SYSTEMATIC EVIDENCE MAP (SEM) FOR TOLUENE

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DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

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1. OBJECTIVES

The aim and scope of the systematic evidence map (SEM) are to:

- Conduct literature searches to identify available relevant studies published since the toluene toxicological profile was last published in June 2017, including studies in humans, animals, *in vitro* models, or *in silico*.

- Screen literature search results using methods consistent with principles of systematic review to determine if identified studies meet the Populations, Exposures, Comparators, and Outcomes (PECO inclusion criteria) outlined below (see Section 2.1).

- Prepare an interactive literature inventory to provide an overview of the new evidence that meets PECO criteria.

- Perform high-level data review and extraction of studies identified during the updated literature search to determine if any could potentially address key data needs or impact existing minimal risk levels (MRLs) for toluene, as identified in the toxicological profile (ATSDR 2017).

2. METHODS

2.1 LITERATURE SEARCH STRATEGY

A literature search was conducted to identify studies examining health effects, toxicokinetics, and mechanisms of action for toluene. The PECO criteria used to identify relevant studies examining the health effects of toluene are presented in Table 2-1.

Table 2-1. PECO Criteria for Screening of ATSDR SEM Literature Search Results

PECO element	Evidence
Population	Humans, laboratory mammals, and other animal models of established relevance to human health (e.g., *Xenopus* embryos); mammalian organs, tissues, and cell lines; and bacterial and eukaryote models of genetic toxicity.
Exposure	*In vivo* (all routes), *ex vivo*, and *in vitro* exposure to the chemical of interest, including mixtures to which the chemical of interest may contribute significantly to exposure or observed effects.
Comparison	Any comparison (across dose, duration, or route) or no comparison for select study types (case reports without controls, acute lethality limit tests without controls).
Outcomes	Any endpoint suggestive of a toxic effect on any bodily system, or mechanistic change associated with such effects. Any endpoint relating to toxicokinetics/ dynamics of the chemical within the body.

ATSDR = Agency for Toxic Substances and Disease Registry; PECO = Populations, Exposures, Comparators, and Outcomes; SEM = systematic evidence map

The current literature search was intended to identify studies not included in the existing toxicological profile for toluene (ATSDR 2017); thus, the literature search was restricted to studies published between January 2015 to February 2024 to capture literature published since the search was conducted for the existing profile. The following main databases were searched in February 2024:

- PubMed
- Embase
- SciFinder
- Scopus

The search strategy used the chemical names, Chemical Abstracts Service Registry Numbers (CASRNs), synonyms, Medical Subject Headings (MeSH) headings, and keywords for toluene. The query strings used for the literature search are presented in Appendix A (Table A-1). These query strings are designed to capture all data potentially relevant to the PECO statement as well as additional data potentially relevant to developing a toxicological profile (e.g., chemistry, production, use, environmental fate, etc.).

These additional data studies that are potentially relevant, but do not meet the PECO criteria, will not be included in the SEM but will be tagged for potential future use in profile development.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), National Toxicology Program (NTP), National Technical Reports Library (NTRL), and Regulations.gov websites using the queries presented in Appendix A (Table A-2). Regulatory documents and review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature from these resources, including unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

2.2 LITERATURE SCREENING STRATEGY

Two screeners independently conducted a title and abstract screening of the search results using DistillerSR[1] to identify study references that met the PECO eligibility criteria (see Table 2-1).

References that were included based on PECO eligibility criteria during title and abstract screen were submitted for reference retrieval. For nonlocal retrieval items (e.g., pay-per-citation, etc.), an additional screening step was conducted based on refined PECO criteria with a narrowed focus to capture only key health hazard information and studies that may fill data gaps (Table 2-2). Citations selected for full-text retrieval were limited to English-language, full-length journal articles or study reports at this stage.

[1]DistillerSR is a web-based systematic review software used to screen studies available at: https://www.evidencepartners.com/products/distillersr-systematic-review-software.

Table 2-2. Refined PECO Criteria for Screening of Nonlocal Citations

PECO element	Evidence
Population	Humans or laboratory mammals.
Exposure	Inhalation, oral, or dermal exposure to the chemical of interest, including mixtures that contain a high percentage of the chemical of interest.
Comparison	Any comparison (across dose, duration, or route) or no comparison (no controls) for select study types (case reports, acute lethality limit tests).
Outcomes	Any endpoint suggestive of a toxic effect on any bodily system or containing information to address data gaps (e.g., PBPK model, toxicokinetics, toxicity or mechanistic data to inform specific effects, etc.)[a].

[a]Data gaps identified in the 2017 profile for toluene included: longer-term exposures by oral and dermal routes in humans and animals; studies in animals that inform low dose levels (values ≤15 ppm for inhalation and 100 mg/kg for oral) or specific neurobehavioral effects; genotoxicity studies *in vivo* and *in vitro* to evaluate clastogenicity; mechanistic or toxicokinetic data on arene oxide (reactive intermediate); multi-generation studies to inform lack of reproductive effects; developmental human studies (occupational); sensitive and specific toxicological or mechanistic studies to inform neurological, neurodevelopmental, and immunological effects; and toxicokinetic data for oral and dermal exposures.

PBPK = physiologically based pharmacokinetic; PECO = Populations, Exposures, Comparators, and Outcomes

References that were included based on title and abstract screening advanced to full-text review using the broad PECO eligibility criteria listed in Table 2-1. Full-text copies of potentially relevant references identified from title and abstract screening were retrieved, embedded in DistillerSR screening forms, and independently assessed by two screeners using DistillerSR to confirm eligibility. If studies were considered PECO-relevant based on full-text review, screeners categorized the studies as one of the following study types: primary health effects studies (human toxicity, animal toxicity) or supporting data studies. Supporting data studies include the following study types: genotoxicity, mechanistic, toxicokinetic, secondary sources, and conference abstracts. Some human toxicity studies were also classified as supporting data studies due to limited information on quantitative dose-response metrics for toluene, including case reports, studies lacking quantitative exposure reporting or estimated exposure using a job-exposure matrix, and studies with combined analysis of benzene-toluene-ethylbenzene-xylene (BTEX) exposure only. For animal studies, exposure routes other than inhalation, oral, and dermal were also classified as supporting studies. Additionally, due to integration across durations, routes, and/or species, which precluded the ability to extract data into DistillerSR forms, meta-analyses were also classified as supporting studies at full text review. Lastly, studies that did not meet PECO criteria but contained other profile-relevant data were categorized as one of the following study types for potential use during later profile development: chemistry, biomarker, interaction, or susceptible populations.

At both the title/abstract and full-text review levels, any screening conflicts were resolved by discussion between the primary screeners, with consultation by a third screener (if needed) to resolve any remaining disagreements.

2.3 HIGH-LEVEL DATA EXTRACTION FOR LITERATURE INVENTORY

References that were categorized as PECO-relevant health effects studies advanced to high-level data extraction in DistillerSR. Information extracted for human toxicity studies included study population, measure of exposure, duration, route, systems evaluated, and whether or not examined systems showed an exposure-related effect. Information extracted for animal toxicity studies included species, strain, animal number and sex, duration, route, number of dose groups, doses/concentrations, systems evaluated, and systems showing an exposure-related effect. Extracted data were exported into Tableau Public[2] for interactive data visualization.

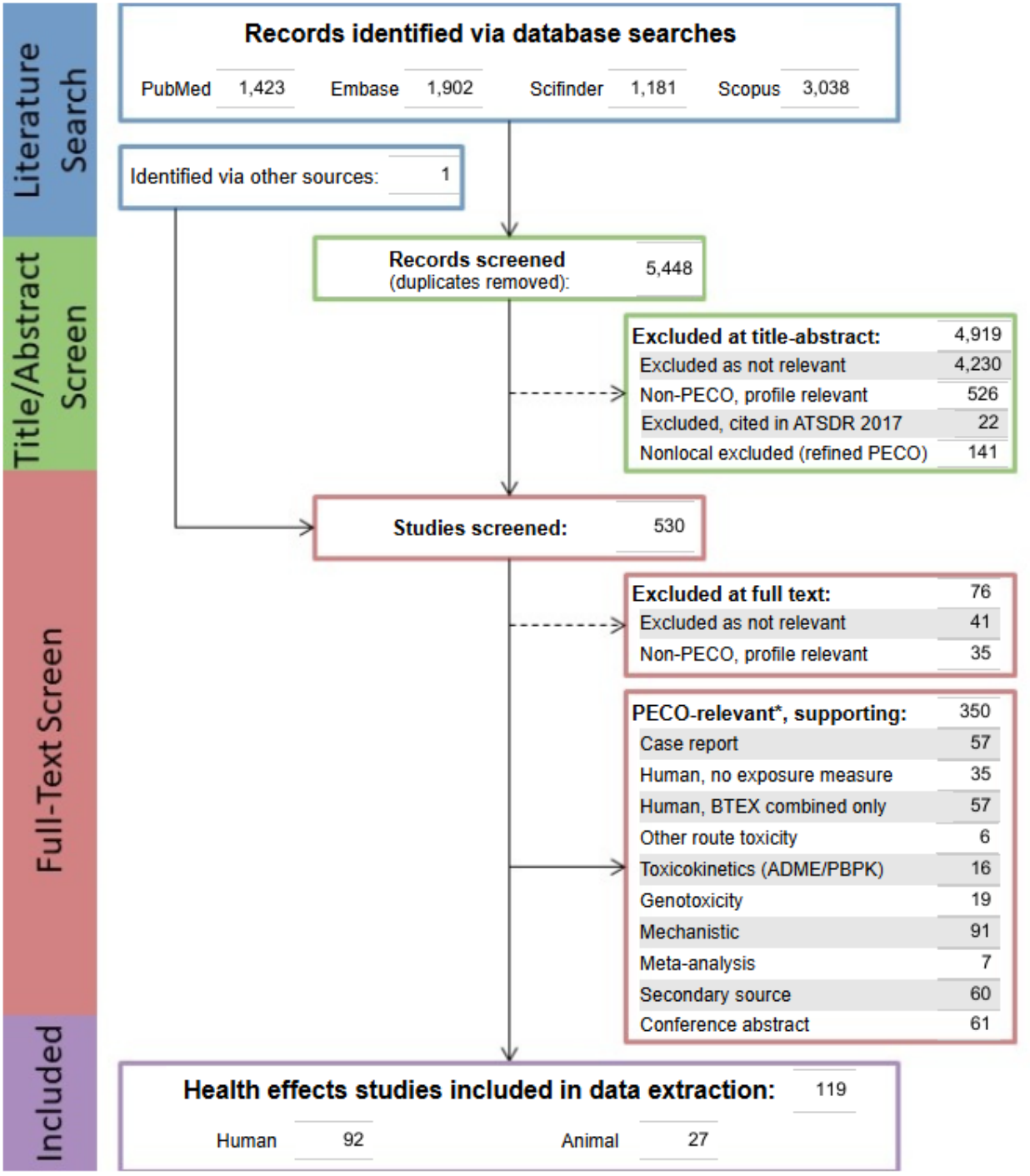
[2]Tableau Public is a web-based data visualization software available at https://public.tableau.com.

3. RESULTS

3.1 LITERATURE SEARCH RESULTS

Literature searches from all bibliographic databases yielded 5,448 unique references (after removal of duplicates). Title and abstract screening identified 4,230 references as not relevant, 526 references as non-PECO profile-relevant items, and 22 references that were already cited in ATSDR (2017); 141 references were nonlocal and identified as not relevant based on refined PECO criteria. After removing not PECO-relevant references, the remaining 529 items proceeded to full-text review. Grey literature search results screened outside of DistillerSR added 1 citation, bringing the total number of citations for full-text review to 531. An additional 76 references were identified as not PECO-relevant during full-text screening; of these, 35 were identified as non-PECO profile-relevant items. The remaining 469 references were identified as PECO-relevant; 104 references included health effects data, 281 references contained other supporting data, and 15 references contained both health effects and supporting data. A summary of the results of the literature search and screening is presented in Figure 3-1.

Figure 3-1. Literature Flow Diagram



*Supporting studies may contain data relevant to multiple supporting categories and/or human or animal health effects data

ADME = absorption, distribution, metabolism, and excretion; PBPK = physiologically based pharmacokinetic; PECO = Populations, Exposures, Comparators, and Outcomes

Interactive literature flow diagram can be accessed at: LitFlow SEM Toluene 2024.

3.2 LITERATURE INVENTORY

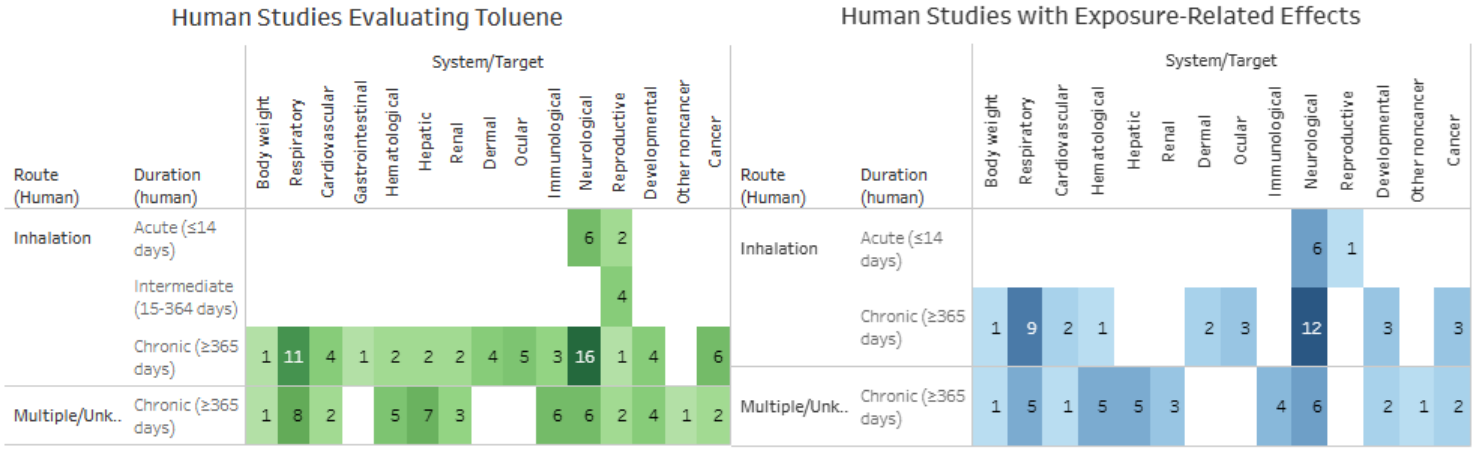
The literature search and screen identified 92 human and 27 animal health effects studies for toluene; some studies included multiple experiments in different species and/or of different durations.

As shown in Figure 3-2 (A: Human Data) and (B: Animal Data), the majority of human studies were chronic-duration and the majority of animal studies were acute-duration. Regarding route, the majority of newly identified studies with toxicity data examined exposure via the inhalation route, with some oral route studies in animals. For human studies, 61% (74) of the data gathered focused on the inhalation route and 39% (47) were multiple/unknown exposure routes (due to exposure assessment via biomarker). For animal studies, 88% (58) of animal studies focused on the inhalation route and 12% (8) of animal studies focused on the oral route. The most-studied endpoints include body weight, respiratory, neurological, and reproductive and developmental. Findings from human and animal studies are consistent with the existing Toxicological Profile for Toluene (ATSDR 2017): (1) the nervous system, respiratory system, and developing organism are potential toxicity targets of toluene based on human and animal data; (2) there is some evidence for immunological effects in humans and animals; (3) animal studies generally report toluene-associated effects in reproductive studies, but findings from human studies are mixed; and (4) evidence for effects in other organ systems are mixed (e.g., hepatic, renal, hematological).

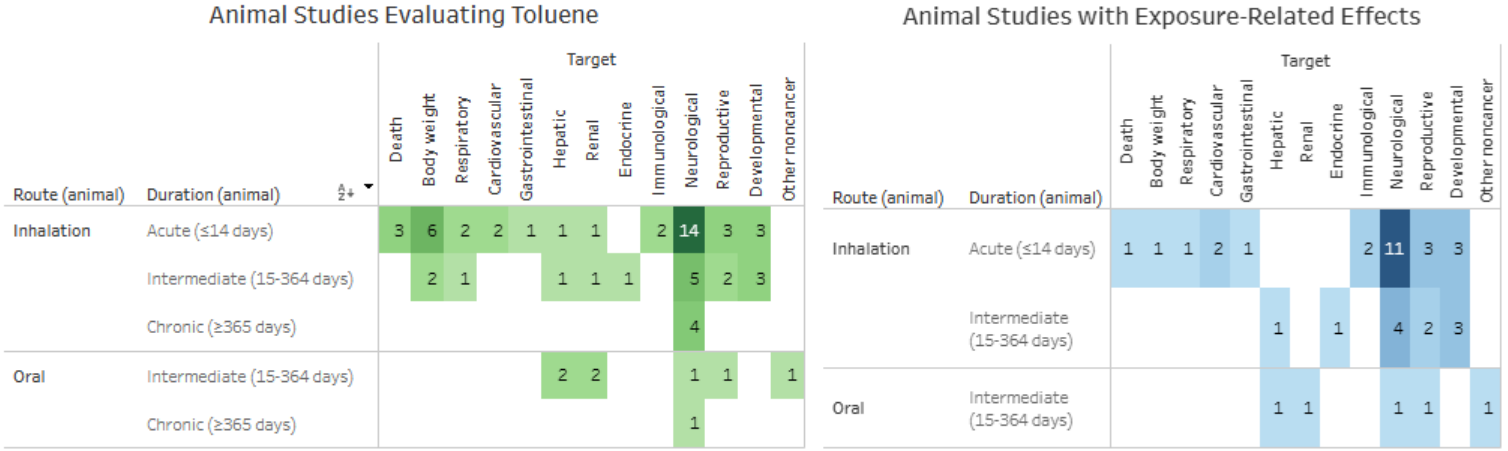
The current toxicological profile (ATSDR 2017) identified several data needs in the toxicological database for toluene. Some of the studies identified during the updated literature search may address these data needs, particularly for the inhalation database (see Table 3-1).

Figure 3-2. Health Effects Studies for Toluene

A:



B:



*Interactive database can be accessed at: [Interactive health effects]

Table 3-1. Data Needs Identified for Toluene by ATSDR (2017)

Exposure route	Data needs	Studies to potentially address data need
Inhalation	Special studies evaluating sensitive neurobehavioral function in animals (<15 ppm)	None
	Intermediate-duration studies in humans	Reproductive study (association reported): Santos et al. 2019 (longitudinal; 3,562 pregnancies; preterm labor)
	Intermediate-duration, low-level inhalation studies in animals (<100 ppm)	Cosnier et al. 2018 (4-week rat study with multiple concentrations ≥5 ppm; multi-endpoint, decreased liver weight at ≥5 ppm)
	Additional neurological studies in groups of toluene-exposed workers in which exposure to other chemicals is minimal	
	Additional occupational/epidemiological studies in large groups of people to better evaluate potential associations between exposure and reproductive effects	Association reported: Santos et al. 2019 (longitudinal; 3,562 pregnancies; preterm labor) No association reported: Namvar et al. 2023 (prospective; 806 women; ovarian function); Wei et al. 2023 (cross sectional; 2,633 females; sex hormones)
	Additional occupational/epidemiological studies in large groups of people to better evaluate potential associations between exposure developmental effects (including neurodevelopmental).	Association reported: Gong et al. 2018 (case-control, 94,106 low-birth weight and 376,424 controls) No association reported: Gonzalez-Casanova et al. 2018 (longitudinal study; 718 mother-child pairs; cognitive development in offspring); Madaniyazi et al. 2022 (cross sectional; 5,017 children; neurodevelopmental delays)
	Additional 2-generation study in a second animal species (e.g., rabbit).	None
	Additional low-exposure neurodevelopmental studies in animals to establish no effect levels (<2,000 ppm)	Armenta-Reséndiz et al. 2019 (neurobehavior evaluated in rats following acute juvenile exposure multiple concentrations ≥500 ppm; reported effects at 8,000 ppm) Svenson et al. 2022 (astrogliosis evaluated in mice following acute juvenile exposure multiple concentrations ≥1,000 ppm; reported effects at ≥2,000 ppm)
	Studies evaluating immunological function in animals	None

Table 3-1. Data Needs Identified for Toluene by ATSDR (2017)

Exposure route	Data needs	Studies to potentially address data need
Oral	Additional low-dose animal studies following intermediate- and chronic-duration, with sensitivity for neurobehavioral alterations and/or mechanisms (<100 mg/kg/day)	Alkaisi et al. 2021 (8-week study in mice, reporting adverse hepatic, renal, and neurological effects at 0.2 mg/kg/day; only tested dose; neurobehavior was not evaluated)
	Additional low-exposure neurodevelopmental studies in animals to establish no effect levels (<106 mg/kg/day)	None
	Studies evaluating immunological function in animals	None

ATSDR = Agency for Toxic Substances and Disease Registry

4. REFERENCES

4.1 CURRENT PROFILE

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4.3 ANIMAL TOXICITY

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APPENDIX A. LITERATURE SEARCH STRATEGIES

Table A-1. Database Query Strings

Database search date	Query string
PubMed	
02/2024	((Toluene[mh:noexp] AND (("Toluene/toxicity"[mh] OR "Toluene/adverse effects"[mh] OR "Toluene/poisoning"[mh] OR "Toluene/pharmacokinetics"[mh] OR ("Toluene"[mh] AND ("environmental exposure"[mh] OR "chemically induced"[sh])) OR ("Toluene"[mh] AND toxicokinetics[mh:noexp])) OR ("Toluene"[mh] AND ((indexingmethod_automated OR indexingmethod_curated) AND ("RNA"[mh] OR "DNA"[mh] OR "DNA Replication"[mh] OR "Salmonella typhimurium"[mh] OR antagonist*[tw] OR inhibitor*[tw] OR "blood"[tw] OR "serum"[tw] OR "plasma"[tw] OR pharmacokinetic*[tw] OR toxicokinetic*[tw] OR "pbpk"[tw] OR "poisoned"[tw] OR "poisoning"[tw] OR "urine"[tw] OR "urinary"[tw] OR "toxicity"[sh] OR "occupational diseases"[mh] OR "hazardous substances"[mh] OR "epidemiology"[sh] OR "epidemiologic studies"[mh]))) OR ("Toluene/blood"[mh] OR "Toluene/cerebrospinal fluid"[mh] OR "Toluene/urine"[mh]) OR ("Toluene"[mh] AND ("endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh])) OR ("Toluene"[mh] AND ("computational biology"[mh] OR "medical informatics"[mh] OR genomics[mh] OR genome[mh] OR proteomics[mh] OR proteome[mh] OR metabolomics[mh] OR metabolome[mh] OR genes[mh] OR "gene expression"[mh] OR phenotype[mh] OR genetics[mh] OR genotype[mh] OR transcriptome[mh] OR ("systems biology"[mh] AND ("environmental exposure"[mh] OR "epidemiological monitoring"[mh] OR analysis[sh])) OR "transcription, genetic "[mh] OR "reverse transcription"[mh] OR "transcriptional activation"[mh] OR "transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, messenger"[mh] OR "RNA, transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "reverse transcriptase polymerase chain reaction"[mh] OR "base sequence"[mh] OR "trans-activators"[mh] OR "gene expression profiling"[mh])) OR ("Toluene/antagonists and inhibitors"[mh]) OR ("Toluene/metabolism"[mh] AND ("humans"[mh] OR "animals"[mh])) OR ("Toluene/pharmacology"[majr]) OR ("Toluene"[mh] AND (("Neoplasms"[mh] OR "Carcinogens"[mh] OR "Lymphoproliferative disorders"[mh] OR "Myeloproliferative disorders"[mh] OR "Toxicity Tests"[mh] OR ((cancer*[tiab] OR carcinogen*[tiab]) AND (risk*[tiab] OR health[tiab]) AND assessment*[tiab]) OR "Mutagens"[mh] OR "Mutagenicity Tests"[mh] OR "Chromosome Aberrations"[mh] OR "DNA Damage"[mh] OR "DNA Repair"[mh] OR "DNA Replication/drug effects"[mh] OR "DNA/drug effects"[mh] OR "DNA/metabolism"[mh] OR "Genomic Instability"[mh] OR "Salmonella typhimurium/drug effects"[mh] OR "Salmonella typhimurium/genetics"[mh] OR "Sister Chromatid Exchange"[mh] OR strand-break*[tiab])))))) OR ((("1-Methylbenzene"[tw] OR "Antisal 1a"[tw] OR "Benzene, methyl"[tw] OR "Benzene, methyl-"[tw] OR "Benzene,methyl"[tw] OR "Methacide"[tw] OR "Methane, phenyl-"[tw] OR "METHYL BENZENE"[tw] OR "Methylbenzene"[tw] OR "Methylbenzol"[tw] OR "Phenylmethane"[tw] OR "TOLOULE"[tw] OR "Tolu-Sol"[tw] OR "Toluene"[tw] OR "tolueno"[tw] OR "Toluol"[tw] OR "Toulene"[tw]) NOT medline[sb]) AND (toxicity[ti] OR death OR lethal OR fatal OR fatality OR necrosis OR LC50* OR "LC 50" OR LD50* OR "LD 50" OR "body weight" OR "body mass index" OR "weight loss" OR "weight gain" OR weight-change* OR overweight OR obesity OR inhal* OR respiratory OR "pulmonary" OR airway OR trachea OR tracheobronchial OR lung OR lungs OR nose OR nasal OR nasopharyngeal OR larynx OR laryngeal OR pharynx OR bronchial OR bronchi OR bronchioles OR bronchitis OR hemothorax OR alveolar OR alveoli OR irritation OR irritant OR sensitization OR sensitizer OR "asthma" OR cilia OR mucocilliary OR cardio OR vascular OR cardiovascular OR "circulatory system" OR "circulatory function" OR "circulatory effect" OR "circulatory effects" OR

Table A-1. Database Query Strings

Database search date	Query string
	"circulatory toxicity" OR "cardiac" OR "coronary" OR "heart rate" OR "heart failure" OR "heart attack" OR "heart muscle" OR "heartbeat" OR "myocardial-infarction" OR "chest pain" OR artery OR arteries OR veins OR venules OR cardiotox* OR "gastro-intestinal" OR gastrointestinal OR "digestive system" OR "digestive organs" OR "digestive function" OR "digestive effect" OR "digestive effects" OR "intestinal" OR intestine* OR "gi tract" OR "gi disorder" OR abdominal OR esophagus OR esophageal OR stomach OR pancreas OR pancreatic OR diarrhea OR nausea OR vomit OR ulcer* OR constipation OR emesis OR "gut microbes" OR "gut flora" OR "gut microflora" OR anorexia OR hematological OR hematology OR hemato OR haemato OR blood OR anemia OR anaemia OR cyanosis OR "cyanotic" OR erythrocytopenia OR leukopenia OR thrombocytopenia OR hemoglobin OR erythrocyte OR hematocrit OR "bone marrow" OR reticulocyte OR methemoglobin OR red-blood-cell OR musculoskeletal OR skeletal OR muscle OR muscular OR arthritis OR "altered bone" OR "joint pain" OR "limb pain" OR hepatic OR "liver" OR hepatocytes OR gallbladder OR cirrhosis OR jaundice OR "hepatocellular degeneration" OR "hepatocellular hypertrophy" OR hepatomegaly OR hepatotox* OR renal OR "kidney" OR "kidneys" OR "urinary" OR "bladder" OR "urine" OR "blood urea nitrogen" OR bun OR nephropath* OR nephrotox* OR dermal OR "skin contact" OR "skin rash" OR "skin irritation" OR "skin redness" OR "skin effect" OR "skin effects" OR "skin exposure" OR "skin contact" OR acanthosis OR dermatitis OR psoriasis OR edema OR acne OR ocular OR "retinal" OR "eye function" OR "eye effects" OR "eye effect" OR "eye irritation" OR "blurred vision" OR blindness OR myopia OR cataracts OR "auditory system" OR ototoxic* OR endocrine OR "hormone changes" OR "hormone excess" OR "hormone deficiency" OR "hormone secretion" OR "hormone toxicity" OR "hormone levels" OR "sella turcica" OR thyroid OR adrenal OR pituitary OR immunological OR immunologic OR immune OR immunotox* OR lymphoreticular OR lymph-node OR spleen OR thymus OR macrophage OR leukocyte* OR lymphocyt* OR white-blood-cell OR immunotox* OR neurological OR neurologic OR neurotoxic OR neurotoxicity OR "neuropathy" OR neurodegenerat* OR "neurodevelopment" OR "nervous system" OR "nerve" OR brain OR "cerebrovascular" OR neurotoxicant OR neurochemistry OR neurophysiology OR neuropathology OR "motor activity" OR motor change* OR behavior-change* OR behavioral-change* OR sensory-change* OR cognitive OR "cognition" OR vertigo OR drowsiness OR headache OR ataxia OR reproductive OR "reproduction system" OR "reproduction function" OR "reproduction effect" OR "reproduction toxicity" OR "infertility" OR "maternal toxicity" OR developmental OR "in utero" OR placenta OR pregnan* OR terata* OR terato* OR embryo* OR fetus* OR foetus* OR fetal* OR foetal* OR prenatal* OR "pre-natal" OR perinatal* OR "post-natal" OR postnatal* OR neonat* OR newborn* OR zygote* OR child OR children OR infant* OR offspring OR weanling* OR elderly OR oocyte OR ovary OR ovarian OR uterus OR uterine OR testes OR testicular OR sperm OR estrogen* OR androgen* OR "human milk" OR "breast milk" OR "altered food consumption" OR "altered water consumption" OR "metabolic effect" OR "metabolic toxicity" OR Fever OR cytotox* OR cancer OR cancerous OR neoplas* OR tumor OR tumors OR tumour* OR malignan* OR carcinoma OR carcinogen OR carcinogen* OR angiosarcoma OR blastoma OR fibrosarcoma OR glioma OR leukemia OR leukaemia OR lymphoma OR melanoma OR meningioma OR mesothelioma OR myeloma OR neuroblastoma OR osteosarcoma OR sarcoma OR mutation OR mutations OR genotoxicity OR genotoxic OR "micronuclei" OR "micronucleus" OR "chromosome aberrations" OR mutagenicity OR mutagenic OR "mechanism of action"[tiab:~0] OR "mode of action"[tiab:~0] OR "mechanism of toxicity"[tiab:~0] OR "adverse effect" OR "adverse effects" OR "health effects" OR noncancer OR poisoning OR morbidity OR inflammation OR "inflammatory response" OR antagonist OR inhibitor OR metabolism OR "environmental exposure" OR toxicokinetics

Table A-1. Database Query Strings

Database search date	Query string
	OR pharmacokinetics OR "pbpk" OR "gene expression" OR "adverse outcome pathway" OR metabolom* OR proteom* OR genomic* OR transcriptom* OR epigenom* OR epigene* OR "transcription factor" OR "transcriptional activation" OR epidemiology OR epidemiological OR case-control* OR case-referent OR case-report OR case-series OR cohort* OR correlation-stud* OR cross-sectional-stud* OR ecological-studies OR ecological-study OR follow-up-stud* OR longitudinal-stud* OR metaanalyses OR metaanalysis OR meta-analysis OR prospective-stud* OR record-link* OR retrospective-stud* OR seroepidemiologic-stud* OR "population health" OR occupation* OR worker* OR workmen* OR workplace* OR "volunteers" OR "human health" OR "dietary" OR "oral intake" OR "oral exposure" OR "oral administration" OR ingest* OR gavage* OR "drinking-water" OR biomarker* OR biomonitor* OR "biological monitoring" OR "environmental fate" OR NHANES OR "Nutrition Examination Survey" OR (cvd NOT "chemical vapor deposition") OR (human AND (risk OR toxic* OR safety)) OR mammal* OR ape OR apes OR baboon* OR balb OR beagle* OR boar OR boars OR bonobo* OR bovine OR C57 OR C57bl OR callithrix OR canine OR canis OR capra OR capuchin* OR cats OR cattle OR cavia OR chicken OR chickens OR chimpanzee* OR chinchilla* OR cow OR cows OR cricetinae OR dog OR dogs OR equus OR feline OR felis OR ferret OR ferrets OR flying-fox OR Fruit-bat OR gerbil* OR gibbon* OR goat OR goats OR guinea-pig* OR guppy OR hamster OR hamsters OR horse OR horses OR jird OR jirds OR lagomorph* OR leontopithecus OR longevans OR macaque* OR marmoset* OR medaka OR merione OR meriones OR mice OR monkey OR monkeys OR mouse OR muridae OR murinae OR murine OR mustela-putorius OR nomascus OR non-human-primate* OR orangutan* OR pan-paniscus OR pan-troglodytes OR pig OR piglet* OR pigs OR polecat* OR pongopygmaeus OR quail OR rabbit OR rabbits OR rat OR rats OR rhesus OR rodent OR rodentia OR rodents OR saguinus OR sheep OR sheeps OR siamang* OR sow OR sows OR Sprague-Dawley OR swine OR swines OR symphalangus OR tamarin* OR vervet* OR wistar OR wood-mouse OR zebra-fish OR zebrafish))) AND (2016/04/01:3000[mhda] OR 2016/04/01:3000[edat] OR 2016/04/01:3000[crdt] OR 2015:3000[dp])
Embase	
02/2024	Limit: 2015-current (toluene/to or toluene/ae or toluene/do or toluene/pk or toluene/ih or toluene/po or toluene/ad or toluene/cr or toluene/it or toluene/pd or toluene/pv) or ((toluene/) and ((exp adverse drug reaction/ or exp toxicity/ or exp poisoning/ or exp death/ or exp occupational exposure/ or exp environmental exposure/ or exp drug interaction/ or exp pregnancy/ or exp pregnancy complications/ or exp fertility/ or exp infertility/ or exp reproduction/ or exp fetus/ or exp embryo/ or exp congenital disorder/ or exp child/ or exp carcinogenicity/ or exp toxicokinetics/) or (exp bioinformatics/ or exp medical informatics/ or exp genomics/ or exp genome/ or exp proteomics/ or exp proteome/ or exp metabolomics/ or exp metabolome/ or exp gene/ or exp gene expression/ or exp phenotype/ or exp genetics/ or exp genotype/ or exp transcriptome/ or exp epidemiological monitoring/ or exp genetic transcription/ or exp messenger RNA/ or exp transfer RNA/ or exp protein synthesis/ or exp reverse transcriptase polymerase chain reaction/ or exp nucleotide sequence/ or exp transactivator protein/ or exp gene expression profiling/))) or ((*toluene/) and ((toxicity.ti. or death.ti,ab,kf. or lethal.ti,ab,kf. or fatal.ti,ab,kf. or fatality.ti,ab,kf. or necrosis.ti,ab,kf. or LC50*.ti,ab,kf. or LC 50.ti,ab,kf. or LD50*.ti,ab,kf. or LD 50.ti,ab,kf. or overweight.ti,ab,kf. or obesity.ti,ab,kf. or weight loss.ti,ab,kf. or weight gain.ti,ab,kf. or weight change.ti,ab,kf. or weight changes.ti,ab,kf. or poisoning.ti,ab,kf. or morbidity.ti,ab,kf. or inflammation.ti,ab,kf. or inflammatory response.ti,ab,kf. or irritation.ti,ab,kf. or irritant.ti,ab,kf. or sensitization.ti,ab,kf. or sensitizer.ti,ab,kf. or asthma.ti,ab,kf. or bronchitis.ti,ab,kf. or heart failure.ti,ab,kf. or heart attack.ti,ab,kf. or myocardial infarction.ti,ab,kf. or chest pain.ti,ab,kf.

Table A-1. Database Query Strings

Database search date	Query string
	or circulatory function.ti,ab,kf. or circulatory effect.ti,ab,kf. or circulatory effects.ti,ab,kf. or circulatory toxicity.ti,ab,kf. or cardiotox*.ti,ab,kf. or digestive function.ti,ab,kf. or digestive effect.ti,ab,kf. or digestive effects.ti,ab,kf. or gi disorder.ti,ab,kf. or diarrhea.ti,ab,kf. or nausea.ti,ab,kf. or vomit*.ti,ab,kf. or ulcer*.ti,ab,kf. or constipation.ti,ab,kf. or emesis.ti,ab,kf. or anorexia.ti,ab,kf. or cyanosis.ti,ab,kf. or cyanotic.ti,ab,kf. or erythrocytopenia.ti,ab,kf. or leukopenia.ti,ab,kf. or thrombocytopenia.ti,ab,kf. or hematocrit.ti,ab,kf. or arthritis.ti,ab,kf. or altered bone.ti,ab,kf. or joint pain.ti,ab,kf. or limb pain.ti,ab,kf. or cirrhosis.ti,ab,kf. or jaundice.ti,ab,kf. or liver toxicity.ti,ab,kf. or liver lesions.ti,ab,kf. or liver weight.ti,ab,kf. or liver weights.ti,ab,kf. or hepatocellular degeneration.ti,ab,kf. or hepatocellular hypertrophy.ti,ab,kf. or hepatomegaly.ti,ab,kf. or hepatotox*.ti,ab,kf. or renal toxicity.ti,ab,kf. or kidney weights.ti,ab,kf. or blood urea nitrogen.ti,ab,kf. or bun.ti,ab,kf. or nephropath*.ti,ab,kf. or nephrotox*.ti,ab,kf. or skin rash.ti,ab,kf. or skin irritation.ti,ab,kf. or skin redness.ti,ab,kf. or skin effect.ti,ab,kf. or skin effects.ti,ab,kf. or acanthosis.ti,ab,kf. or dermatitis.ti,ab,kf. or psoriasis.ti,ab,kf. or eczema.ti,ab,kf. or edema.ti,ab,kf. or acne.ti,ab,kf. or eye function.ti,ab,kf. or eye effects.ti,ab,kf. or eye effect.ti,ab,kf. or eye irritation.ti,ab,kf. or blurred vision.ti,ab,kf. or blindness.ti,ab,kf. or myopia.ti,ab,kf. or cataracts.ti,ab,kf. or ototoxic*.ti,ab,kf.) or ((hormone changes or hormone excess or hormone deficiency or hormone secretion or hormone toxicity or hormone levels or infertility or sella turcica or immunotox* or neurotoxic or neurotoxicity or neuropathy or neurodegenerat* or neurodevelopment or neurotoxicant or neuropathology or motor changes or tremor or tremors or behavior changes or sensory changes or vertigo or drowsiness or headache or ataxia or endocrine disruption or reproduction function or reproduction effect or reproduction toxicity or maternal toxicity or placenta* or terata* or terato* or embryo* or fetus* or foetus* or fetal* or foetal* or neonat* or newborn* or zygote* or child or children or infant* or offspring or elderly or weanling* or altered food consumption or altered water consumption or metabolic effect or metabolic toxicity or fever or histopathology or cytotox* or cancer or cancerous or neoplas* or tumor* or tumour* or malignan* or carcinoma* or carcinogen or carcinogen* or angiosarcoma* or blastoma* or fibrosarcoma* or glioma* or leukemia* or leukaemia* or lymphoma* or melanoma* or meningioma* or mesothelioma* or myeloma* or neuroblastoma* or osteosarcoma* or sarcoma* or mutation or mutations or genotoxicity or genotoxic or micronuclei or micronucleus or chromosome aberrations or mutagenicity or mutagenic or gene expression).ti,ab,kf.) or ((adverse effect or adverse effects or health effects or noncancer or NHANES or Nutrition Examination Survey or body weight or body mass index or respiratory or pulmonary or airway or trachea or tracheobronchial or lung or lungs or nose or nasal or nasopharyngeal or larynx or laryngeal or pharynx or bronchial or bronchi or bronchioles or hemothorax or alveolar or alveoli or cilia or mucocilliary or cardio or heart muscle or heart rate or heartbeat or circulatory system or vascular or cardiovascular or cardiac or coronary or artery or arteries or veins or venules or gastro intestinal or gastrointestinal or digestive system or digestive organs or intestinal or intestine* or gi tract or abdominal or esophag* or stomach or pancreas or pancreatic or gut microbes or gut flora or gut microflora or hematological or hematology or hemato or haemato or blood or anemia or anaemia or hemoglobin or erythrocyte* or bone marrow or reticulocyte* or methemoglobin or red blood cell or musculoskeletal or skeletal or muscle or muscular or hepatic or liver or hepatocytes or gallbladder or renal or kidney* or urinary or bladder or urine or dermal or ocular or retinal or auditory system or endocrine or thyroid or thyroxine or adrenal or pituitary or immunological or immunologic or immune or lymphoreticular or lymph node or lymph nodes or spleen or thymus or macrophage* or leukocyt* or lymphocyt* or white blood cell or white blood cells or neurological or neurologic or nervous system or nerve or brain or cerebrovascular or neurochemistry or

Table A-1. Database Query Strings

Database search date	Query string
	neurophysiology or motor activity or behavioral or cognitive or cognition or reproductive or reproduction system or developmental or oocyte or ovary or ovarian or uterus or uterine or testes or testicular or sperm or estrogen* or androgen* or human milk or breast milk).ti,ab,kf.) or (((toxic* and mechanism of action) or (toxic* and mode of action)).ti,ab,kf.) or ((mechanism of toxicity or antagonist or inhibitor or inhibition or metabolism or toxicokinetic or toxicokinetics or pharmacokinetic or pharmacokinetics or pbpk or adverse outcome pathway or metabolom* or proteom* or genomic* or transcriptom* or epigenom* or epigene* or transcription factor or transcriptional activation or human health or exposure or exposures or exposed or inhal* or dermal exposure or dermal absorption or dermal penetration or dermal application or dermal applications or dermally applied or cutaneous application or skin contact or dietary or oral intake or oral exposure or oral administration or ingest* or gavage* or drinking-water or epidemiology or epidemiological or case-control* or case-referent or case report or case reports or case series or cohort or cohorts or correlation study or correlation studies or cross-sectional study or cross-sectional studies or ecological studies or ecological study or follow-up study or follow-up studies or longitudinal study or longitudinal studies or metaanalyses or metaanalysis or meta-analysis or prospective study or prospective studies or record linked or record linkage or retrospective study or retrospective studies or seroepidemiologic study or population health or occupation* or worker* or workmen* or workplace* or volunteers or pregnan* or in utero or prenatal* or pre natal or perinatal* or post natal or postnatal* or environmental exposure or occupational exposure or biomarker* or biomonitor* or biological monitoring or environmental fate).ti,ab,kf.) or ((mammal* or ape or Apes or baboon* or balb or beagle* or boar or boars or bonobo* or bovine or C57 or C57bl or callithrix or canine or canis or capra or capuchin* or cats or cattle or cavia or chicken or chickens or chimpanzee* or chinchilla* or cow or cows or cricetinae or dog or dogs or equus or feline or felis or ferret or ferrets or flying-fox or fruit-bat or gerbil* or gibbon* or goat or goats or guinea pig* or guppy or hamster or hamsters or horse or horses or jird or jirds or lagomorph* or leontopithecus or longevans or macaque* or marmoset* or medaka or merione or meriones or mice or monkey or monkeys or mouse or muridae or murinae or murine or mustela putorius or nomascus or non human primate* or orangutan* or pan paniscus or pan troglodytes or pig or piglet* or pigs or polecat* or pongopygmaeus or quail or rabbit or rabbits or rat or rats or rhesus or rodent or rodentia or rodents or saguinus or sheep or sheeps or siamang* or sow or sows or Sprague Dawley or swine or swines or symphalangus or tamarin* or vervet* or wistar or wood-mouse or zebra-fish or zebrafish).ti,ab,kf.)))
Scifinder	
02/2024	Search References: CAS Registry Number: 108-88-3 Filtered By: Document Type: Journal, Review, Book, Clinical Trial, Commentary, Conference, Dissertation, Editorial, Historical, Letter, Preprint, Report Substance Role: Adverse Effect, Biological Study, Pharmacokinetics, Pharmacological Activity Publication Year: 2015 to 2024 CA Section: Air Pollution and Industrial Hygiene, Toxicology, Water, Waste Treatment and Disposal Database: CAplus
Scopus	
02/2024	(((TITLE-ABS ({antisal 1a} OR {benzene, methyl} OR {benzene, methyl-} OR {benzene,methyl} OR {methacide} OR {methane, phenyl-} OR {1-methylbenzene} OR {methyl benzene} OR {methylbenzene} OR {methylbenzol} OR {phenylmethane} OR

Table A-1. Database Query Strings

Database search date	Query string
	{toloule} OR {tolu-sol} OR {toluene} OR {toluol})) AND (TITLE-ABS-KEY ("death" OR "lethal" OR "fatal" OR "fatality" OR "necrosis" OR lc50* OR "lc 50" OR ld50* OR "ld 50" OR "overweight" OR "obesity" OR "weight loss" OR "weight gain" OR "weight change" OR "weight changes" OR "poisoning" OR "morbidity" OR "inflammation" OR "inflammatory response" OR "irritation" OR "irritant" OR "sensitization" OR "sensitizer" OR "asthma" OR "bronchitis" OR "heart failure" OR "heart attack" OR "myocardial infarction" OR "chest pain" OR "circulatory function" OR "circulatory effect" OR "circulatory effects" OR "circulatory toxicity" OR cardiotox* OR "digestive function" OR "digestive effect" OR "digestive effects" OR "gi disorder" OR "diarrhea" OR "nausea" OR vomit* OR ulcer* OR "constipation" OR "emesis" OR "anorexia" OR "cyanosis" OR "cyanotic" OR "erythrocytopenia" OR "leukopenia" OR "thrombocytopenia" OR "hematocrit" OR "arthritis" OR "altered bone" OR "joint pain" OR "limb pain" OR "cirrhosis" OR "jaundice" OR "liver toxicity" OR "liver lesions" OR "liver weight" OR "liver weights" OR "hepatocellular degeneration" OR "hepatocellular hypertrophy" OR "hepatomegaly" OR hepatotox* OR "renal toxicity" OR "kidney weights" OR "blood urea nitrogen" OR "bun" OR nephropath* OR nephrotox* OR "skin rash" OR "skin irritation" OR "skin redness" OR "skin effect" OR "skin effects" OR "acanthosis" OR "dermatitis" OR "psoriasis" OR "eczema" OR "edema" OR "acne" OR "eye function" OR "eye effects" OR "eye effect" OR "eye irritation" OR "blurred vision" OR "blindness" OR "myopia" OR "cataracts" OR ototoxic* OR "hormone changes" OR "hormone excess" OR "hormone deficiency" OR "hormone secretion" OR "hormone toxicity" OR "hormone levels" OR "infertility" OR "sella turcica" OR immunotox* OR "neurotoxic" OR "neurotoxicity" OR "neuropathy" OR neurodegenerat* OR "neurodevelopment" OR "neurotoxicant" OR "neuropathology" OR "motor changes" OR "tremor" OR "tremors" OR "behavior changes" OR "sensory changes" OR "vertigo" OR "drowsiness" OR "headache" OR "ataxia" OR "endocrine disruption" OR "reproduction function" OR "reproduction effect" OR "reproduction toxicity" OR "maternal toxicity" OR placenta* OR terata* OR terato* OR embryo* OR fetus* OR foetus* OR fetal* OR foetal* OR neonat* OR newborn* OR zygote* OR "child" OR "children" OR infant* OR "offspring" OR "elderly" OR weanling* OR "altered food consumption" OR "altered water consumption" OR "metabolic effect" OR "metabolic toxicity" OR "fever" OR "histopathology" OR cytotox* OR "cancer" OR "cancerous" OR neoplas* OR tumor* OR tumour* OR malignan* OR carcinoma* OR "carcinogen" OR carcinogen* OR angiosarcoma* OR blastoma* OR fibrosarcoma* OR glioma* OR leukemia* OR leukaemia* OR lymphoma* OR melanoma* OR meningioma* OR mesothelioma* OR myeloma* OR neuroblastoma* OR osteosarcoma* OR sarcoma* OR "mutation" OR "mutations" OR "genotoxicity" OR "genotoxic" OR "micronuclei" OR "micronucleus" OR "chromosome aberrations" OR "mutagenicity" OR "mutagenic" OR "gene expression" OR "adverse effect" OR "adverse effects" OR "health effects" OR "noncancer" OR "nhanes" OR "nutrition examination survey"))) OR ((TITLE-ABS ({antisal 1a} OR {benzene, methyl} OR {benzene, methyl-} OR {benzene,methyl} OR {methacide} OR {methane, phenyl-} OR {1-methylbenzene} OR {methyl benzene} OR {methylbenzene} OR {methylbenzol} OR {phenylmethane} OR {toloule} OR {tolu-sol} OR {toluene} OR {toluol})) AND (TITLE-ABS (*algia OR *asthenia OR *emia OR *itis OR *oma OR *opathy OR *orrhagia OR *orrhea OR (*osis AND NOT osmosis) OR *paresis OR *plasia OR *plegia OR *pnea OR *megaly OR *pathy OR *otoxic*))) OR ((TITLE-ABS ({antisal 1a} OR {benzene, methyl} OR {benzene, methyl-} OR {benzene,methyl} OR {methacide} OR {methane, phenyl-} OR {1-methylbenzene} OR {methyl benzene} OR {methylbenzene} OR {methylbenzol} OR {phenylmethane} OR {toloule} OR {tolu-sol}

Table A-1. Database Query Strings

Database search date	Query string
	OR {toluene} OR {toluol})) AND (TITLE-ABS-KEY ("body weight" OR "body mass index" OR "respiratory" OR "pulmonary" OR "airway" OR "trachea" OR "tracheobronchial" OR "lung" OR "lungs" OR "nose" OR "nasal" OR "nasopharyngeal" OR "larynx" OR "laryngeal" OR "pharynx" OR "bronchial" OR "bronchi" OR "bronchioles" OR "hemothorax" OR "alveolar" OR "alveoli" OR "cilia" OR "mucocilliary" OR "cardio" OR "heart muscle" OR "heart rate" OR "heartbeat" OR "circulatory system" OR "vascular" OR "cardiovascular" OR "cardiac" OR "coronary" OR "artery" OR "arteries" OR "veins" OR "venules" OR "gastro intestinal" OR "gastrointestinal" OR "digestive system" OR "digestive organs" OR "intestinal" OR intestine* OR "gi tract" OR "abdominal" OR esophag* OR "stomach" OR "pancreas" OR "pancreatic" OR "gut microbes" OR "gut flora" OR "gut microflora" OR "hematological" OR "hematology" OR "hemato" OR "haemato" OR "blood" OR "anemia" OR "anaemia" OR "hemoglobin" OR erythrocyte* OR "bone marrow" OR reticulocyte* OR "methemoglobin" OR "red blood cell" OR "musculoskeletal" OR "skeletal" OR "muscle" OR "muscular" OR "hepatic" OR "liver" OR "hepatocytes" OR "gallbladder" OR "renal" OR kidney* OR "urinary" OR "bladder" OR "urine" OR "dermal" OR "ocular" OR "retinal" OR "auditory system" OR "endocrine" OR "thyroid" OR "thyroxine" OR "adrenal" OR "pituitary" OR "immunological" OR "immunologic" OR "immune" OR "lymphoreticular" OR "lymph node" OR "lymph nodes" OR "spleen" OR "thymus" OR macrophage* OR leukocyt* OR lymphocyt* OR "white blood cell" OR "white blood cells" OR "neurological" OR "neurologic" OR "nervous system" OR "nerve" OR "brain" OR "cerebrovascular" OR "neurochemistry" OR "neurophysiology" OR "motor activity" OR "behavioral" OR "cognitive" OR "cognition" OR "reproductive" OR "reproduction system" OR "developmental" OR "oocyte" OR "ovary" OR "ovarian" OR "uterus" OR "uterine" OR "testes" OR "testicular" OR "sperm" OR estrogen* OR androgen* OR "human milk" OR "breast milk"))) OR ((TITLE-ABS ({antisal 1a} OR {benzene, methyl} OR {benzene, methyl-} OR {benzene,methyl} OR {methacide} OR {methane, phenyl-} OR {1-methylbenzene} OR {methyl benzene} OR {methylbenzene} OR {methylbenzol} OR {phenylmethane} OR {toloule} OR {tolu-sol} OR {toluene} OR {toluol})) AND (TITLE-ABS-KEY ((toxic* AND "mechanism of action") OR (toxic* AND "mode of action") OR "mechanism of toxicity" OR "antagonist" OR "inhibitor" OR "inhibition" OR "metabolism" OR "toxicokinetic" OR "toxicokinetics" OR "pharmacokinetic" OR "pharmacokinetics" OR "pbpk" OR "adverse outcome pathway" OR metabolom* OR proteom* OR genomic* OR transcriptom* OR epigenom* OR epigene* OR "transcription factor"))) OR ((TITLE-ABS ({antisal 1a} OR {benzene, methyl} OR {benzene, methyl-} OR {benzene,methyl} OR {methacide} OR {methane, phenyl-} OR {1-methylbenzene} OR {methyl benzene} OR {methylbenzene} OR {methylbenzol} OR {phenylmethane} OR {toloule} OR {tolu-sol} OR {toluene} OR {toluol})) AND (INDEXTERMS ("bioinformatics" OR "medical informatics" OR "genomics" OR "genome" OR "proteomics" OR "proteome" OR "metabolomics" OR "metabolome" OR "genes" OR "gene expression" OR "phenotype" OR "genetics" OR "genotype" OR "transcriptome" OR "epidemiological monitoring" OR "genetic transcription" OR "messenger rna" OR "transfer rna" OR "protein synthesis" OR "reverse transcriptase polymerase chain reaction" OR "nucleotide sequence" OR "transactivator protein" OR "gene expression profiling"))) AND PUBYEAR > 2014 AND PUBYEAR < 2025) AND (LIMIT-TO (SUBJAREA , "ENVI") OR LIMIT-TO (SUBJAREA , "MEDI") OR LIMIT-TO (SUBJAREA , "BIOC") OR LIMIT-TO (SUBJAREA , "PHAR") OR LIMIT-TO (SUBJAREA , "AGRI") OR LIMIT-TO (SUBJAREA , "IMMU") OR LIMIT-TO (SUBJAREA , "EART") OR LIMIT-TO (SUBJAREA , "NEUR") OR LIMIT-TO (SUBJAREA , "HEAL") OR LIMIT-TO (

Table A-1. Database Query Strings

Database search date	Query string
	SUBJAREA , "VETE") OR LIMIT-TO (SUBJAREA , "PSYC") OR LIMIT-TO (SUBJAREA , "NURS") OR LIMIT-TO (SUBJAREA , "DENT"))

Table A-2. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS via ChemView	
02/2024	Compound searched: 108-88-3
NTP	
02/2024	Limited to 2010 to present and not dated "108-88-3" "Toluene" "methyl benzene" "Methylbenzene" "Toluol" "Phenylmethane" "Benzene, methyl" "Benzene, methyl-" "1-Methylbenzene" "Methacide" "Antisal 1a" "Methylbenzol" "Methane, phenyl-" "toloule" "Tolu-Sol" "tolueno"
NTRL	
02/2024	Limited to 2015 to present terms appearing in title or keyword "Antisal 1a" OR "Benzene, methyl" OR "Benzene, methyl-" OR "Benzene,methyl" OR "Methacide" OR "Methane, phenyl-" OR "1-Methylbenzene" OR "methyl benzene" OR "Methylbenzene" OR "Methylbenzol" OR "Phenylmethane" OR "toloule" OR "Tolu-Sol" OR "Toluene" OR "tolueno" OR "Toluol" OR "touleno"
Regulations.gov	
02/2024	"Toluene" "Methylbenzene" "108-88-3"
Other	Identified throughout the assessment process

APPENDIX B. SUPPLEMENTAL STUDIES

B.1. HUMAN STUDIES, BTEX COMBINED ANAYLSIS ONLY

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B.7. TOXICOKINETIC (ADME/PBPK)

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B.10. META-ANALYSIS

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