Report on Carcinogens (RoC) Concept: *ortho*-Toluidine

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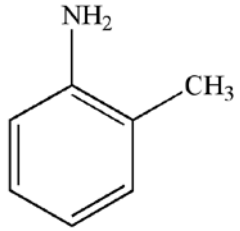

1. Rationale

ortho-Toluidine (CASRN 95-53-4) is an arylamine used (either directly or as an intermediate) to make dyes, rubber chemicals, and herbicides. It has been listed as *reasonably anticipated to be a human carcinogen* since 1983 in the *Report on Carcinogens* (RoC) based on sufficient evidence of carcinogenicity from studies in experimental animals and significant U.S. exposure (12th RoC, NTP 2011). Since that time, several cancer studies in humans have been published in the peer-reviewed literature, and the International Agency for Research on Cancer (IARC 2010, 2012) has concluded that *ortho*-toluidine is carcinogenic to humans (Group 1). Exposure to this chemical is a public health concern, especially for people who are or have been exposed in the workplace; it is a high production volume chemical in the United States. For these reasons *ortho*-toluidine is proposed for review for possible change in listing status.[1]

In January 2012, the NTP solicited information on *ortho*-toluidine and other nominated substances (77FR2728, see *http://ntp.niehs.nih.gov/go/rocnom* for comments). The only comment that was received (see *http://ntp.niehs.nih.gov/go/37663*) supported the evaluation of *ortho*-toluidine for the RoC and provided information on "two significant studies which were not published in time for consideration by IARC." These included a cancer study in humans (Pira *et al.* 2010) and a mechanistic study finding adducts in human urinary bladder tissue or tumors (Richter *et al.* 2006, Böhm *et al.* 2011).

[1] If selected as a candidate substance, the scientific evaluation of *ortho*-toluidine will be captured in the draft RoC monograph, which consists of a cancer evaluation component and draft substance profile (for more details see *http://ntp.niehs.nih.gov/go/rocprocess*). The proposed approach, delineated in this concept document, for preparing the cancer evaluation of the draft monograph is tailored to the nature, extent, and complexity of the scientific information on this chemical. This concept document also discusses information supporting the rationale and the proposed approach including (1) data on human exposure, (2) an overview of the nature and extent of the scientific information for evaluating carcinogenicity in humans and/or animals, (3) scientific issues and questions relevant to the evaluation of *ortho*-toluidine carcinogenicity, and (4) the proposed approach for conducting the scientific evaluation including the literature search strategy, the scope and focus of the monograph, and the approaches to obtain scientific and public input to address the key scientific questions and issues.

2. Overview of Data Related to Human Exposure

Significant U.S. exposure to *ortho*-toluidine is inferred via its use, high production volume, and biomonitoring data.

The single largest use of *ortho*-toluidine is in the synthesis of 6-ethyl-*ortho*-toluidine, which is an intermediate used to manufacture the herbicides metolachlor and acetochlor (IARC 2010). It is also used in the manufacture of a rubber antioxidant and as an intermediate in the manufacture of more than 90 dyes and pigments and of rubber chemicals, pharmaceuticals, and pesticides (IARC 2010, NTP 2011). According to the 2006 update for chemicals listed in the U.S. EPA Toxic Substances Control Act Inventory, production or importation of *ortho*-toluidine in the United States was in the range of 10 million to < 50 million pounds (EPA 2006).

Evidence for exposure to *ortho*-toluidine comes from studies reporting its presence (either the chemical itself or hemoglobin adducts) in blood, breast milk, and urine in humans. People are potentially exposed to *ortho*-toluidine in the workplace, from the environment, smoking, food (albeit at low levels), consumer products, and medical use. Occupational exposure to *ortho*-toluidine can occur by inhalation or skin contact during its production, or during the production of dyes, pigments, and rubber chemicals manufactured from this chemical (Lüersen *et al.* 2006, NTP 2011). Historical data from the 1980s (National Occupational Exposure Survey) estimated that 30,000 workers potentially were exposed to *ortho*-toluidine (NIOSH 1990). Ambient and breathing zone air levels were < 1 ppm in a U.S. plant producing thioindigo dyes in the 1940s (IARC 2010) and in a U.S. plant producing rubber chemicals (Teass *et al.* 1993, Ward *et al.* 1996). Levels in urine were reported to be as high as 1.7 mg/L [1,700 μg/L] for individual thioindigo dye workers (Ott and Langner 1983), and up to a mean of 99 μg/L for rubber-chemical production workers in post-shift samples taken in the 1990s (Ward *et al*. 1996).

Evidence indicating the potential for environmental exposure comes from the EPA's Toxics Release Inventory, which reported environmental releases of *ortho*-toluidine between 1988 and 2009 ranged from 6,900 to 55,000 lb (TRI 2009). *ortho*-Toluidine has also been found in hair dyes, commercial dyes, and cigarettes; higher concentrations of *ortho*-toluidine have been found in the urine of smokers compared with non-smokers. Prilocaine, a local anesthetic, is metabolized to *ortho*-toluidine, and increased *ortho*-toluidine hemoglobin adducts have been found in patients treated subcutaneously with this anesthetic (IARC 2010).

3. Overview of the Scientific Information Regarding Carcinogenicity

3.1. Human cancer studies

The human cancer studies available when *ortho*-toluidine was evaluated for listing in the *Third Annual Report on Carcinogens* were primarily case reports of urinary-bladder cancer occurring among workers exposed to dyes in addition to *ortho*-toluidine. These studies were inadequate for evaluating effects specifically from exposure to *ortho*-toluidine. Since then, additional studies of occupational exposure to *ortho*-toluidine have been identified. These (based on preliminary literature searches) include two population-based case-control studies, one of childhood acute lymphoblastic leukemia (Castro-Jiménez and Orozco-Vargas 2011), and the second on urinary-bladder cancer (Richardson *et al.* 2007), and several cohort studies (Case and Pearson 1954, Ott and Langner 1983, Stasik 1988,

Ward *et al.* 1991, Sorahan 2008, Carreón *et al.* 2010, Pira *et al.* 2010) of workers (or subcohorts of exposed workers) involved in five different types of industries including: (1) magenta manufacturing, (2) aniline manufacturing and processing (3) bromoindigo or thioindigo dye production, (4) 4-chloro-*ortho*-toluidine production and processing, and (5) rubber chemicals production. Multiple publications were reported on many of the cohorts, especially the NIOSH cohort of workers manufacturing rubber chemicals. Most of the cohort studies focused on urinary-bladder cancer and did not report findings for cancers at other tissue sites. The database consists of studies reporting both incidence and mortality findings, incidence only, and mortality only findings for urinary-bladder cancer.

The major co-exposures in these industries of initial concern – that is, those substances that are suspected carcinogens – include *ortho*-nitrotoluene, 4,4'-methylenebis(2-methylaniline), *ortho*-aminoazotoluene, aniline, 4-chloro-*ortho*-toluidine, nitrobenzene, hydroquinone, *N*-phenyl-2-naphthylamine (phenyl-β-naphthylamine), and 2-mercaptobenzothiazole. Also, in the NIOSH cohort study of rubber chemical workers, there is the possibility that 4-aminobiphenyl, a known human bladder carcinogen, was a low-level contaminant based on the reactions used and process chemistry (Ward *et al.* 1996). Biomonitoring data and personal air sampling data for *ortho*-toluidine, 4-aminobiphenyl, and aniline are available for a subset of workers in one of the cohort studies. The studies did not evaluate whether any of these co-exposures were effect modifiers and did not have adequate statistical power to do these analyses.

3.2. Cancer studies in experimental animals

The NTP has concluded that there is sufficient evidence for the carcinogenicity of *ortho*-toluidine from dietary studies in rodents (12th RoC, NTP 2011). In rats, *ortho*-toluidine caused sarcomas in the spleen and other organs in both sexes; tumors of the urinary bladder and mammary gland in females; and mesothelioma of the abdominal cavity and epididymis, and fibromas in males. In mice, it caused hemangioma and hemangiosarcoma in both sexes and liver tumors in females (Weisburger *et al.* 1978, NCI 1979).

Two additional studies in experimental animals have been identified since the 1983 listing: (1) a dietary toxicity study that reported mesothelioma of the epididymis occurring in male rats exposed for 13 weeks and necropsied at 26 weeks (13 weeks after exposure was stopped) (NTP 1996), and (2) a study, published in Russian, conducted in rats, mice, and dogs (Pliss 2004); documentation of this study is limited.

3.3. Mechanistic and other relevant data

ortho-Toluidine is well absorbed following ingestion, inhalation, or contact with the skin (IARC 2010). Metabolism has not been fully characterized. A study in rats suggests that *N*-acetylation and hydroxylation at the 4-position are the major metabolic pathways. Other pathways include oxidation of the amino and methyl groups, and hydroxylation at the 6-position. Metabolites are primarily excreted as sulfate conjugates. Little is known about metabolism in humans. The metabolite, *N*-acetyl-*ortho*-toluidine, and hemoglobin adducts have been detected in people exposed to *ortho*-toluidine (Ward *et al.* 1996). Adducts to hemoglobin, albumin, and DNA (in the liver) are formed in rodents after exposure to *ortho*-toluidine (IARC 2010, Duan *et al.* 2008). *ortho*-Toluidine releasing DNA adducts were detected in urinary-bladder tumors from cancer patients and in bladder tissue from sudden death victims (exposure history for both subjects not known) (Böhm *et al.* 2011). *ortho*-

Toluidine has been tested in *in vitro* and *in vivo* in experimental animals studies measuring genetic damage from *ortho*-toluidine exposure (IARC 2010).

4. Key Scientific Questions and Issues for the Cancer Evaluation

The carcinogenicity of *ortho*-toluidine in experimental animals has been well established since the early 1980s, and no new data have been identified to challenge these conclusions or to suggest that the findings in experimental animals are not relevant to humans. The key questions and issues in the re-review of *ortho*-toluidine concern the evaluation of human cancer studies and mechanistic data.

Questions related to the evaluation of human cancer studies

- What is the level of evidence (sufficient, limited, or inadequate) for the carcinogenicity of *ortho*-toluidine from studies in humans? What are the tissue sites?
- What are the major potential confounders for evaluating urinary bladder cancer risk in these studies?
- Can the relationship between bladder cancer and exposure to *ortho*-toluidine be explained by exposure to these substances?

Questions related to the evaluation of mechanistic data

- What are the potential mechanisms by which *ortho*-toluidine may cause cancer?
- Is there evidence that these mechanisms occur in humans? If so, what is the level of the evidence (strong, moderate, weak)?

5. Proposed Approach for Conducting the Cancer Evaluation

5.1. Scope and focus of the draft RoC monograph

ORoC will prepare the draft RoC monograph on *ortho*-toluidine which will consist of two parts, the cancer evaluation component and the substance profile. The cancer evaluation component of the draft monograph will review and assess the scientific literature, provide a discussion of scientific issues, and assess and integrate the relevant scientific evidence applying the listing criteria to reach a preliminary recommendation on whether the listing classification of *ortho*-toluidine should be changed. The substance profile of the draft monograph will give the NTP's preliminary listing recommendation and a summary of the key supportive evidence. Details on the methods for writing the draft RoC monograph and topics typically covered in the monograph are outlined in the NTP process for the preparation of the RoC (*http://ntp.niehs.nih.gov/go/rocprocess*).

The cancer evaluation will focus on human cancer studies and mechanistic data. It will not re-evaluate the level of evidence in experimental animals but will assess the evidence from any new studies and integrate the findings from the animal studies into the overall synthesis of cancer studies in humans and mechanistic data. Information on properties, use and production, and exposure was recently updated in the *12th RoC*; thus, these chapters will be limited to an update of this information published since 2011. When relevant, the recent IARC monographs on *ortho*-toluidine will be used as a resource. Details of the preliminary literature search strategy including data sources and literature search terms that are consistent with this approach are discussed in Appendix 1.

As discussed in Section 4, a key question in the review of *ortho*-toluidine is the evaluation of potential confounding from co-exposures in the workplace. Thus, the RoC evaluation will include a concise summary of toxicological and epidemiologic evidence for the occupational chemicals identified in the epidemiologic studies. The review will also discuss evidence related to the hypothesis that the presence of the methyl group at the *ortho*-position relative to the amino group (*ortho*-methyl aniline) in *ortho*-toluidine enhances carcinogenicity because aniline is one of the more common co-exposures in the epidemiologic studies. This information will help identify substances that may be risk factors for bladder cancer and should be considered as potential confounders. It will also help in determining whether any epidemiologic studies should be excluded from the review.

5.2. Proposed approach for obtaining scientific and public input

Public comments on scientific issues have been requested[2] on *ortho*-toluidine at several times prior to the development of the draft RoC monograph including the request for information on the nomination, and the request for comment on the draft concept. The ORoC will consider this information and experts suggested by the public in drafting the cancer evaluation component of the draft monograph. The ORoC will create a webpage for the candidate substances currently under review. The webpage will typically include the following: (1) RoC documents related to the review of the substance (e.g., concept document, draft RoC monograph), (2) citations for references identified from literature searches, (3) public comments, (4) an input box for the public to provide information (such as new literature) or comment (such as the identification of additional scientific issues), and (5) information on public meetings or listening sessions. The NTP will communicate when new information is added or updated (such as updated literature searches) to the website via the NTP list serve. Additional scientific issues may be identified during the preparation of the monograph. Future forums (such as a listening session) for receiving public comment on any additional scientific issues may be considered depending on public interest; these would be announced via the *Federal Register* notice, NTP list serve[3] and the RoC website.

ORoC will attempt to identify experts in dye chemistry and/or manufacturing for information on the current or historical use of *ortho*-toluidine to manufacture various dyes (such as magenta manufacturing) or dye intermediates (such as *para*-chloro-*ortho*-toluidine). This information may be useful for understanding the exposure scenarios in the human epidemiologic studies. Sources for identifying dye chemistry experts include web searches for dye manufacturing experts, textile departments or schools (e.g., the Institute of Textile Technology and the College of Textiles at North Carolina State University), dye industry (e.g., American Association of Textile Chemists and Colorists located in Research Triangle Park, NC and the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers [ETAD] headquartered in Basel, Switzerland).

ORoC will consult with appropriate technical advisors, external or internal to the government (such as NIOSH), with knowledge related to *ortho*-toluidine, dyes, or arylamines. Sources for identifying these advisors include, but are not limited to, peer-reviewed literature databases, and recommendations from the scientific community and the public. Advisors with knowledge of occupational hygiene or epidemiology will be consulted to critically review the ORoC assessment of the human cancer studies and will be asked to

[2] Federal Register notice and public comments are available at *http://ntp.niehs.nih.gov/go/rocnom*.

[3] Persons can subscribe to the NTP list serve free-of-charge at *http://ntp.niehs.nih.gov/go/getnews*.

focus on whether potential confounding from co-exposures can be ruled out in the cancer studies in humans. Advisors with knowledge in genotoxicity and carcinogenesis will be consulted to help identify relevant literature, and to provide critical comments on the ORoC assessment of the mechanistic data.

6. Public Release and Peer Review of the Draft Monograph

Once completed, the draft RoC monograph on *ortho*-toluidine will undergo interagency review followed by release for public comment and public peer review. The NTP will convene an external scientific panel[4] to peer review the draft monograph in a public forum. Members of the panel will be from the public and private sectors with expertise in disciplines related to the cancer evaluation of *ortho*-toluidine such as epidemiology, exposure assessment, urinary-bladder cancer, toxicology, biotransformation of aromatic amines, and mechanisms of carcinogenesis. The NTP will set aside time at the peer-review meeting for a discussion of scientific issues raised in the public comments.

[4] NTP panels are federally chartered technical and scientific advisory groups convened as needed to provide advice on specific scientific issues and peer review. Members of NTP panels are scientists with relevant expertise and knowledge from the public and private sectors. The final selection of membership is based upon providing a balanced and unbiased group of highly qualified individuals and is made in accordance with Federal Advisory Committee Act and HHS implementing guidelines; *http://ntp.niehs.nih.gov/go/166.*

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Appendix 1

Preliminary Literature Search Strategy: *ortho*-Toluidine

This document identifies the data sources and search terms and preliminary search strategies for identifying literature for the draft monograph on *ortho*-toluidine. The literature search will be updated approximately every three months, and prior to preparing the draft monograph for interagency review. Additional literature searches will be conducted as needed to identify information to address scientific issues that arise during the review. Citations retrieved from literature searches will be uploaded to web-based systematic review software and screened using inclusion and exclusion criteria. Multi-level reviews of the literature are conducted, with initial reviews based on titles and abstracts only, and later reviews based on full-text.

1. Data Sources

Identification of synonyms and metabolites for ortho-*toluidine (CASRN 95-53-4)*

- *Synonyms*- IARC and National Library of Medicine databases (e.g., ChemIDplus, Hazardous Substances Data Bank)
- *Metabolites*- Son *et al.* (1980) and IARC (2010)

Citation databases (searches for titles, abstracts, and key words)

- PubMed
- Web of Science
- Scopus

Additional data sources

- Authoritative reviews or general sources for exposure and other information (e.g., Toxnet; U.S. Government agencies websites, publications and databases; International Agency for Research on Cancer)
- Citations in authoritative reviews, and primary references located by literature search
- QUOSA library of occupational case-control studies (full text search for *ortho*-toluidine)

Data sources for potential confounders (See ortho-*toluidine concept document)*

- ATSDR Toxicological Profiles
- European Union Risk Assessments
- International Agency for Research on Cancer (IARC) monographs
- National Toxicology Program
- Environmental Protection Agency (EPA) IRIS
- California Environmental Protection Agency (EPA)
- World Health Organization Concise International Chemical Assessment Documents (CICAD)

Data sources for dye intermediates (manufacture of magenta and other dyes)

- *The Chemistry of Dyestuffs, a Manual for Students of Chemistry and Dyeing* by L.L. Lloyd and M. Fort, 1919
- *Colour Index Heritage Edition* on DVD 2005

2. Preliminary Literature Searches:

Literature searches in the three databases (see Data Sources, Section 1) are conducted using search terms specific for *ortho*-toluidine (synonyms, chemical class, metabolites, and exposure scenario) and for the topics covered by the monograph (See Table 1).

As mentioned in the concept document for *ortho*-toluidine, the monograph will focus on human cancer studies and mechanistic data, and thus extensive literature searches will be conducted to identify this information. Searches will also be conducted for studies of cancer in experimental animals published since the first listing of *ortho*-toluidine in the RoC (1982). The monograph will rely primarily on authoritative reviews for information on exposure, toxicokinetics, and genotoxicity, but literature searches will be conducted to update that information from the date of the most recent comprehensive review by IARC (Working group met in 2008, literature searches will be conducted from 2007). Searches will be conducted to identify other major authoritative reviews (See Table 1).

Searches for human cancer studies are somewhat unique because they involve the identification of search terms for exposure scenarios for which people may be exposed to *ortho*-toluidine in addition to search terms specific for *ortho*-toluidine. Potential exposure to *ortho*-toluidine may occur during (1) dye manufacturing, (2) magenta manufacturing, (3) aniline manufacturing, (4) production of rubber chemicals, (5) chloro-*ortho*-toluidine manufacturing, and (6) production of metolachlor or acetochlor. Literature searches are conducted by combining substance search terms (*ortho*-toluidine or exposure scenarios) with search terms for cancer and search terms for epidemiologic studies as outlined below.

Substance-specific search terms: ("dyestuff" OR (dye AND (manufacturing OR manufacture)) OR rubber chemicals OR ortho toluidine OR o-toluidine OR chloro-o-toluidine OR chloro-*ortho* toluidine OR aniline OR ((manufacture OR manufacturing OR production) AND magenta) OR metolachlor OR acetochlor)

AND

Cancer search terms: (cancer OR tumors)

AND

Epidemiologic search terms: (epidemiolog* OR case-control OR cohort OR case-report OR case-series OR workers OR workmen)

In addition to the human cancer studies identified from the above searches, a full-text search for *ortho*-toluidine is conducted using a QUOSA library of occupational case-control studies.

Table 1: Preliminary Literature Search Approach

Topic	Combined with	Date/limits
Human exposure	*ortho*-toluidine synonyms	Reviews Primary literature: since 2007
Studies in experimental animals	*ortho*-toluidine synonyms *ortho*-toluidine hydrochloride and CASRN	Primary literature since 1982
ADME and Toxicokinetics	*ortho*-toluidine synonyms *ortho*-toluidine metabolites[a]	Reviews Primary literature: since 2007

Appendix 1

Topic	Combined with	Date/limits
Genotoxicity	*ortho*-toluidine synonyms *ortho*-toluidine metabolites[a]	Reviews Primary literature: since 2007
Toxicity	*ortho*-toluidine synonyms *ortho*-toluidine metabolites[a]	Reviews Primary literature: since 2007
Mechanisms	ortho-toluidine synonyms ortho-toluidine metabolitesa aromatic amines	No limits Includes searches for animal cancer studies of metabolites

ADME = adsorption, distribution, metabolism and excretion

[a] Major metabolites include azoxytoluene, *o*-nitrosotoluene, *N*-acetyl-*o*-toluidine, *N*-acetyl-*o*-aminobenzyl alcohol, 4-amino-*m*-cresol, *N*-acetyl-4-amino-*m*-cresol, anthranilic acid, and *N*-acetylanthranilic acid.