

Draft RoC Monograph on o-Toluidine

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o-Toluidine presentations

- Rationale and human exposure
- Cancer studies in experimental animals
- Cancer studies in humans
- Metabolism and mechanisms of carcinogenicity
- Overall cancer evaluation
- Substance profile



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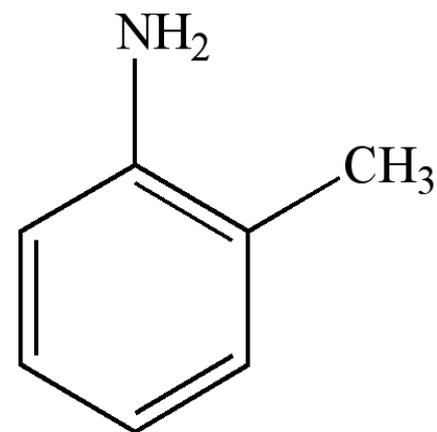
Draft RoC monograph on *o*-toluidine

Rationale and human exposure



o-Toluidine was selected for re-review for the RoC

- Aromatic amine used to make dyes, rubber chemicals, and herbicides
- Listed in the RoC since 1983 as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals
- Numerous human cancer studies published since 1983
 - IARC concluded o-toluidine is carcinogenic to humans (2010, 2012)
- Widespread U.S. exposure



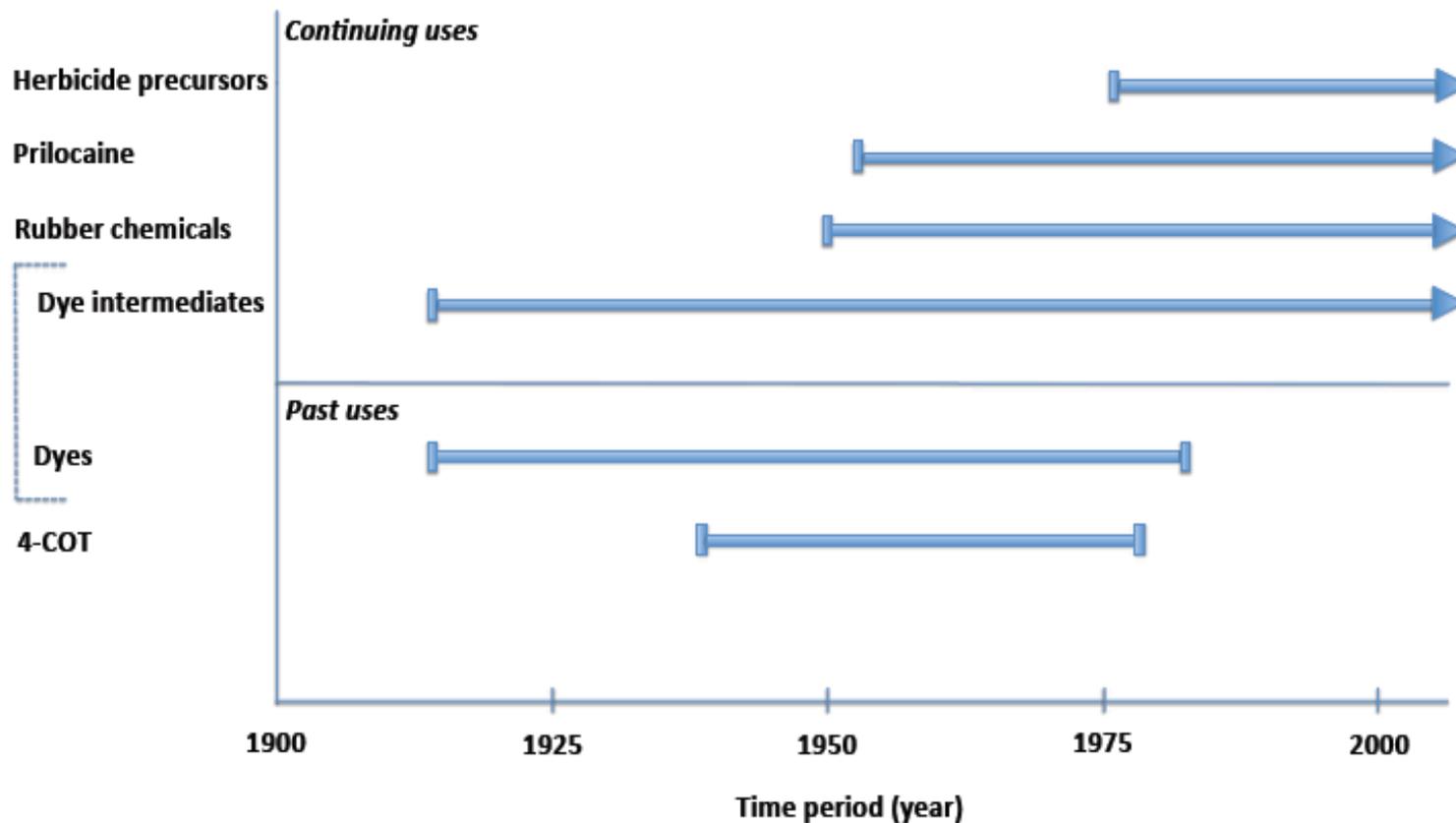
Human exposure: Key questions

- Are a significant number of people living in the United States exposed to *o*-toluidine?
- How are people (sources, settings, and levels) exposed to *o*-toluidine?
- What additional exposure information from the dye and rubber chemical industries can help facilitate the evaluation of the human cancer studies (Appendix C)?

Significant number of persons living in the United States are (or were) exposed to o-toluidine

- Evidence of past and present widespread usage
- High production volume (between 10 and 50 million lbs.)
- Biological monitoring studies indicate that exposure to o-toluidine is widespread, occurring in workers, smokers and non-smokers
- Sources of exposure: Workplace, medical and consumer products, cigarette smoking and the environment

Uses for *o*-toluidine in the United States have changed over time



4-COT = 4-chloro-*ortho*-toluidine

Occupational exposure to *o*-toluidine occurs in a variety of industries

- Major industries
 - *o*-Toluidine production
 - Production of pesticides or pesticide intermediates such as 6-ethyl-*o*-toluidine or 4-chloro-*o*-toluidine
 - Rubber chemical industry
 - Dye industry such as magenta manufacturing
- Workers exposed via inhalation or dermally
- Average levels in U.S. have been typically 0.1 ppm or less and have decreased over time
 - OSHA sampling data from the 1980's to 2000's: 0.06-0.11 ppm
 - Rubber chemical industry data: ~0.1 ppm (late 1970's) to ~0.02 (late 1990's to early 2000's)

Exposure to o-toluidine occurs outside the workplace

- Evidence for widespread exposure includes detection of o-toluidine in the urine and breast milk, Hb adducts in the blood, and o-toluidine releasing DNA adducts in urinary bladder tissue or tumors
- Potential sources of exposure include:
 - Cigarette smoking: Measured in cigarette smoke, biomonitoring data
 - Dental products: Levels of Hb adducts increased 41-fold after surgery using prilocaine as an anesthetic; also found in urine
 - Commercial products: Hair and other dyes
 - Food: Found in German food surveys; no information found in U.S. Total Diet Study (1991 to 2003)
 - Environment: TRI data in the United States, occurrence in water and sediment, levels of Hb adducts vary with geographical location

Human exposure: Reviewers' questions

- Comment on whether the chemical identity and description of *o*-toluidine (Section 1: Properties and Human Exposure) are clear and technically accurate.
- Comment on whether the information on use, production, and human exposure for *o*-toluidine (Section 1: Properties and Human Exposure and Appendix C) is clear and technically accurate.
 - Identify any information that should be added or deleted.
- Comment on whether adequate information is presented to document past and/or current human exposure to *o*-toluidine in the United States. Exposure can be inferred by data on usage, production, or evidence for exposure in the workplace, from the environment or consumer products, diet, or other sources due to lifestyle choices (such as tobacco smoking).

Appendix C: Reviewers' questions

Many of the human epidemiologic studies do not have detailed information on exposure to *o*-toluidine or other co-exposures; however, some of this information can be inferred from knowledge of the manufacturing process, or dye chemistry. Appendix C provides information about the nature of the potential for exposure to *o*-toluidine and to other intermediates or end products in these manufacturing processes that may be useful to interpret the human epidemiologic studies.

- Comment on whether the information in this section is clear and technically accurate.
- Comment on whether the information was useful for characterizing exposure to *o*-toluidine and other occupational chemicals in the human epidemiologic studies.



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Studies of cancer in experimental animals



Cancer studies in experimental animals: Background information and key questions

- RoC listing of *o*-toluidine based on several feeding studies in different strains of rats and mice (Weisburger 1978, NCI 1979)
- Additional studies published since that time
- Key questions
 - What is the level of evidence of carcinogenicity from studies in experimental animals?
 - What are the tissue sites?

Cancer studies in experimental animals: Methods

- Literature search to identify “new” studies (Appendix A)
- Assessment of reporting and study quality (Appendix E, 4.2)
 - Adequacy of reporting quality
 - Evaluated quality of several performance elements: Substance characterization, animal husbandry, study design, endpoint assessment, and data interpretation
 - Utility of the study to inform the cancer evaluation
- Assessment of the neoplasm findings: Integration across studies (4.3)
 - Identification of exposure-related tissue sites
- Preliminary level of evidence conclusion
 - Sufficient, not sufficient (RoC criteria)

Adequate database for evaluation the potential carcinogenicity of o-toluidine in experimental animals

Route of exposure	Species (sex)	Study quality assessment	Reference
feed	F344 rat (M & F)	minimal concerns	NCI 1979
feed	F344 rat (M)	minimal concerns	Hecht 1982
feed	F344 rat (M)	minimal concerns	NTP 1996
feed	CD rat (M)	minimal concerns	Weisburger 1978
feed	B6C3F ₁ mouse (M & F)	minimal concerns	NCI 1979
feed	albino CD-1 mouse (M & F)	minimal concerns	Weisburger 1978
feed	dog	inadequate: no controls	Pliss 2004
<i>per os</i>	fish	inadequate reporting	Anders 1991
sc	rat (M & F)	some concerns	Pliss 2004
sc	mice (M & F)	inadequate reporting	Pliss 2004
sc	Syrian golden hamster (M)	some concerns	Hecht 1983

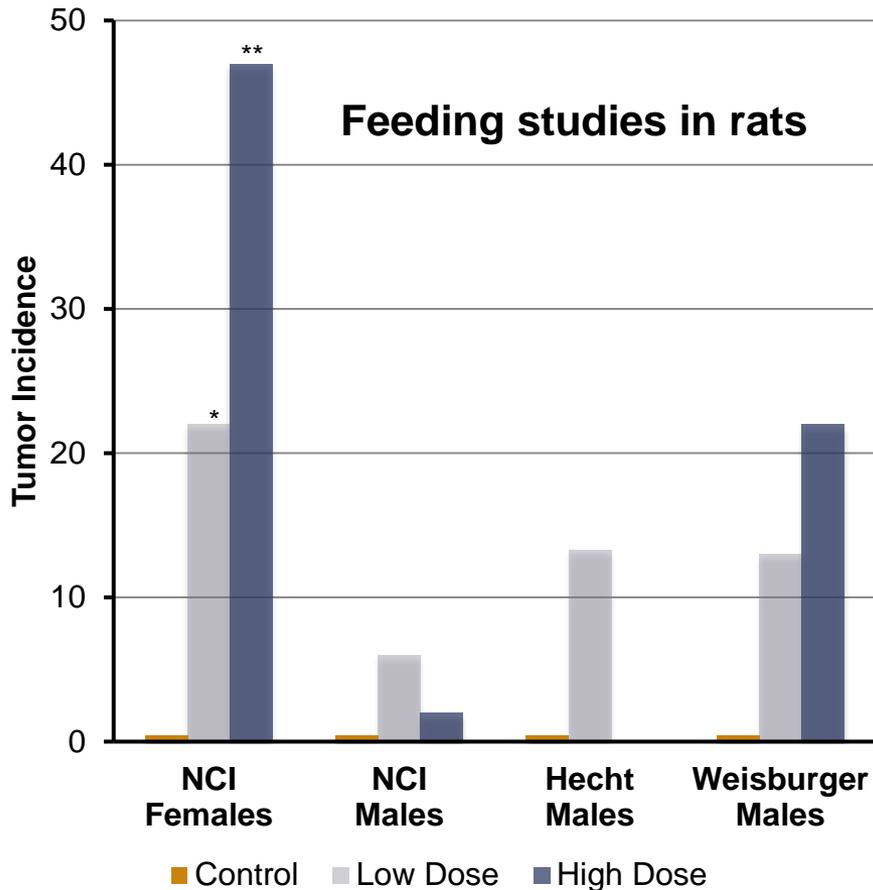
Evaluation of the feeding studies in rats

Strain (sex)	Doses (ppm) # of animals	Duration Exp./total	Ref
<i>1982/83 Evaluation</i>			
CD (M)	0, 4000, 8000 25, 25, 25	18 /24 mo	Weis- burger 1978
F344 (M & F)	0, 3000, 6000 20, 50, 50	~ 2 yr	NCI 1979
<i>"New" Studies</i>			
F344 (M)	0, 4000 30, 30	73/93 wk	Hecht 1982
F344 (M)	0, 5000 10, 20	13/26 wk*	NTP 1996

* 13 wk interim, 13 wk. stop exposure, 26 wk. continuous exposure

- 3 Informative chronic studies
 - Near lifetime exposure
 - Doses approaching toxicity
 - Complete histopathology of all major organs
- 5 Types of neoplasms
 - Urinary bladder, connective tissue, subcutaneous tissue, mesothelium, mammary gland
- Consistent finding across studies
 - Some neoplasms found in both sexes
 - Some neoplasms found in both strains
 - New studies support original studies

o-Toluidine causes urinary bladder neoplasms in rats



Controls – 0 tumors

* = $P < 0.001$; ** = $P < 0.0001$

Carcinoma and papilloma combined

- Rare tumor
- Robust finding in only study of females
 - High incidence (47%)
 - Decreased time to 1st tumor
 - Dose response
- Findings in males
 - Consistent findings in 3 chronic studies in 2 different strains
 - Low incidences; statistically not significant
 - Hyperplasia seen after short exposure (13 & 26 wk) (NTP 1996)

o-Toluidine also causes other neoplasms in rats

Neoplasm	Findings (increased neoplasm incidences)	Comments
<i>connective tissues:</i> <i>sarcoma</i> multiple types in spleen and other organs	both chronic studies of F344 rats dose-response: both sexes (NCI) increased incidences: specific types of sarcomas in all organs (males and females) and in the spleen (females)	sarcomas grouped together because of similar appearance
<i>subcutaneous tissue:</i> fibroma and fibrosarcoma	males in all 3 chronic studies two different strains: F344 & CD dose-response (NCI)	
<i>mesothelioma: tunica vaginalis</i> or abdominal cavity and organs	F344 males (NCI) low incidences after short (13 wk) exposure/duration (NTP)	
<i>mammary gland:</i> fibroadenoma	both sexes of F344 rats: females (NCI), males (Hecht) dose-response: females (NCI)	typically do not progress to malignancy

Dietary exposure to o-toluidine causes neoplasms in mice

Species (sex)	Dose (ppm) # of animals	Duration	Neoplasms	Ref
B6C3F ₁ mouse (M & F)	0, 1000, 3000 20, 50, 50	~ 2 yr	<i>blood vessels</i> (hemangiosarcoma or hemangioma): M outside historical control range dose response <i>liver</i> (hepatocellular adenoma or carcinoma): F dose response	NCI 1979
albino CD-1 mouse (M & F)	0, 8000, 16000 25, 25, 25	18/24 mo*	<i>blood vessel</i> (hemangiosarcoma or hemangioma): M & F significant increased incidence at both doses	Weisburge r 1978

* exposure/total duration

- No “new” studies
- Both studies considered to be informative because of near lifetime exposure using doses that approach toxicity and complete histopathological examination of all major organs

Inadequate evidence of carcinogenicity from studies of subcutaneous injection of o-toluidine

Species, strain (sex)	Dose # of animals	Duration*	Neoplasms	Ref
Syrian golden hamster (M)	0, 203 mg/kg/wk 15,15	52/87 wk	“no statistically significant increased incidences”	Hecht 1993
Rat (not specified) (M &F)	0, 30 mg/injection 25, 25	18-24 mo	increased incidences of combined neoplasms	Pliss 2004

* exposure/total

- Study limitations: less than lifetime exposure or observation duration, smaller numbers of animals, uncertainty about whether necropsies were completed on all major organs
- Less relevant route of exposure

Preliminary level of evidence conclusion: Vote

- Sufficient evidence of carcinogenicity from studies in experimental animals based on increased incidence of tumors in two species at multiple tissue sites
- Dietary exposure to *o*-toluidine caused tumors of the
 - Urinary bladder and connective tissue (sarcoma) in rats of both sexes
 - Subcutaneous tissue and mesothelium in male rats
 - Blood vessels in male and female mice, and liver in female mice
- Supporting evidence
 - Increased incidences in benign tumors of the mammary gland (fibroadenoma) in male and female rats

Cancer studies in animals: Reviewers' questions

- Please comment on the overall approach for preparing the cancer assessment of the studies in experimental animals. Specifically, are the methods for evaluating study quality reasonable, transparent, and clearly presented? Is the assessment of the utility of the studies for informing the cancer evaluation systematic, transparent, and clearly presented?
- Comment on whether the scientific information from cancer studies in experimental animals for *o*-toluidine (Section 4: Studies of Cancer in Experimental Animals and Appendix E) is clear, technically correct, and objectively presented
 - Identify any information that should be added or deleted
- Provide any scientific criticisms of NTP's cancer assessment of the experimental animal studies of exposure to *o*-toluidine and how findings from the scientific evidence across studies were synthesized



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Cancer studies in humans



Cancer studies in humans

- Key Questions
 - What is the level of evidence of carcinogenicity from studies in humans?
 - What are the potential confounders?
 - Can any association between o-toluidine and cancer be explained by chance, bias or confounding?
- Methods: Protocol posted on the RoC website
 - Literature search strategy (3.1)
 - Description of studies (3.2)
 - Evaluation of study quality (3.3)
 - Cancer assessment: individual studies, integration of evidence across studies (3.4)
 - Preliminary level of evidence conclusion (3.5)

Identification and selection of studies

- Literature search strategy/inclusion and exclusion criteria
 - Appendix A, protocol
 - Excluded studies: Case-report and case series studies, not externally peer-reviewed
- Most studies only reported on urinary bladder cancer
 - Excluded childhood ALL case-control study
- Description of studies
 - (Appendix D tables)

Reference	Cancer endpoint	Industry
Case and Pearson 1954	urinary bladder mortality	UK dyeworkers
Pira 2010	urinary bladder mortality	Italian dyeworkers
Ott and Langner 1983	multiple cancer sites mortality	US dyeworkers
Stasik 1988	urinary bladder mortality	4-COT production
Carreón 2010, 2013	urinary bladder mortality & incidence	US rubber chemical workers
Sorahan 2008	urinary bladder mortality & incidence	UK rubber chemical workers
Richardson 2007	urinary bladder incidence	occupational exposure
Castro-Jiménez 2011	childhood ALL incidence	potential exposure

All = acute lymphoblastic leukemia

Blue background: historical cohort studies

Tan background: population based case-control studies

Blue font – new update

Methods for evaluating study quality: Potential for biases

- Questions and guidelines in protocol used to reach conclusions for the potential (both differential, non-differential) for selection and information bias (Section 3.3.1/Appendix D)
- Adequacy of statistical methods, statistical power and other factors also evaluated
- When possible, guidelines are specific for endpoint: Urinary bladder cancer
 - Example: Incidence data considered more informative, adequate follow-up 20 years or greater
- Consideration of whether there was information to determine the direction of the bias
 - Example: Non-differential misclassification usually biases towards the null
- Potential for a bias does not always mean the study was biased

Methods for evaluating study quality: Potential for confounding and utility of studies

- Evaluation of potential confounding (protocol/Appendix D, Sections 3.3.2-3)
 - Major risk factors for urinary bladder cancers are tobacco smoking, arsenic, radiation, some drugs and occupational exposures, mostly dyes
 - Many occupational studies have limited methodologies to formally evaluate potential confounding
 - Approach: Identify which co-exposures were potential confounders, consider methods to evaluate confounding, relative levels of confounders compared to *o*-toluidine, and magnitude of risk estimates
- Identification of most informative studies (Section 3.3.4)

Latest update of NIOSH study of US rubber workers: Improvements of several design elements

- Carreón 2010 was considered to be a high quality study
- What Carreón 2013 adds:
 - Longer follow up: 18 more years
 - Improved case ascertainment: 6 states vs. NY only
 - Greater statistical power: 37 vs. 13 cases of urinary bladder cancer
 - Improved exposure assessment: exposure ranks
 - More detailed analyses:
 - External and internal analyses for (1) probability of exposure , (2) duration of exposure, (3) time since first exposure, (4) cumulative exposure (unlagged and lagged analysis)
 - Cox regression models, lagged 10 years, evaluated both categorical and continuous exposure distribution and interaction with age
- Planned changes to monograph: replace 2010 study with 2013 study (primarily pages 43, Table 3-3, delete Table 3-4, Appendix D tables)

Study quality evaluation of the individual studies: Informative studies

Study	Exposure assessment	Analyses Information on confounding
NIOSH rubber chemical cohort Carreón 2010, 2013 1875 workers	work history/industrial hygiene surveys (job, time period) exposure rank	SIR, SMR, SRR, HR, probability of exposure, employment duration, time since 1 st employment, cumulative rank exposure indirect smoking adjustment measurement of co-exposures, internal analysis
UK rubber chemical cohort Sorahan 2008 53 workers	work history/expert knowledge	SIR, SMR, RR employment duration RR adjusted for exposure to 2-mecaptobenzothiazole (MBT) PBN phenol beta naphthylamine (PBM) and aniline in internal analysis
Italian dyeworkers cohort/magenta or o-toluidine mfg. Pira 2010 47 male workers	work history/expert knowledge	SMR excluded workers with exposure to benzidine, α -naphthylamine, or β -naphthylamine.

Study quality evaluation of the individual studies: Limited or inadequate quality

Study	Exposure assessment	Analyses Information on confounding
UK dyeworkers cohort/magenta mfg. Case & Pearson 1954 85 male workers	inferred via knowledge of industrial process	SMR excluded workers with exposure to auramine, benzidine, α -naphthylamine, or β -naphthylamine
US dyeworkers cohort/brom- or thioindigo mfg. Ott & Langner 1983 117 male workers	working in dept. that o -toluidine may have been used; only used to make bromindigo dyes	SMR excluded individuals with high exposure to arsenicals, vinyl chloride, or asbestos
population-based case –control study Richardson 2007 1062 male cases	JEM/self reported job history/population based; probability of exposure	RR adjusted for smoking and other non-occupational factors but not occupational exposures
4-COT production cohort Stasik 1988 335 workers	working in 4-COT production or process dept.	SMR exposure to 4-chloro- o -toluidine was predominant

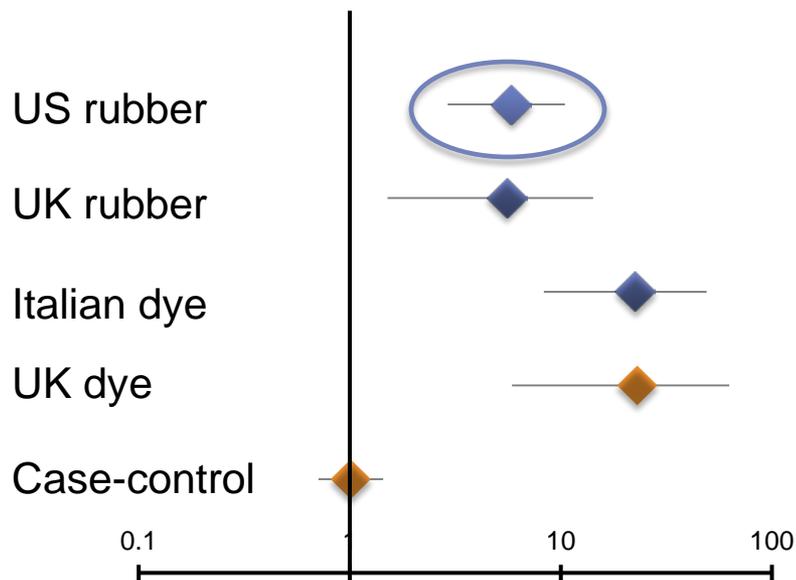
Grey= inadequate study

Carreón 2013 confirms and strengthens the findings of previous update (Carreón 2010)

- Confirms findings of previous publication
 - Statistically significant elevated risks (~4 fold compared to ~6 fold) of urinary bladder were observed among workers definitely exposed to *ortho*-toluidine
 - Positive, statistically significant exposure-duration response relationship
 - Risks unlikely to be due to smoking
- Strengthens findings
 - Positive exposure-response relationships with cumulative exposure in internal analyses, unlagged ($P < 0.01$) and lagged for 10 ($P < 0.01$) and 20 years ($P = 0.037$); SRR among highest exposure group ~7 fold
 - Cox regression analyses: Positive association for cumulative rank of exposure using both categorical and continuous models of exposure
 - Interaction between exposure rank and age (higher risk < 60 years)
 - Robust findings with sensitivity analysis for identification of cases

Credible evidence of an association between increased urinary bladder cancer risk and exposure to o-toluidine

Risk estimates (SIR, SMR or OR)



no urinary bladder cancer deaths observed in US dyeworker cohort

incidence: US and UK rubber chemical cohorts, case control study

mortality: UK and Italian dyeworkers cohorts

◆ = informative studies

◆ = lower quality studies

- Consistent findings across studies
- Significant exposure-response employment duration in rubber both chemical studies
- Significant exposure-response relationship with cumulative exposure rank in both unlagged and 10 and 20 lagged analysis
- Large magnitudes of effect across studies
- US dye workers and case control studies: Null

– Limited statistical power and/or misclassification of exposure probable

Blue font – new information from Carreón 2013

Are there alternative explanations to explain the increased risk of urinary bladder cancer?

- Small number of cases in most studies, imprecise estimates
 - Carreón 2013 update is based on a larger number of urinary bladder cases, increasing the precision for the SIR for definitely exposed and power for detecting exposure-response relationships
- Association unlikely to be explained by selection or information bias
 - No major concerns about selection bias
 - Potential for non-differential misclassification of exposure in some studies
 - High magnitude of risk estimates mitigates concern
- Smoking unlikely to explain results
 - NIOSH: Estimated SIR from smoking = 1.08 based on subset of workers
 - UK rubber chemical study: No excess risk of lung cancer
 - Large magnitude of effect in other studies

Potential confounding from occupational co-exposures can be reasonably ruled out across studies

- Rubber chemical industries
 - Levels of co-exposures well characterized in NIOSH cohort
 - Aniline common exposure: no evidence for increased risk of urinary bladder cancer
 - RR for urinary bladder cancer and *o*-toluidine remained elevated in UK study after adjustment for exposure to aniline, MBT and PBN
- Dye industries – co-exposures are more of a potential concern
 - Animal carcinogens, other aromatic compounds, potential for effect modification
- *o*-Toluidine common exposure across studies
 - No independent evidence that any co-exposures are known human bladder carcinogens
 - Co-exposures in other cohorts vary

Preliminary level of evidence conclusion: Vote

- Sufficient evidence of carcinogenicity from studies in humans
- Several epidemiologic studies have found an increased risk of urinary bladder cancer among workers exposed to o-toluidine
- Risk of urinary bladder increased with **increasing** level or longer duration of exposure to *ortho*-toluidine
- Unlikely to be explained by chance, bias or confounding

Human Cancer studies: Reviewers' questions

- Please comment on the overall approach for preparing the cancer assessment of the epidemiologic studies. Specifically, are the methods for evaluating study quality and potential confounding systematic and transparent? Is the assessment of the utility of the studies for informing the cancer evaluation reasonable and clearly presented?
- Comment on whether the scientific information from the cancer studies in humans for *o*-toluidine (Section 3: Human Cancer Studies and Appendices C and D) is clear, technically correct, and objectively presented.
 - Identify any information that should be added or deleted.
- Provide any scientific criticisms of NTP's cancer assessment of the epidemiologic studies of exposure to *o*-toluidine, including how the findings from the individual studies were interpreted and the evidence across studies was synthesized.



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Metabolism and mechanistic data



Metabolism and mechanistic data: Outline

- Metabolism data from Section 2 of the monograph
- Key questions
- Aromatic amines (background information)
- Key mechanistic events for urinary bladder cancer
- Structural comparison studies (QSARs)

Metabolism and mechanistic data: Key questions

- What is the scope of the mechanistic literature for o-toluidine in humans and experimental animals?
- What are the potential mechanisms of carcinogenicity?
- Do these mechanisms operate in humans and experimental animals?

Aromatic amines

- Many genotoxic and carcinogenic compounds
 - Monocyclics (*o*-toluidine, alkyl anilines, Cl anilines)
 - Bicyclics and polycyclics (2-AAF, 4-ABP, 2-naphthylamine)
- Common metabolic and mechanistic schemes
- Common target tissues

Metabolism and mechanistic data: Scope of literature

- Metabolism
 - Data limited to a few studies in male rats (Son *et al.* 1980, Cheever *et al.* 1980, Kulkarni *et al.* 1983, Brock *et al.* 1990)
 - No definitive studies identifying P450s
 - Human data limited to measurement in urine or hemoglobin adducts in occupational and non-occupational exposed workers (Korinth *et al.* 2006, 2007, Ward *et al.* 1996, Brown *et al.* 1995, Stettler *et al.* 1992)
- Mechanistic studies
 - Extensive genetic toxicology database for *o*-toluidine
 - Rely on secondary literature (IARC 2010, 2000, Danford 1991)
 - Extensive database for bicyclic and polycyclic aromatic amines (less for monocyclic compounds)

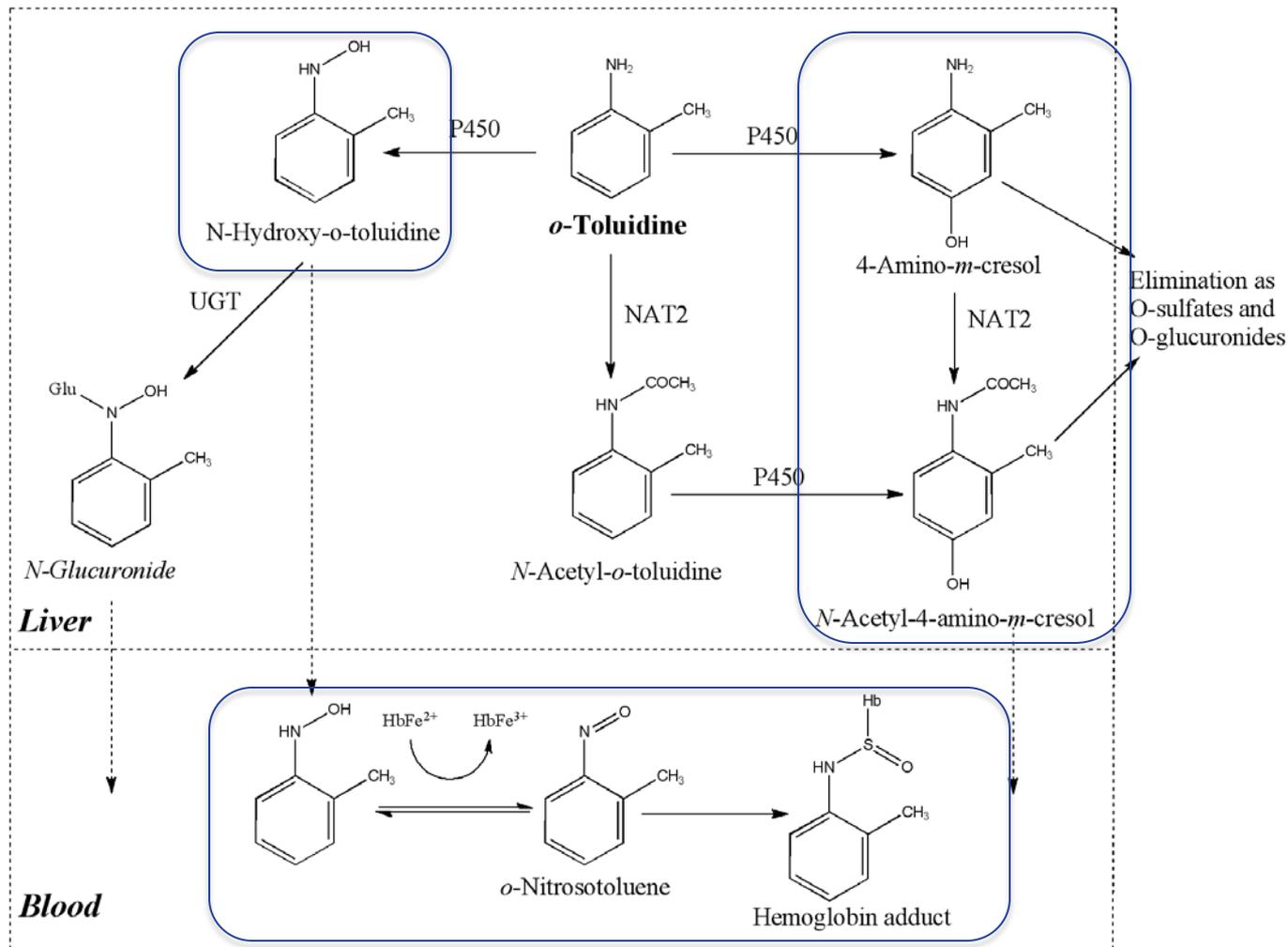
Mechanistic data support the carcinogenicity of o-toluidine

- Metabolic activation
 - Hemoglobin adducts are biological markers of bioactivation
- DNA damage in several mammalian cell types including human and rat urinary bladder mucosa
- Oxidative DNA damage and cell proliferation

Key events: *o*-Toluidine metabolism

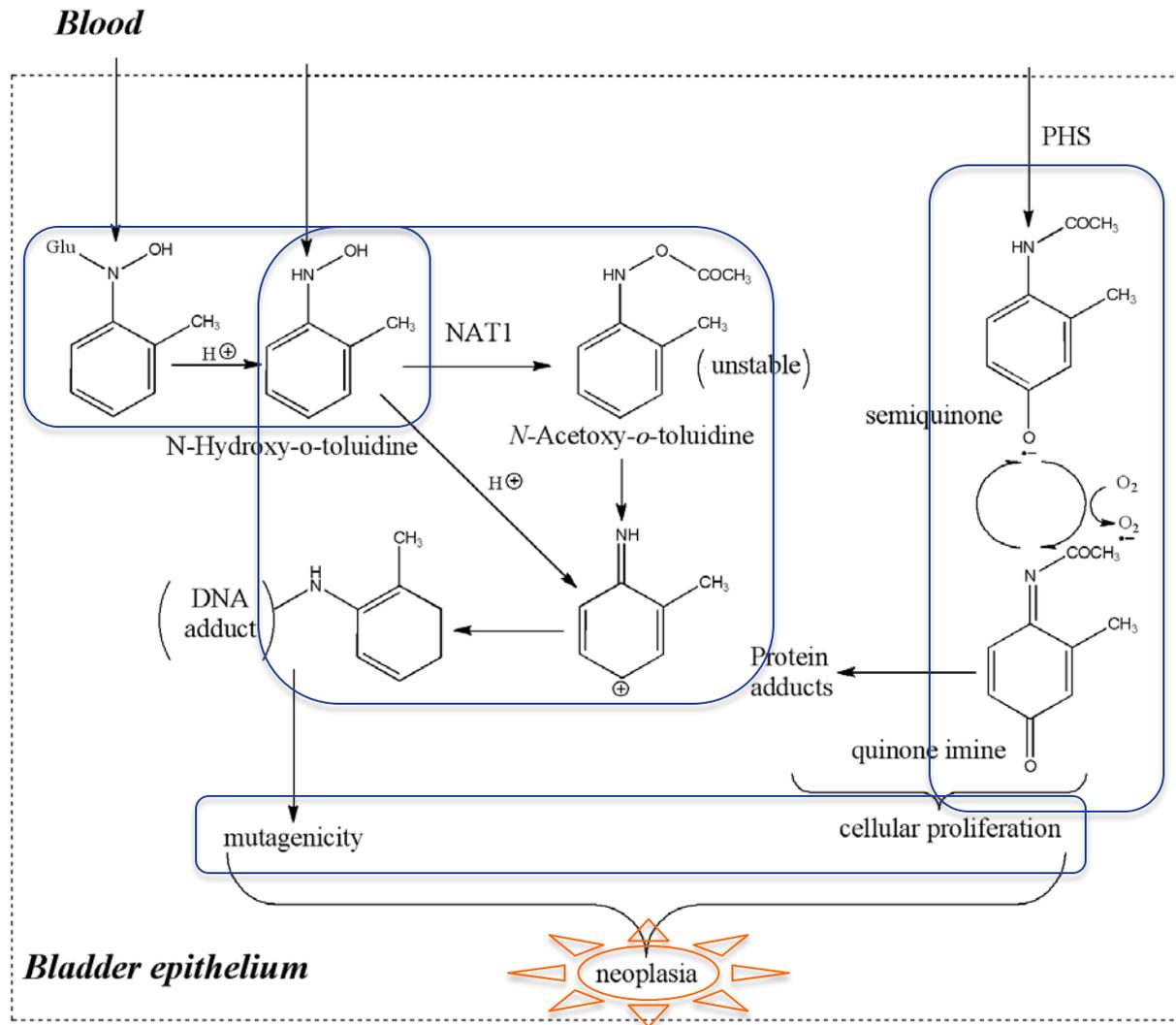
- Liver
 - N-hydroxylation (P450)
 - Ring oxidation (P450)
 - N-acetylation (NAT2, slow acetylator = greater risk)
 - Sulfate/glucuronide conjugation (6/1 ratio)
- Blood
 - Oxidation to *o*-nitrosotoluene
 - Methemoglobin and hemoglobin adducts
- Urinary bladder
 - Hydrolysis of glucuronides (acidic pH or glucuronidase)
 - O-acetylation (NAT1)
 - Oxidation of phenolic metabolites (peroxidases)

Metabolic activation: liver and blood



Source: English et al. 2012, Kadlubar and Badawi 1995

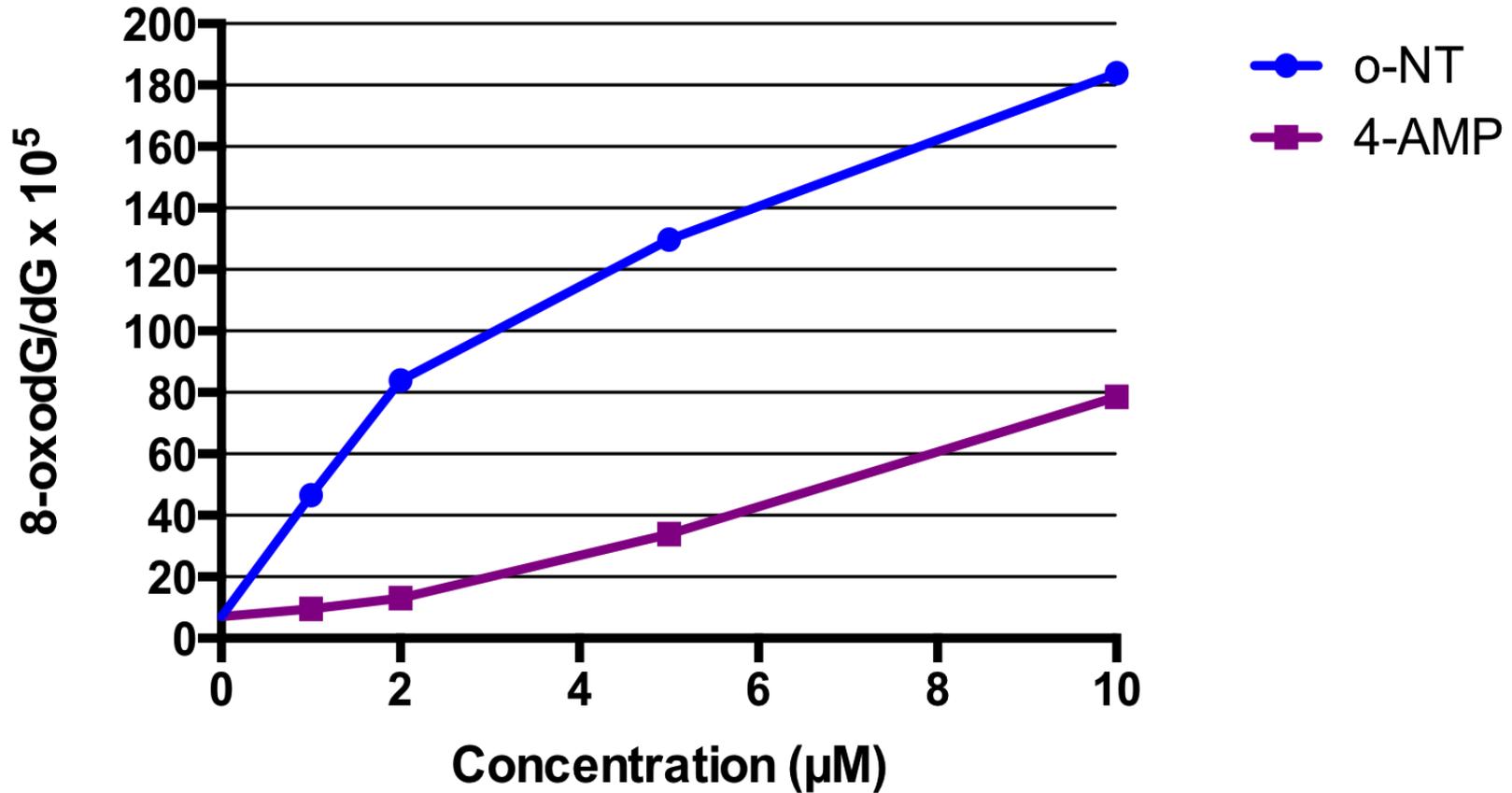
Reactive metabolites in the urinary bladder



o-Toluidine is genotoxic

- DNA adducts
 - Rats (liver and nasal mucosa but not in bladder)
 - Humans (releasing adducts in bladder)
- Mutations
 - N-oxidized metabolites Ames test
 - Human cells
- DNA damage
 - Rodent & human cells
 - Mouse (liver, kidney)
 - Rat (urinary bladder)
- Clastogenic
 - Sister chromatid exchange in rodent/human cells, hamster and mouse bone marrow
 - Micronuclei induction in rodent/human cells, rat blood
 - Chromosomal aberrations & aneuploidy in rodent cells

Oxidative damage in calf thymus DNA



Source: Ohkuma et al. 1999

Structural comparison: Carcinogenic activity and potency of aromatic amines

- Carcinogenic activity: chemical reactivity (metabolic activation) and steric properties (bulk/shape)
 - N-oxidation
 - monocyclics lower probability
- Carcinogenic potency: hydrophobicity most important, reactivity and steric properties also involved
 - *o*-toluidine more hydrophobic than aniline
 - *o*-methyl group enhances potency
 - *N*-hydroxy metabolites of *o*-toluidine and not aniline were mutagenic
 - conformation of adducts affects mutagenic potency

Metabolism and mechanistic data: Summary

- Metabolic activation is required
 - N-hydroxylation
- *o*-Toluidine and its *N*-hydroxylated metabolites form protein and DNA adducts and are genotoxic
- Non-genotoxic mechanisms (cytotoxicity) contribute to carcinogenicity
- ROS and oxidative damage likely involved

Reviewers' questions: Metabolism and mechanistic data

- Was the mechanistic data (Section 5 and Appendix F) presented in a clear, technically correct, and objective manner?
- Are the available mechanistic data relevant for identifying and evaluating the potential mechanisms of action for the carcinogenic effects of *o*-toluidine?
 - Provide any scientific criticisms of the NTP's interpretation and application of the genetic and related effects (Section 5.1, Appendix F).
 - Provide any scientific criticisms of the NTP's interpretation and application of the mechanistic considerations (Section 5.2) or the structural comparison studies (Section 5.3).
 - Identify any information that should be added or deleted.



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Overall cancer evaluation: Integration of human, animal, and mechanistic data



Preliminary listing recommendation: o-toluidine is a known human urinary bladder carcinogen

- Increased risks of urinary bladder cancer among o-toluidine exposed workers in concert with
 - Cancer studies showing site concordance for urinary bladder cancer in female and male rats and humans
 - Mechanistic data demonstrating biological plausibility in humans

Mechanistic data demonstrating biological plausibility in humans

- Proposed mechanism for urinary bladder carcinogenicity similar to that of other aromatic amines causing urinary bladder cancer in humans
- Metabolic activation resulting in binding of reactive metabolites to DNA and proteins, oxidative DNA damage, and genotoxicity
- Data suggesting that key metabolic steps and genotoxic effects occur in humans
 - Hemoglobin adducts – suggest that proposed metabolic pathways occur in humans
 - Genotoxic in human lymphocytes and urinary bladder mucosa cells
 - *o*-Toluidine releasing DNA adducts found in urinary bladder tissue and tumors

Overall evaluation: Reviewers' questions:

- Comment on the overall cancer evaluation (Section 6: Overall Cancer Evaluation - Synthesis of Human, Animal, and Mechanistic Data) and whether the available metabolic, genotoxicity, and mechanistic data provide support for the relevance of the cancer studies in humans and experimental animals to human carcinogenicity.
 - Provide any scientific criticism of the NTP's overall assessment and integration of the human cancer, experimental animal, and mechanistic data.



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Substance profile



Draft substance profile

- Contains NTP's preliminary recommendation of the listing status of the substance.
- Summarizes the scientific information that is key to reaching a recommendation.
- Provides information on properties, use, production and exposure.
- Provides information on existing federal regulations and guidelines.

Substance profile: Proposed changes

- Changes to be made based on comments on cancer evaluation component
 - Overall language Cancer studies in animals, including site concordance for urinary bladder cancer in female and male rats and humans
 - Replace NIOSH 2010 with 2013, add additional information on exposure-response relationship with cumulative exposure ranks
 - Adjust language on DNA releasing adducts
 - Add cell transformation information

Draft substance profile: Reviewers' questions:

- Comment on whether the information on use, production, and human exposure for *o*-toluidine is clear and technically accurate.
- Comment on whether the information presented regarding cancer studies in humans is clear, technically correct, and objectively stated.
 - Comment on whether the substance profile highlights the information from the cancer studies in humans that are considered key to reaching the listing recommendation.
- Comment on whether the information presented regarding cancer studies in experimental animals is clear, technically correct, and objectively stated.
 - Comment on whether the substance profile highlights the key information from the cancer studies in experimental animals that supports the listing recommendation.
- Comment on whether the information presented regarding studies on mechanisms of carcinogenicity and other relevant data is clear, technically correct, and objectively stated.
 - Comment on whether the substance profile highlights the studies on mechanisms of carcinogenicity and other relevant data that are key to providing support for the carcinogenicity of *o*-toluidine in humans.