transitional epithelium of the rat urinary bladder (OECD, 2006). o-Toluidine causes the transformation of cultured mammalian cells, again, in the presence of S9 or in cells with intrinsic metabolism. In a bone marrow sister chromatid exchange assay in mice, o-toluidine produced positive results after high intraperitoneal doses (McFee et al., 1989). Ohkuma et al. (1999) reported that o-toluidine metabolites caused oxidative DNA damage in vitro in the presence of Cu(II). DNA adducts of o-toluidine (as a major metabolite of prilocaine) were detected in human urothelial cells obtained from the urine of patients following prilocaine anesthesia (Richter et al., 2006; Gaber et al., 2007), and were later detected in 11 out of 12 human bladder tumors (Böhm et al., 2011). When evaluated in male rats, DNA adducts were obtained in the liver (Brock et al., 1990) and nasal mucosa (Duan et al., 2008).

3.5. Mode of action evaluation

Mode of action considerations are an integral component of risk assessment frameworks designed to establish the human relevance of animal carcinogenicity data (Boobis et al., 2006; Meek et al., 2003). Mode of Action is defined as the sequence of measurable key events and cellular and biochemical events, starting with the interaction of an agent with the target cell, through functional and anatomical changes, resulting in adverse health effects (US EPA, 2005a). Key events are empirically observable, necessary steps in the disease

Table 2
Summary of repeated dose studies with *o*-toluidine hydrochloride and LOAEL and NOAEL values for noncancer effects.

Study type (species)	Exposure route	NOAEL[a] (mg/kg-day)	LOAEL[a] (mg/kg-day)	Critical effect(s)	Reference
14-Day (F344 rat)	Diet	None	23.7 (males) 25.5 (females)	Methemoglobinemia (males, females); ↓Body weight gain (females)	Dupont (1994) OECD (2006)
20-Day with 5 and 10 day interim groups (F344 rat)	Gavage	None	225 (males)	↓Body weight gain; ↓ Survival; Cyanosis; ↑Spleen organ weight Splenic congestion, hemosiderosis, and hematopoiesis; Bone marrow hypercellularity	Short et al. (1983)
4-Week (Wistar rat)	Diet	None	40 (males)	↑Hepatic microsomal lipid peroxidation, GSH concentrations, GSH peroxidase and catalase activities. ↓Hepatic superoxide dismutase activity	Jodynis-Liebert and Murias (2002)
7-Week[b] (F344 rat)	Diet	37(males) 149 (females)	75 (males) 224 (females)	↓Body weights	NCI (1979)
7-Week[b] (B6C3F1 mouse)	Diet	None	301 (males and females)	↓Body weight gain (males and females)	NCI (1979)
26-Week with 13 week interim, stop and continuous exposure groups (F344 rat)	Diet	None	213 (males)	↓Body weight gain; ↑Spleen absolute and relative organ weight Hyperplasia of the urinary bladder transitional epithelium (completely reversible). Hemosiderin accumulation in the spleen, liver Kupffer cells, and renal tubule epithelium (partly reversible) Fibrosis of the splenic capsule (irreversible). Mesothelial hyperplasia (continuous exposure) of the tunica vaginalis	NTP (1996)
18 Month[c] (CD-1 rat)	Diet	None	162 (males)	↓Body weight gain; ↓Survival	Weisburger et al. (1978)
72 Week[d] (F344 rat)	Diet	None	98 (males)	↓Body weight gain; ↓Survival	Hecht et al. (1982)
2-Year (F344 rat)	Diet	None	178 (males) 92 (females)	↓Body weight gain; ↓Survival; ↑Incidence of epithelial hyperplasia of urinary bladder; ↑Incidence of mesothelial hyperplasia of splenic capsule	NCI (1979)
18 Month[e] (CD-1 mouse)	Diet	None	905 (males and females)	↓Body weight gain; ↓Survival	Weisburger et al. (1978)
2-Year (B6C3F1 mouse)	Diet	None	93 (males) 97 (females)	↓Body weight gain; ↑Incidence of splenic hematopoesis (males); ↑Incidence of ovarian cysts (females)	NCI (1979)

[a] Dose of the hydrochloride multiplied by 0.746 to obtain the dose of the free base, *o*-toluidine.
[b] Animals were held for 1 week on control diets following the 7 week exposure period.
[c] Animals were held for 6 months on control diets following the 18 month exposure period.
[d] Animals were held for 20 weeks on control diets following the 72 week exposure period.
[e] Animals were held for 3 months on control diets following the 18 month exposure period.

Table 3
Summary of rodent oral carcinogenicity studies with *o*-toluidine hydrochloride.

Test system	Route	Tumors	Reference
CD rats (males) 18 mo/24 mo ob	Diet	Subcutaneous fibromas and fibrosarcomas; urinary bladder transitional cell carcinomas (n.s.)	Weisburger et al. (1978)
F344 rats 101–104 wks	Diet	Both sexes: sarcomas of multiple organs. Males: mesotheliomas in multiple organs or the tunica vaginalis; urinary bladder transitional-cell carcinomas (n.s.). Females: splenic sarcomas, urinary bladder transitional-cell carcinomas, mammary gland fibroadenomas or adenomas.	NCI (1979)
F344 rats (males) 72 wks	Diet	Urinary bladder papillomas and carcinoma (n.s.); mesotheliomas and sarcomas of the peritoneum; liver and mammary tumors	Hecht et al. (1982)
CD-1 mice 18 mo/24 mo ob	Diet	Hemangiosarcomas and hemangiomas of abdominal viscera (both sexes)	Weisburger et al. (1978)
B6C3F1 mice 103 week	Diet	Hemangiosarcomas and hemangiomas (males) Hepatocellular carcinomas (females)	NCI (1979)

n.s. = not significant.
ob = observation.

process. While the mode or modes of action for *o*-toluidine toxicity and carcinogenicity in various target tissues are not completely understood, we propose that the following set of key events is involved in the mode of carcinogenic action of *o*-toluidine to the human and rodent urinary bladder (see Fig. 2):

1. Initial bioactivation by hepatic microsomal cytochrome P450 enzymes.
2. Further bioactivation in the urinary bladder transitional epithelium via NAT1 yielding reactive nitrenium ions and forming DNA adducts.
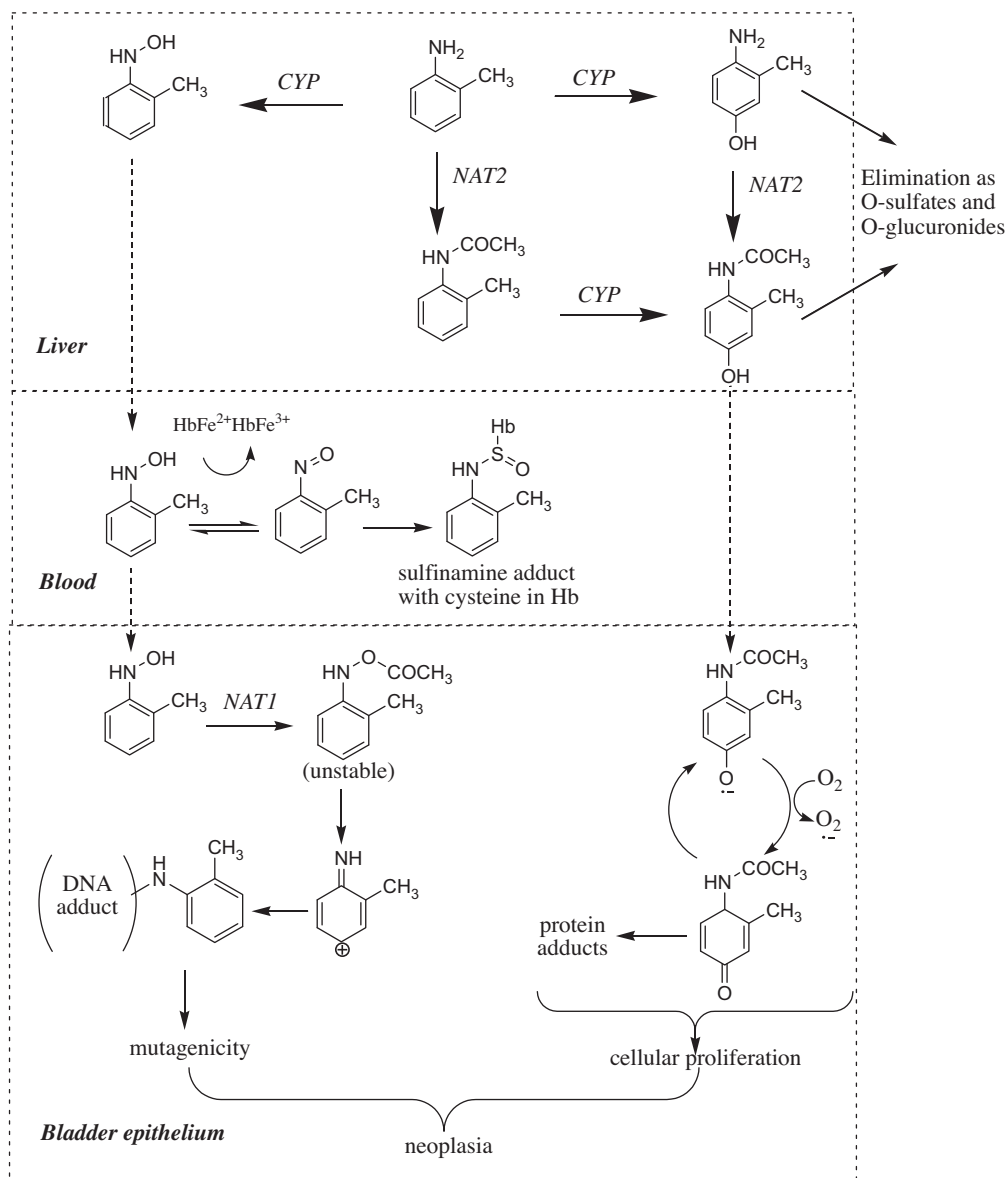
Fig. 2. Proposed key events involved in the mode of carcinogenic action of *o*-toluidine to the transitional epithelium.

3. Semiquinone/quinone redox cycling in the transitional epithelium producing oxidative cellular damage and compensatory cell proliferation.
4. Mutation resulting from direct or indirect DNA damage to urinary bladder transitional epithelial cells.
5. Human and rodent urinary bladder tumors.

The urinary bladder is the only suspected target in humans for *o*-toluidine carcinogenicity. Hecht et al. (1982) distinguished between the mechanism involved in the tumor induction in the urinary bladder and liver (*N*-oxidation) and that responsible for effects observed in the spleen, peritoneum, skin, and mammary gland. A comparison of the effects of chronic administration of *o*-toluidine hydrochloride, and a minor metabolite, *o*-nitrosotoluene, showed *o*-nitrosotoluene to be more potent than *o*-toluidine hydrochloride in the male F-344 rat urinary bladder and liver. This was not the case for effects observed in the peritoneum, skin, and spleen, where *o*-nitrosotoluene and *o*-toluidine induced comparable numbers of tumors, nor in the mammary gland, where fibroadenomas were induced to a greater extent by *o*-toluidine. The

authors concluded that induction of bladder tumors by *o*-toluidine hydrochloride depends upon *N*-oxidation and resulting interaction of *o*-nitrosotoluene or the corresponding hydroxylamine with cellular macromolecules in the bladder epithelium, whereas *N*-oxidation may not be a key step for the induction of tumors of the skin, spleen, peritoneum, and mammary gland.

The data for *o*-toluidine suggest that multiple modes of action likely contribute to the carcinogenicity of this compound, with some target organ specificity. Mutagenic *N*-oxidized metabolites participate in the development of urinary bladder neoplasias, and stimulation of cell division in the transitional epithelium may also be involved (see Fig. 2).

3.5.1. Metabolic activation

N-oxidation catalyzed by cytochrome P450 appears to be a necessary if not sufficient step in the activation of *o*-toluidine to a bladder and liver carcinogen. Hepatic cytochrome P450 enzymes catalyze the formation of *N*-hydroxyl-*o*-toluidine (Stillwell et al., 1987; Cheever et al., 1992; Teass et al., 1993; Sabbioni and Sepai, 1995); the specific cytochrome P450 isoforms responsible for this

activation have not be elucidated. Further oxidation to nitrosotoluene may take place in the blood, generating methemoglobin (Kiese, 1966). The N-oxidized intermediates form adducts with blood hemoglobin, as demonstrated in vitro (Teass et al., 1993) and in vivo (Stillwell et al., 1987; Cheever et al., 1992; Richter et al., 2001). Alternatively, the N-hydroxyarylamine can potentially be metabolized to a reactive N-sulfonyloxyarylamine, N-acetoxyarylamine and/or N-hydroxy arylamine N-glucuronide, which are capable of reacting with protein and DNA (Sabbioni and Sepai, 1995) and may be responsible for the cytotoxicity and genotoxicity of o-toluidine.

N-hydroxy-o-toluidine and its various conjugates produced in the liver can enter the circulation and reach the lumen of the bladder, where they can form highly electrophilic nitrenium ions, which may in turn lead to DNA adducts in the transitional epithelium (Kadlubar and Badawi, 1995; Taylor et al., 1998; Riedel et al., 2006). Nonenzymatic activation of N-hydroxyarylamines to reactive electrophiles is favored under the mildly acidic conditions (e.g., pH 5–6) found in human and dog urine (Beland et al., 1983), but this activation can also take place at pH 7.5 (Kulkarni et al., 1983). NAT1 in the urinary bladder is thought to catalyze acetylation of the N-hydroxyarylamine to form the reactive N-acetoxyarylamine that ultimately hydrolyzes and binds to DNA (Talaska, 2003) and initiates DNA damage.

While N-oxidation appears to be a critical step in generation of DNA reactive metabolites, intermediates formed via alternate pathways are likely to contribute to the expression of o-toluidine toxicity. CYP2E1 is implicated in the ring-hydroxylation of o-toluidine at positions para- and ortho- to the amine. Ring-hydroxylation reactions are catalyzed by liver microsomes and liver nuclei from naïve rats, and induced by ethanol treatment (Diaz Gomez et al., 2006). The major pathway of o-toluidine metabolism is ring hydroxylation in the para (4)-position to form 4-amino-m-cresol, and N-acetylation of the latter to form N-acetyl-4-amino-m cresol; both metabolites are excreted as sulfates and glucuronides in the urine (Cheever et al., 1980; Son et al., 1980; Williamson et al., 1995). The unconjugated phenolic metabolites are sensitive to oxidation (Son et al., 1980), potentially forming reactive quinone imines which can undergo redox cycling and generate reactive oxygen species. This mechanism may explain the observation that the N-acetyl-4-amino-m-cresol concentration in urine was positively correlated with urinary bladder cell proliferation in rats (Dupont, 1994).

3.5.2. Genotoxic modes of action

N-oxidation of o-toluidine is necessary for the expression of mutagenicity, as well as the development of bladder tumors. When tested for mutagenicity in S. typhimurium, the hydroxylamine, C-nitroso and hydroxamic acid metabolites of o-toluidine were found to have significant mutagenic activity exclusively in base-pair mutagen sensitive strains of S. typhimurium (Hecht et al., 1979), but only in the presence of rat liver homogenate (Hecht et al., 1979; Gupta et al., 1987). The parent compound has repeatedly been negative in standard bacterial reverse mutation assays. The mutagenicity was proposed to arise from activation of o-toluidine to N-acetyl-N-hydroxy-o-toluidine, which can further generate reactive electrophilic nitrenium ions and covalently bind DNA (Gupta et al., 1987). Previous reviews (Danford, 1991; IARC, 2000; OECD, 2006) of the extensive body of genetic toxicity data have detailed the lack of consistency in assay results, but conclude that o-toluidine exhibits mutagenic, clastogenic, and DNA damaging activity under certain conditions.

DNA damage may alternatively be mediated by the generation of free radical intermediates from phenolic or N-hydroxyl metabolites of o-toluidine. Quinoneimines can undergo a one-electron reduction catalyzed by flavoenzyme NADPH-cytochrome P450

reductase and spontaneously reoxidize with the resultant formation of reactive oxygen species. The generation of free radicals by o-toluidine was confirmed by Ohkuma et al. (1999) who showed that 4-amino-3-methylphenol (major metabolite) is autooxidized to form aminomethylphenoxyl radical, and o-nitrosotoluene (minor metabolite) is reduced by NADH to the o-toluolhydronitroxide radical. These authors proposed that oxidative damage to DNA via these radical metabolites of o-toluidine participates in the carcinogenic action of o-toluidine. 8-Oxo-7,8-dihydro-2′-deoxyguanosine is formed efficiently in vitro by o-toluidine metabolites (4-amino-3-methylphenol and o-nitrosotoluene plus NADH) in the presence of Cu(II) and is known to cause GC to TA transversions, a common DNA mutation found in bladder carcinoma (Ohkuma et al., 1999). It is of interest that ring hydroxylation of o-toluidine to 2-amino-m-cresol and 4-amino-m-cresol can be catalyzed by rat liver nuclei as well as microsomes (Diaz Gomez et al., 2006). Thus o-toluidine metabolites and reactive oxygen species might be generated in proximity to potential DNA targets.

There is also evidence of a direct interaction between o-toluidine metabolites and DNA in vivo. o-Toluidine-releasing DNA adducts in the human bladder were preliminarily reported by Richter et al. (2006) and Gaber et al. (2007) by capillary gas chromatography–mass spectrometric analysis of hydrolyzed adducts. Subsequently these adducts were detected in 11 out of 12 human bladder tumors at a mean concentration of 8.72 ± 4.49 fmol/μg DNA. This concentration was >100-fold higher than 4-aminobiphenyl-releasing DNA adducts in the same tissues, and >30-fold higher than concentrations of o-toluidine-releasing DNA adducts in epithelial and submucosal bladder tissues of 46 sudden death victims (Böhm et al., 2011).

When evaluated in male rats, DNA adducts were obtained in the liver (Brock et al., 1990) and also in the nasal mucosa (Duan et al., 2008), but surprisingly, none were detected in the urinary bladder epithelium by the nucleotide ^{32}P-postlabeling assay (detection limit ~1 adduct in 10^8 normal nucleotides) following single or 7-day gavage dosing (Duan et al., 2008). The authors noted that some adducts are significantly underestimated by the postlabeling assay used. Lifetime bioassays with o-toluidine identified the liver as a target in male and female mice (NCI, 1979); in male rats (Hecht et al., 1982), and 1 rat out of 50 (sex not specified) developed a hepatic sarcoma after subcutaneous injection (Pliss, 2004). Nasal pathology was not evaluated in the chronic studies. On the basis of relative macromolecular binding of ortho- and para- isomers of toluidine, Brock et al. (1990) concluded that the degree of binding did not correlate directly to carcinogenic potency. When the urinary bladder epithelium of the rat was specifically investigated following 14-day feeding with o-toluidine, both unscheduled DNA synthesis and cellular proliferation were found to be significantly elevated when compared with controls (Dupont, 1994).

3.5.3. The urinary bladder epithelium

Toxicity to the urinary bladder epithelium was investigated in male and female F344 rats fed o-toluidine for 14-days (Dupont, 1994). There was a dose related increase in cell proliferation, which was positively correlated with the urinary excretion of N-acetyl-4-amino-m-cresol in both sexes. At the high dose level, mild urothelial hyperplasia was observed in females that received an estimated 321 mg/kg-day, and minimal thickening of the urothelial layer was observed in males that received an estimated 300 mg/kg-day (after correcting for the test substance stability). The high dose level in this study also induced unscheduled DNA synthesis in urinary bladder cells evaluated ex vivo. Cell proliferation is indicative of a regenerative response of the transitional epithelium to cellular damage caused by o-toluidine metabolites. Toxicity to the urinary bladder epithelium may be a consequence of reactive oxygen species generated by the redox cycling of

N-acetyl-4-amino-*m*-cresol and its quinone imine counterpart, or damage mediated by the electrophilic quinone imine itself (see Fig. 2). *N*-acetyl-4-amino-*m*-cresol is a major *o*-toluidine metabolite (Williamson et al., 1995) and is sensitive to spontaneous oxidation (Son et al., 1980).

4. Exposure characterization

4.1. Sources of exposure

The general population may be exposed to low *o*-toluidine concentrations through ambient air, tobacco smoke, or food (HSDB, 2011), as well as drinking water. No reports were located in the published literature referencing the measurement of *o*-toluidine in drinking water. However, *o*-toluidine has occasionally been detected during extraction testing of drinking water system components at low ppb concentrations, normalized to static at-the-tap conditions (NSF International, personal communication). *o*-Toluidine occurs in tobacco leaves, black tea, and in beans (*Phaseolus vulgaris*) (Neurath et al., 1977). Unspecified toluidine isomers that are likely to include *o*-toluidine were detected in kale (*Brassica oleracea*), celery (*Apium graveolens*), and carrots (*Daucus carota*) (OECD, 2006). *o*-Toluidine is ubiquitous in the human environment because it is a major component of tobacco smoke (OECD, 2006). In the main-stream smoke of cigarettes, 30–208 ng/cigarette of *o*-toluidine was measured, and in the unfiltered side-stream smoke it was the most abundant of the aromatic amines measured at 2–4 µg/cigarette (Luceri et al., 1993). In spite of the relatively higher level of *o*-toluidine found in side-stream cigarette smoke, the contribution from passive smoke to *o*-toluidine hemoglobin adduct levels was concluded to be small relative to region-specific factors, such as traffic density-related air pollution in larger cities as compared to rural environments (Richter et al., 2001). The *o*-toluidine concentration in ambient air from a variety of indoor air sources, including nonsmoking zones and rooms where smoking was allowed, ranged from 3.0 to 16.9 ng/m^3. The concentration of an outdoor air source in Florence, Italy (location not specified) was 2.5 ng/m^3 (Luceri et al., 1993).

Environmental exposure can occur during the manufacture and processing of *o*-toluidine, either via wastewater effluent or atmospheric emissions. Raw wastewater from a pigment manufacturing factory in Korea contained *o*-toluidine at 25.3 mg/L, a level acutely toxic to aquatic organisms, but the substance was not detected in the effluent indicating that it was removed effectively by the factory wastewater treatment plant (Jo et al., 2008). *o*-Toluidine was present in surface-water samples taken from three rivers in Germany, at levels of 0.3–1 µg/L (Neurath et al., 1977). According to the Official Emission Declaration for the year 2000, <25 kg/annum of *o*-toluidine was emitted into the atmosphere at the five German production and processing sites (OECD, 2006). Annual environmental releases from industrial facilities in the United States were 16,229, 18,797, and 16,240 lb in 2005, 2006, and 2007, respectively, as reported in the Toxics Chemical Release Inventory (TRI, 2009).

Releases of *o*-toluidine may occur from products like coal oil and gasoline. It is formed by the reductive degradation of *o*-nitrotoluene at former ammunition sites (OECD, 2006), but degrades more rapidly than its precursor (Van Aken and Agathos, 2002). The estimated half-life in air of *o*-toluidine due to indirect photodegradation is 2.9 h (OECD, 2006) assuming 500,000 hydroxyl radicals/cm^3 (AOPWIN, v. 1.91). Significant hydrolysis of *o*-toluidine in aquatic compartments is not expected due to the lack of hydrolysable functional groups. The results of several standard biodegradation tests, indicated that *o*-toluidine is readily biodegradable under aerobic conditions (OECD, 2006).

4.2. Exposure assessment

o-Toluidine has been detected in most urine samples analyzed from the general population and the levels are generally higher in smokers compared to non-smokers (Riffelmann et al., 1995; Riedel et al., 2006). OECD (2006) concluded that tobacco smoke, including environmental tobacco smoke, is the predominant source of *o*-toluidine exposure in non-occupationally exposed humans. Diet and region-specific factors such as air pollution have also been identified as possible contributing sources (DeBruin et al., 1999; Richter et al., 2001).

Biomonitoring data in humans confirmed that population exposure to *o*-toluidine occurs in both smokers and non-smokers, under various occupational and environmental settings (Weiss et al. 2000; Weiss and Angerer, 2002; Talaska, 2003; Riedel et al. 2006).

5. Determination of dose response relationship

5.1. Key study and critical effect

5.1.1. Key study

Considering the proposed mode of urinary bladder carcinogenicity and the lack of adequate exposure information among occupationally exposed cohorts, quantitative assessment of cancer risk associated with *o*-toluidine relies on urinary bladder carcinogenicity data from laboratory animals. Several studies on the carcinogenicity of *o*-toluidine were located and the two-year study of *o*-toluidine hydrochloride in diet (NCI, 1979) with rats and mice was selected as the key study. It was considered to be the most suitable for risk assessment, based on the chronic duration of exposure and thoroughness of reporting. The study was conducted prior to the development of current regulatory guidelines (US EPA, 2009), and the protocol lacked clinical chemistry, urinalysis, and organ weight measurements, although these deficiencies did not impact the assessment. As discussed in *6.2.2, Critical effect*, the compromised health status and low survival of the males rats in this study hindered meaningful interpretation of male tumor data; however, considering the concordance of target organ (i.e. urinary bladder) between humans and rodents, the fact that the maximum tolerated dose may have been exceeded was not considered to impact the assessment. Since the reaction of *o*-toluidine with stomach acid will yield the hydrochloride salt, the use of the hydrochloride salt as the test article rather than *o*-toluidine itself is not a concern (US EPA, 1984). Chronic dietary administration to rats resulted in sarcomas of multiple organs in each sex, fibromas of the subcutaneous tissue and mesotheliomas in multiple organs or the tunica vaginalis in male rats, and sarcomas of the spleen, transitional-cell carcinomas of the urinary bladder, and fibroadenomas or adenomas of the mammary gland in female rats (Table 4).

The results of chronic testing in mice of both sexes showed increased hepatocellular adenomas and carcinomas and vascular tumors (hemangiosarcoma and hemangioma) particularly in high dose males (Table 5). These studies in rodents had adequate test group sizes of 50 per sex, per dose level, and appropriate exposure duration of 101–104 weeks. The dietary route of exposure was also relevant. The other carcinogenicity studies, reviewed previously (IARC, 2000), were less completely reported; however, the tumor findings from other oral studies (Weisburger et al., 1978; Hecht et al., 1982; Pliss, 2004) were concordant with the results reported by NCI (1979).

The epidemiology studies were also assessed and subsequently discounted for their potential use in quantifying *o*-toluidine risk. Several confounding factors were identified; notably, the numerous co-exposures that occurred with *o*-toluidine in the

Table 4
Neoplastic and nonneoplastic effects in F344 rats after chronic dietary administration of *o*-toluidine hydrochloride[a] (NCI, 1979).

Dose, ppm in feed	0		3000		6000	
	Males	Females	Males	Females	Males	Females
Survival[b]	90%[g]	80%[g]	26%	57%	0%[c]	22%
Mean body weights compared to controls[d]	NA	NA	↓13%	↓12%	↓22%	↓23%
Neoplastic effects						
Urinary bladder – transitional cell carcinoma or papilloma	0/20	0/20[f]	3/50	10/45[h]	1/44	22/47[i]
Spleen – sarcoma, NOS; angiosarcoma, or osteosarcoma	0/20	0/20[f]	8/49	9/49[h]	4/42	12/49[h]
Subcutaneous tissue – fibroma	0/20[f]	0/20	28/50[i]	4/50	27/49[i]	2/49
Mammary gland – fibroadenoma or adenoma, NOS	0/20	7/20[f]	7/50	20/50	1/50	35/49[i]
Multiple organs lesions of mesenchymal origin[e] – malignant mesothelioma; mesothelioma, NOS; fibrosarcoma; angiosarcoma; or osteosarcoma	0/20[f]	0/20[f]	17/49[i]	2/50	29/42[i]	19/49[i]
Non-neoplastic effects						
Urinary bladder – epithelial hyperplasia	0/20	0/20	9/50	21/45	7/44	13/47
Spleen capsule mesothelial hyperplasia	0/20	0/20	18/49	32/49	5/42	12/49

Statistical analysis of non-neoplastic effects was not performed.
NA = not applicable.
  [a] Only effects observed at an increased incidence compared to controls were included with the following exceptions; if observed in only one sex, results for the other sex were included for comparison; ^rare tumors were included.
  [b] Survival data were not provided, and were thus estimated from the available graphs at 104 weeks except where otherwise indicated.
  [c] Survival at 101 weeks.
  [d] Mean body weight data were not provided, and were thus estimated from the available graphs.
  [e] Tumors are most frequently associated with the spleen; excludes lesions NOS that were too undifferentiated to classify.
  [f] Cochran-Armitage test, $p < 0.05$.
  [g] Significant Tarone test for dose-related trend in mortality $p < 0.001$.
  [h] Significant Fischer exact test compared to control, $p < 0.05$.
  [i] Significant Fischer exact test compared to control, $p < 0.01$.

epidemiology studies. These included aniline, 4-aminobiphenyl (Ward et al., 1991); 4,4′-methylene bis(2-methylaniline) and smoking (Rubino et al., 1982); *N*-acetyl-*o*-toluidine, and 6-chloro-*o*-toluidine (Stasik, 1988); and 2-mercaptobenzothiazole and phenyl-β-naphthylamine (Sorahan et al., 2000), among others. Many of these substances are considered carcinogenic or possibly carcinogenic to humans, and it is possible that these exposures interacted with *o*-toluidine exposure, thus influencing the incidences of bladder cancer discovered. While the disease occurrence was ultimately attributed primarily to the effect of *o*-toluidine exposure, disease rates could not be used to establish *o*-toluidine risk values.

The exposure information available from Ward et al. (1996) was valuable for establishing an association between *o*-toluidine exposure and bladder cancer in the worker cohort studied (Ward et al., 1991; Markowitz and Levin, 2004; Markowitz, 2005), but there are several confounds to using these data for the purpose of quantitative risk assessment. Elevated urinary concentrations and hemoglobin adducts were measured in both exposed and unexposed cohorts, and there is insufficient information to quantitatively describe the relationship between external and internal dose. Further, temporal trends and variations in exposure concentrations from 1972 to 2003 occurred due to improvements in workplace exposure controls and job reassignments. Thus, extrapolation beyond the bounds of the observation period (Ward et al., 1996) to estimate previous and subsequent exposure concentrations was not considered appropriate. In the absence of a quantitative exposure assessment, the epidemiology studies could not be used to establish reliable risk values.

5.1.2. Critical effect

The target tissues in which malignant neoplasms developed following lifetime oral exposure of rodents to *o*-toluidine were principally the urinary bladder, spleen, liver and vasculature. In identifying the critical effect from the NCI (1979) studies, in general, tumors that occurred in more than one species and or sex were assessed as being more significant than single-species and sex tumors that were observed. Additionally, tumors with

significantly elevated incidences at both low and high doses were assessed as more significant than tumors that were induced only after high-dose treatment. *o*-Toluidine targets the urinary bladder in multiple species (dog, rabbit, guinea pig, and rat) and the urinary bladder is the only suspected target in humans for *o*-toluidine carcinogenicity. The incidence of transitional cell neoplasia of the urinary bladder was significantly increased in female F344 rats at both low and high dose levels, and was consequently selected as the critical effect. Also considered but not selected as the critical effect were sarcomas of multiple organs (the spleen most frequently involved), which were also observed in rats of each sex. The splenic hyperplasia, fibrosis, and neoplasia reported in male and female rats are considered to be sequelae to red blood cell destruction and heme pigment accumulation (Goodman et al., 1984; NTP, 1996). Such effects would become manifest only when the capacity of the spleen to remove and process damaged red blood cells is exceeded, suggesting that these tumors are not suitable for low-dose linear extrapolation, and do not represent the critical effect. The incidences of mammary, vascular, and hepatocellular tumors were increased only in high dose groups, and were therefore not critical.

Increased incidences of urinary bladder tumors were found in male and female rats in the NCI (1979) study, females showing a significant dose-related trend and significant pairwise comparisons with the control at both low and high dose levels. Transitional cell epithelial hyperplasia, potentially a precursor lesion that progresses to neoplasia, was present in both males (0/20, 9/50, and 7/44) and females (0/20, 21/45, and 13/47), and there appeared to be a dose-related progression from transitional-cell epithelial hyperplasia to transitional-cell carcinomas of the urinary bladder, particularly in females. The lack of dose–response relationship in males may be attributable to the high mortality rate in this study, with 100% mortality in high-dose males at 101 weeks. Based on this study and other chronic studies in male rats, it is evident that the urinary bladder is a target organ in both sexes. In male CD rats fed *o*-toluidine hydrochloride for 18 months and held for 6 months on control diets, the incidences of transitional cell carcinomas were 3/23 in the low dose (162 mg/kg-day) group and 4/23 in the high

Table 5
Neoplastic and nonneoplastic effects in mice after chronic dietary administration of *o*-toluidine hydrochloride[a] (NCI, 1979).

	0		1000		3000	
	Males	Females	Males	Females	Males	Females
Dose, ppm in feed						
Survival	75%	95%	86%	78%	68%	86%
Mean body weights compared to controls[b]	NA	NA	↓6%	↓15%	↓15%	↓33%
Neoplastic effects						
Hepatocellular carcinoma	4/19	0/20[†]	16/50	2/49	11/50	7/50
Hepatocellular adenoma	1/19	0/20	3/50	2/49	3/50	6/50
Hepatocellular carcinoma or adenoma	5/19	0/20[†]	19/50	4/49	14/50	13/50[**]
Hemangiosarcoma	1/19[†]	1/20	1/49	1/49	10/50	2/50
Hemangioma	1/19	0/20	1/48	0/49	2/50	1/50
Hemangiosarcoma or hemagioma	1/19[†]	1/20	2/50	1/49	12/50	3/50
Non-neoplastic effects						
Splenic hematopoiesis	1/19	9/20	6/49	4/49	11/50	6/47
Ovarian cyst	NA	1/20	NA	7/46	NA	6/47

Statistical analysis of non-neoplastic effects was not performed.
NA, not applicable.
[a] Only effects observed at an increased incidence compared to controls were included with the following exceptions; if observed in only one sex, results for the other sex were included for comparison; ^rare tumors were included.
[b] Mean body weight data were not provided, and were thus estimated from the available graphs.
[†] Cochran-Armitage test, *p* < 0.05.
[**] Significant Fischer exact test compared to control, *p* < 0.01.

dose (350 mg/kg-day) group, compared with concurrent (0/16) or pooled (5/111) controls (Weisburger et al., 1978). In male F344 rats fed *o*-toluidine hydrochloride for 72 weeks and held for 20 weeks (Hecht et al., 1982), the incidence of transitional cell carcinomas was 4/30 (13%) in treated rats (98 mg/kg-day) compared with 0/27 in controls. While the incidences of transitional cell carcinomas of the bladder in male rats do not achieve statistical significance, the similarity in response among the three bioassays suggests biological significance of these tumors. The lower incidence of bladder tumors in male rats compared with female rats may be related to sex-specific patterns of arylamine activation and detoxication. For example, glutathione *S*-transferase M1 (GSTM1), a phase II enzyme linked to arylamine detoxification and bladder cancer risk (Yu et al., 2002) is more abundant in male than female mouse livers. If arylamine detoxification activity is also higher in male than female rats, the expected result would be lower bladder tumor incidence in male rats. In support of a sex-based difference in *o*-toluidine activation/detoxication, urinary bladder epithelial changes observed in a 14-day oral study with rats (Dupont, 1994) was more pronounced in females (LOAEL = 170 mg/kg-day) than in males (LOAEL = 300 mg/kg-day). In a study of gender- and smoking-related bladder cancer risk in the United States, women were found to have a higher propensity for arylamine activation compared to men and, consequently, experience a higher level of bladder cancer risk relative to men with comparable arylamine exposure (Castelao et al., 2001). Consistent with this result, the levels of blood hemoglobin adducts of toluidine isomers in male children were slightly lower than in female children (Richter et al., 2001).

In sum, of all the tumor types induced by chronic administration of *o*-toluidine to laboratory animals, transitional tumors of the urinary bladder epithelium are the only malignant tumors that were observed in more than one species and sex, indicating consistency of results across different assays and animal models. Tumors were significant at low as well as high doses, and the tumor response was larger in female rats than in male rats. The relevance of this neoplasia to humans is inferred from evidence in epidemiology studies and a common cytochrome P450-dependent bioactivation pathway in rats and humans.

According to McConnell et al. (1986) and Brix et al. (2010), the incidences of bladder tumors of the same tissue of origin observed in chronic animal bioassays may be combined for risk assessment purposes. Thus, transitional cell neoplasia of the urinary bladder was considered the critical effect, and papilloma and carcinoma incidences were combined for benchmark dose modeling. A separate histomorphogenic origin was assumed for the single squamous cell carcinoma observed in low dose males (data not shown), and this tumor type was not combined with the transitional cell tumors.

5.2. Identification of susceptible populations

For carcinogens that act through a mutagenic mode of action, there can be greater susceptibility for the development of tumors when exposures occur in early life stages as compared with later life stages (US EPA, 2005b). The key study selected for the quantitative estimation of *o*-toluidine cancer risk is a standard two-year bioassay in which exposure in the diet commenced at the young adult stage of life; 6 weeks in rats and mice (NCI, 1979). To address the potential impact of early-life exposure, the cancer risk derived from the standard study was modified according to US EPA (2005b) with potency adjustments for early-life exposures. Susceptibility to *o*-toluidine will depend on the maturity of arylamine bioactivation capacity, which develops early in life. Thus while the neonate is considered inherently more sensitive to mutagenic carcinogens, it would not be susceptible to *o*-toluidine until arylamine bioactivation capacity develops. Susceptibility to methemoglobinemia, generally greater in infants (Denshaw-Burke and Savior, 2008), would also be dependent upon the capacity for bioactivation of *o*-toluidine. Based on these considerations, default age-specific adjustment factors were selected for age groups between 6 months and 16 years, because no toxicokinetic or toxicodynamic data were available for *o*-toluidine to directly assess cancer susceptibility during this period. A quantitative analysis of lifetime unit risk is presented in *6.4 Dose–Response Assessment* that incorporates the recommended factors for early life stage susceptibility as well as early life stage exposure.

A principal route of metabolism for *o*-toluidine is via *N*-acetylation, a primary detoxication mechanism in humans for aromatic amines (Beland and Kadlubar, 1985). Genetic polymorphisms have been identified for N-acetylation enzymes, NAT1 and NAT2, and epidemiological evidence indicates that acetylator phenotype affects an individual's susceptibility to aromatic amine-induced bladder cancer (Freudenthal et al., 1999). The mean increased risk

of bladder cancer in NAT2 slow acetylators is 1.5–1.75 times higher relative to fast acetylators in cases of documented exposure to aromatic amines (Golka et al., 2002; Talaska, 2003). Thus it is possible that slow acetylators may be more susceptible to *o*-toluidine toxicity than rapid acetylators, however this possibility has not been confirmed in animal models.

5.3. Benchmark dose modeling

To evaluate the dose–response relationship for the combined transitional cell carcinomas and papillomas of the urinary bladder of female rats (NCI, 1979) a benchmark dose (BMD) was calculated. The BMD is the statistical lower confidence limit on the dose producing a predetermined level of change for an adverse effect compared with the response in untreated animals (US EPA, 2000a, 2011a). BMDs, benchmark dose lower bounds (BMDLs) and their associated slope factors were determined at the 10% response level with 95% confidence, an appropriate default given the tumor incidences of 22% and 47%, at low and high dose levels, respectively. For the purposes of the present assessment, the estimated mean achieved dietary doses in animals were converted to human equivalent doses, consistent with the default assumption that the human is more sensitive to *o*-toluidine than the rat (Table 6). The quantal linear model with the slope restricted to $\geqslant 1$ had the best statistical fit with the fewest parameters, and the statistical 95% lower confidence limit on a 10% effect level (BMDL$_{10}$) is 13 mg/kg-day (see Appendix). The corresponding slope factor of 0.0077 (mg/kg-day)$^{-1}$ is an upper-bound estimate of risk per increment of dose that can be used to estimate risk probabilities for different exposure levels.

The unit risk, defined as the upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 µg/L (US EPA, 2011c), may be calculated from the slope factor using the default 70 kg body weight and 2 L/day drinking water consumption of an adult:

$$\text{Unit Risk} = \frac{0.0077 \text{ kg-day}}{\text{mg}} \times \frac{1}{70 \text{ kg}} \times \frac{2 \text{ L}}{\text{day}} \times \frac{1}{1000 \text{ µg}}$$
$$= 0.22 \times 10^{-6} (\text{µg/L})^{-1}$$

The derived cancer slope factor used in the above Unit Risk calculation was based on a standard chronic adult rodent bioassay, which did not address the impacts of early-life exposures. For carcinogens acting through a mutagenic mode of action, US EPA (2005b) recommends that a potency adjustment should be used to assess cancer risks associated with early-life exposures. We used the slope factor first to calculate unit risks for individual life stages, and then summed the lifestage unit risks to derive the lifetime, lifestage-adjusted unit risk, as recommended by US EPA (2005b) supplemental guidance.

Chemical-specific data were not available to assess cancer susceptibility from early-life exposure directly, and the recommended default adjustment factors of "10" for the risk from 6 months to <2 years of life; "3" for the risk for ages two through <16; and "1" for ages 16 until 70, were applied. For the periods from birth to <6 months of age, an adjustment factor of "1" was used instead of the default of "10", based on deficiencies in cytochrome P450s involved in the metabolic activation of aromatic amines (Ginsberg et al., 2002). Xenobiotic metabolizing cytochrome P450s are generally immature at birth and reach full maturation between 6 months and one year of age (Vieira et al., 1996; Cresteil, 1998; Kurata et al., 1998; Nakamura et al., 1998; Tanaka, 1998; Valcke and Krishnan, 2011). Although the specific CYP isoforms that bioactivate *o*-toluidine have yet to be defined, given the overlapping substrate specificity within this family of enzymes, it is reasonable

to conclude that the degree of metabolic activation of *o*-toluidine by neonates until six months of age is low relative to adults. Thus selection of a 1x adjustment factor for ages 0 (birth) to 6 months is health-protective. After six months of age, the rate of cytochrome P450-catalyzed activities is comparable to adult levels. The use of default potency adjustment factors for lifestages beginning at six months is therefore consistent with the capacity for metabolic activation of *o*-toluidine and associated risks at these lifestages (Ginsberg et al., 2002).

Water ingestion rates also vary as a function of age, and children drink more water relative to their body weights than do adults. Thus, age-specific drinking water ingestion rates were applied to the various age range groupings (US EPA, 2005b). Recommended water ingestion rates were used, specifically, consumer-only ingestion rates, which represent the quantity of water consumed only by individuals who reported water intake during the survey period (US EPA, 2011b). Central estimates of the 90th percentile ingestion rates (L/kg-day) were chosen to correspond approximately with the default standard water ingestion quantity of 2 L for a 70 kg adult (88th percentile, US EPA, 2000). Using the recommended age groups, potency adjustment (p.a.) factors and exposure factors, listed in Table 7, and the derived slope factor, the individual age group unit risks (Unit Risk$_{age}$) associated with the corresponding life stage were derived according to the following:

$$\text{Unit Risk}_{age}(\text{µg/L})^{-1} = \underbrace{\frac{0.0077 \text{ kg} - \text{day}}{\text{mg}}}_{} \times \underbrace{\frac{1}{\text{bw(kg)}}}_{} \times \underbrace{\frac{\text{ingested water(L)}}{\text{day}}}_{}$$
$$\times \underbrace{\frac{1 \text{ mg}}{1,000 \text{ µg}}}_{} \times \underbrace{\frac{\text{exposure(y)}}{70 \text{y}}}_{} \times \underbrace{\frac{\text{p.a.}}{1}}_{}$$

The lifetime age group-adjusted Unit Risk (Unit Risk$_{AGE}$) for a population with average life expectancy of 70 years is obtained by summation of individual Unit Risk$_{age}$ values (Table 7), thus:

$$\sum \text{individual Unit Risk}_{age} = \text{lifetime Unit Risk}_{AGE}$$
$$= 4.7 \times 10^{-7} (\text{µg/L})^{-1} \text{or } 0.47$$
$$\times 10^{-6} (\text{µg/L})^{-1}$$

For *o*-toluidine, the lifetime Unit Risk$_{AGE}$ of 0.47×10^{-6} (µg/L)$^{-1}$ is approximately 2-fold higher than the unadjusted Unit Risk value of 0.22×10^{-6} (µg/L)$^{-1}$ for a 70 kg adult consuming 2 L of drinking water per day (Table 8). Based on the ratio of the Unit Risk$_{AGE}$ to the unadjusted Unit Risk, a lifestage-adjusted oral slope factor and 10^{-5} cancer risk level can be derived. The values and derivation of these parameters are presented in Table 8. The lifestage-adjusted 10^{-5} cancer risk level is 0.61 µg/kg-day; ~2-fold lower than the unadjusted cancer risk level of 1.3 µg/kg-day.

6. Risk characterization

The total allowable concentration (TAC) is a mean value used to evaluate the results of product extraction testing to static at-the-tap conditions (NSF/ANSI 61, 2011). For a compound considered to be a genotoxic carcinogen, the TAC is set at the 10^{-5} cancer risk level, a level considered to be safe and protective of public health (US EPA, 1991). The cancer risk level is calculated from the unit risk. The derived lifestage-adjusted unit risk (Unit Risk$_{AGE}$) was used for the calculation of the TAC, as follows:

$$\frac{10^{-5}}{\text{TAC}} = \frac{0.47 \times 10^{-6}}{\text{µg/L}}$$
$$\text{TAC} = 21 \text{ µg/L or } 0.021 \text{ mg/L}$$
$$= 0.02 \text{ mg/L or } 20 \text{ µg/L (rounded)}$$

Table 6
Dose–response data for benchmark dose modeling of combined neoplasia of urinary bladder in female rats administered *o*-toluidine hydrochloride (NCI, 1979).

Average Dietary Level[a] (ppm)	F344 female rat dose, *o*-toluidine hydrochloride[b,c] (mg/kg-day)	F344 female rat dose, *o*-toluidine[d] (mg/kg-day)	Human equivalent dose[e], *o*-toluidine mg/kg-day	Urinary bladder transitional cell neoplasia[f], combined Incidence/total
0	0	0	0	0/20
3,000	257	192	48	10/45[*]
6,000	530	395	95	22/47[**]

[a] Based on gravimetric formulation of the diets.
[b] Doses were estimated using the standard allometric relationship of food consumption (*F*, kg/day) and body weight (W, kg) for all animal species combined (US EPA, 1988a), i.e., $[F = 0.065 \, W^{0.7919}]$.
[c] Based on mean study body weights of 0.265 kg and 0.230 kg, respectively, at the low- and high-dose levels, obtained from female body weight data graphs at Week 75.
[d] The dose of *o*-toluidine as the free base was calculated by multiplying the dose of the hydrochloride by the ratio of molecular weights for the free base (107.155) to the hydrochloride (143.616), equal to 0.746.
[e] Human Equivalent Dose = dose (mg/kg-day) × (kg rat/70 kg human)$^{0.25}$.
[f] Lesions include papilloma and carcinoma.
[*] $p < 0.05$.
[**] $p \leqslant 0.01$.

This risk assessment accounts for the relatively higher drinking water intake on a body weight basis (L/kg-day) of early lifestage populations by using exposure factors for these populations in determining a lifestage-adjusted lifetime Unit Risk estimate. It also accounts for the potential for greater susceptibility in life stages between 6 months and 16 years of age relative to adults by using recommended potency adjustment factors (US EPA, 2005c).

Urinary measurements provide a reasonable indication of general population exposure to *o*-toluidine for comparison with the 10^{-5} cancer risk level derived in this risk assessment. An adult consuming 2 L of drinking water per day containing *o*-toluidine at the total allowable concentration (TAC) of 20 μg/L would consume 40 μg/day. The mean total exposure to *o*-toluidine from all sources was determined by Riedel et al. (2006) from urinary measurements to be 0.11 μg/day in non-smokers (*n* = 9) and 0.20 μg/day in smokers (*n* = 10) (Riedel et al., 2006). Similar results were obtained by Weiss and Angerer, 2002, who detected *o*-toluidine in 16 out of 20 subjects (smoking status was not reported) at urinary concentrations of 0.12 μg/L (median) and 2.7 μg/L (95th percentile); concentrations ranged from <0.05 to 3.1 μg/L. Assuming 1 L of urine is excreted per person per day (West, 1990), the median exposure from all sources was 0.12 μg/day and the 95th percentile exposure was 2.7 μg/day. The latter value is comparable to the calculated daily uptake of *o*-toluidine by smokers of 3.6 μg/day (Talaska, 2003). When compared with the 10^{-5} cancer risk value of 40 μg per day, high level general population exposure, represented by the 95th percentile or individuals smoking 25 cigarettes per day, is approximately 7–9% of the 10^{-5} cancer risk value. Mean or median exposures in the populations studied by Weiss and Angerer (2002) and Riedel et al. (2006) are <1% of the 10^{-5} cancer risk value.

7. Discussion and conclusions

o-Toluidine and its hydrochloride salt are listed in the NTP Report on Carcinogens, 12th Edition, as *reasonably anticipated to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in experimental animals (NTP, 2011a). The lifestage-adjusted cancer potency factor determined in the current risk assessment is 0.016 (mg/kg-day)$^{-1}$ and the corresponding unit risk value is 0.47×10^{-6} (μg/L)$^{-1}$. These values and the basis for their derivation may be compared with previous assessments and conclusions reached by government agencies. Thus, the US EPA (1987) classified *o*-toluidine as a Group B2, probable human carcinogen and calculated an oral cancer slope factor of 0.24 (mg/kg-day)$^{-1}$ and corresponding unit risk value of 6.9×10^{-6} (μg/L)$^{-1}$. In this assessment, the key study selected by US EPA was that

of Hecht et al. (1982) in which male rats were fed a single dose of *o*-toluidine in the diet for 72 weeks. The slope factor was determined based on the incidence of skin fibromas in treated rats (US EPA, 1997). In a later assessment (US EPA, 1988b) a cancer slope value of 0.069 (mg/kg-day)$^{-1}$ was derived from the NCI (1979) chronic study with male rats. Doses of 0, 150 and 300 mg/kg-day were assumed, and human potency was calculated using a mean rat body weight of 408 g (mean of mean body weights of 450 g, 400 g, and 375 g in control, low and high dose groups, respectively). The incidences of sarcomas in combined target organs (0/20, 15/50, and 37/49 in control, low dose and high dose groups, respectively) were used to derive the slope factor (US EPA, 1988b). The US EPA (1987, 1988b) values were not adopted for this risk assessment because of the compromised health status of the male rats, the benign nature of the skin fibromas used in the initial assessment, and the nonlinear mechanism pertaining to aromatic amine-induced sarcomas used in the later assessment (Goodman et al., 1984).

The State of California Office of Environmental Health Hazard Assessment (OEHHA) has listed *o*-toluidine and the hydrochloride as chemicals known to the state to cause cancer under the Safe Drinking Water and Toxic Enforcement Act of 1986 (OEHHA, 2009). Using an expedited method for conducting risk assessments, "no significant risk levels" (levels calculated to result in one excess case of cancer in an exposed population of 100,000 assuming lifetime exposure) of 4 μg/day for *o*-toluidine and 5 μg/day for *o*-toluidine hydrochloride were derived, and the cancer potency value was 0.18 (mg/kg-day)$^{-1}$ (OEHAA, 2008). The methodology and critical effect selected by California's OEHHA (2008) differ from the current assessment, in that OEHHA (2008) determined that the male rat was the most sensitive test system, and the potency estimate was based on the geometric mean of human potency values derived from the male rat in relevant studies (NCI, 1979; Russfield et al., 1973; Hecht et al., 1982; Weisburger et al., 1978). In the NCI study, potency was derived using "time-to-tumor" analysis (Crump et al., 1991) due to the decreased survival of male rats. The site and histopathology used for dose–response data used in the potency calculation was "subcutaneous tissue and skin – site with varying histopathology." These single species and sex tumor types consist primarily of benign subcutaneous fibromas. The OEHHA value was not adopted for this risk assessment, because female rats were more sensitive to the development of urinary bladder tumors that were considered more relevant to humans, particularly in light of the epidemiology data and IARC (2009) conclusion that exposure to *o*-toluidine is carcinogenic for human urinary bladder epithelial cells. Indeed, the only malignant tumor type observed in both sexes of rat and in multiple species including humans were transitional cell neoplasias of the urinary bladder epithelium, and

Table 7
Unit Risk analysis by lifestage.

Age grouping[a]	Exposure duration (years)	Potency adjustment[a]	90th% Ingestion of drinking Water, consumer only[b] (L/kg bw)	Unit risk$_{age}$ (μg/L)-1
0–<1 month	0.083	1×	0.235	2.15×10^{-09}
1–<3 months	0.167	1×	0.228	4.19×10^{-09}
3–<6 months	0.25	1×	0.148	4.07×10^{-09}
6 month–<1 year	0.5	10×	0.112	6.16×10^{-08}
1–<2 years	1	10×	0.056	6.16×10^{-08}
2–< 3 years	1	3×	0.052	1.72×10^{-08}
3–<6 years	3	3×	0.043	4.26×10^{-08}
6–<11 years	5	3×	0.035	5.78×10^{-08}
11–<16 years	5	3×	0.026	4.29×10^{-08}
16–70 years[c]	54	1×	0.029[c]	1.72×10^{-07}
Total lifetime unit risk$_{AGE}$	70	–	–	4.66×10^{-07}

[a] Recommended set of childhood age groups and default potency adjustment factors for Agency exposure assessments (US EPA, 2005c).
[b] US EPA (2011b) exposure factors handbook (Estimates of Direct and Indirect Water Ingestion: Community Water) except where noted otherwise.
[c] The 16-<21 year age grouping was collapsed with adults into one age-group based on equivalent sensitivity and similar drinking water consumption rates. Adult default body weight and drinking water consumption values of 70 kg and 2 L/day, respectively, were used, according to NSF procedures.

Table 8
Comparison of lifestage-adjusted and unadjusted risk derivations.

Parameter – units	Unadjusted for lifestage (default)		With lifestage adjustment	
	Formula	Value	Formula	Value
Slope factor (mg/kg-day)-1	$\frac{0.1}{BMDL_{10}}$ [a]	0.0077	$\frac{0.1 \times LAF}{BMDL_{10}}$ [b]	0.016
10^{-5} cancer risk level (mg/kg-day)	$\frac{0.00001}{\text{slope factor}}$	1.3×10^{-3}	$\frac{10^{-5} \text{ cancer risk level}_D}{LAF}$ [c]	0.61×10^{-3}
Unit risk (μg/L)$^{-1}$	$\text{slope factor}^d \times \frac{2\,L}{day} \times \frac{1\,mg}{1000\mu g}$ [d]	0.22×10^{-6}	Unit risk$_{AGE}$ (see Table 7 for derivation)	0.47×10^{-6}

[a] BMDL$_{10}$ = 13 mg/kg-day (see Appendix).
[b] LAF (lifestage adjustment factor) = $\frac{\text{unit risk}_{AGE}}{\text{unit risk}}$ = 2.14 = 2 (rounded).
[c] 10^{-5} cancer risk level$_D$ = Default (unadjusted) 10^{-5} cancer risk level.
[d] Default adult body weight and drinking water consumption.

the bladder is therefore the relevant target organ. Recognizing this and the reduced survival observed in treated female rats, some of which developed bladder tumors, a mortality-adjusted analysis (NTP, 2011b; Portier and Bailer, 1989), could not be performed for the bladder tumor incidence in female rats for the present assessment since the necessary individual animal survival and tumor data were not reported by NCI (1979). However, a mortality-adjusted BMDL estimate was considered unlikely to appreciably deviate from that estimated in the present assessment, because survival remained high well beyond the 65-week time to first urinary bladder tumor.

Based on the evidence of increased risk of bladder cancer in humans occupationally exposed to *o*-toluidine, measures to reduce occupational exposures to the lowest feasible concentrations have been recommended (NIOSH, 1990; UK HSE, 1996). The existing occupational exposure limits are not health-based but rather based on technological control and measurement capabilities, and consequently vary considerably. A Maximum Exposure Limit of 0.2 ppm (0.9 mg/m^3; 8-h time-weighted average) was established in the United Kingdom based on a level of control that was deemed to be reasonably practicable, with ongoing reductions in exposure levels as reasonably practicable with the currently available technology (UK HSE, 1996). The American Conference of Governmental Industrial Hygienists identified *o*-toluidine as a confirmed animal carcinogen with unknown relevance to humans (ACGIH, 2001) and recommended an occupational exposure limit of 8.8 mg/m^3 (2 ppm) as an 8-h time-weighted average, with a skin notation (sk), reflecting the fact that dermal absorption is an important occupational exposure pathway. It was recognized that occupational exposure occurred, as shown by elevated levels of *o*-toluidine in urine and *o*-toluidine hemoglobin adducts in workers,

despite workplace air concentrations of <1 ppm (4.38 mg/m^3) which was the study limit of detection (Ward et al., 1991; Teass et al., 1993). In 2006, the German MAK-Commission considered *o*-toluidine to be a proven human carcinogen (cited by Gaber et al., 2007), where it had previously been classified as "to be regarded as carcinogenic for humans" (Class 2) for the workplace. For occupational settings in Germany, a Technical Exposure Limit (TRK) based on analytical capabilities of 0.5 mg/m^3 (∼0.1 ppm) was assigned (OECD, 2006).

In conclusion, contemporary risk assessment methodology (NSF/ANSI 61 Annex A, 2011; US EPA, 2005a) were used to determine an acceptable level for *o*-toluidine in drinking water. The drinking water action level of 20 μg/L derived in this risk assessment is protective of public health, since it was based on chronic oral data for *o*-toluidine (as the hydrochloride) from an endpoint and laboratory animal species predictive of the human target, and further adjusted for early lifestage susceptibility and exposure.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.yrtph.2012.08.011.

References

ACGIH (American Conference of Governmental Industrial Hygienists), 2001. Documentation of the threshold limit values and biological exposure indices, 7th ed. American Conference of Governmental Industrial Hygienists. Cincinnati, OH.

Baan, R., Straif, K., Grosse, Y., Secretan, B., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Cogliano, V., 2008. Carcinogenicity of some aromatic amines, organic dyes, and related exposures. Lancet Oncol. 9, 322–323.

Beland, F.A., Beranek, D.T., Dooley, K.L., Heflich, R.H., Kadlubar, F.F., 1983. Arylamine-DNA adducts in vitro and in vivo: their role in bacterial mutagenesis and urinary bladder carcinogenesis. Environ. Health Perspect. 49, 125–134.

Beland, F.A., Kadlubar, F.F., 1985. Formation and persistence of arylamine DNA adducts in vivo. Environ. Health Perspect. 62, 19–30.

Böhm, F., Schmid, D., Denzinger, S., Wieland, W.F., Richter, E., 2011. DNA adducts of ortho-toluidine in human bladder. Biomarkers 16 (2), 120–128.

Boobis, A.R., Cohen, S.M., Dellarco, V., McGregor, D., Meek, M.E., Vickers, C., Willcocks, D., Farland, W., 2006. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Crit. Rev. Toxicol. 36, 781–792.

Brix, A.E., Hardisty, J.F., McConnell, E.E., 2010. Combining neoplasms for evaluation of rodent carcinogenesis studies. In: Hsu, C.H., Stedeford, T. (Eds.), Cancer Risk Assessment: Chemical Carcinogenesis, Hazard Evaluation and Risk Quantification. John Wiley and Sons, Hoboken, New Jersey.

Brock, W.J., Hundley, S.G., Lieder, P.H., 1990. Hepatic macromolecular binding and tissue distribution of ortho- and para-toluidine in rats. Toxicol. Lett. 2 (3), 317–325.

Brown, K.K., Teass, A.W., Simon, S., Ward, E.M., 1995. A biological monitoring method for o-toluidine and aniline in urine using high performance liquid chromatography with electrochemical detection. Appl. Occup. Environ. Hyg. 10, 557–565.

Castelao, J.E., Yuan, J.-M., Skipper, P.L., Tannenbaum, S.R., Gago-Dominguez, M., Crowder, J.S., Ross, R.K., Yu, M.C., 2001. Gender- and smoking-related bladder cancer risk. J. Nat. Cancer Inst. 93 (7), 538–545.

Cesarone, C.F., Bolognesi, C., Santi, L., 1982. Evaluation of damage to DNA after in vivo exposure to different classes of chemicals. Arch. Toxicol. Suppl. 5, 355–359.

Cheever, K.L., Richards, D.E., Plotnick, H.B., 1980. Metabolism of ortho-, meta-, and para-toluidine in the adult male rat. Toxicol. Appl. Pharmacol. 56, 361–369.

Cheever, K.L., Debord, D.G., Swearengin, T.F., Booth-Jones, A.D., 1992. Ortho-toluidine blood protein adducts: HPLC analysis with fluorescence detection after a single dose in the adult male rat. Fundam. Appl. Toxicol. 18 (4), 522–531.

ChemIDplus, 2011. National Library of Medicine on-line database. <http://chem.sis.nlm.nih.gov/chemidplus/>.

Cresteil, T., 1998. Onset of xenobiotic metabolism in children: toxicological implications. Food Addit. Contam. 15 (Suppl.), 45–51.

Crump, K.S., Howe, R.B., Van Landingham, C., Fuller, W.G., 1991. Tox-Risk (Toxicology Risk Assessment Program) version 3. KS Crump Division, Clement International Corp., Ruston, LA.

Danford, N., 1991. The genetic toxicology of ortho-toluidine. Mutat. Res. 258 (3), 207–236.

DeBord, D.G., Swearengin, T.F., Cheever, K.L., Booth-Jones, A.D., Wissinger, L.A., 1992. Binding characteristics of ortho-toluidine to rat hemoglobin and albumin. Arch. Toxicol. 66 (4), 231–236.

DeBruin, L.S., Pawliszyn, J.B., Josephy, P.D., 1999. Detection of monocyclic aromatic amines, possible mammary carcinogens, in human milk. Chem. Res. Toxicol. 12 (1), 78–82.

Denshaw-Burke, M., Savior, D.C., 2008. Methemoglobinemia. <http://emedicine.medscape.com/article/204178-print>.

Diaz Gomez, M.I., Fanelli, S.L., Delgado de Layño, A.M.A., Castro, J.A., Castro, G.D., 2006. Liver nuclear and microsomal CYP2E1-mediated metabolism of xenobiotics in rats chronically drinking an alcohol-containing liquid diet. Toxicol. Ind. Health 22 (9), 367–374.

Duan, J.D., Jeffrey, A.M., Williams, G.M., 2008. Assessment of the medicines lidocaine, prilocaine, and their metabolites, 2,6-dimethylaniline and 2-methylaniline, for DNA adduct formation in rat tissues. Drug Metab. Dispos. 36 (8), 1470–1475.

DuPont, 1994. Urinary bladder toxicity - 14-day feeding study with o-toluidine in rats. Report DuPont HLR 699-93, TS0557449, as cited in OECD, 2006; Screening Information Data Set for o-Toluidine. <http://www.oecd.org/document/55/0,2340,en_2649_34379_31743223_1_1_1_1,00.html>.

El-Bayoumy, K., Donahue, J.M., Hecht, S.S., Hoffmann, D., 1986. Identification and quantitative determination of aniline and toluidines in human urine. Cancer Res. 46, 6064–6067.

Freudenthal, R.I., Stephens, E., Anderson, D., 1999. Determining the potential of aromatic amines to induce cancer of the urinary bladder. Int. J. Toxicol. 18 (5), 353–359.

Gaber, K., Harréus, U.A., Matthias, C., Kleinsasser, N.H., Richter, E., 2007. Hemoglobin adducts of the human bladder carcinogen o-toluidine after treatment with the local anesthetic prilocaine. Toxicology 229, 157–164.

Gatehouse, D., 1981. Mutagenic activity of 42 coded compounds in the microtiter fluctuation test. In: de Serres, F.J., Ahsby, J. (Eds.), Progress in Mutation Research, Evaluation of Short-term Tests for Carcinogenesis, vol. 1. Report of the International Collaborative Program. Elsevier/North Holland, New York, Amsterdam, Oxford, pp. 376-386 (Chapter 35).

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., Goble, R., 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicol. Sci. 66, 185–200.

Golka, K., Prior, V., Blaszkewicz, M., Bolt, H.M., 2002. The enhanced bladder cancer susceptibility of NAT2 slow acetylators toward aromatic amines: a review considering ethnic differences. Toxicol. Lett. 128, 229–241.

Goodman, D.G., Ward, J.M., Reichardt, W.D., 1984. Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, p-chloroaniline, azobenzene, o-toluidine hydrochloride, 4,4′-sulfonyldianiline, or D & C red No. 9. J. Natl. Cancer Inst. 73 (1), 265–273.

Gnojkowski, J., Baer-Dubowska, W., Klimek, D., Chmiel, J., 1984. Effect of toluidines on drug metabolizing enzymes in rat liver, kidney and lung. Toxicology 32, 335–342.

Gupta, R.L., Gupta, A.K., Pathak, D.P., Juneja, T.R., 1987. Mutagenic studies of ortho-toluidine and its potential metabolites. Indian J. Exp. Biol. 25, 618–622.

Hammons, G.J., Dooley, K.L., Kadlubar, F.F., 1991. 4-Aminobiphenyl-hemoglobin adduct formation as an index of in vivo N-oxidation by hepatic cytochrome P-450IA2. Chem. Res. Toxicol. 4, 144–147.

Hecht, S.S., El-Bayoumy, K., Tulley, L., LaVoie, E., 1979. Structure-mutagenicity relationships of N-oxidized derivatives of aniline, o-toluidine, 2′-methyl-4-aminobiphenyl and 3,2′-dimethyl-4-aminobiphenyl. J. Med. Chem. 22 (8), 981–987.

Hecht, S.S., El-Bayoumy, K., Rivenson, A., Fiala, E., 1982. Comparative carcinogenicity of o-toluidine hydrochloride and o-nitrosotoluene in F-344 rats. Cancer Lett. 16 (1), 103–108.

HSDB (Hazardous Substances Database), 2011. On-line database. <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB> (accessed 27.10.2011).

Hubbard, S.A., Green, M.H.L., Bridges, B.A., Wain, A.J., and Bridges, J.W., 1981. Fluctuation test with S9 and hepatocyte activation. In: de Serres, F.J., Ashby, J. (Eds.), Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program (Progress in Mutation Research Vol. 1), Amsterdam, Elsevier, pp. 361–370.

IARC (International Agency on Research on Cancer), 1982. Some aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in drinking water and dental preparations. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 87. International Agency for Research on Cancer, Lyon, France.

IARC (International Agency on Research on Cancer), 1987. Monographs on the evaluation of carcinogenic risks to humans. Supplement 7. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs, vols. 1–42. World Health Organization, Lyon, France. <http://monographs.iarc.fr/ENG/Monographs/suppl7/index.php>.

IARC (International Agency on Research on Cancer), 2000. O-Toluidine. Monogr. Evaluat. Carcinog. Risk. Hum. 77, 267–322.

IARC (International Agency on Research on Cancer), 2009. A review of human carcinogens-Part F: Chemical agents and related occupations. Summary of Evaluations, vol. 100F. <http://monographs.iarc.fr/pdfnews/WG-100F.pdf> (accessed 25.10.2011).

IARC (International Agency on Research on Cancer), 2010. Some Aromatic Amines, Organic Dyes, and Related Exposures, IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 99. International Agency for Research on Cancer, Lyon, France.

Jo, H.J., Park, E.J., Cho, K., Kim, E.-H., Jung, J., 2008. Toxicity identification and reduction of wastewaters from a pigment manufacturing factory. Chemosphere 70 (6), 949–957.

Jodynis-Liebert, J., Matuszewska, A., 1999. Effect of toluidines and dinitrotoluenes on caffeine metabolic ratio in rat. Toxicol. Lett. 104, 159–165.

Jodynis-Liebert, J., Murias, M., 2002. Modulation of antioxidant defense system by dietary fat in rats intoxicated with o-toluidine. Hum. Exp. Toxicol. 21 (12), 659–665.

Kadlubar, F.F., Badawi, A.F., 1995. Genetic susceptibility and carcinogen-DNA adduct formation in human urinary bladder carcinogenesis. Toxicol. Lett. 82/83 (1995), 627–632.

Kawalek, J.C., Hallmark, R.K., Andrews, A.W., 1983. Effect of lithocholic acid on the mutagenicity of some substituted aromatic amines. J. Natl. Cancer Inst. 71, 293–298.

Kiese, M., 1966. The biochemical production of ferrihemoglobin-forming derivatives from aromatic amines, and mechanisms of ferrihemoglobin formation. Pharmacol. Rev. 18, 1091–1161.

Kim, D., Guengerich, F., 2005. Cytochrome P450 activation of arylamines and heterocyclic amines. Annu. Rev. Pharmacol. Toxicol. 45, 27–49.

Kulkarni, B., Fiala, E.S., Weisburger, J.H., 1983. Estimation of N-hydroxy-o-toluidine, a urinary metabolite of o-toluidine and o-nitrosotoluene, by high performance liquid chromatography with electrochemical detection. Carcinogenesis 4 (10), 1275–1280.

Kurata, N., Nishimura, Y., Iwase, M., Fischer, N.E., Tang, B.K., Inaba, T., Yasuhara, H., 1998. Trimethadione metabolism by human liver cytochrome P450: evidence for the involvement of CYP2E1. Xenobiotica 28, 1041–1047.

Leslie, C., Reidy, G.F., Murray, M., Stacey, N.H., 1988. Induction of xenobiotic biotransformation by the insecticide chlordimeform, a metabolite 4-chloro-o-toluidine and a structurally related chemical o-toluidine. Biochem. Pharmacol. 37, 2529–2535.

Luceri, F., Pieraccini, G., Moneti, G., Dolara, P., 1993. Primary aromatic amines from side-stream cigarette smoke are common contaminants of indoor air. Toxicol. Ind. Health 9 (3), 405–413.

Markowitz, S.B., Levin, K., 2004. Continued epidemic of bladder cancer in workers exposed to ortho-toluidine in a chemical factory. J. Occup. Environ. Med. 46 (2), 154–160.

Markowitz, S.B., 2005a. Corrections to: Markowitz, S.B., Levin, K. Continued epidemic of bladder cancer in workers exposed to ortho-toluidine in a chemical factory. J. Occup. Environ. Med. 46, 154–160.

Markowitz, S.B., 2005b. Corrections to: Markowitz, S.B., Levin, K. Continued epidemic of bladder cancer in workers exposed to ortho-toluidine in a chemical factory. J. Occup. Environ. Med. 47 (9), 875–877.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., Boorman, G.A., 1986. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76 (2), 283–289.

McFee, A.F., Jauhar, P.P., Lowe, K.W., MacGregor, J.T., Wehr, C.M., 1989. Assays of three carcinogen/non-carcinogen chemical pairs for in vivo induction of chromosome aberrations, sister chromatid exchanges and micronuclei. Environ. Mol. Mutagen. 14 (4), 207–220.

Meek, M.E., Bucher, J.R., Cohen, S.M., Dellarco, V., Hill, R.N., Lehman-McKeeman, L.D., Longfellow, D.G., Pastoor, T., Seed, J., Patton, D.E., 2003. A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33, 591–653.

Nagao, M., Takahashi, Y., 1981. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay. In: de Serres, F.J., Ashby, J. (Eds.), Evaluation of Short-Term Tests for Carcinogens. Report of the International Collaborative Program (Progress in Mutation Research Vol. 1). Elsevier, Amsterdam, pp. 302–313.

Nakamura, H., Tanaka, E., Ishikawa, A., Fukao, K., Tsuji, K., Ohkawa, H., 1998. Age-related changes in hepatic drug-oxidizing activity using trimethadione as a probe drug in humans. Hepatol. Res. 12, 85–92.

NCI (National Cancer Institute), 1979. Bioassay of o-toluidine hydrochloride for possible carcinogenicity. CAS No. 636-21-5. NCI-CG-TR-153. US Department of Health, Education, and Welfare. Available on-line at: <http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr153.pdf>.

Neurath, G.B., Dünger, M., Pein, F.G., Ambrosius, D., Schreiber, O., 1977. Primary and secondary amines in the human environment. Food Cosmet. Toxicol. 15, 275–282.

NIOSH (National Institute for Occupational Safety and Health), 1990. Request for Assistance in Preventing Bladder Cancer from Exposure to o-Toluidine and Aniline NIOSH ALERT: December 1990 DHHS (NIOSH) Publication No. 90-116; see <http://www.cdc.gov/NIOSH/90-116.html> (accessed 01.07.2009).

NSF/ANSI 61, 2011. Drinking Water System Components-Health Effects. NSF International, Ann Arbor, MI.

NTP (National Toxicology Program), 1996. NTP Technical Report on Comparative Toxicity and Carcinogenicity Studies of o-Nitrotoluene and o-Toluidine Hydrochloride (CAS Nos. 88-72-2 and 636-21-5) Administered in Feed to Male F344/N Rats. NIH Publication 96-3936.

NTP (National Toxicology Program), 2011a. 12th Report on Carcinogens. <http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf> (accessed 27.10.2011).

NTP (National Toxicology Program), 2011b. Statistical Procedures for NTP Studies, Expanded Overview. <http://ntp.niehs.nih.gov/?objectid=72015E2C-BDB7-CEBA-F17F9ACA7AE5346D> (accessed 01.12.2011).

OECD (Organisation for Economic Co-Operation and Development), 2004. The 2004 OECD list of high production volume chemicals. OECD, Paris. Available at: <http://www.oecd.org/dataoecd/55/38/33883530.pdf> (accessed 11.02.2010).

OECD (Organisation for Economic Co-operation and Development), 2006. SIDS Initial Assessment Report o-Toluidine. <http://www.chem.unep.ch/irptc/sids/OECDSIDS/95534.pdf> (accessed 03.06.2009).

OEHHA (State of California Office of Environmental Health Hazard Assessment), 2008. Title 27, California Code of Regulations Article 7. No Significant Risk Levels. <http://www.oehha.ca.gov/prop65/law/pdf_zip/RegsArt7.pdf> (accessed 14.07.2009).

OEHHA (State of California Office of Environmental Health Hazard Assessment), 2009. Chemicals Known to the State to Cause Cancer or Reproductive Toxicity. Safe Drinking Water and Toxic Enforcement Act of 1986. <http://oehha.ca.gov/prop65/prop65_list/Newlist.html> (accessed 14.07.2009).

Ohkuma, Y., Hiraku, Y., Oikawa, S., Yamashita, N., Murata, M., Kawanishi, S., 1999. Distinct mechanisms of oxidative DNA damage by two metabolites of carcinogenic o-toluidine. Arch. Biochem. Biophys. 1, 97–106.

Pliss, G.B., 2004. Experimental study of ortho-toluidine carcinogenicity. Vopr. Onkol. 50 (5), 567–571, Translated by Lena Hope, NSF International.

Prince, M.M., Ward, E.M., Ruder, A.M., Salvan, A., Roberts, D.R., 2000. Mortality among rubber chemical manufacturing workers. Am. J. Ind. Med. 37, 590–598.

Portier, C.J., Bailer, A.J., 1989. Testing for increased carcinogenicity using a survival-adjusted quantal response test. Fundam. Appl. Toxicol. 12 (4), 731–737.

Richter, E., Rösler, S., Scherer, G., Gostomzyk, J.G., Grübl, A., Krämer, U., Behrendt, H., 2001. Haemoglobin adducts from aromatic amines in children in relation to area of residence and exposure to environmental tobacco smoke. Int. Arch. Occup. Environ. Health 74 (6), 421–428.

Richter, E.K., Gaber, K., Harréus, A., Matthias, C., Kleinsasser, N.H., 2006. o-Toluidine adducts in human bladder DNA and hemoglobin by the local anesthetic prilocaine. Toxicol. Lett. 164S, S255.

Riedel, K., Scherer, G., Engl, J., Hagedorn, H.-W., Tricker, A.R., 2006. Determination of three carcinogenic aromatic amines in urine of smokers and nonsmokers. J. Anal. Toxicol. 30 (3), 187–195.

Riffelmann, M., Müller, G., Schmieding, W., Popp, W., Norpoth, K., 1995. Biomonitoring of urinary aromatic amines and arylamine, hemoglobin adducts in exposed workers and nonexposed control persons. Int. Arch. Occup. Environ. Health 68, 36–43.

Rubino, G.F., Scansetti, G., Piolatto, G., Pira, E., 1982. The carcinogenic effect of aromatic amines: an epidemiological study of the role of o-toluidine and 4,4′-methylene bis(2-methylaniline) in inducing bladder cancer in man. Environ. Res. 27, 241–254.

Russfield, A.B., Hornburger, F., Weisburger, E.K., Weisburger, J.H., 1973. Further studies on carcinogenicity of environmental chemicals including simple aromatic amines. Toxicol. Appl. Pharmacol. 25, 446. Abstract.

Sabbioni, G., Sepai, O., 1995. Comparison of hemoglobin binding, mutagenicity, and carcinogenicity of arylamines and nitroarenes. Chimia 49, 374–380.

Short, C.R., King, C., Sistrunk, P.W., Kerr, K.M., 1983. Subacute toxicity of several ring-substituted dialkylanilines in the rat. Fundam. Appl. Toxicol. 3 (4), 285–292.

Son, O.S., Everett, D.W., Fiala, E.S., 1980. Metabolism of o-[methyl-^{14}C] o-toluidine in the F344 rat. Xenobiotica 10 (7-8), 457–468.

Sorahan, T., Hamilton, L., Jackson, J.R., 2000. A further cohort study of workers employed at a factory manufacturing chemicals for the rubber industry, with special reference to the chemicals 2-mercaptobenzothiazole (MBT), aniline, phenyl-beta-naphthylamine and o-toluidine. Occup. Environ. Med. 57, 106–115.

Sorahan, T., 2008. Bladder cancer risks in workers manufacturing chemicals for the rubber industry. Occup. Med. (Lond.) 58 (7), 496–501.

Stasik, M.J., 1988. Carcinomas of the urinary bladder in a 4-chloro-o-toluidine cohort. Int. Arch. Occup. Environ. Health 60, 21–24.

Stillwell, W.G., Bryant, M.S., Wishnok, J.S., 1987. GC analysis of biologically important aromatic amines – application to human dosimetry. Biomed. Environ. Mass Spectrom. 14 (5), 221–227.

Suzuki, H., Ikeda, N., Kobayashi, K., Terashima, Y., Shimada, Y., Suzuki, T., Hagiwara, T., Hatakeyama, S., Nagaoka, K., Yoshida, J., Saito, Y., Tanaka, J., Hayashi, M., 2005. Evaluation of liver and peripheral blood micronucleus assays with 9 chemicals using young rats. A study by the Collaborative Study Group for the Micronucleus Test (CSGMT)/Japanese Environmental Mutagen Society (JEMS)-Mammalian Mutagenicity Study Group (MMS). Mutat. Res. 583 (2), 133–145.

Talaska, G., 2003. Aromatic amines and human urinary bladder cancer: exposure sources and epidemiology. J. Environ. Sci. Health C 21 (1), 29–43.

Tanaka, E., 1998. In vivo age-related changes in hepatic drug-oxidizing capacity in humans. J. Clin. Pharm. Ther. 23, 247–255.

Taylor, J.A., Umbach, D.M., Stephens, E., Castranio, T., Paulson, D., Robertson, G., Mohler, J.L., Bell, D.A., 1998. The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene–gene-exposure three-way interaction. Cancer Res. 58, 3603–3610.

Teass, A.W., DeBord, D.G., Brown, K.K., Cheever, K.L., Stettler, L.E., Savage, R.E., Weigel, W.W., Dankovic, D., Ward, E., 1993. Biological monitoring for occupational exposures to o-toluidine and aniline. Int. Arch. Occup. Environ. Health 65, S115–S118.

TRI (Toxics Chemical Release Inventory), 2009. <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TRI> (accessed 09.06.2009).

UK HSE (United Kingdom Health and Safety Executive), 1996. o-Toluidine; Criteria document for an occupational exposure limit. London, HSE Books (ISBN 0 7176 1057 8) see <http://www.inchem.org/documents/cicads/cicads/cicad07.htm#PartNumber:12> (accessed 01.07.2009).

US Environmental Protection Agency, 1984. ETD Production/Exposure Profile: o-Toluidine and o-Toluidine Hydrochloride. US Environmental Protection Agency, Office of Pesticide Programs and Toxic Substances, Washington, DC.

US Environmental Protection Agency, 1987. Health and Environmental Effects Profile for 2-Methylaniline and 2-Methylaniline Hydrochloride. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Cincinnati, OH.

US Environmental Protection Agency, 1988a. Recommendations for and documentation of biological values for use in risk assessment. EPA 600/6-87/008, NTIS PB88-179874/AS, February 1988.

US Environmental Protection Agency, 1988b. Evaluation of the potential carcinogenicity of o-toluidine (95-53-4) In support of reportable quantity adjustments pursuant to CERCLA section 102. EPA 600/8-91/187, June 1988.

US Environmental Protection Agency, 1991. National primary drinking water regulations: final rule. Fed. Regist. 56 (20), 3526–3614.

US Environmental Protection Agency, 1997. Health Effects Assessment Summary Tables. FY 1997 Update. Office of Research and Development, Office of Emergency and Remedial Response, Washington, DC. EPA/540/R-97-036.

US Environmental Protection Agency, 2000a. Benchmark dose technical guidance document-External review draft. EPA/630/R-00/ 001. Risk assessment forum. Available at: <http://www.epa.gov/iris/backgrd.html>.

US Environmental Protection Agency, 2000b. 2-Methylaniline (o-Toluidine) 95-53-4 Hazard Summary. Last updated on Monday, December 19, 2011. See <http://www.epa.gov/ttn/atw/hlthef/methylan.html#ref2> (accessed 20.12.2011).

US Environmental Protection Agency, 2000c. Estimated per capita water ingestion in the United States. EPA-822-00-008. Office of Science and Technology/Office of Water, US Environmental Protection Agency, Washington DC.

US Environmental Protection Agency, 2005a. Guidelines for carcinogen risk assessment. EPA/630/P-03/001B. March 2005. <http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283>.

US Environmental Protection Agency, 2005b. Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. EPA/630/R-03/003F. March 2005. <http://www.epa.gov/cancerguidelines/sup-guidance-early-life-exp-carcinogens.htm>.

US Environmental Protection Agency, 2005c. Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants. EPA/630/P-03/003F. November 2005. <http://www.epa.gov/raf/publications/pdfs/AGEGROUPS.PDF>.

US Environmental Protection Agency, 2007. Non-confidential 2006 Inventory Update Rule (IUR) records by chemical including manufacturing, processing and use information. Washington, DC. <http://cfpub.epa.gov/iursearch/index.cfm?s=chem&err=t> (last updated 12.05.2010).

US Environmental Protection Agency, 2009. Health effects testing guidelines. US Code of Federal Regulations, Title 40, Part 798. US Environmental Protection Agency.

US Environmental Protection Agency, 2010. Office of Pollution Prevention and Toxics. ChemRTK HPV Challenge Program. Available on-line at: <http://www.epa.gov/chemrtk/pubs/update/hpv_1990.htm> (last updated 10.08.2010).

U.S. Environmental Protection Agency, 2011a. Benchmark Dose Software Version 2.2 (Build 66) Released September 6, 2011. National Center for Environmental Assessment, Washington DC. Available at: <http://epa.gov/ncea/bmds/> (last updated 13.09.2011).

US Environmental Protection Agency, 2011b. Exposure Factors Handbook, 2011 ed. National Center for Environmental Assessment, Washington, DC, EPA/600/R-09/052F. Available from the National Technical Information Service, Springfield, VA, and online at <http://www.epa.gov/ncea/efh>.

US Environmental Protection Agency, 2011c. Glossary of IRIS terms. Integrated Risk Information System (IRIS). <http://www.epa.gov/ncea/iris/help_gloss.htm> (last updated 26.07.2011).

Valcke, M., Krishnan, K., 2011. An assessment of the impact of physico-chemical and biochemical characteristics on the human kinetic adjustment factor for systemic toxicants. Toxicology 286, 36–47.

Van Aken, B., Agathos, S.N., 2002. Implication of manganese (III), oxalate, and oxygen in the degradation of nitroaromatic compounds by manganese peroxidase (MnP). Appl. Microbiol. Biotechnol. 58, 345–351.

Vian, L., Bichet, N., Gouy, D., 1993. The *in vitro* micronucleus test on isolated human lymphocytes. Mutat. Res. 291 (1), 93–102.

Vieira, I., Sonnier, M., Cresteil, T., 1996. Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. Eur. J. Biochem. 238, 476–483.

Ward, E., Carpenter, A., Markowitz, S., Roberts, D., Halperin, W., 1991. Excess number of bladder cancers in workers exposed to *ortho*-toluidine and aniline. J. Natl. Cancer Inst. 83, 501–506.

Ward, E.M., Sabbioni, G., DeBord, D.G., Teass, A.W., Brown, K.K., Talaska, G.G., Roberts, D.R., Ruder, A.M., Streicher, R.P., 1996. Monitoring of aromatic amine exposure in workers at a chemical plant with a known bladder cancer excess. J. Natl. Cancer Inst. 88, 1046–1052.

Weisburger, E.K., Russfield, A.B., Homburger, F., Weisburger, J., Boger, E., Van Dongen, C.G., Chu, K.C., 1978. Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J. Environ. Pathol. Toxicol. 2 (2), 325–356.

Weiss, T., Ewers, U., Fliegner, A., Angerer, J., 2000. Innere Belastung der Allgemeinbevoelkerung mit Amino- und Nitroaromatischen Verbindungen. Umweltmed. Forsch. Prax. 5 (2), 101–106.

Weiss, T., Angerer, J., 2002. Simultaneous determination of various aromatic amines and metabolites of aromatic nitro compounds in urine for low level exposure using gas chromatography–mass spectrometry. J. Chromatogr. B 778, 179.

Weiss, T., Kaefferlein, H.U., Bruening, T., Angerer, J., 2005. Ambient and biological monitoring of exposure to aromatic amines in the rubber industry. Toxicol. Sci. 84 (1-S), 263. Abstract.

West, J.B., 1990. Regulation of volume and osmolality of the body fluids. In: West, J.B. (Ed.), Best and Taylor's Physiological Basis of Medical Practice, 11th ed. Williams and Wilkins, Baltimore, pp. 478–485.

Williamson, J.A., Lieder, P.H., Amegashitsi, L., 1995. Development of a biomonitoring assay for ortho-toluidine or its metabolites in human urine. J. Anal. Toxicol. 19, 256–260.

WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 1998. Concise International Chemical Document No. 7. *o*-Toluidine. Geneva, 1998. Available on-line at: <http://www.inchem.org/documents/cicads/cicads/cicad07.htm>.

Yu, M.C., Skipper, P.L., Tannenbaum, S.R., Chan, K.K., Ross, R.K., 2002. Arylamine exposures and bladder cancer risk. Mutat. Res. 506–507 (2002), 21–28.

Zeiger, E., Haworth, S., 1985. Tests with a preincubation modification of the Salmonella/microsome assay. In: Ashby, J. et al. (Eds.), Evaluation of Short-Term Tests for Carcinogenesis, Progress in Mutation Research, vol. 5. Elsevier, Amsterdam, pp. 187–199, Chapter 14.