Review

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The human health effects of unconventional oil and gas (UOG) chemical exposures: a scoping review of the toxicological literature

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Abstract: Many chemicals associated with unconventional oil and natural gas (UOG) are known toxicants, leading to health concerns about the effects of UOG. Our objective was to conduct a scoping review of the toxicological literature to assess the effects of UOG chemical exposures in models relevant to human health. We searched databases for primary research studies published in English or French between January 2000 and June 2023 on UOG-related toxicology studies. Two reviewers independently screened abstracts and full texts to determine inclusion. Seventeen studies met our study inclusion criteria. Nine studies used solely *in vitro* models, while six conducted their investigation solely in animal models. Two studies incorporated both types of models. Most studies used real water samples impacted by UOG or lab-made mixtures of UOG chemicals to expose their models. Most *in vitro* models used human cells in monocultures, while all animal studies were conducted in rodents. All studies detected significant deleterious effects associated with exposure to UOG chemicals or samples, including endocrine disruption, carcinogenicity, behavioral changes and metabolic alterations. Given the plausibility of causal relationships between UOG chemicals and adverse health outcomes highlighted in this review, future risk assessment studies should focus on measuring exposure to UOG chemicals in human populations.

Keywords: unconventional oil and gas; hydraulic fracturing; fracking; review; toxicology; human health

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Introduction

Hydraulic fracturing is a fossil fuel extraction technique that consists of injecting large volumes of fracking fluid (a mixture of water, sand or other proppants, and a variety of chemicals used as, for example, biocides, friction reducers, scale inhibitors, clay stabilizers, surfactants, acids, corrosion inhibitors, gelling agents, foaming agents or pH adjustors [1]) in the rock formation to create fractures, freeing the trapped fossil fuel (e.g., natural gas) for extraction [2]. Unconventional oil and gas (UOG) operations generate large quantities of wastewaters (flowback fluids and produced water) whose physical properties vary considerably depending on the rock formation [3]. These wastewaters contain hydraulic fracturing chemicals, and constituents naturally present in the oil and gas deposits such as volatile organic compounds (VOCs; such as benzene, toluene, ethylbenzene, xylenes [commonly referred as BTEX], acetate, acetone, dichloromethane and chloroform [4–6]), radioactive elements, and trace metals (e.g., arsenic, strontium, barium, manganese) [3, 6–11]. Local contamination of soil, air and water in proximity to unconventional oil and gas (UOG) operations has been demonstrated in various studies [12–18].

As highlighted in recent reviews [19, 20], a growing number of epidemiological studies are showing deleterious

health effects in communities living in the vicinity of UOG operations. Health effects associated with proximity to UOG include birth outcomes (e.g., preterm birth, low birth weight, and congenital abnormalities) [21–28], self-reported health symptoms (e.g., rashes/skin problems, nose bleeds, stuffy nose, cough, blocked sinuses and fatigue) [29, 30], asthma exacerbations [31, 32], as well as adverse cardiovascular [33] and mental health [34, 35] outcomes. A number of studies found that exposure to ambient air pollutants (e.g., carbon monoxide, nitrogen dioxide, particulate matter and VOCs) in various areas of the world not necessarily impacted by UOG, is associated with many of these health outcomes, including birth outcomes [36, 37], and respiratory and cardiovascular diseases [38, 39]. Although limited in number, toxicological research initiatives are working towards elucidating some of the underlying mechanisms of toxicity explaining the associations between proximity to UOG and health outcomes. Indeed, some chemicals associated with UOG, such as acrylamide, benzene, bisphenol A, dibutyl phtalate and strontium, are reproductive and developmental toxicants in humans [40], carcinogenic and mutagenic [41, 42], endocrine disruptors [43–46], and can promote oxidative stress [47–53]. All of these mechanisms of toxicity are known to be implicated in the etiology of multiple diseases identified in the UOG epidemiological literature. A review used FracFocus data to describe the chemicals used in hydraulic fracturing for oil production in California, their frequency, function, and potential acute toxicity for aquatic and mammalian species. Of the approximately 300 chemicals used for unconventional oil production in California, many of these, including solvents and surfactants, lacked toxicity data. For example, the authors found that toxicity data was unavailable for five of the most frequently used chemicals. For the chemicals with available toxicity data, the data was often incomplete [54].

An assessment of acute and chronic health hazards of hydraulic fracturing chemicals published in 2015 compiled health hazard information for 113 individual chemicals reported to be used in hydraulic fracturing fluids in North Dakota. The health hazard acute endpoints found to be the most associated with hydraulic fracturing fluids constituents included respiratory tract irritation, as well as eye and skin irritation or damage. Chronic toxicity endpoints were not available for the majority of chemicals [55]. It is important to note that health hazards were identified based on the information reported for individual chemicals in Safety Data Sheets and publicly available databases, such as the Agency for Toxic Substances and Disease Registry and the European Chemicals Agency. A review of the toxicological literature published in 2020 was limited to experimental studies evaluating the endocrine disrupting potential of exposure to a mixture of 23 UOG chemicals [46]. Our primary objective was to update and expand on these reviews to include toxicological studies published between January 2000 to June 2023 examining toxicity on multiple endpoints associated with UOG chemicals whose effects were measured specifically in the context of UOG (i.e., using mixtures of UOG chemicals, samples of UOG wastewater) in models (*in vivo* or *in vitro*) relevant for human health.

Methods

Our review is a companion paper of our recent scoping review of epidemiological studies regarding the human health effects of UOG [56].

Data sources and searches

We defined UOG using hydraulic fracturing as the injection of fluids under pressure great enough to fracture shale and tight rock formations. An experienced biomedical librarian (MDW) conducted comprehensive searches in MEDLINE, and Embase (through OVID) for all published studies in English or French from 2000 to June 16, 2023. The toxicology search concepts are listed in Supplementary Material Table S1.

Study selection

We included toxicological studies that investigated harmful effects in *in vitro* (cellular) or animal assays exposed to UOG chemicals in the context of UOG, i.e., that chemicals (either individual or in mixtures) had to mimic the UOG components (e.g., components of hydraulic fracturing fluid; air emissions; water contamination by UOG activities; or UOG wastewaters). We excluded studies that assessed the impact of UOG chemicals (1) individually and in studies not related to UOG; (2) on *in vitro* and animal models not relevant to human health (e.g., yeast, aquatic species models, birds). We further excluded studies that had no control group and were not peer-reviewed. Reviews and conference abstracts were also excluded.

Titles and abstracts of studies were first screened to determine initial eligibility for full-text review using COVIDENCE [57], a web-based screening and data extraction tool. Both screening and full text review were completed by two independent reviewers. Disagreements at either stage were resolved through discussion until achieving consensus or, if required, the input of a third reviewer.

Due to the wide heterogeneity of models, exposures (i.e., chemicals used, pathways, duration), outcomes and

methodological approaches, a formal bias tool evaluating the quality of selected studies was not applied.

Data extraction, synthesis, and analysis

Given the variation in models, exposure (i.e., chemicals, pathways, duration) and outcome definitions, data synthesis was descriptive. Relevant information extracted from *in vitro* toxicological studies included: first author; publication year; journal; funding source; study objective; model (i.e., cell line, tissue culture, source); UOG chemicals or samples tested; endpoints of interest; main findings. Relevant information extracted from animal toxicological studies included: first author; publication year; journal; funding source; study objective; model (i.e., animal species, strain, sex, age); exposure treatments (UOG chemicals or samples tested; concentrations, route and duration of exposure); endpoints of interest; and main findings.

Data were extracted directly into the Health Assessment Workplace Collaborative (HAWC) platform (https://hawcproject.org). HAWC is a publicly available web-based tool designed for data extraction of animal bioassay and *in vitro* studies [58, 59]. Data were independently extracted first by one member of the study team for all selected studies. A second member of the team then verified the exactitude of the data extraction process for each study, and summarized the extracted data descriptively in order to highlight main findings. The main findings from each study from each stream (*in vitro* and animal models) were synthesized in tables.

A PRISMA flow diagram visually summarises the screening and selection process is presented in Figure 1. One screening and selection process was conducted for both this scoping review and our companion paper [56].

Results

6,886 records were identified through the searches. After duplicates were removed, 4,367 titles and abstracts were reviewed independently by two reviewers, of which 17 met our inclusion criteria. Nine studies used solely *in vitro* models to study the toxicity of UOG chemicals, while six conducted their

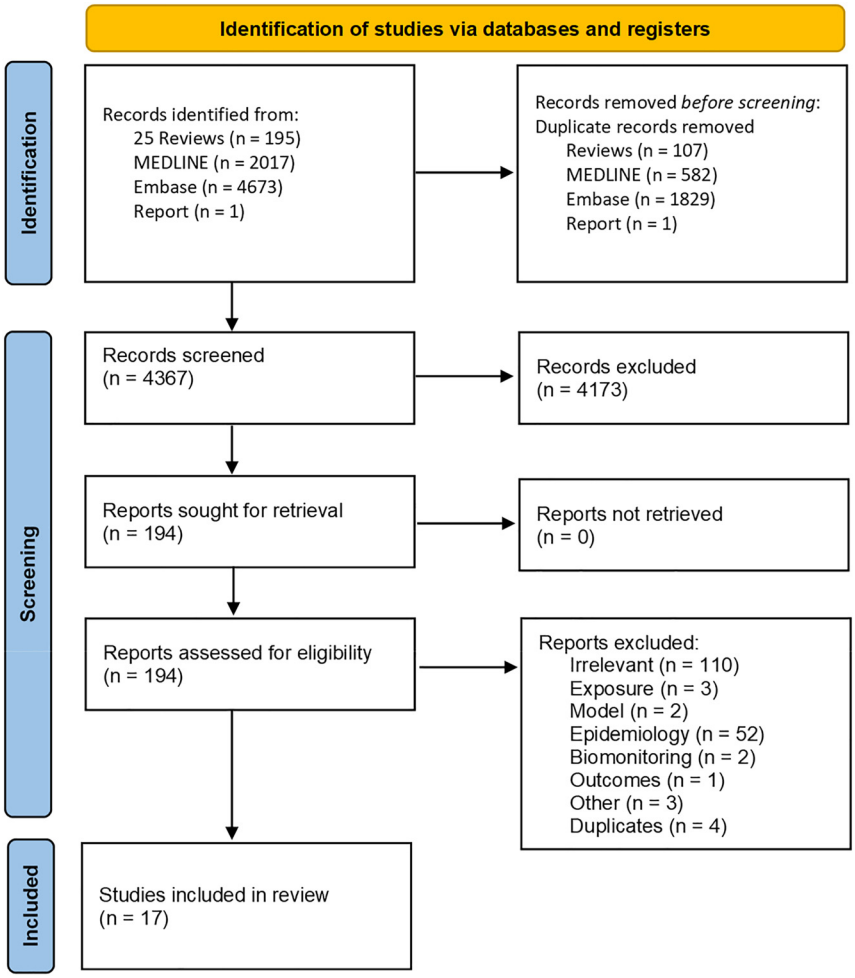

Figure 1: Summary of selection of studies included in this scoping review.

investigation solely in mice. Two studies [44, 60] incorporated both *in vitro* and animal models in their design. All studies were published after 2014.

Relevant information extracted from the 11 *in vitro* toxicological studies are presented in Table 1. In terms of exposure conditions, four studies used real water samples impacted by UOG (e.g., produced, flowback or wastewater, drinking water, surface water, groundwater) [45, 61–63]. Three studies used various lab-made mixtures of UOG chemicals [44, 45, 64]. One study investigated the effects of three biocides, a surfactant, a friction reducer and a coal seam geogenic [65]. One recent study used UOG wastewater

Table 1: Characteristics (UOG chemicals or samples tested; models; endpoints; main findings; deleterious effects) of included *in vitro* toxicological studies.

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
Abraham (2023)	– Impacted water: Produced water diluted 100-fold with raw river water – Chlorine impacted drinking water: 13.5 mg/L chlorine in impacted water – Chlorine river water: 6.0 mg/L chlorine in raw river water – Chloramine impacted drinking water: 13.5 mg/L chloramine in impacted water – Chloramine river water: 6.0 mg/L chloramine in raw river water	Toxicity values based on mammalian cell assays (CHO cells; hamster; ovary)	– Calculated cytotoxicity and genotoxicity using the TIC-Tox method based on toxicity values established in mammalian cell assays	– **Chlorine impacted drinking water:** 5X calculated cytotoxicity compared to chlorine river water; 2X calculated genotoxicity compared to chlorine river water – **Chloramine impacted drinking water:** 5X calculated cytotoxicity compared to chloramine river water; 2X calculated genotoxicity compared to chloramine river water	– Cytotoxicity – Genotoxicity
Bain (2018)	– Biocides: Bronopol (BP), glutaraldehyde (GA), tetrakis(hydroxymethyl) phosphonium sulfate (THPS) – Surfactant: 2-Butoxyethanol – Friction reducer: Polyacrylamide – Coal seam geogenic: *o*-cresol	Luciferase report assays (CALUX®) using U-2 OS cell line (human; epithelial; osteosarcoma)	– Activity of the estrogen (ER), androgen (AR), progesterone (PR), glucocorticoid (GR) and PPARγ receptors	– **BP:** Disruption of all receptors activity at cytotoxic concentrations – **GA:** Disruption all receptors activity at cytotoxic conc; ↑ of ER-CALUX at 6.25 µM – **THPS:** Disruption all receptors activity at cytotoxic concentrations; ↑ of ER-CALUX at 6.25–25 µM – **2-Butoxyethanol:** no significant effects – **Polyacrylamide:** no significant effects – **o-cresol:** no significant effects	Endocrine disruption
Bamberger (2019)	– Surface water samples collected in Susquehanna County – Groundwater samples collected in Susquehanna County	– YCM3 strain for ligand-induced Aryl hydrocarbon receptor (AhR) signaling – Transfected Ishikawa cells (human; epithelial,	– Activity of Ah receptor in YCM3 – Activity of ER, AR, PR and GR receptors	– **Surface water:** ↑ AhR in 8 samples close to impaired natural gas wells[a] – **Ground water:** ↑ AhR in 14 samples close to impaired natural gas wells	Endocrine disruption

Table 1: (continued)

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
		dometrial carcinoma)		– **Endocrine receptor antagonism (type of water samples not mentioned):** 17/53 samples exhibited ↓ activity for at least one receptor; no association with presence of impaired natural gas wells – **Endocrine receptor agonism:** 20 surface water samples and 2 groundwater samples exhibited ↑ ER activity; no association with presence of impaired natural gas wells [a]*reported compromised integrity of casing and cement*	
Crosby (2018)	– Wastewaters from unconventional oil/gas well	– Liver cells (HepG-2 cells (human; epithelial; hepatoma) and rat hepatocytes) – HK-2 cells (human; epithelial; kidney)	– Gene expression (cell-cell communication) – Quantification of proteins implicated in cellular metabolism: AhR; CYP1A1; NQO1; GST – Wound healing inhibition	– **Gene expression:** ↓ genes implicated in gap junction formation, tight junction formation, cellular matrix adhesion, focal adhesion, apoptosis, cell-cell adhesion and communication (1:100 dilution); ↑ gene implicated in purine biosynthesis (1:100 dilution) – **Proteins:** ↓ NQO1 – **Wound healing:** ↓ wound closure with increasing concentrations; no wound closure at highest concentration	– Cytotoxicity – Impaired cellular communication
Kassotis (2014)	– Surface and groundwater samples from drilling-dense region – 12 chemicals used in natural gas operations	Firefly luciferase reporter gene assay in transfected HepG-2 (human; epithelial; hepatoma) and MCF-7 cells (human; epithelial; adenocarcinoma of the mammary gland)	– Activity of AR (in HepG-2 cells) and ER (in MCF-7 cells)	– **Chemicals:** Exhibited anti-ER and anti-AR activities – **Water samples:** Exhibited ER (89 % of samples) anti-ER (41 % of samples), AR (12 % of samples) and anti-AR (46 % of samples) activities. Large differences amongst sites. Agonist or antagonist receptor activities were higher in drilling-dense regions compared to reference sites.	Endocrine disruption

Table 1: (continued)

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
Kassotis (2015)	– 24 commonly used UOG chemicals – Equimolar mixture of 9 chemicals (9-mix) – Equimolar mixture of 23 chemicals (23-mix (no BPA)) – Equimolar mixture of 24 chemicals (24-mix (contains BPA))	Reporter gene assay in transfected Ishikawa (human; epithelial, endometrial carcinoma) and HepG-2 cells (human; epithelial; hepatoma)	– Activity of ER, AR, PR, TR, GR	– **Individual chemicals:** 21 chemicals anti-ER, 21 chemicals anti-AR, 12 anti-PR, 7 anti-TR, 10 anti-GR. 1 ↑ ER, 1 ↑PR, 2 ↑ TR. Most potent antagonist activities for ER. – **Mix-9:** No agonist activities. No antagonist activities for PR, TR and GR. Disruption of ER and AR. – **Mix-23:** No agonist activities. Disruption of ER, AR, PR, TR and GR. – **Mix-24:** Disruption of ER, AR, PR, TR and GR.	Endocrine disruption
Kassotis (2016)	– Surface water at a West Virginia injection well disposal site – Surface water upstream of a West Virginia injection well disposal site – Surface water downstream of a West Virginia injection well disposal site	Reporter gene assay in transfected Ishikawa cells (human; epithelial, endometrial carcinoma)	– Activity of ER, AR, PR, TR, GR – Toxicity via CMV-β-Gal activity in the ER activity screen in Ishikawa human cells	– **At disposal site:** Anti-ER, AR, PR, TR and GR. No agonist activities. Moderate to high toxicity at 40X. – **Upstream:** No antagonist activities. Low agonist activities for PR. No toxicity. – **Downstream:** Anti-ER, AR, PR, TR and GR. No agonist activities. Moderate to high toxicity at 40X.	– Endocrine disruption – Cytotoxicity
Kassotis (2018)	– Mixture of 23 commonly used UOG chemicals – UOG wastewaters – Surface water downstream from UOG well pad and retention pond – Surface water on private property with historical UOG spill – Surface water at UOG well pad	– 3T3-L1 cells (mouse; fibroblast; embryo) – PPARγ reporter assay in HEK-293-cells (human; epithelial; kidney)	– Triglyceride accumulation – Pre-adipocyte proliferation – Cell viability	– **23-mix:** ↑ triglyceride accumulation; ↑ pre-adipocyte proliferation; ↓ cell viability; no activation of PPARγ – **Water samples:** ↑ triglyceride accumulation; ↑ pre-adipocyte proliferation; ↓ cell viability; ↑ PPARγ activity – **Field blanks:** no effects	Impaired metabolic health
Kassotis (2020)	– Surface and groundwater samples from drilling-dense region in Colorado – Reference water sample from area with no UOG – Wastewater samples	Reporter gene assay in transfected Ishikawa cells (human; epithelial, endometrial carcinoma)	– Activity of ER, AR, PR, TR and GR	– **Water samples from medium drilling-intensity:** ↑ agonist activity for ER, AR, PR and TR compared to reference; ↑ anti-ER and AR activity compared to reference – **Water samples from high drilling-intensity:** ↑ agonist activity for ER, AR and TR	Endocrine disruption

Table 1: (continued)

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
				compared to reference; ↑ anti-ER, AR and GR activity compared to reference – **Water samples upstream of UOG:** ↑ agonist activity for ER, and AR compared to reference; ↑ anti-ER, AR, PR and GR activity compared to reference – **Wastewater samples:** ↑ anti-ER, AR and PR activity compared to reference	
Yao (2015)	– Flowback water samples	BEAS-2B cells (human; epithelial; lung)	– Cytotoxicity – Colony formation in soft agar (carcinogenicity) – Cell migration – Transcription profile – Trace metals update in cells	– **Flowback water:** ↓ cell viability after 10 days exposure to 4 %; ↑ colony numbers at 0.5 %; different cell morphology and growth rate after exposure; ↑ migration capacity; ↑ expression of genes in inflammation, cell migration, cell proliferation and *wnt* signaling pathway; ↓ expression of genes implicated in apoptosis, adherent junction and endocytosis – **Barium:** ↓ cell viability; uptake in cells – **Strontium:** no effect on cell viability; uptake in cells	Carcinogenic potential
Zhuang (2023)	– Produced water samples from China treated or not by oxidation technology	V79 cells (Chinese hamster; fibroblast; lung)	– Cytotoxicity – DNA damage assessed by Comet assay	– **Produced water:** ↑ cell shrinkage after 48 h at 5 and 30 mg/mL; ↓ cell viability after 48 h at 3, 5 and 7 mg/mL; ↓ cell viability after 12, 24 and 48 h at 9–30 mg/mL; no DNA damage – **Treated produced water:** ↑ cell shrinkage after 48 h at 5 and 30 mg/mL; ↓ cell viability after 12, 24 and 48 h at 7–30 mg/mL; ↑ DNA damage after 12, 24 and 48 h at 50–800 µg/mL	– Acute cytotoxicity – Acute genotoxicity

samples treated by various oxidation processes [66]. Another recent study used drinking water samples containing UOG wastewater and then treated by chlorination or chloramination, two disinfectant treatments used in municipal water treatment plants [61].

In vitro models ranged from mammalian cells to reporter assays, and to transfected cells. Most studies used human cells, while a few used hamster and mouse cells. All studies used monocultures. Endpoints included cytotoxicity (6 of 11 studies), genotoxicity (2/11), hormone receptor activity (6/11), gene and protein expression (2/11), wound healing inhibition (1/11), uptake of chemicals in cells (1/11), triglyceride accumulation (1/11), cell proliferation (1/11), carcinogenicity (1/11) and cell migration (1/11).

All studies found deleterious effects associated with exposure to UOG chemicals or samples. In particular, Abraham et al. [61] found significant cytotoxicity and genotoxicity associated with chlorinated or chloraminated drinking water samples containing UOG wastewater using the TIC-Tox method. Bain et al. [65] used the CALUX® assay and reported endocrine disruption on multiple hormone receptors (estrogen, androgen, progesterone, glucocorticoid and PPARγ) following exposure to biocides, surfactant, friction reducer and coal seam geogenic compounds. Bamberger et al. [62] found that surface and groundwater samples collected in a dense UOG region exhibited endocrine disruption activity, including an increase in the activity of the aryl-hydrocarbon receptor (AhR) in samples close to impaired natural gas wells, as well as several endocrine receptor antagonism and agonism. Crosby et al. [63] found that exposure to wastewater samples in liver and kidney cells led to impaired expression of genes implicated in cellular communication. Kassotis et al. [43–45, 67] found evidence of endocrine disruption in multiple studies, with impaired receptor activities both in reporter gene assays and in transfected cells exposed to surface and groundwater samples, either from drilling-dense regions or in mixtures of commonly-used UOG chemicals. To our knowledge, Kassotis et al. [45] is the first study investigating the toxicity of UOG, specifically the endocrine disruption potential of UOG. Notably, in Kassotis et al. [43], the antagonist activities of several hormone receptors were seen in surface water at an injection well disposal site and downstream. In Kassotis et al. [64], impaired metabolic health – with triglyceride accumulation and pre-adipocyte proliferation, was observed in mouse fibroblast cells exposed to a mixture of 23 UOG chemicals, UOG wastewater and surface water at a UOG well pad. Yao et al. [60] investigated the carcinogenic effects of flowback water samples in human lung cells and found that exposed cells had higher migration capacity and increased expression of genes implicated in inflammation, proliferation and migration. Genes promoting apoptosis, endocytosis and adherent junctions were inhibited. Additionally, Zhuang et al. [66] measured the cytotoxicity and DNA damage caused by exposure of Chinese hamster lung fibroblast cells to UOG produced water, when treated or not by oxidation. The authors found that treated produced water induced greater DNA damage than untreated produced water.

Relevant information extracted from the eight toxicological studies using animal assays are presented in Table 2. In terms of exposure conditions, seven studies used lab-made mixtures of UOG chemicals [44, 68–73] and one study used BEAS-2B cells exposed to flowback water and then injected subcutaneously in female mice [60]. Animals were exposed to lab-made mixtures of UOG chemicals in drinking water for varied amounts of time.

All studies used mice, including C57BL/6 J (7 of eight studies) or athymic nude mice (1/8). Two studies used both males and females, while five and one studies used only females and males, respectively. Six studies used offspring, while two studies used adult mice. Endpoints included body weight (3/8); energy, activity and behavior (2/8); metabolic health endpoints such as glucose tolerance, liver triacylglycerol, insulin and pancreas analysis (2/8); immune cells populations and immune response (2/8); endocrine endpoints such as anogenital distances, serum hormones, sperm and ovarian follicle assessments and mammary gland development (3/8); heart assessment (1/8); and tumor formation (1/8).

All studies found deleterious effects associated with exposure to UOG chemicals. In particular, Balise et al. [68] found that exposure to a mixture of 23 commonly used UOG chemicals at concentrations from 1.5 to 1,500 µg/kg/day in drinking water prior to mating, and between gestational day 0 until postnatal day 21 (PND21), led to disruption of energy expenditure in female mice offspring. A follow-up study in adult female mice challenged with a high fat, high sugar diet also showed impaired behavior (decreased sleep, increased exploratory behavior) and metabolic health (decreased fat pad weight) [69]. Results from Boulé et al. [70] showed that exposure to a mixture of 23 commonly used UOG chemicals at 30 and 300 µg/kg/day in drinking water from gestational day 0 to PND21 in male and female mice offspring challenged with various infectious agents led to disruptions in immune cell populations. A follow-up study conducted by O'Dell et al. [72] in adult male and female mice challenged with the same infectious agents also noted altered immune cells populations, with distinct effects in males and females. Kassotis et al. [44] found that exposure to the same mixture at concentrations from 30 to 3,000 µg/kg/day in drinking water from gestational day 11 until birth led to disruption in testis and heart weight, and a decrease in sperm count and anogenital distance in male offspring. A study from the same group conducted in female offspring

Table 2: Characteristics (UOG chemicals or samples tested; models; endpoints; main findings; deleterious effects) of included animal toxicological studies.

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
Animal assays					
Balise (2019)	Mixture of 23 commonly used UOG chemicals at 1.5, 150 of 1,500 ug/kg/day in drinking water for 5 weeks prior to mating, and between gestational day 0 until PND21.	C57BL/6J female mice offspring aged to 7 months	– Body weight and composition – Spontaneous activity – Energy expenditure – Glucose tolerance	– **1.5 ug/kg/day:** ↓ total and resting energy expenditure during dark cycle. ↓ resting energy expenditure during light cycle. – **150 ug/kg/day:** ↓ total and resting energy expenditure during dark cycle. ↓ spontaneous activity during dark cycle. – **1,500 ug/kg/day:** ↓ total energy expenditure during light cycle. ↓ spontaneous activity during light cycle. – No difference between exposure groups in body weight, body composition and in glucose tolerance.	Behavioral changes
Balise (2019)	Mixture of 23 commonly used UOG chemicals at 1.5, 15, 150 and 1,500 µg/kg/day in drinking water for 5 weeks prior to mating, and between gestational day 0 until PND21.	C57BL/6J female mice offspring aged to 12 months and given a 3 day high fat, high sugar diet (HFHSD) challenge	– Body weight and composition – Spontaneous activity – Energy expenditure – Glucose tolerance – Liver triacylglycerol – Serum insulin – Pancreas analysis	– **1.5 µg/kg/day:** Alterations in exploratory behavior; ↑ non-resting energy expenditure and activity during light cycle; ↓ periuterine fat pad weight – **15 µg/kg/day:** Alterations in exploratory behavior; ↑ non-resting energy expenditure and activity during light cycle; ↓ periuterine fat pad weight – **150 µg/kg/day:** Alterations in exploratory behavior; ↑ non-resting energy expenditure and activity during light cycle; ↓ periuterine fat pad weight – **1,500 µg/kg/day:** ↑ non-resting energy expenditure and activity during light cycle; ↓ body weight; ↓ periuterine fat pad weight; ↑ brown fat pad weight	– Behavioral changes – Impaired metabolic health

Table 2: (continued)

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
				No difference between exposure groups in fat and lean mass; total food consumption and food bouts; insulin concentrations; estrus cyclicity.	
Boule (2018)	Mixture of 23 commonly used UOG chemicals at 30 and 300 µg/kg/day in drinking water from gestational day 0 until PND21.	Male and female C57BL/6J mice offspring challenged with infectious agents (house dust mite (HDM) extract-induced allergic airway disease; influenza A virus (IAV); autoimmune encephalomyelitis (EAE)) after exposure	– Immune cells populations – Onset of symptoms	– **30 µg/kg/day:** ↑ granulocyte monocyte precursors in females – **30 µg/kg/day** + **HDM challenge:** ↓ $CD4^+$ T cells in females; ↑ Th2and Th17 cells in females; ↓ Treg: Th2 cells radio in males and females; ↑ airway macrophages in females; ↑ airway eosinophils and lymphocytes in males and females – **30 µg/kg/day** + **IAV challenge:** no significant effects – **30 µg/kg/day** + **EAE challenge:** ↑ Th1 cells in females; ↓ Treg: Th1 cells ratio in females; ↑ Treg: Th17 cells ratio in females; earlier disease onset in females; higher disease scores in females – **300 µg/kg/day:** ↓ bone marrow cells in females; ↑ hematopoietic stem cells in females; ↑ $Gr1^+CD11b^+$ myeloid cells in male and female spleen – **300 µg/kg/day** + **HDM challenge:** ↑ Th17 cells in females; ↓ Treg: Th2 cells radio in females; ↑ airway macrophages in females – **300 µg/kg/day** + **IAV challenge:** ↑ viral nucleoprotein (NP)-specific $CD8^+$ T cells – **300 µg/kg/day** + **EAE challenge:** Earlier disease onset in females	Immune dysregulation

Table 2: (continued)

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
				No difference between exposure groups in body weights, number of pups per litter or sex ratio among exposure groups.	
Kassotis (2015)	Mixture of 23 commonly used UOG chemicals at 3, 30, 300, and 3,000 µg/kg/day in drinking water, from gestational day 11 until birth	Male C57BL/6J mice offspring	– Anogenital distances – Fully retained nipples – Body and organ weights – Serum testosterone – Sperm assessment	– **3 µg/kg/day:** ↑testis weights at PND21 and 85; ↑heart weight and cardiac myocyte diameter at PND21; non-significant ↓ anogenital distance – **30 µg/kg/day:** ↓sperm count at PND85; non-significant ↓ anogenital distance – **300 µg/kg/day:** ↑testis weights at PND21; ↓sperm count at PND85; ↑body and thymus weight at PND21; non-significant ↓ anogenital distance – **3,000 µg/kg/day:** ↑testis weights at PND85; ↑serum testosterone at PND85; non-significant ↓ anogenital distance No difference between exposure groups in sperm morphology.	Endocrine disruption
Kassotis (2016)	Mixture of 23 commonly used UOG chemicals at 3, 30, 300, and 3,000 µg/kg/day in drinking water, from gestational day 11 until birth	Female C57Bl/6 mice offspring	– Ovarian follicle assessment – Serum hormones – Heart assessment	– **3 µg/kg/day:** ↓FSH and LH. ↓prolactin. Disrupted folliculogenesis. ↑body weight at PND7, PND13 and PND21. ↓uterine weight at PND85. ↑ovary weight at PND85. ↑collagen deposition in heart at PND85. – **30 µg/kg/day:** ↓FSH and LH. ↓prolactin. Disrupted folliculogenesis. ↑collagen deposition in heart at PND85. – **300 µg/kg/day:** ↑GH and TSH. ↓prolactin. Disrupted folliculogenesis. ↑ body weight at PND7, PND13 and PND21. ↓ovary weight at PND85	– Endocrine disruption – Developmental disruption

Table 2: (continued)

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
				– **3,000 µg/kg/day:** ↓FSH and LH. ↓prolactin. Disrupted folliculogenesis. No difference between exposure groups in estradiol serum levels.	
O'Dell (2021)	Mixture of 23 commonly used UOG chemicals in drinking water at a final concentration of 0.1 µg/mL for each chemical for at least 8 weeks	Adult (6–8 weeks) male and female C57Bl/6 mice challenged with infectious agents (house dust mite (HDM) extract-induced allergic airway disease; influenza A virus (IAV); autoimmune encephalomyelitis (EAE)) after exposure	– Cellularity of primary and secondary immune organs – Immune system function	– **Immunologically-naïve female mice:** ↑ in thymocytes. ↑ peripheral lymph node cells. ↓ percentage of CD19+ cells in spleen. – **Immunologically-naïve male mice:** ↓ bone marrow cells. – **HDM female mice:** ↑ number of Th2 cells in lymph nodes. ↑ airway eosinophils. ↓ airway macrophages and lymphocytes – **HDM male mice:** ↑ airway macrophages. – **Influenza A female mice:** no difference in morbidity of immune cells populations. – **Influenza A male mice:** ↓ CD8+ T-cells, CTL and virus NP-specific specific CD8+ T cells. ↓ number of cells in lymph nodes. ↓ number of CD4+ cells. No difference in morbidity. – **EAE female mice:** ↑ severity of disease. ↓ number of Treg cells. – **EAE male mice:** ↓ number of TH1 cells.	Altered immune system
Sapouckey (2018)	Mixture of 23 commonly used UOG chemicals at 3, 30, 300, and 3,000 µg/kg/day in drinking water, from gestational day 11 to birth	Female C57Bl/6 mice offspring	– Morphology of mammary glands – Immunohistochemical analysis for ERα and Ki67 (marker of cell proliferation) – Apoptotic cells in mammary tissue	– **3 µg/kg/day:** ↑Ki67--positive cells; ↑ proliferation/apoptosis ratio. – **300 µg/kg/day:** ↑ducts in adult mice; ↑volume mammary epithelium in adult mice – **3,000 µg/kg/day:** ↑volume mammary epithelium No difference between exposure groups in prepubertal mammary gland morphology.	Mammary gland development

Table 2: (continued)

Lead author, year	UOG chemicals/samples tested	Models	Endpoints	Main findings	Deleterious effects
Yao (2015)	BEAS-2B cells transformed by flowback water injected subcutaneously in left and rank flanks	Female athymic nude mice (Nu/J) aged 6–8 weeks with BEAS-2-B cells tumor xenografts	– Tumor formation	– **Injection of flowback water-treated BEAS-2-B cells:** 5/6 mice developed tumors with diameters ranging from 0.2 to 1 cm – **Injection of control BEAS-2B cells:** no tumor (n=1)	Carcinogenic potential

showed altered levels of various hormones such as FSH, LH, prolactin, GH and TSH, as well as disrupted folliculogenesis and collagen deposition in the heart [71]. Using the same experimental design, Sapouckey et al. [73] also noted an increase in mammary ducts and volume of the mammary epithelium in these female offspring. The study conducted by Yao et al. [60] injected human lung epithelial cells exposed to UOG flowback water into female athymic nude mice to generate solid human tumor xenografts. The authors found that five out of six mice injected with the flowback water-treated cells developed tumor xenografts. As well, one control mouse was injected with normal cells, which did not form any tumors.

Discussion

A scoping review published in 2020 that examined the human health outcomes associated with exposure to UOG activity highlighted that very few studies investigated mechanisms of toxicity underpinning the associations between UOG exposure and health outcomes [20]. Our review included only 17 studies, comprising nine using solely *in vitro* models, six conducted solely in animal models, and two using both *in vitro* and animal models. Notably, among the *in vitro* studies included in the review, all found deleterious effects associated with exposure to UOG chemicals or samples on a variety of endpoints, such as cytotoxicity, endocrine disruption, triglyceride accumulation and carcinogenesis. In terms of exposure, most studies used drinking, surface or groundwater samples from UOG-intensive regions [43, 45, 61, 62, 64, 67], while five studies used wastewater or produced water samples [60, 63, 64, 66, 67] and four studies were conducted with lab-made mixtures of UOG chemicals [44, 45, 64, 65]. All animal studies included in this review also reported significant impacts of UOG exposure on a range of endpoints, including behavioral changes, metabolic health, immune dysregulation, endocrine disruption, developmental disruption, carcinogenesis and mammary gland development. In terms of exposure, most studies used lab-made mixtures of UOG chemicals to exposure animals through drinking water [44, 68–73]. One study used BEAS-2B cells previously exposed to flowback water to induce tumor xenografts in mice [60].

More than a 1,000 different chemicals have been identified as commonly-used compounds in UOG operations for a variety of functions, such as proppants, biocides, friction reducers, scale inhibitors, clay stabilizers, surfactants, acids, corrosion inhibitors, gelling agents, foaming agents and pH adjustors [1]. Assessing the toxicity of UOG chemicals is a complicated enterprise, in part due to the lack of toxicological information for the majority of chemicals used in this industry [40] and the withholding of information on proprietary or trade secret grounds. Elliott et al. [40] evaluated 1,021 chemicals used in hydraulic fracturing for their reproductive and developmental toxicity and found that for the 24 % of chemicals with available toxicity information, 43 and 40 % of them were reproductive and developmental toxicants, respectively. Trickey et al. [74] investigated disclosure forms submitted to FracFocus, the US hydraulic fracturing chemical registry, and determined that 18 % of the chemicals imported in FracFocus were not identifiable and marked as confidential, proprietary or trade secret. Yost et al. (2016) used a list of 1,173 chemicals associated with the UOG industry (1,076 chemicals used in the hydraulic fluid, and 134 chemicals identified in wastewaters) and compiled the available chronic oral reference values (i.e., amount of the chemical that can be ingested every day without significantly increasing the risk of adverse health effects over a lifetime) for these 1,173 chemicals. Their analysis revealed chronic oral reference values were available for only 90 of the 1,076 chemicals reported in hydraulic fracturing fluids and 83 of the 134 chemicals reported in wastewaters. Chemicals for which chronic oral reference values were available included well studied heavy metals (e.g., arsenic, cobalt, cadmium), organic compounds (e.g., benzene, phosphine, benzyl chloride), polycyclic aromatic hydrocarbons

(e.g., benzo [a] pyrene) and pesticides (e.g., heptachlor, aldrin, dieldrin). The authors also identified 36 chemicals frequently used in UOG operations: chronic oral reference values were not available for 28 of these chemicals (including, but not limited to, quartz-alpha, hydrochloric acid, isopropanol, diammonium peroxydisulfate, guar gum, sodium hydroxide, glutaraldehyde, sodium chloride, potassium hydroxide, ethanol, solvent naphtha, ammonium chloride) [75].

The main deleterious effects identified in the toxicological studies included in our review are aligned with the current epidemiological literature on the associations between exposure to UOG activity and human health outcomes. A recent review conducted by our team identified 52 studies where investigated health outcomes were plausibly attributable to exposure to UOG chemicals, with birth outcomes being the most widely studied health effects. The majority of studies found significant deleterious effects, including on maternal, birth and infant outcomes, respiratory and cardiovascular outcomes, childhood cancer, hospital admissions, self-reported health symptoms, and mortality [56].

Endocrine disruption, as identified in multiple toxicological studies included in this review, is a plausible mechanism that could explain the higher odds of negative maternal and birth outcomes observed in multiple epidemiological studies conducted in the UOG context [21, 23, 25–28, 76, 77]. Toxicological data on individual chemicals known to be used or emitted by UOG operations can be used to contextualize the results of the studies included in this review, and the plausibility of the epidemiological literature findings. For example, certain VOCs (e.g., BTEX) and trace elements (e.g., barium, strontium, arsenic) are emitted by UOG operations [3, 6, 8, 10, 11, 14, 78–85] with some congeners being known or suspected endocrine disruptors and human carcinogens [86–91].

Limitations and future opportunities

The wide range of models, exposures and outcomes measured in the various studies included in our review made it challenging to apply a formal bias tool to evaluate the quality of studies, which is a limitation of our review. Furthermore, our review focuses on the toxicity of UOG mixtures and environmental samples and does not provide a comprehensive overview of the toxicological data relevant to all individuals chemicals. All *in vitro* studies identified in this review used monocultures. This is an important limitation of the identified studies, since cell-cell communication and interactions with the extracellular matrix, which cannot be evaluated in monocultures systems, play important roles in cellular function, behavior, and the development of disease [92]. More complex *in vitro* systems, such as co-cultures and organ-on-chip systems, are more physiologically-relevant and accurate to study how cells interact with each other and their environment and should be prioritized in future research endeavors [93–97].

Rodents are not always an accurate model to use in toxicity testing. For example, studies have shown that the metabolism of environmental contaminants, including endocrine disruptors, differ between rodent species and humans [98–100]. Researchers should ensure that the animal models used are relevant for the human disease of interest and share similar mechanisms of action [100]. Another important limitation of the animal studies included in the review is the fact that they all used drinking water as the pathway of exposure to UOG chemicals or samples, therefore not considering other relevant pathways of exposure. Indeed, studies have demonstrated impacts of UOG activity on local air quality [79, 101–104], making inhalation an appropriate additional exposure pathway to use in future studies. Future toxicological studies could focus on the inhalation pathway by using innovative cellular models at the Air-Liquid Interface (ALI). Indeed, pulmonary cell systems at the ALI allow for a better replication of the airway physiology, the possibility of repeating and chronic exposure scenarios and the delivery of contaminants in aerosol similar to inhalation in humans [105]. In addition, animal models can be exposed to air pollutants in various types of exposure chambers [106]. Other considerations to improve the appropriate selection of models include: selection of the most sensitive species possible, accounting for species differences in metabolism of contaminants (for example, by using humanized transgenic rodents; conducting prior testing of contaminants metabolism in cellular models of various species, and using physiologically based pharmacokinetic [PBPK] modeling to predict the contaminants metabolism and behavior in humans [107, 108]); ensuring that the animal model presents the relevant target and outcome; ensuring animals of both sexes are included when relevant; and consideration of the latency period between exposure and effect in the design of the study [100].

The studies we identified offered little background on their choice of exposure concentrations, highlighting the need for more toxicological studies using physiologically-relevant models, environmentally-relevant concentrations of UOG chemicals based on biomonitoring studies, and exposure pathways relevant to real-life exposure scenarios.

Conclusions

There is a growing body of evidence of an association between proximity to UOG operations and adverse human health outcomes. Our review highlighted multiple mechanisms of toxicity associated with UOG chemicals, including endocrine disruption, genotoxicity, impaired cellular communication, impaired metabolic health biomarkers, behavioral changes, immune dysregulation, and carcinogenesis. This review also highlights the sparsity of studies using mixtures of UOG chemicals and environmentally-relevant samples, and suggests models and exposure methods that could be prioritized in future research.

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References

1. Wollin KM, Damm G, Foth H, Freyberger A, Gebel T, Mangerich A, et al. Critical evaluation of human health risks due to hydraulic fracturing in natural gas and petroleum production. Arch Toxicol 2020;94:967–1016.
2. Norris JQ, Turcotte DL, Moores EM, Brodsky EE, Rundle JB. Fracking in tight shales: what is it, what does it accomplish, and what are its consequences? Annu Rev Earth Planet Sci 2016;44:321–51.
3. Pichtel J. Oil and gas production wastewater: soil contamination and pollution prevention. Appl Environ Soil Sci 2016;2016:24.
4. Goldstein BD, Brooks BW, Cohen SD, Gates AE, Honeycutt ME, Morris JB, et al. The role of toxicological science in meeting the challenges and opportunities of hydraulic fracturing. Toxicol Sci 2014;139:271–83.
5. Hecobian A, Clements AL, Shonkwiler KB, Zhou Y, MacDonald LP, Hilliard N, et al. Air toxics and other volatile organic compound emissions from unconventional oil and gas development. Environ Sci Technol Lett 2019;6:720–6.
6. Luek JL, Gonsior M. Organic compounds in hydraulic fracturing fluids and wastewaters: a review. Water Res 2017;123:536–48.
7. Brown VJ. Radionuclides in fracking wastewater: managing a toxic blend. Environ Health Perspect 2014;122:A50–5.
8. Chittick EA, Srebotnjak T. An analysis of chemicals and other constituents found in produced water from hydraulically fractured wells in California and the challenges for wastewater management. J Environ Manag 2017;204:502–9.
9. Fontenot BE, Hunt LR, Hildenbrand ZL, Carlton JDD, Oka H, Walton JL, et al. An evaluation of water quality in private drinking water wells near natural gas extraction sites in the Barnett Shale Formation. Environ Sci Technol 2013;47:10032–40.
10. Lester Y, Ferrer I, Thurman EM, Sitterley KA, Korak JA, Aiken G, et al. Characterization of hydraulic fracturing flowback water in Colorado: implications for water treatment. Sci Total Environ 2015;512–513:637–44.
11. Srebotnjak T, Rotkin-Ellman M. Fracking fumes: air pollution from hydraulic fracturing threatens public health and communities. Natural Resources Defense Council; 2014.
12. Crowe E, Patton S, Thomas D, Thorpe B. When the wind blows: tracking toxic chemicals in gas fields and impacted communities. Battleboro, VT: Coming Clean Inc.; 2016. [Internet]. Available from: https://aglaw.psu.edu/wp-content/uploads/2020/06/document_gw_01.pdf.
13. Gilman JB, Lerner B, Kuster W, De Gouw J. Source signature of volatile organic compounds from oil and natural gas operations in northeastern Colorado. Environ Sci Technol 2013;47:1297–305.
14. Macey GP, Breech R, Chernaik M, Cox C, Larson D, Thomas D, et al. Air concentrations of volatile compounds near oil and gas production: a community-based exploratory study. Environ Health 2014;13:82.
15. Vengosh A, Jackson RB, Warner N, Darrah TH, Kondash A. A critical review of the risks to water resources from unconventional shale gas development and hydraulic fracturing in the United States. Environ Sci Technol 2014;48:8334–48.
16. Werner AK, Vink S, Watt K, Jagals P. Environmental health impacts of unconventional natural gas development: a review of the current strength of evidence. Sci Total Environ 2015;505:1127–41.
17. Wisen J, Chesnaux R, Wendling G, Werring J, Barbecot F, Baudron P. Assessing the potential of cross-contamination from oil and gas hydraulic fracturing: a case study in northeastern British Columbia, Canada. J Environ Manag 2019;246:275–82.
18. Wisen J, Chesnaux R, Werring J, Wendling G, Baudron P, Barbecot F. A portrait of wellbore leakage in northeastern British Columbia, Canada. Proc Natl Acad Sci 2019;117:913–22.
19. Bamber AM, Hasanali SH, Nair AS, Watkins SM, Vigil DI, Van Dyke M, et al. A systematic review of the epidemiologic literature assessing health outcomes in populations living near oil and natural gas operations: study quality and future recommendations. Int J Environ Res Publ Health 2019;16:2123.
20. Deziel NC, Brokovich E, Grotto I, Clark CJ, Barnett-Itzhaki Z, Broday D, et al. Unconventional oil and gas development and health outcomes: a

scoping review of the epidemiological research. Environ Res 2020;182: 109124.
21. Caron-Beaudoin É, Whitworth KW, Bosson-Rieutort D, Wendling G, Liu S, Verner MA. Density and proximity to hydraulic fracturing wells and birth outcomes in Northeastern British Columbia, Canada. J Expo Sci Environ Epidemiol 2020;31:53–61.
22. Casey JA, Savitz DA, Rasmussen SG, Ogburn EL, Pollak J, Mercer DG, et al. Unconventional natural gas development and birth outcomes in Pennsylvania, USA. Epidemiology 2016;27:163–72.
23. Currie J, Greenstone M, Meckel K. Hydraulic fracturing and infant health: new evidence from Pennsylvania. Sci Adv 2017;3:e1603021.
24. Gonzalez DJ, Sherris AR, Yang W, Stevenson DK, Padula AM, Baiocchi M, et al. Oil and gas production and spontaneous preterm birth in the San Joaquin Valley, CA: a case – control study. Environ Epidemiol 2020;4:7.
25. Hill EL. Shale gas development and infant health: evidence from Pennsylvania. J Health Econ 2018;61:134–50.
26. Whitworth KW, Marshall AK, Symanski E. Drilling and production activity related to unconventional gas development and severity of preterm birth. Environ Health Perspect 2018;126:8.
27. Whitworth KW, Marshall AK, Symanski E. Maternal residential proximity to unconventional gas development and perinatal outcomes among a diverse urban population in Texas. PLoS One 2017; 12:e0180966.
28. Willis MD, Hill EL, Boslett A, Kile ML, Carozza SE, Hystad P. Associations between residential proximity to oil and gas drilling and term birth weight and small-for-gestational-age infants in Texas: a difference-in-differences analysis. Environ Health Perspect 2021;129:077002.
29. Rabinowitz PM, Slizovskiy IB, Lamers V, Trufan SJ, Holford TR, Dziura JD, et al. Proximity to natural gas wells and reported health status: results of a household survey in Washington Country, Pennsylvania. Environ Health Perspect 2015;123:21–6.
30. Tustin AW, Hirsch AG, Rasmussen SG, Casey JA, Bandeen-Roche K, Schwartz BS. Associations between unconventional natural gas development and nasal and sinus, migraine headache, and fatigue symptoms in Pennsylvania. Environ Health Perspect 2017;125:189.
31. Rasmussen SG, Ogburn EL, McCormack M, Casey JA, Bandeen-Roche K, Mercer DG, et al. Association between unconventional natural gas development in the Marcellus Shale and asthma exacerbations. JAMA Intern Med 2016;176:1334–43.
32. Willis M, Hystad P, Denham A, Hill E. Natural gas development, flaring practices and paediatric asthma hospitalizations in Texas. Int J Epidemiol 2020;49:1883–96.
33. Denham A, Willis MD, Croft DP, Liu L, Hill EL. Acute myocardial infarction associated with unconventional natural gas development: a natural experiment. Environ Res 2021;195:7.
34. Aker AM, Whitworth KW, Bosson-Rieutort D, Wendling G, Ibrahim A, Verner MA, et al. Proximity and density of unconventional natural gas wells and mental illness and substance use among pregnant individuals: an exploratory study in Canada. Int J Hyg Environ Health 2022;242. https://doi.org/10.1016/j.ijheh.2022.113962.
35. Casey JA, Wilcox HC, Hirsch AG, Pollak J, Schwartz BS. Associations of unconventional natural gas development with depression symptoms and disordered sleep in Pennsylvania. Sci Rep 2018;8:1–10.
36. Bergstra AD, Brunekreef B, Burdorf A. The influence of industry-related air pollution on birth outcomes in an industrialized area. Environ Pollut 2021;269:7.
37. Stieb DM, Chen L, Eshoul M, Judek S. Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. Environ Res 2012;117:100–11.
38. Hoek G, Krishnan RM, Beelen R, Peters A, Ostro B, Brunekreef B, et al. Long-term air pollution exposure and cardio-respiratory mortality: a review. Environ Health 2013;12:1–16.
39. Vawda S, Mansour R, Takeda A, Funnell P, Kerry S, Mudway I, et al. Associations between inflammatory and immune response genes and adverse respiratory outcomes following exposure to outdoor air pollution: a HuGE systematic review. Am J Epidemiol 2014;179: 432–42.
40. Elliott EG, Ettinger AS, Leaderer BP, Bracken MB, Deziel NC. A systematic evaluation of chemicals in hydraulic-fracturing fluids and wastewater for reproductive and developmental toxicity. J Expos Sci Environ Epidemiol 2016;27:90–9.
41. Elliott EG, Trinh P, Ma X, Leaderer BP, Ward MH, Deziel NC. Unconventional oil and gas development and risk of childhood leukemia: assessing the evidence. Sci Total Environ 2017;576:138–47.
42. Xu X, Zhang X, Carrillo G, Zhong Y, Kan H, Zhang B. A systematic assessment of carcinogenicity of chemicals in hydraulic-fracturing fluids and flowback water. Environ Pollut 2019;251:128–36.
43. Kassotis CD, Iwanowicz LR, Akob DM, Cozzarelli IM, Mumford AC, Orem WH, et al. Endocrine disrupting activities of surface water associated with a West Virginia oil and gas industry wastewater disposal site. Sci Total Environ 2016;557:901–10.
44. Kassotis CD, Klemp KC, Vu DC, Lin CH, Meng CX, Besch-Williford CL, et al. Endocrine-disrupting activity of hydraulic fracturing chemicals and adverse health outcomes after prenatal exposure in male mice. Endocrinology 2015;156:4458–73.
45. Kassotis CD, Tillitt DE, Davis JW, Hormann AM, Nagel SC. Estrogen and androgen receptor activities of hydraulic fracturing chemicals and surface and ground water in a drilling-dense region. Endocrinology 2014;155:897–907.
46. Nagel S, Kassotis CD, Vandenberg L, Lawrence B, Robert J, Balise V. Developmental exposure to a mixture of unconventional oil and gas chemicals: a review of experimental effects on adult health, behavior, and disease. Mol Cell Endocrinol 2020;513:11.
47. Andersson H, Piras E, Demma J, Hellman B, Brittebo E. Low levels of the air pollutant 1-nitropyrene induce DNA damage, increased levels of reactive oxygen species and endoplasmic reticulum stress in human endothelial cells. Toxicology 2009;262:57–64.
48. Bayil S, Cicek H, Geyikli Cimenci I, Hazar M. How volatile organic compounds affect free radical and antioxidant enzyme activity in textile workers. Arh Hig Rad Toksikol 2008;59:283–7.
49. Huang W, Wang G, Lu SE, Kipen H, Wang Y, Hu M, et al. Inflammatory and oxidative stress responses of healthy young adults to changes in air quality during the Beijing Olympics. Am J Respir Crit Care Med 2012; 186:1150–9.
50. Kim JH, Moon JY, Park EY, Lee KH, Hong YC. Changes in oxidative stress biomarker and gene expression levels in workers exposed to volatile organic compounds. Ind Health 2010;49:8–14.
51. Kim SS, Meeker JD, Keil AP, Aung MT, Bommarito PA, Cantonwine DE, et al. Exposure to 17 trace metals in pregnancy and associations with urinary oxidative stress biomarkers. Environ Res 2019;179:9.
52. Lu CY, Ma YC, Lin JM, Li CY, Lin RS, Sung FC. Oxidative stress associated with indoor air pollution and sick building syndrome-related symptoms among office workers in Taiwan. Inhal Toxicol 2007;19: 57–65.
53. Peluso M, Munnia A, Ceppi M, Giese RW, Catelan D, Rusconi F, et al. Malondialdehyde–deoxyguanosine and bulky DNA adducts in schoolchildren resident in the proximity of the Sarroch industrial estate on Sardinia Island, Italy. Mutagenesis 2013;28: 315–21.

54. Stringfellow WT, Camarillo MK, Domen JK, Sandelin WL, Varadharajan C, Jordan PD, et al. Identifying chemicals of concern in hydraulic fracturing fluids used for oil production. Environ Pollut 2017; 220:413–20.
55. Wattenberg EV, Bielicki JM, Suchomel AE, Sweet JT, Vold EM, Ramachandran G. Assessment of the acute and chronic health hazards of hydraulic fracturing fluids. J Occup Environ Hyg 2015;12: 611–24.
56. Aker AM, Friesen M, Ronald LA, Doyle-Waters MM, Takaro TJ, Thickson W, et al. The human health effects of unconventional oil and gas development (UOGD): a scoping review of epidemiologic studies. Can J Public Health 2024;1–22. https://doi.org/10.17269/s41997-024-00860-2.
57. Covidence. Covidence systematic review software. Melbourne, Australia: Veritas Health Innovation; 2021. [Internet] Available from: www.covidence.org.
58. Shapiro A, Antoni S, Guyton K, Lunn R, Loomis D, Rusyn I, et al. HAWC: health assessment Workspace collaborative. Research Triangle Park, NC: National Toxicology Program; 2015.
59. Shapiro AJ, Antoni S, Guyton KZ, Lunn RM, Loomis D, Rusyn I, et al. Software tools to facilitate systematic review used for cancer hazard identification. Environ Health Perspect 2018;126:104501.
60. Yao Y, Chen T, Shen SS, Niu Y, DesMarais TL, Linn R, et al. Malignant human cell transformation of marcellus shale gas drilling flow back water. Toxicol Appl Pharmacol 2015;288:121–30.
61. Abraham DG, Liberatore HK, Aziz MT, Burnett DB, Cizmas LH, Richardson SD. Impacts of hydraulic fracturing wastewater from oil and gas industries on drinking water: quantification of 69 disinfection by-products and calculated toxicity. Sci Total Environ 2023;882:7.
62. Bamberger M, Nell M, Ahmed AH, Santoro R, Ingraffea AR, Kennedy RF, et al. Surface water and groundwater analysis using aryl hydrocarbon and endocrine receptor biological assays and liquid chromatography-high resolution mass spectrometry in Susquehanna County, PA. Environ Sci: Process Impacts 2019;21:988–98.
63. Crosby L, Tatu CA, Varonka M, Charles KM, Orem WH. Toxicological and chemical studies of wastewater from hydraulic fracture and conventional shale gas wells. Environ Toxicol Chem 2018;37:2098–111.
64. Kassotis CD, Nagel SC, Stapleton HM. Unconventional oil and gas chemicals and wastewater-impacted water samples promote adipogenesis via PPARγ-dependent and independent mechanisms in 3T3-L1 cells. Sci Total Environ 2018;640:1601–10.
65. Bain PA, Kumar A. *In vitro* nuclear receptor inhibition and cytotoxicity of hydraulic fracturing chemicals and their binary mixtures. Chemosphere 2018;198:565–73.
66. Zhuang Y, Ji Y, Kuang Q, Zhang Z, Li P, Song J, et al. Oxidation treatment of shale gas produced water: molecular changes in dissolved organic matter composition and toxicity evaluation. J Hazard Mater 2023;452:11.
67. Kassotis CD, Harkness JS, Vo PH, Vu DC, Hoffman K, Cinnamon KM, et al. Endocrine disrupting activities and geochemistry of water resources associated with unconventional oil and gas activity. Sci Total Environ 2020;748:16.
68. Balise VD, Cornelius-Green JN, Kassotis CD, Rector RS, Thyfault JP, Nagel SC. Preconceptional, gestational, and lactational exposure to an unconventional oil and gas chemical mixture alters energy expenditure in adult female mice. Front Endocrinol 2019;10:10.
69. Balise VD, Cornelius-Green JN, Parmenter B, Baxter S, Kassotis CD, Rector RS, et al. Developmental exposure to a mixture of unconventional oil and gas chemicals increased risk-taking behavior, activity and energy expenditure in aged female mice after a metabolic challenge. Front Endocrinol 2019;10:13.
70. Boulé LA, Chapman TJ, Hillman SE, Kassotis CD, O'Dell C, Robert J, et al. Developmental exposure to a mixture of 23 chemicals associated with unconventional oil and gas operations alters the immune system of mice. Toxicol Sci 2018;163:639–54.
71. Kassotis CD, Bromfield JJ, Klemp KC, Meng CX, Wolfe A, Zoeller RT, et al. Adverse reproductive and developmental health outcomes following prenatal exposure to a hydraulic fracturing chemical mixture in female C57Bl/6 mice. Endocrinology 2016;157:3469–81.
72. O'Dell CT, Boule LA, Robert J, Georas SN, Eliseeva S, Lawrence BP. Exposure to a mixture of 23 chemicals associated with unconventional oil and gas operations alters immune response to challenge in adult mice. J Immunot 2021;18:105–17.
73. Sapouckey SA, Kassotis CD, Nagel SC, Vandenberg LN. Prenatal exposure to unconventional oil and gas operation chemical mixtures altered mammary gland development in adult female mice. Endocrinology 2018;159:1277–89.
74. Trickey K, Hadjimichael N, Sanghavi P. Public reporting of hydraulic fracturing chemicals in the USA, 2011–18: a before and after comparison of reporting formats. Lancet Planet Health 2020;4:e178–85.
75. Yost EE, Stanek J, DeWoskin RS, Burgoon LD. Overview of chronic oral toxicity values for chemicals present in hydraulic fracturing fluids, flowback, and produced waters. Environ Sci Technol 2016;50:4788–97.
76. Cairncross ZF, Couloigner I, Ryan MC, McMorris C, Muehlenbachs L, Nikolaou N, et al. Association between residential proximity to hydraulic fracturing sites and adverse birth outcomes. JAMA Pediatr 2022;176:585–92.
77. Stacy SL, Brink LL, Larkin JC, Sadovsky Y, Goldstein BD, Pitt BR, et al. Perinatal outcomes and unconventional natural gas operations in Southwest Pennsylvania. PLoS One 2015;10:15.
78. Brown VJ. Industry issues: putting the heat on gas. Environ Health Perspect 2007;115:A76.
79. Caron-Beaudoin É, Whyte KP, Bouchard MF, Chevrier J, Haddad S, Copes R, et al. Volatile organic compounds (VOCs) in indoor air and tap water samples in residences of pregnant women living in an area of unconventional natural gas operations: findings from the EXPERIVA study. Sci Total Environ 2022;805:150242.
80. Caron-Beaudoin É, Valter N, Chevrier J, Ayotte P, Frohlich K, Verner MA. Gestational exposure to volatile organic compounds (VOCs) in Northeastern British Columbia, Canada: a pilot study. Environ Int 2018;110:131–8.
81. Ferrer I, Thurman EM. Chemical constituents and analytical approaches for hydraulic fracturing waters. Trends Environ Anal Chem 2015;5:18–25.
82. Franklin M, Chau K, Cushing LJ, Johnston JE. Characterizing flaring from unconventional oil and gas operations in South Texas using satellite observations. Environ Sci Technol 2019;53:2220–8.
83. Johnston JE, Chau K, Franklin M, Cushing L. Environmental justice dimensions of oil and gas flaring in south Texas: disproportionate exposure among hispanic communities. Environ Sci Technol 2020;54: 6289–98.
84. Sun Y, Wang D, Tsang DC, Wang L, Ok YS, Feng Y. A critical review of risks, characteristics, and treatment strategies for potentially toxic elements in wastewater from shale gas extraction. Environ Int 2019; 125:452–69.
85. Vengosh A, Kondash A, Harkness J, Lauer N, Warner N, Darrah TH. The geochemistry of hydraulic fracturing fluids. Proc Earth Planet Sci 2017; 17:21–4.

86. Caserta D, Graziano A, Monte GL, Bordi G, Moscarini M. Heavy metals and placental fetal-maternal barrier: a mini-review on the major concerns. Eur Rev Med Pharmacol Sci 2013;17:2198–206.
87. Faniband M, Lindh CH, Jönsson BA. Human biological monitoring of suspected endocrine-disrupting compounds. Asian J Androl 2014; 16:5.
88. Iavicoli I, Fontana L, Bergamaschi A. The effects of metals as endocrine disruptors. J Toxicol Environ Health, Part B 2009;12:206–23.
89. Loomis D, Guyton KZ, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, et al. Carcinogenicity of benzene. Lancet Oncol 2017;18:1574–5.
90. Reutman SR, LeMasters GK, Knecht EA, Shukla R, Lockey JE, Burroughs GE, et al. Evidence of reproductive endocrine effects in women with occupational fuel and solvent exposures. Environ Health Perspect 2002;110:805–11.
91. Webb E, Bushkin-Bedient S, Cheng A, Kassotis CD, Balise V, Nagel SC. Developmental and reproductive effects of chemicals associated with unconventional oil and natural gas operations. Rev Environ Health 2014;29:307–18. 2014/12/06 ed.
92. Miki Y, Ono K, Hata S, Suzuki T, Kumamoto H, Sasano H. The advantages of co-culture over mono cell culture in simulating in vivo environment. J Steroid Biochem Mol Biol 2012;131:68–75.
93. Berg EL, Hsu YC, Lee JA. Consideration of the cellular microenvironment: physiologically relevant co-culture systems in drug discovery. Adv Drug Deliv Rev 2014;69:190–204.
94. Bogdanowicz DR, Lu HH. Studying cell-cell communication in co-culture. Biotechnol J 2013;8:395.
95. Clift MJ, Fytianos K, Vanhecke D, Hočevar S, Petri-Fink A, Rothen-Rutishauser B. A novel technique to determine the cell type specific response within an *in vitro* co-culture model via multi-colour flow cytometry. Sci Rep 2017;7:1–15.
96. Danoy M, Shinohara M, Rizki-Safitri A, Collard D, Senez V, Sakai Y. Alteration of pancreatic carcinoma and promyeloblastic cell adhesion in liver microvasculature by co-culture of hepatocytes, hepatic stellate cells and endothelial cells in a physiologically-relevant model. Integr Biol 2017;9:350–61.
97. Wikswo JP. The relevance and potential roles of microphysiological systems in biology and medicine. Exp Biol Med 2014;239:1061–72.
98. Burkina V, Rasmussen MK, Pilipenko N, Zamaratskaia G. Comparison of xenobiotic-metabolising human, porcine, rodent, and piscine cytochrome P450. Toxicology 2017;375:10–27.
99. Martignoni M, Groothuis GM, de Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expet Opin Drug Metabol Toxicol 2006;2:875–94.
100. Patisaul HB, Fenton SE, Aylor D. Animal models of endocrine disruption. Best Pract Res Clin Endocrinol Metabol 2018;32:283–97.
101. Colborn T, Schultz K, Herrick L, Kwiatkowski C. An exploratory study of air quality near natural gas operations. Hum Ecol Risk Assess 2014;20: 86–105.
102. Litovitz A, Curtright A, Abramzon S, Burger N, Samaras C. Estimation of regional air-quality damages from Marcellus Shale natural gas extraction in Pennsylvania. Environ Res Lett 2013;8:9.
103. Swarthout RF, Russo RS, Zhou Y, Miller BM, Mitchell B, Horsman E, et al. Impact of Marcellus Shale natural gas development in southwest Pennsylvania on volatile organic compound emissions and regional air quality. Environ Sci Technol 2015;49:3175–84.
104. Vinciguerra T, Yao S, Dadzie J, Chittams A, Deskins T, Ehrman S, et al. Regional air quality impacts of hydraulic fracturing and shale natural gas activity: evidence from ambient VOC observations. Atmos Environ 2015;110:144–50.
105. Upadhyay S, Palmberg L. Air-liquid interface: relevant *in vitro* models for investigating air pollutant-induced pulmonary toxicity. Toxicol Sci 2018;164:21–30.
106. Chen L, Lippmann M. Inhalation toxicology methods: the generation and characterization of exposure atmospheres and inhalational exposures. Curr Protoc Toxicol 2015;63:24–4.
107. Indorf P, Patzak A, Lichtenberger F. Drug metabolism in animal models and humans: translational aspects and chances for individual therapy. Acta Physiol 2021;233:5.
108. Zhuang X, Lu C. PBPK modeling and simulation in drug research and development. Acta Pharm Sin B 2016;6:430–40.

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