

Effects of environmental stressors on stem cells

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Abstract

Environmental toxicants are ubiquitous, and many are known to cause harmful health effects. However, much of what we know or think we know concerning the targets and long-term effects of exposure to environmental stressors is sadly lacking. Toxicant exposure may have health effects that are currently mischaracterized or at least mechanistically incompletely understood. While much of the recent excitement about stem cells (SCs) focuses on their potential as therapeutic agents, they also offer a valuable resource to give us insight into the mechanisms and risks of toxicant effects. Not only as a response to the increasing ethical pressure to reduce animal testing, SC studies allow us valuable insight into the true effects of human exposure to environmental stressors under controlled conditions. We present a review of the history of publications on the effects of environmental stressors on SCs, followed by a consolidation of the literature over the past five years on a subset of key environmental stressors of importance to human health and their effects on both embryonic and tissue SCs. The review will make constructive suggestions as to areas of toxicant research where further studies are needed, as well as making indications of the potential utility for advancing knowledge and directing research on environmental toxicology.

Key words: Environmental substances; Toxic; Stem cells; Endocrine disruptors; Alcohols; Tobacco smoking; Metals; Heavy; Particulate matter; Volatile organic compounds; Ozone

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Core tip: Environmental toxicants can cause health effects. While most research and discussion of stem cells focuses on their potential as therapeutic agents, they also offer a valuable resource to give us insight into the mechanism and incidence of the effects of environmental toxicants. We present a review of the history of relevant publications, followed by a consolidation of the literature over the past five years on a subset of key environmental stressors of importance to human health. Constructive suggestions as to the areas of toxicant research where further studies are needed, and indications of the

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potential for advancing knowledge are made.

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INTRODUCTION

Humans are chronically exposed to environmental stressors, pollutants of natural or anthropic origin which can have the effect of altering normal biological processes. While we have long known that many environmental toxicants, such as Lead (Pb) and Mercury (Hg), have deleterious effects on human health, the mechanisms by which these occur are not fully understood^[1]. Further compelling the imperative to more thoroughly understand the biological mechanisms behind these environmental toxicants are recent findings that their effects are transgenerational, echoing resulting outcomes far beyond initial exposure^[2].

While much attention has been given to the therapeutic potential of stem cells (SCs)^[3], their ability to serve as barometers of the toxic effects of environmental stressors should not be understated^[4]. As the body's raw materials, SCs and their responses to environmental insult serve as windows into the pathways of disease. In this review, we highlight this much overlooked intersection of the study of environmental stressors and their impact on SC health.

Both tissue and embryonic SCs (ESCs) are seen as resources for the repair and regeneration of human tissues^[5]. SCs are also thought to maintain these tissues for the lifespan of the individual through their key characteristics of self-renewal and differentiation into specialized cells. Healthy SC function includes balanced cell proliferation and sufficient capacity for appropriate differentiation. We focus on the effects of environmental stressors that impact these operations. We also examine cell viability to analyze toxicity and provide a fuller picture of a toxicant's effect, by correlating cell number to cell behavior^[6,7].

We began by a careful search to numerate the publications examining the effects of environmental stressors on SC populations. The goal was to achieve a better understanding of the history of this research as well as understand how research on individual stressors has changed over that time. We then made a more focused search of PubMed for research in the last five years on known environmental toxicants and SCs.

A list of known environmental agents whose exposure is known to cause adverse health effects in humans was drawn from the National Institute of Environmental Health Sciences (NIEHS)^[8]. These environmental toxicants were cross-referenced with the United States Comprehensive Environmental Response, Compensation, and Liability Act Priority List of Hazardous Substances as outlined in the Agency for Toxic Substances and Disease Registry (ATSDR) 2017 substance priority list (SPL)- "the government's list, in order of priority, of substances most commonly found at waste facility sites on the National Priorities List (NPL) that are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure"^[9]. Each substance on the list is given an impact score, derived from an algorithm, with higher scores denoting those substances that most frequently appear at NPL sites, their known toxicity, and potential for human exposure, and then ranked by order of highest to lowest impact. Findings were summarized into a list of 20 environmental toxicants, including four heavy metals, ten endocrine disruptors, and six other important substances.

Table 1 outlines these toxicants with the corresponding highest rank of each substance within its class in the 2017 ATSDR SPL. For example, Arsenic (As) is listed at a rank of #1, with a score of 1674, derived from an algorithm that factors its ubiquity at NPL sites, its high toxicity, and the large risk for human exposure. The heavy metals, Pb, As, and Hg claim the top three spots on the list while the Volatile Organic Compound (VOC), Vinyl Chloride, ranks #4 on the list. Several other VOCs, like Benzene and Trichloroethylene, are also included on the ATSDR SPL. However, we record the highest ranked substance (Vinyl Chloride) for each toxicant class in our table, along with their score.

LITERATURE SEARCH

A keyword search was then performed in PubMed including the name of the environmental toxicant and SCs, *e.g.*, "Cadmium and stem cells" or "Particulate matter and stem cells" that were published from 2014 - June 2019. For each query, counts were recorded of total number of articles, review articles, and original research articles, and recorded in **Table 2**. On the topic of radiation and SCs, only those articles which treated radiation as an environmental exposure, as opposed to a tool for directed differentiation, were included. Our findings are classified again into three different classes: heavy metals, endocrine disruptors, and other environmental toxicants known to have deleterious human health effects. A separate PubMed search was

Table 1 Toxicants and their highest rank on the 2017 ATSDR substance priority list¹

Toxicant	Rank of highest scored class member	ATSDR score	Toxicant	Rank of highest scored class member	ATSDR score
Heavy metals:					
Lead (Pb)	2	1531	Mercury	3	1458
Arsenic	1	1674	Cadmium	7	1320
EDs:					
PAHs	8	1306	Organotins	Not Rated	-
OCs	37	1049	DDT	13	1183
BPA	Not Rated	-	DES	Not Rated	-
Dioxins	72	941	PCBs	5	1345
Phthalates	58	995	PFAS	143	788
Other environmental toxicants:					
Radiation	Not Rated	-	Particulate Matter	Not Rated	-
Alcohol	Not Rated	-	Ozone	Not Rated	-
Tobacco Smoking	Not Rated	-	VOCs	4	1358

¹Toxicants listed in order of number of PubMed counts listed in Table 2. ATSDR: Agency for Toxic Substances and Disease Registry; EDs: Endocrine disruptors; PAHs: Polycyclic aromatic hydrocarbons; OCs: Organophosphorus compounds; BPA: Bisphenol A; DDT: Dichlorodiphenyltrichloroethane; DES: Diethylstilbestrol; PCBs: Polychlorinated biphenyls; PFAS: Per- and polyfluoroalkyl substances; PM: Particulate matter; VOCs: Volatile organic compounds.

Table 2 Counts from PubMed¹ searches from January 2014 - June 2019

Toxicant	Total number of articles	Review articles	Original research articles	Toxicant	Total number of articles	Review articles	Original research articles
Metals:							
Lead (Pb)	4436	1239	3197	Mercury	22	0	22
Arsenic	108	11	97	Cadmium	44	2	42
EDs:							
PAHs	944	22	922	Organotins	24	0	24
OCs (Pesticides)	430	31	399	DDT	11	1	10
BPA	84	15	69	DES	10	5	5
Dioxins	44	9	35	PCBs	8	1	7
Phthalates	36	2	34	PFAS	2	0	2
Other environmental toxicants:							
Radiation	4302	589	3713	PM	61	3	58
Alcohol	1760	112	1648	Ozone	17	0	17
Tobacco Smoking	188	34	154	VOCs	11	0	11

¹PubMed.gov, the United States National Library of Medicine National Institutes of Health. ED: Endocrine disruptors; PAHs: Polycyclic aromatic hydrocarbons; OCs: Organophosphorus compounds; BPA: Bisphenol A; DDT: Dichlorodiphenyltrichloroethane; DES: Diethylstilbestrol; PCBs: Polychlorinated biphenyls; PFAS: Per- and polyfluoroalkyl substances; PM: Particulate matter; VOCs: Volatile organic compounds.

conducted for each of the 20 toxicants, including the substance name and SC, *e.g.*, “Phthalates and stem cells” without a time limitation. Key class members’ data are presented in Figure 1-3: Separated as Figure 1. Heavy metals, Figure 2. Endocrine disruptors, and Figure 3. Other environmental toxicants. The figures illustrate PubMed publication counts for the substances plotted against time in years, from 1953, the earliest publication on SCs and one of the listed environmental toxicants, up until June 2019. The findings of our PubMed literature review from 2014 - June 2019 are presented in Table 3-5, with toxicants organized again into three classes: Table 3; Heavy metals, Table 4; Endocrine disruptors, and Table 5. Other environmental toxicants. The results are presented by mechanism of action as pertaining to SC viability, differentiation, and proliferation. Each table is then followed by a more focused discussion of key references, the mechanism of action and their associated health outcomes.

HEAVY METALS

Heavy metals are ubiquitous in the human environment and their toxicity is associated with varied adverse health effects depending on the dose, route and duration of exposure. Of the top ten chemicals on the 2017 ATSDR SPL, four of these are heavy metals: As, Pb, Hg, and Cadmium (Cd)^[9].

Pb is a persistent environmental toxin that for more than a hundred years has been known to have harmful human health effects^[10]. Pb exposure in neural SCs (NSCs) was shown to slightly reduce cell proliferation^[11]. Pb exposure was also shown to induce changes in microgliosis and astrogliogenesis in the hippocampus of mice, interfering with normal

Table 3 Heavy metals and their effects on stem cells

Environmental Toxicant	Type of stem cell	Model	In vivo/ In vitro	Parameters ¹	Ref.	Environmental Toxicant	Type of stem cell	Model	In vivo/ In vitro	Parameters ¹	Ref.
Lead	Fetal germ	H	<i>In vivo</i>	↑DNA methylation changes	[2]	Arsenic	Adipose-derived MSC	M	<i>In vivo</i>	↓Differentiation	[15]
Lead	Neural progenitor	H	<i>In vitro</i>	↓Proliferation	[11]	Arsenic	Induced pluripotent stem cell	H	<i>In vitro</i>	↓Viability, ↑DNA damage	[16]
Lead	ESC	H	<i>In vitro</i>	↑Neuronal differentiation changes	[77]	Mercury	HSC	M	<i>In vivo</i>	↓Proliferation at high-doses ↑Proliferation at low-doses	[17]
Lead	Bone marrow-derived MSC	R	<i>In vitro</i>	↓Osteogenesis	[13]	Mercury	Neural progenitor	M	<i>In vivo</i>	↓Differentiation	[18]
Lead	Neural stem	M	<i>In vitro</i>	↑Astroglialogenesis, ↑Microgliosis	[12]	Cadmium	Neural progenitor	H	<i>In vitro</i>	↓Proliferation, ↑Apoptosis	[19]
Arsenic	ESC	M	<i>In vitro</i>	↓Differentiation	[78]	Cadmium	HSC	M	<i>In vivo</i>	↓Differentiation potential, ↑Myelopoiesis	[20]

¹For detailed information on parameters, see text below. H: Human; R: Rat; M: Mouse; ESC: Embryonic stem cells; HSC: Hematopoietic stem cells; MSC: Mesenchymal stem cells.

Table 4 Endocrine disruptors and their effects on stem cells

Environmental Toxicant	Type of stem cell	Model	In vivo/ In vitro	Parameters ¹	Ref.	Environmental Toxicant	Type of stem cell	Model	In vivo/ In vitro	Parameters ¹	Ref.
PAHs	Neural progenitor	R	<i>In vitro</i>	↑Proliferation, ↓Cell size	[23]	Dioxins	HSC	M	<i>In vivo</i>	↑Cell number, ↓Lymphocyte differentiation	[33]
PAHs	HSC	H	<i>In vitro</i>	↓Osteoblast differentiation, ↓Self-renewal	[24]	Dioxins	HSC	M	<i>In vitro</i>	↓Long-term self-renewal	[34]
PAHs	Spermatogonial stem	M	<i>In vivo</i>	↑Mutations	[25]	Phthalates	HSC	H	<i>In vitro</i>	↓Viability	[6]
PAHs	Adipose-derived MSC	C	<i>In vitro</i>	↓Adipocyte differentiation potential	[26]	Phthalates	Neural progenitor	M	<i>In vitro</i>	↓Viability, ↑ROS, ↑Apoptosis	[36]
PAHs	Skeletal muscle-derived progenitor	H	<i>In vitro</i>	↓Myogenic differentiation	[27]	Phthalates	ESC	M	<i>In vitro</i>	↓Viability	[37]
OCs	Neural progenitors derived from human embryonal carcinoma stem	H	<i>In vitro</i>	↓Viability	[28]	Organotins	Spermatogonial stem	H	<i>In vitro</i>	↑Apoptosis	
Bisphenol A	Mammary epithelial stem	H	<i>In vitro</i>	↑Proliferation, ↑Sphere-forming capability	[30]	Organotins	Bone marrow MSC	M	<i>In vitro</i>	↑Adipogenesis, ↓Osteogenesis	[38]
Bisphenol A	Prostate epithelial stem	R	<i>In vitro</i>	↑Proliferation	[31]	Organotins	Bone marrow MSC	M	<i>In vitro</i>	↑Adipogenesis	[39]
Bisphenol A	Bone marrow MSC	H	<i>In vitro</i>	↑Cytotoxicity	[79]	DDT	Bone marrow MSC	H	<i>In vitro</i>	↑Proliferation, ↑Differentiation, ↓Morphological changes	[41]
Dioxins	Umbilical cord blood-derived iPSC	H	<i>In vitro</i>	↑Differentiation	[80]	DES	Spermatogonial stem	M	<i>In vitro</i>	↑DNA damage, ↑Apoptosis	[43]
Dioxins	Cord blood derived HSC	H	<i>In vitro</i>	↓Lymphopoiesis	[32,81]	PCBs	Liver epithelial stem-like	R	<i>In vitro</i>	↑Alterations in gene signaling	[46]
Dioxins	Bone marrow MSC	M	<i>In vitro</i>	↓Osteogenesis	[35]	PFAS	Spermatogonial stem	H	<i>In vitro</i>	↓Expression of spermatogonial markers	[48]

¹For detailed information on parameters, see text below. PAHs: Polycyclic aromatic hydrocarbons; OCs: Organophosphorus compounds; BPA: Bisphenol A; DDT: Dichlorodiphenyltrichloroethane; DES: Diethylstilbestrol; HSC: Hematopoietic stem cells; MSC: Mesenchymal stem cells; iPSC: Induced pluripotent stem cells; ESC: Embryonic stem cells; PCBs: Polychlorinated biphenyls; PFAS: Per- and polyfluoroalkyl substances; H: Human; R: Rat; C: Canine; M: Mouse; ROS: Reactive oxygen species.

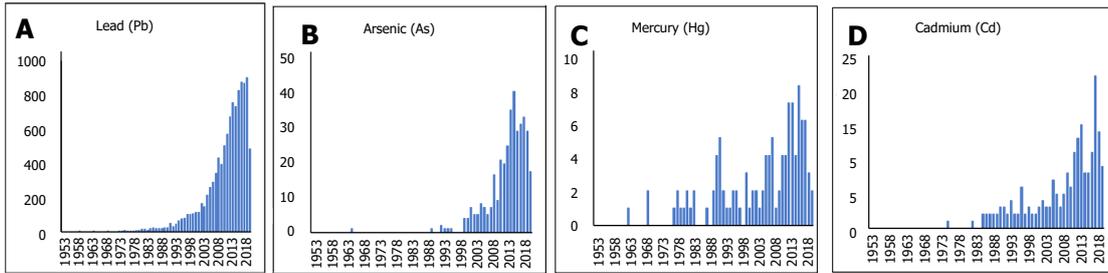


Figure 1 Heavy metal PubMed publication counts from 1953 - June 2019. A: Counts resulting from a PubMed search of the term, “Lead Pb and Stem Cells” plotted against time in years; B: Counts resulting from a PubMed search of the term, “Arsenic and Stem Cells” plotted against time in years; C: Counts resulting from a PubMed search of the term, “Mercury and Stem Cells” plotted against time in years; D: Counts resulting from a PubMed search of the term, “Cadmium and Stem Cells” plotted against time in years.

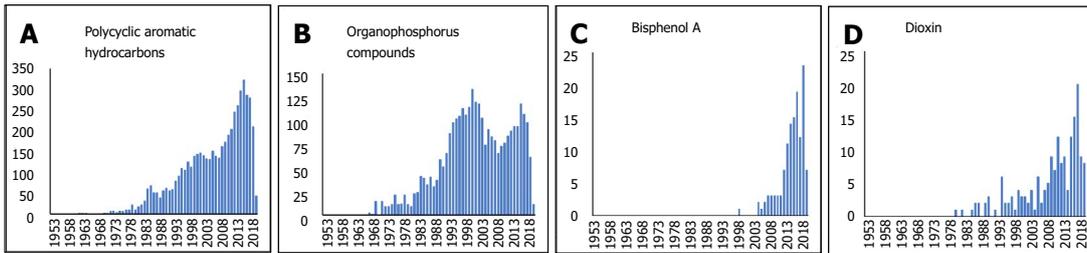


Figure 2 Endocrine disruptor PubMed publication counts from 1953 - June 2019. A: Counts resulting from a PubMed search of the term, “Polycyclic Aromatic Hydrocarbons and Stem Cells” plotted against time in years; B: Counts resulting from a PubMed search of the term, “Organophosphorus Compounds and Stem Cells” plotted against time in years; C: Counts resulting from a PubMed search of the term, “Bisphenol A and Stem Cells” plotted against time in years; D: Counts resulting from a PubMed search of the term, “Dioxins and Stem Cells” plotted against time in years.

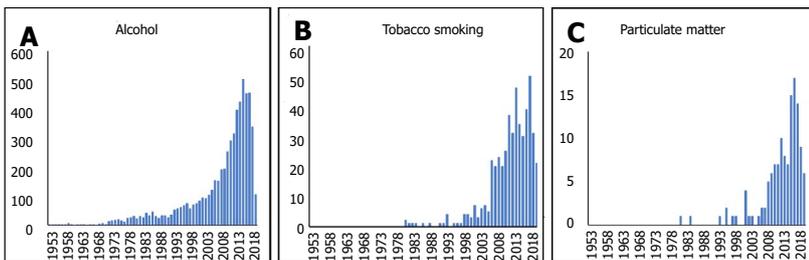


Figure 3 Other environmental toxicants PubMed publication counts from 1953 - June 2019. A: Counts resulting from a PubMed search of the term, “Alcohol and Stem Cells” plotted against time in years; B: Counts resulting from a PubMed search of the term, “Smoking and Stem Cells” plotted against time in years; C: Counts resulting from a PubMed search of the term, “Particulate Matter and Stem Cells” plotted against time in years.

neurogenesis^[12]. Bone marrow-derived mesenchymal SCs (BM-MSCs) in rats showed an inversely proportional relationship between osteocalcin expression, a gene key to osteogenesis and Pb intake^[13]. In a striking finding, DNA methylation changes in the fetal germ cells of pregnant mothers exposed to Pb were carried over to her grandchildren^[2].

As is a known human carcinogen and is rated as the number one substance of concern on the ATSDR’s 2017 SPL. It is a naturally occurring element that when combined with oxygen, chlorine, or sulfur can form inorganic As compounds. In embryonic mouse SCs, As inhibited differentiation into neurons and myotubes^[14]. Differentiation, specifically osteogenesis and chondrogenesis, was also decreased in murine adipose-derived MSCs (AD-MSCs) after exposure to inorganic As^[15]. In human induced pluripotent SCs, As exposure was shown to create a dose-dependent sequence of morphology changes, a decrease in viability, and induced genotoxicity^[16].

Hg is a heavy metal with known associations to neuroinflammation, immunotoxicity, behavioral disorders, and adverse kidney effects. Mice exposed to 50 µmol of HgCl₂ experienced an increase in hematopoietic SC (HSC) proliferation, while those exposed to the higher dose of 100 µmol HgCl₂ saw HSC suppression^[17]. Hg exposure suppressed embryonic murine NSCs neural differentiation at as low as 10 pmol concentration within 7 d and inhibited neural and glial differentiation by day 14. Moreover, Hg concentrations over 100 pmol suppressed NSC differentiation to motor or dopaminergic neurons^[18].

Cd is a naturally occurring toxic metal in the earth’s crust, typically extracted as a byproduct in the mining for other metals. It is commonly found in batteries, dyes, and some metal and plastic products. Low levels of Cd exposure decreased cell number and proliferation and induced apoptosis in adult human neural progenitor cells (NPCs)^[19]. HSCs exposed to Cd over three months experienced an increase in long-term HSCs, a loss in long-term potential, and promoted

Table 5 Other environmental toxicants and their effects on stem cells

Environmental Toxicant	Type of stem cell	Model	In vivo/In vitro	Parameters ¹	Ref.	Environmental Toxicant	Type of stem cell	Model	In vivo/In vitro	Parameters ¹	Ref.
Radiation, Ionizing	HSC	H	In vitro	↑ROS, ↑Apoptosis, ↑Senescence, ↓Long-term renewal	[49,50]	Particulate Matter	Bone marrow MSC	M	In vivo	↑ROS, ↓Proliferation	[61]
Radiation, Radiofrequency	HSC	H	In vitro	↓DNA damage	[51]	Particulate Matter	HSC	H	In vivo	↓Telomere length	[62]
Alcohol	HSC	M	In vivo	↑DNA double stranded breaks, ↑Chromosome rearrangement, ↑Myelopoiesis	[52]	Ozone (O3)	Adipose-derived MSC	H	In vitro	↑ROS, ↑Lipid accumulation	[64]
Alcohol	Intestinal stem	M	In vivo	↓Differentiation	[53]	VOCs	Bone marrow HSC	M	In vivo	↑Apoptosis, ↓Nucleated bone marrow cells	[67]
Alcohol	ESC	H	In vitro	↑Differentiation	[54]	VOCs	Enhanced eosinophil/basophil progenitor	H	In vivo	↑Differentiation	[65]
Cigarette smoke	ESC	M	In vitro	↑Apoptosis, ↓Viability	[59]	VOCs	Neural progenitor	M	In vitro	↑Cytotoxicity	[66]
Cigarette smoke	Bone marrow MSC	H	In vitro	↓Differentiation, ↓Morphological changes	[58]						

¹For detailed information on parameters, see text below. HSC: Hematopoietic stem cells; ESC: Embryonic stem cells; MSC: Mesenchymal stem cells; VOCs: Volatile organic compounds; H: Human; M: Mouse; ROS: Reactive oxygen species.

myelopoiesis^[20].

Endocrine disruptors are chemicals or chemical mixtures that interfere with the proper function of hormones and can be naturally occurring, such as phytoestrogens, or synthesized as in plastics, plasticizers, pesticides, fungicides, and pharmaceuticals^[21]. In this review, we include organophosphorus compounds (OPs), polycyclic aromatic hydrocarbons (PAHs), bisphenol A (BPA), dioxins, phthalates, organotins, dichlorodiphenyltrichloroethane (DDT), diethylstilbestrol (DES), polychlorinated biphenyls (PCBs), and per- and polyfluoroalkyl substances (PFAS). PAHs are highly persistent organic compounds primarily released through both naturally occurring and man-made combustion, such as smoking or burning of fuel^[22]. NSCs exposed to the PAH, benzo(a)pyrene (BaP), showed impairment in the transition from cell replication to neurodifferentiation, resulting in higher cell number, but reduced cell size and damaged neuronal features such as neurite formation and the development of dopamine and acetylcholine phenotypes^[23]. BaP also decreased self-renewal and osteoblast differentiation of human BM-MSCs^[24]. Mice exposed orally to BaP experienced spermatogonial SC (SSC) mutations with different phases of spermatogenesis exhibiting varying sensitivities to BaP^[25]. In AD-MSCs, BaP did not inhibit cell proliferation, but did significantly inhibit adipocyte differentiation potential^[26]. In human skeletal muscle-derived progenitor cells, low doses of BaP repressed myogenic differentiation without causing cell toxicity. When BaP exposure was withdrawn, the inhibitory effects on myogenesis were reversed^[27].

OPs include the highly toxic nerve agent, sarin, as well as commonly used pesticides because of their inhibition of acetylcholinesterase. NPCs exposed to the OP pesticides paraoxon and mipafox during retinoic acid-induced differentiation showed reduced cell viability at high concentrations. Only paraoxon was shown to alter the process of neurodifferentiation^[28].

The concern over the harmful health effects of BPA, a xenoestrogen used in the making of plastics, has been widely popularized in the media, leading the United States Food and Drug Administration to abandon its endorsement of its use in baby bottles^[29]. BPA is also commonly found in sports equipment, food and beverage packaging, and thermal paper products. In BM-MSCs, BPA exposure led to a dose-responsive increase in cytotoxicity, along with increased lipid peroxidation. BPA altered the response of proteins key in the regulation of fate and differentiation of human mammary epithelial SCs^[30]. Low level BPA exposure altered differentiation of prostate epithelial SCs toward basal progenitors, reducing commitment to luminal progenitor cells, while increasing SC size and proliferation^[31].

Dioxins include chlorinated dibenzo-p-dioxins, chlorinated dibenzofurans and certain PCBs. Dioxins are highly toxic, persistent compounds that are typically released into the environment through industrial incineration and bleaching processes. Exposure to the dioxin 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the most toxic dioxin, was shown to impair human B cell development by reducing lineage commitment in HSCs^[32]. TCDD was also

shown to suppress hematopoietic progenitor cells and accelerate their differentiation to mature lineages^[32]. HSCs in the fetal livers of offspring of TCDD-exposed mice experienced increased HSC proliferation, but B and T lymphocyte differentiation was significantly decreased^[33]. TCDD exposure activated the aryl hydrocarbon receptor (AHR) in the fetus of pregnant mice, leading to impairment of the long-term self-renewal of HSCs^[34]. In BM-MSCs, AHR activation by TCDD decreased osteoblast differentiation, possibly by suppressing the expression of the protein, β -catenin^[35].

Phthalates are a group of chemicals primarily used to soften plastics and due to the prevalence of plastic products, exposure is widespread in the United States population. HSCs were exposed to four phthalates: dibutyl phthalate (DBP), benzylbutyl phthalate, diethyl phthalate (DEP), and diethylhexyl phthalate (DEHP), and all four compounds were shown to reduce cell viability^[6]. NSCs exposed to DEHP also showed reduced cell viability, along with increased apoptosis due to reactive oxidative stress^[36]. DEP and DBP also reduced cell viability in the ESCs of mice^[37].

Organotins include tributyltin (TBT) and triphenyltin, substances with strong antibacterial and fungicidal properties that were historically used as marine anti-fouling additives in paint. Baker *et al.*^[38] showed that in BM-MSCs, TBT activated adipogenesis and reduced osteogenesis. This finding was also supported in primary BM-MSCs for both TBT and triphenyltin^[39].

DDT, an organochlorine developed as an insecticide was banned in the United States in 1972 after public outcry over its health effects, most notably outlined in Rachel Carson's famous work, *Silent Spring*^[40]. However, DDT is a persistent organic pollutant, maintaining long-term exposure to organisms in the soil. When BM-MSCs were exposed to DDT, they exhibited altered morphology and inhibited self-renewal capacity, along with dose-dependent increased proliferation and differentiation^[41].

DES is a synthetic estrogen that was prescribed by the United States physicians from 1938-1971, to prevent miscarriages and avoid other pregnancy problems, but was later found to cause a rare vaginal cancer to girls exposed in utero^[42]. DES exposure in SSCs increased DNA damage, induced apoptosis, and increased intracellular superoxide anions^[43].

PCBs are intentionally produced, stable aromatic chlorinated hydrocarbons commonly used as coolants in electrical equipment, as lubricants and plasticizers. Some PCBs are "dioxin-like" (DL-PCBs) and others are non-dioxin-like (NDL-PCBs), with the distinction owing to the site of chlorine substitution on the phenyl rings. DL-PCBs have congeners with no or only one chlorine substitution in the ortho position, have toxic effects similar to dioxins and bind strongly to the AHR^[44]. The remaining NDL-PCB congeners, who have been linked through epidemiological studies with prostate cancer, have unique toxic effects and thus we examine them as a separate category of toxicant^[45]. Human exposure occurs from improper storage and spillage of PCBs, where they bind strongly to soil and enter food sources. Liver progenitor cells exposed to the NDL-PCB, PCB153, experienced significant changes in the S1P/ceramide (Cer) ratio, known to be crucial in determining cell fate^[46].

PFAS are a group of manmade chemicals that are used in including stain- and water-repellent fabrics, nonstick coatings, polishes, paints, and fire-fighting foams. They are very persistent compounds and thus can be found in water, soil, and organisms. Epidemiological studies have linked PFAS exposure to increased cholesterol levels^[47]. SSCs exposed to PFAS did not experience a decrease in germ cell viability, an increase in reactive oxygen species (ROS), or reduced cell viability^[48].

RADIATION

Ionizing radiation, a common treatment for cancer, is known to induce ROS by altering cellular metabolism and is known to reduce numbers of bone marrow HSCs and alter differentiation^[49,50]. However, the lower-intensity radiofrequency radiation, now rampant in society due to increased cell-phone and electronic use was shown to have only a mild effect on DNA damage and no effect on HSC apoptosis, ROS, cell cycle or DNA repair^[51].

ALCOHOL

Alcohol, known by its chemical name, ethanol, is mainly consumed via liquor, wine, and beer in order to create a psychoactive effect. Acetaldehyde, an endogenous and alcohol-derived metabolite, was shown to create DNA double-stranded breaks in HSCs. These breaks altered homeostasis by stimulating recombination repair, causing chromosome rearrangements, and inducing myelopoiesis^[52]. Chronic alcohol consumption in mice was also shown to disrupt homeostasis in intestinal SCs (ISCs), in part, through the β -catenin pathway, suppressing proliferation of ISCs^[53]. In human ESCs, alcohol was shown to stimulate differentiation by increasing the influx and metabolism of retinol^[54].

TOBACCO SMOKING

Cigarette smoking and the associated nicotine exposure is a notorious carcinogen and is the leading cause of preventable death in the United States^[55]. While tobacco smoke is also considered a VOC and its smoke is known to contain PAHs, we treat it separately here due to its known severe health implications. Smoking is known to induce oxidative damage in SCs^[56]. AD-MSCs exposed to cigarette smoke extract had significant impairment to cell viability, proliferation, and experi-

enced genetic level variations in differentiation^[57]. Moreover, electronic cigarette extract exposure showed detrimental effects on BM-MSC morphology and proliferation^[58]. ESCs exposed to cigarette smoke condensate produced altered gene expressions, reduced viability, and induced apoptosis^[59].

It is important to note that PAHs are known to be present in cigarette smoking. However, for the purpose of this review, we have examined the effects of cigarette smoking and PAH exposure on SCs separately.

PARTICULATE MATTER

Particulate matter (PM) are fine particles of air pollution whose potential to harm health is directly related to their size and capacity for inhalation^[60]. Cui and colleagues showed that PM induces ROS, causing a suppression of *in vivo* proliferation in BM-MSCs^[61]. Moreover, more recent and higher concentrations of PM_{2.5} exposure in workers *via* welding fumes were shown to significantly reduce telomere length in HSCs^[62].

OZONE

Ozone (O₃) is a highly unstable toxic gas present in low levels in the atmosphere known to cause oxidative stress. However, there has been recent mechanistic evidence to suggest that low concentrations of O₃ may be therapeutic in some diseases^[63]. AD-MSCs exposed to high concentrations of O₃ experienced cell damage *via* ROS, but low concentrations (5, 10 µg O₃/mL) had no effect on viability. Further O₃ exposure promoted adipogenesis^[64].

VOCS

VOCs are a large group of organic chemicals who disperse easily into surrounding air due to their high vapor pressure at standard air pressure. They are abundant in building materials, paints, and are produced in the burning of fossil fuels, and include third-hand smoke, the residue left behind on surfaces after smoking.

Infant eosinophil/basophil progenitor cell (Eo/B) viability was positively associated with VOC exposure, which contrasted from maternal Eo/B cells, which showed few to no associations^[65]. This increase of HSCs in infants due to environmental exposure suggests an enhanced risk of the development of respiratory outcomes. NSCs exposed to acrolein, a VOC present in third hand smoke, experienced high rates of cytotoxicity, altered regulatory gene expression, inhibited proliferation at low doses, and cell death at high doses^[66].

Formaldehyde (FA), a VOC commonly found in building materials and paints, significantly reduced nucleated bone marrow cells, and increased apoptosis in HSCs. These results suggest that FA's toxic effects operate by altering myeloid progenitor growth and survival through oxidative damage and reduced gene expression levels^[67].

DISCUSSION

The figures illustrating PubMed publication counts for key environmental toxicants attempt to describe the onset and subsequent pattern of research interest. There was little interest in Pb from the 1960s until the late 80s' and then a precipitous explosion of publications that is still climbing. The capture of the public's attention with the water supply crisis which started in Flint, Michigan in 2014, coupled with the headline grabbing work exploring the transgenerational effects^[2], guarantee that Pb will remain a highly investigated toxicant for the foreseeable future. Compare now the recent onset of research on the heavy metal with an even higher ATSDR substance priority score, As. At the peak of publication number, six years ago in 2013, there was not even a twentieth of the number of publications on Pb. Hg and Cd have even fewer publications, with almost as high ATSDR substance priority scores as Pb and As. The difference is even more striking when we switch attention to publication in just the last five years. In that time, no review articles exist on the effect of Hg on SCs compared to over a 1000 on the effects of Pb.

Research on PAHs and OCs began in the late 60s' and show an interesting hump of activity peaking in the late 90s' followed by another peak in 2012-2013. BPA has only very recently begun to capture public attention and we confidently predict a steep increase in publications over the next few years. It is less clear why the dioxins on which research has existed for longer has yet to see an increase in publication activity.

Publications on the effects of alcohol on SC populations began in the 50s and show a broadly similar increase to that of Pb until a peak in 2015. More surprising is that the research on tobacco smoking garners only a tenth of the publications of alcohol in spite of constant public attention to the deleterious effects, with research appearing to have plateaued as of 2013. No reviews have been published on the effects of O₃ or VOCs on SCs in the last five years. To further emphasize the disparity in publication activity, Figure 4 plots the number of PubMed Original Research Articles published between January 2014 to June 2019 against the toxicant's ATSDR substance priority score. The regression line hopefully helps indicate those substances that are under-researched.

There are a number of reasons that contribute to the disparity including, but not limited to: Difficulty of isolation and maintenance of a given toxicant; Issues related to organic *vs.* inorganic form; Difficulty of administration in an *in vitro in vivo* preparation; and of course,

Table 6 Proposed under-researched opportunities for stem cell models on environmental exposures

Environmental toxicant	Health outcomes	Stem cell model	Environmental toxicant	Health outcomes	Stem cell model
Heavy metals					
Pb	Decreased child cognition	Neural progenitor and SC-derived organoids	Cd	Kidney	Renal epithelial stem Nephron progenitor
Pb	Adult liver function	SC-derived organoids	Cd	Lung damage	Alveolar epithelial progenitor
As	Carcinogen: all tissues	Epigenetic analysis of different tissue populations	Cd	Lower bone strength	MSC
Hg	Cognitive function	Neural progenitor and SC-derived organoids			
Endocrine disruptors					
PAHs	Carcinogen: lung, skin	Epigenetic analysis of different tissue populations	Organotins	Liver	SC-derived organoids
OCs (Pesticides)	Cognition	Neural progenitor and SC-derived organoids		Kidney	Renal epithelial stem Nephron progenitor
BPA	Unclear		DDT	Carcinogen	Epigenetic analysis of different tissue SC populations
Dioxins	Carcinogen	Epigenetic analysis of different tissue SC populations	PCBs	Immune system	HSC derived populations
Phthalates	Carcinogen	Epigenetic analysis of different tissue SC populations	PCBs	Carcinogen	Epigenetic analysis of different tissue SC populations
	Cognition	Neural progenitor and SC-derived organoids		Cognition	Neural progenitor and SC-derived organoids
Organotins	Carcinogen	Epigenetic analysis of different tissue SC populations	PFAS	Unclear	
Organotins	CNS	Neural progenitor and SC-derived organoids			
Other toxicants					
Particulate matter	Unclear		VOCs	Unclear	
Ozone	Constricted breathing	SC-derived smooth muscle			

PAHs: Polycyclic aromatic hydrocarbons; OCs: Organophosphorus compounds; Pb: Lead; As: Arsenic; Cd: Cadmium; Hg: Mercury; BPA: Bisphenol A; DDT: Dichlorodiphenyltrichloroethane; DES: Diethylstilbestrol; PCBs: Polychlorinated biphenyls; PFAS: Per- and polyfluoroalkyl substances; VOCs: Volatile organic compounds.

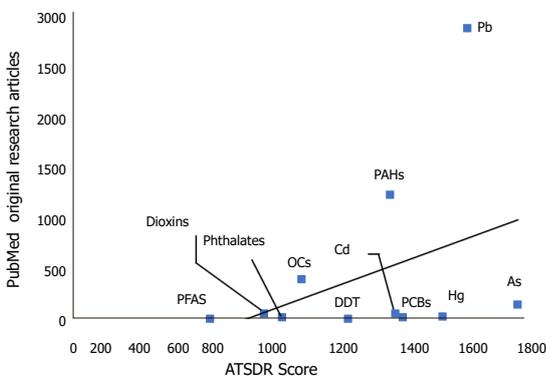


Figure 4 Agency for toxic substances and disease registry score vs number of PubMed original research articles 2014 - June 2019. ATSDR: Agency for Toxic Substances and Disease Registry; PAHs: Polycyclic Aromatic Hydrocarbons; OCs: Organophosphorus Compounds; Pb: Lead; As: Arsenic; Cd: Cadmium; Hg: Mercury; DDT: Dichlorodiphenyltrichloroethane; PCBs: Polychlorinated Biphenyls; PFAS: Per- and polyfluoroalkyl substances.

obtaining of funding to pursue research on a given toxicant.

Finally, in Table 6, we consider what if any are the clear and most prevalent health outcome(s) associated with each toxicant exposure and identify the most appropriate SC or SC-derived model for further research given the phase of life associated with that health outcome.

The tables of the toxicants and their effects on SCs represent a comprehensive consolidation of the references on the effects of environmental toxicants on SCs over the last five years. A note of qualification is required. It is tempting to conclude that any effect of a toxicant on a cell population is negative. Anyone who has worked in this field for any length of time likely, like one of us, has entire data sets that could not attract funding for further analysis because the interpretation of the data suggested that the “toxicant” exposure had what *prima facie* appeared a positive effect on the population under examination (Parker, Unpub. Obsn.)^[68]. However, it is important to note that, for example, an increase in proliferation in a cell population does not necessarily mean the exposure’s effect is beneficial. The result without a proper developmental investigation defies proper interpretation. But for the purposes of this review, and for the field of toxicology, that the toxicant has an effect at a level that can be reasonably be expected to be experienced by the target tissues is sufficient to indicate that the toxicant requires further study.

One of the major challenges to environmental toxicology today is quantifying the joint impact of environmental mixtures on health outcomes to more closely resemble real-world exposure^[69]. We have tried to be as structured as possible in our classifications and groupings but inevitably certain of our “environmental toxicants” are themselves a mixture of active ingredients. An excellent recent review on the suitability of *in vitro* SC preparation for high throughput screening of mixtures was published by Liu *et al.*^[70].

A relatively recently acknowledged challenge is studying the combined effects of chemical and social stressors^[71]. Such issues appear to be a problem not tractable by an *in vitro* cell preparation. However, one can imagine comparing cell samples obtained from carefully selected subject populations to begin to ask questions of how socioeconomic status, proximity to industrial pollutants, and occupation, affects response to a subsequent stressor. Further, how cells obtained from subjects during specific stages of life may usefully inform particular risk.

Finally, the effects of radiation and SC populations rightly have focused on the role of radiation in the treatment of patients with cancer. However, particularly as attention shifts again to exploration of low earth orbit travel and beyond, researchers are turning their attention to how such travel and potential settlement will affect human physiology^[72,73].

Almeida-Porada *et al.*^[74] explored how the effects of radiation on the bone marrow niche can negatively impact hematopoiesis due to changes in MSC function independently of direct effect on HSCs.

It is worth emphasizing that our intent is not to say that all environmental toxicant effects on human health are mediated by SCs. But understanding the effects, or lack thereof, are an important part of the process of dermining mechanism, and potential interventions to ameliorate or prevent deleterious health impacts of exposure. Such models might in this regard be the “canary in the coal mine” as a more sensitive test for toxicity than other cell populations, *e.g.*, fibroblasts^[75]. In this direction, Liang, Yin, and Faiola present a comprehensive review on developmental neural toxicity and environmental toxicants^[76].

Industries manufacturing chemicals have a financial and legal duty to their shareholders to develop their technologies to maximize profit. The rapidity with which new chemicals appear in our environment outpaces the ability of interested parties to test and demonstrate potential deleterious impact using existing models. SC models offer a fast and robust model that can be at least a first indicator of the need for more laborious, time consuming and resource-expensive testing.

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