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Review

Cellular and physiological mechanisms of halogenated and organophosphorus flame retardant toxicity

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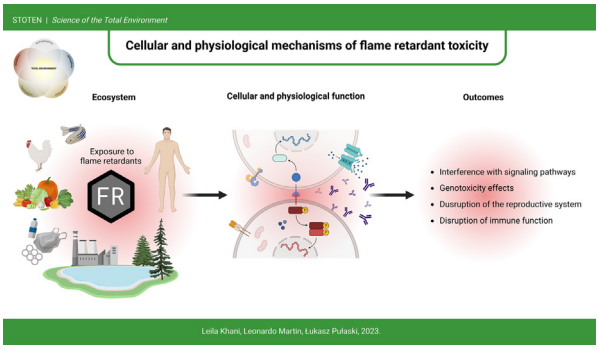
HIGHLIGHTS

- Flame retardants (halogenated or organophosphorus) have common biological toxicity targets despite their chemical diversity.
- Hydrophobic flame retardants can alter membrane parameters and signaling pathways in cells, leading to multi-tissue toxicity.
- DNA damage, impaired repair and epigenetic patterns can result from flame retardant bioactivity and cause genotoxic effects.
- Cellular differentiation, reproductive function and embryonic development can be impacted by flame retardant exposure.
- Flame retardants disrupt innate and adaptive immune systems causing too weak or too strong response toward pathogens.

GRAPHICAL ABSTRACT



Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; ASD, autism spectrum disorders; BCEP, bis (2-chloroethyl) phosphate; BCIPP, bis(1-chloro-2-propyl)phosphate; BDCIPP, bis(1,3-dichloro-2-propyl)phosphate; BDE-3, bromodiphenyl ether; BDE-4, 2,2′,4,4′-tetrabromodiphenyl ether; BDE-47, brominated diphenyl ethers-47; BDE-49, brominated diphenyl ethers-49; BDE-153, brominated diphenyl ethers-153; BDE-209/DBDE, decabromodiphenyl ether; BMP, 2,2-bis(bromomethyl)-1,3-propanediol; BPA, bisphenol A; BPDP, *tert*-butylphenyl diphenyl phosphate; BTBPE, 1,2-bis(2,4,6-tribromophenoxy) ethane; CNS, central nervous system; CVB3, coxsackievirus B3; DBDPE, decabromodiphenyl ethane; DCs, dendritic cells; DE-71/DE-79, polybrominated diphenyl ether mixtures; DSBs, double-strand breaks; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; FR, flame retardant; HBCD, hexabromocyclododecane; HSA, human serum albumin; MOMP, mitochondrial outer membrane permeabilization; MQ, macrophage; MSCs, mesenchymal stromal cells; mtDNA, mitochondrial DNA; NHEJ, non-homologous end joining; OBDE, octabromodiphenylether; PBB, polybrominated biphenyl; PBDE, polybrominated diphenyl ether; PBMCs, peripheral blood mononuclear cells; PBP, pentabromophenol; PCB-153, polychlorinated biphenyls 153; POP, persistent organic pollutant; RBCs, red blood cells; ROS, reactive oxygen species; RSV, respiratory syncytial virus; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; sncRNA, small non-coding RNA; SSBs, single-strand breaks; TBBPA, tetrabromobisphenol A; TBBPS, tetrabromobisphenol S; TBCO, 1,2,5,6-tetrabromocyclooctane; TBECH, 1,2-dibromo-4-(1,2 dibromoethyl) cyclohexane; TBEP, tris(2-butoxyethyl) phosphate; TBP, tribromophenol; TBPH, bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate; TCBPA, tetrachlorobisphenol A; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TCEP, tris(2-chloroethyl) phosphate; TCP, tricresyl phosphate; TCPP, tris(1-chloro-2-propyl) phosphate; TDBPP, (2,3-dibromopropyl) phosphate; TDCPP, tris(1,3-dichloro-2-propyl) phosphate; TeDB-DiPhOBz, tetradecabromo-1,4-diphenoxybenzene; TNBP, tri-n-butyl phosphate; TPHP, triphenyl phosphate.

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ABSTRACT

Flame retardants (FRs) are chemical substances used to inhibit the spread of fire in numerous industrial applications, and their abundance in modern manufactured products in the indoor and outdoor environment leads to extensive direct and food chain exposure of humans. Although once considered relatively non-toxic, FRs are demonstrated by recent literature to have disruptive effects on many biological processes, including signaling pathways, genome stability, reproduction, and immune system function. This review provides a summary of research investigating the impact of major groups of FRs, including halogenated and organophosphorus FRs, on animals and humans in vitro and/or in vivo. We put in focus those studies that explained or referenced the modes of FR action at the level of cells, tissues and organs. Since FRs are highly hydrophobic chemicals, their biophysical and biochemical modes of action usually involve lipophilic interactions, e.g. with biological membranes or elements of signaling pathways. We present selected toxicological information about these molecular actions to show how they can lead to damaging membrane integrity, damaging DNA and compromising its repair, changing gene expression, and cell cycle as well as accelerating cell death. Moreover, we indicate how this translates to deleterious bioactivity of FRs at the physiological level, with disruption of hormonal action, dysregulation of metabolism, adverse effects on male and female reproduction as well as alteration of normal pattern of immunity. Concentrating on these subjects, we make clear both the advances in knowledge in recent years and the remaining gaps in our understanding, especially at the mechanistic level.

Contents

1. Introduction

Flame retardants (FRs) are a complex group of organic chemicals which are defined by their common industrial use purpose rather than their structure: they are used in numerous manufactured goods (household furniture, clothing, electronics etc.) in order to convey the functionally important characteristics of decreased flammability and/or slowing down and quenching of an already ignited fire. These capabilities are mediated by several elaborate physico-chemical mechanisms that function in solid phase or in gas phase, and are usually activated by thermal decomposition of the flame retardant molecule itself. These mechanisms include (but are not limited to) quenching of reactive radicals emitted within the gaseous flame and providing a thermally insulating layer of charred material around the source of combustion (TriDung, 2020; Shen et al., 2021). Most organic flame retardants can be divided into halogenated organic compounds or organophosphorus compounds, but these groups can overlap (e.g., tris(2,3-dibromopropyl) phosphate contains both halogen and phosphorus atoms) and the classification is not comprehensive or exclusive (Yang et al., 2019). The most widely used types of flame retardants include brominated ethers (e.g. decabromodiphenyl ether (DBDE)), brominated alkanes (e.g. hexabromocyclododecane (HBCD)), brominated phenols (e.g. tetrabromobisphenol A (TBBPA)), phenyl phosphates (e.g. tricresyl phosphate (TCP)) and halogenated organophosphates (e.g. tris(1,3-dichloro-2-propyl)phosphate (TDCPP)) (Fig. 1).

The global annual consumption of FRs was estimated to be around 680,000 tons in 2015, which was predicted to increase to over 2,000,000 tons by 2025 (Hu et al., 2021). This indicates a significant rise in the usage of flame retardants worldwide. FRs can contaminate different environmental reservoirs including air, water, soil via degradation and leaching during production, use, and disposal, and even by accidental release in industrial accidents or fires (Reemtsma et al., 2006). For example, Yin et al. studied the levels of PBDEs in Chinese rivers and reported median concentrations of 0.09 up to 2.19 μg/kg (Yin et al., 2020). FRs can enter the body through inhalation, dermal contact, and unintentional ingestion. Indoor

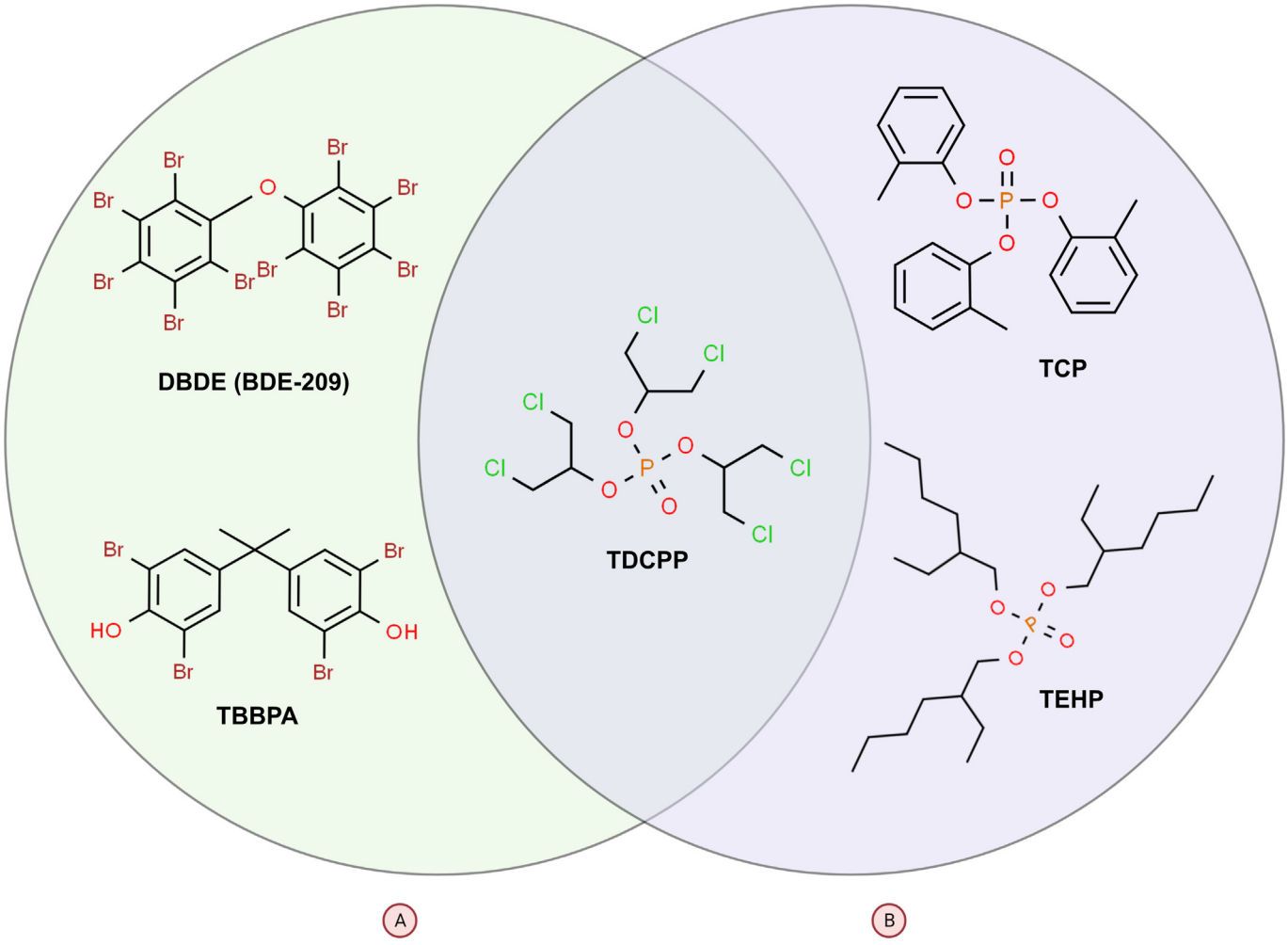

Fig. 1. Examples of molecular structures of commonly used flame retardants (FR) from the halogenated organic compound group (A) and from the organophosphorus compound group (B): decabromodiphenyl ether (DBDE), tetrabromobisphenol A (TBBPA), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), tricresyl phosphate (TCP), tris (2-ethylhexyl)phosphate (TEHP).

environments containing these chemicals serve as the most common source of exposure (Li et al., 2023). Research summarized by Chen et al. indicates that inhalation and ingestion are the primary routes through which humans are exposed to FRs (Chen et al., 2020). Predictably, high levels of some FRs including PBDEs have been reported in human serum (36 ng/g lipid) (Law et al., 2014). In addition, a study of women in California reported a median concentration of PBDEs in breast milk at 31 ng/g lipid weight (Stapleton and Dodder, 2008). In recent years, the European Union prohibited production and use of specific BFRs, such as PBDEs and HBCD, in commercial goods. The use of some PBDEs will be limited under restricted guidelines, e.g. OBDE will be phased out until 2030 and BDE-209 until 2036. Other countries like China, Japan, India, and the United States have made significant progress in regulating POP FRs, aligning with the provisions of the Stockholm Convention (Sharkey et al., 2020; Syed et al., 2020). Establishing legislation can directly decrease the environmental release and exposure to FRs, thereby minimizing the potential risks to human health and ecosystems. Regulations against PBDEs were introduced in 2011 and led to detectable environmental effect within the decade (Sharkey et al., 2020).

Despite considerable chemical diversity of FRs, some features which are important from the biological point of view are common features for this group of chemicals. They are relatively hydrophobic compounds, with very limited solubility in water and aqueous media. The abiogenic 1modifications to organic backbones make them biorthogonal to most catabolic pathways, rending them relatively resistant to biotransformation, even by otherwise efficient microbial biodegraders (Chaudhry and Chapalamadugu, 1991; Chen et al., 2015). For the same reason, most, if not all FRs are not acutely toxic either at the cellular or at the organismal level, and for some time the prevailing view was that they were mostly harmless both for humans and for the environment. However, with increased awareness of environmental and health dangers posed by compounds with related structure (e.g. polychlorinated biphenyls which were identified as dangerous pollutants relatively early) (Ngoubeyou et al., 2022), FRs also became scrutinized by toxicologists. Since the 1990s, many studies have been performed to identify the safety margins of FR exposure, determine their ecological impact and select the congeners with lowest harmful bioactivity (Santillo and Johnston, 2003).

Current toxicological interest in flame retardants stems mostly from four facts:

1. They are abundant in man-made products and readily released to the environment. Use of additive type FRs is driven up constantly by increasingly stringent fire safety regulatory requirements. Since most everyday use organic polymers are naturally flammable, products such as clothing, furniture, electronics etc. must be rendered fire-resistant by addition of FRs. The worldwide consumption of FRs is measured in millions of tons yearly (Ekpe et al., 2020). Humans can be exposed to them by several pathways: by direct contact with household items, by leaching of FRs from used or discarded products, by occupational exposure of especially vulnerable professions (electronics manufacturing,

firefighters etc.), from the increasing load of FRs in the external environment and in the food chain.

2. They are highly resistant to chemical and biological degradation, making them bona fide members of the class of persistent organic pollutants (POPs). In the case of FRs, the same physico-chemical qualities that make them efficient fire suppressors/delayers provide molecular protection against quick decomposition. FR molecules are relatively resistant to spontaneous oxidation by atmospheric oxygen (Anioł and Jankowski, 2018), to degradation by UV from sunlight (Koch et al., 2016), and to enzymatic oxidation or decomposition by most living organisms, including microbes which specialize in degrading complex organic molecules (Yang et al., 2022). These properties are due to their molecular structure with few delocalized electrons and a relatively high proportion of atoms which are uncommon in biogenic hydrocarbons (halogens, phosphorus, sulfur). It makes them capable of concentrating in biomass/tissue of living organisms with little to no chemical modifications, and of persistence in the food chain with selective deposition in higher level consumers (mostly in fatty tissues due to their hydrophobicity).
3. They share structural similarity with other classes of POPs that have previously identified as harmful to human/animal health, such as some pesticides, plasticizers etc. Both polyhalogenated hydrocarbons and organophosphorus compounds are represented among FRs, but also among more bioactive pollutants that have clearly identified biological targets of toxicity. Examples include chlorinated pesticides such as mirex, lindane or aldrin (Lal and Saxena, 1982), polychlorinated biphenyls used as coolant or dielectric fluids in electronics (Fishbein, 1974) or organophosphate biocides such as parathion or chlorpyrifos (Mali et al., 2023). Since these groups of POPs have been extensively studied by toxicologists, the scientific interest naturally extended to chemically similar FRs, especially since the biological models and analytical tools are already at hand.
4. Finally, a growing body of direct evidence exists for actual disruption of biological processes caused by exposure to FRs. While early toxicological literature had to contend with the widespread conviction (especially in industry) about the harmless character of these compounds (Pijnenburg et al., 1995), accumulating evidence of specific, identifiable health hazards linked to their use led to a boom in research in the 2000s which pushed flame retardant toxicology into the mainstream of environmental chemistry and biology. Paradoxically, while this led to regulatory movements to limit the use of FRs and to phase out the ones that are thought to be most harmful to the environment and public health, the industry naturally reacted by exponentially increasing the diversity of produced FR types to circumvent the bans on specific chemical structures, making an exhaustive toxicological characterization of every compound an arduous and long-term task (Santillo and Johnston, 2003).

The toxicological literature on FRs, while voluminous and growing, is avowedly fragmentary and lacking in explanations of mechanism of action, especially at the cellular and molecular level. The purpose of this review is to summarize the current knowledge on targets of FR action, with special regard to potential harmful effects on humans (also derived from studies on other model organisms). Our main intention is to underline those studies which took care to identify some of the mechanisms of action at more than a phenomenological level, as these studies are most valuable from the point of view of potential generalizations and predictions of harmful effects from compounds that have not yet been studied. At the cellular level, we will be mostly talking about targets related to signaling and gene expression. At the physiological level, the most important targets (as a consequence of the molecular and cellular mechanisms) are reproductive and immune functions. These complex actions of FRs on receptors, pathways etc. makes is possible to call them classical endocrine disruptors (Bajard et al., 2021), but this label is simplistic and requires more explanation at the mechanistic level, which we will try to provide. Our additional goal is to point out important gaps in knowledge and show promising directions for further studies. Since our goal is to concisely, but comprehensively describe the mechanisms of toxicity of various FRs at the molecular, cellular and physiological level, with special emphasis on mechanisms common to FRs with different chemical structure, we have organized our review accordingly, providing references to exemplary studies on specific toxicity targets impacted by FRs from various groups. This information is also more concisely summarized in Table 1.

2. Cellular signaling pathways affected by FR exposure

Biochemical signals are crucial for regulating the homeostasis and function of every cell type, and are the main form of information exchange between different cells, tissues and organs in the body. Thus, any external interference in their functioning can have deep and long-term consequences to health of the whole organism, and potential molecular interactions between FRs and dedicated signaling molecules are obviously a valid mechanism of toxicity of these compounds. It is also important to stress that interactions with structural or metabolism-related cellular elements, such as membranes, organelles and enzymes, likewise can have secondary impact on signaling pathways that may be even more important with regard to secondary toxicological impact at the physiological level than potential direct impact on these cellular targets.

2.1. FRs bind to and disrupt hydrophobic cell components

Most FRs are highly hydrophobic chemicals which easily enter the cell and partition predominantly into its lipidic constituents, i.e. membranes and hydrophobic structural elements in proteins. The most important membrane component of the cell is the cell membrane, and FRs can bind to its constituents and change its biophysical and biochemical characteristics, sometimes going as far as to compromise its integrity. These interactions may also disrupt the normal function of cell organelles, such as mitochondria and endoplasmic reticulum, and modulate signaling pathways within the cells which are linked to membranes and hydrophobic proteins. It is thought that FR exposure can result in reduction of cell viability mostly due to impact on crucial pathways of proliferation and apoptosis (Saquib et al., 2021; Zhang et al., 2019a; Barańska et al., 2022a). FRs can also disrupt elements of the normal cellular homeostasis in treated cells, including energy metabolism (such as catabolism of lipids and glucose), antioxidative capacity and others.

Even though all FRs are hydrophobic and readily partition into lipidic compartments such as the cell membrane, the exact mode of interaction and impact on membrane lipids and proteins has not been extensively studied. Liu et al. performed a combined in vitro and in silico study using a model membrane bilayer made of dipalmitoyl phosphatidylcholine (DPPC). Fluorescence anisotropy experiment demonstrated a decrease in liposome membrane fluidity by incorporation of decabromodiphenyl ethane (DBDPE), while molecular dynamics revealed that DBDPE molecules moved toward the membrane surface and bound lipid tails via hydrophobic interactions in an aquatic environment, decreasing lipid disorder in the inner bilayer of model membrane (Fig. 2) (Liu et al., 2019). A similar experimental study was performed on TBBPA which was found to distribute throughout the phospholipid layers, predominantly in favor of lipophilic regions, mostly as intercalator between fatty acid side chains, but avoiding cholesterol-rich regions. This leads to an increase in membrane viscosity, especially in the inner bilayer, and a decrease in permeability to ions. Although this study used liposomes made of model lipid bilayer, the described biophysical changes may also happen in plasma membrane of cells and affect their biological functions (Ogunbayo et al., 2007). In red blood cells (RBCs) which have no internal membranes, only the plasma membrane, TBBPA and tetrabromobisphenol S (TBBPS) treatment increased fluidity of surface membrane monolayer, which was reflected in the morphology and survival (membrane integrity, hemolysis) of treated RBCs (Jarosiewicz et al., 2021). However, octabromodiphenylether (OBDE)

Table 1
Synopsis of toxicity mechanisms and targets for halogenated and organophosphorus FRs. Mitochondrial DNA (mtDNA), endoplasmic reticulum (ER), reactive oxygen species (ROS), DNA double-strand breaks (DSBs), DNA single-strand breaks (SSBs), macrophages (MQ), decabromodiphenyl ether (BDE-209), brominated diphenyl ethers-47 (BDE-47), brominated diphenyl ethers-49 (BDE-49), bisphenol A (BPA), *tert*-butylphenyl diphenyl phosphate (BPDP), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE), hexabromocyclododecane (HBCD), polybrominated diphenyl ether (PBDE), polychlorinated biphenyls (PCB), polychlorinated biphenyls 153 (PCB-153), tetrabromobisphenol A (TBBPA), tetrabromobisphenol S (TBBPS), 1,2,5,6-tetrabromocyclooctane (TBCO), 1,2-dibromo-4-(1,2 dibromoethyl) cyclohexane (TBECH), tris(2-butoxyethyl) phosphate (TBEP), tetrachlorobisphenol A (TCBPA), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCPP), 1,3-dichloro-2-propyl) phosphate (TDCPP), tri-n-butyl phosphate (TNBP), tri-o-cresyl phosphate (TCP), triphenyl phosphate (TPHP).

General target	Specific target/mechanism	Outcome	FRs	CAS registry numbers	References
Cellular signaling pathways	Cell components	Changes in membrane fluidity	DBDPE, TBBPA, TBBPS	84852-53-9, 79-94-7, 39635-79-5	(Liu et al., 2019; Ogunbayo et al., 2007; Jarosiewicz et al., 2021)
		Distortion of morphology and polarity; inhibition of oxidative phosphorylation pathways; increase in mtDNA copy number in mitochondria	BDE-49	243982-82-3	(Napoli et al., 2013)
		ER stress	DBDPE, TDCPP, TPHP	84852-53-9, 13674-87-8, 115-86-6	(Jing et al., 2022; Xiang et al., 2017; Yue et al., 2023)
	Cell homeostasis	Increased ROS formation	TBBPA, TBCO, TBECH, BTBPE, DBDPE, TCEP, TDCPP, PBDE	79-94-7, 3194-57-8, 3322-93-8, 37853-59-1, 84852-53-9, 115-96-8, 13674-87-8	(Saquib et al., 2021; Huang et al., 2010; Wu et al., 2018; Shi et al., 2021; Jing et al., 2022; Lu et al., 2021; Zhang et al., 2016; Saquib et al., 2022; Costa et al., 2015; Reistad et al., 2007)
		Induction of insulin resistance (by ER stress)	TPHP	115-86-6	(Yue et al., 2023; Li et al., 2022a)
		Inhibition of autophagy homeostasis	TPHP, PBDE	115-86-6	(Li et al., 2019; Yue et al., 2023; Li et al., 2022a)
	Differentiation	Adipocyte differentiation	BDE-47, PBDEs	5436-43-1	(Sales et al., 2013; Kamstra et al., 2014)
		Alteration in expression of genes involved in neuronal development (downregulation of the WNT pathway)	TBBPS, TCBPA, BDE-47	39635-79-5, 79-95-8, 5436-43-1	(Liang et al., 2019)
		Alteration in expression of genes involved in neuronal development (upregulation of the NOTCH pathway)	TBBPA, TBBPS, TCBPA	79-94-7, 39635-79-5, 79-95-8	(Liang et al., 2019)
	Visual signaling	Modification of the expression of critical eye proteins; Interference of the visual maintenance function	TPhP	115-86-6	(Shi et al., 2019)
		Visual deformations	TBBPA	79-94-7	(Parsons et al., 2019)
		Disrupting visual behavior	BDE-47, TBCO	5436-43-1, 3194-57-8	(Xu et al., 2017); (Sun et al., 2016)
Genome	DNA damage	DNA damage by higher ROS production	BDE-47	5436-43-1	(Zhang et al., 2019b)
		DSBs induction	TDCPP, TCEP, TCPP, PBDE	13674-87-8, 115-96-8, 13674-84-5	(Zhang et al., 2019b; Bukowski et al., 2019; Lamkin et al., 2022)
		SSBs induction	PBDE	e.g. 32534-81-9	(Gao et al., 2009)
	DNA repair responses	Inhibition of genes involved in DNA repair	HBCD	25637-99-4	(Li et al., 2017)
		Impaired DNA repair	PBDE, TDCPP, TPHP	13674-87-8, 115-86-6	(Chen et al., 2018; Du et al., 2016; Lamkin et al., 2022)
		misregulation of DNA repair system by inducing SSB repair protein and suppressing DBS repair protein	PBDEs	e.g. 32534-81-9	(Gao et al., 2009)
	Chromatin and epigenome	Interference with normal epigenetic pattern of thyroid hormone receptor gene (histone acetylation and methylation)	TBBPA	79-94-7	(Otsuka et al., 2014)
		Suppression of Prm1, Prm2 gene and disruption of chromatin packaging	PBDE	e.g. 32534-81-9	(Khalil et al., 2017)
		Inducing global DNA demethylation	TCPP, TCEP, BPA, TCDD, BDE-47, PCB-153	13674-84-5, 115-96-8, 80-05-7, 1746-01-6, 5436-43-1, 35065-27-1	(Sales et al., 2013; Bukowski et al., 2019)
		Increased DNA methylation	BPA	80-05-7	(Strakovsky et al., 2015; Stel and Legler, 2015)
		DNA hypomethylation	PBDE, TDCPP, TPHP	13674-84-5, 115-86-6	(Shafique et al., 2023; Woods et al., 2012; Volz et al., 2016)
Reproductive system	Male reproductive system	Abnormal sperm morphology; reduced glandular weights	HBCD, PBDE	25637-99-4	(Khalil et al., 2017; van der Ven et al., 2009)
		Disrupted normal transcriptome of sperm; reduced testes size	PBDE	e.g. 32534-81-9	(Khalil et al., 2017)
		Decrease in sperm count; structural abnormalities; decrease weight of the testes and prostate	TBBPA	79-94-7	(Wu et al., 2021)
	Female reproductive system	Diminished follicle number	TPHP	115-86-6	(Ma et al., 2021b)
		Dysregulation of folliculogenesis	PBDEs, HBCD	25637-99-4	(Lefèvre et al., 2016)
		Impaired oocyte development	TBBPA	79-94-7	(Guo et al., 2022)
	Embryonic development	Neurodevelopmental abnormalities	TPHP, TCBPA, DBDPE	115-86-6	(Zhang et al., 2023b; Gustafsson et al., 2023; Hua et al., 2022)
		Disturbance in limb development	BPDP	56803-37-3	(Yan and Hales, 2020)
		Decreased life span and body mass	BDE-209	1163-19-5	(Han et al., 2017)
Immune system	Innate immune responses	Reduced adhesion capacity in macrophages	TPHP, TDCPP	115-86-6	(Li et al., 2020)
		Increased adhesion capacity in macrophages	TNBP, TCP	126-73-8, 78-30-8	(Li et al., 2020)

(continued on next page)

Table 1 (*continued*)

General target	Specific target/mechanism	Outcome	FRs	CAS registry numbers	References
		Decreased MQ' responses to LPS stimulation; impaired immunological synapse of MQ	BDE-47	5436-43-1	(Longo et al., 2019; Longo et al., 2021; Longo et al., 2023)
		Induction of type 2 inflammation in neutrophils and eosinophiles	dechlorane 602	31107-44-5	(Zhou et al., 2020)
	Adaptive immune responses	Th1/Th17 cells Imbalance	PBDEs	e.g. 32534-81-9	(Mynster Kronborg et al., 2016)
		Immunosuppression by induction of Treg and inhibition of CD3+ T cells	TCBPA	79-95-8	(Wang et al., 2021a)
		Increased immunoglobulin level; decreased proliferation of B cells; atrophy of the thymus and spleen	BDE-209	1163-19-5	(Liao et al., 2021; Liu et al., 2012)
	Allergic responses	Th1/Th2 cells imbalance	BDE-209	1163-19-5	(Liao et al., 2021)
		Allergy exacerbation	TBEP, TBBPA, HBCD	78-51-3, 79-94-7, 25637-99-4	(Yanagisawa et al., 2020; Koike et al., 2013)
		Upregulation of IgE receptor	TBBPA	79-94-7	(Tribondeau et al., 2022)
	Response to pathogens	Suppression of antiviral immune responses	TBBPA	79-94-7	(Watanabe et al., 2010; Pullen et al., 2003)
		Suppression of antibacterial immune responses	PBDE	e.g. 32534-81-9	(Arkoosh et al., 2010)
		Decreased vaccine immunization	PCB	1336-36-3	(Heilmann et al., 2006)

treatment did not result in membrane fluidity changes in cellular membranes of thymocytes (Sandal et al., 2004).

A direct effect on membrane properties mediated by partitioning of FR molecules may not be the only mode of membrane-directed bioactivity of these compounds. Interestingly, Tian et al. observed that tetrachlorobisphenol A (TCBPA) exposure changed a range of metabolic pathways in *Saccharomyces cerevisiae*, including inhibiting tricarboxylic acid cycle and hexose monophosphate pathway, which led to directing carbon flux toward glycerol and saturated fatty acids (FAs), and accumulation of hexadecenoic and octadecanoic acid. These saturated FAs are involved in preserving membrane integrity by decreasing bilayer fluidity. This highlights how biochemically mediated metabolome changes may potentially impact membrane composition and properties (Tian et al., 2017). Genes involved in cholesterol biosynthesis were observed to be downregulated in codfish exposed to tris-2-chloroisopropyl phosphate (TCPP). The cholesterol pathway has profound importance in marine creatures, because

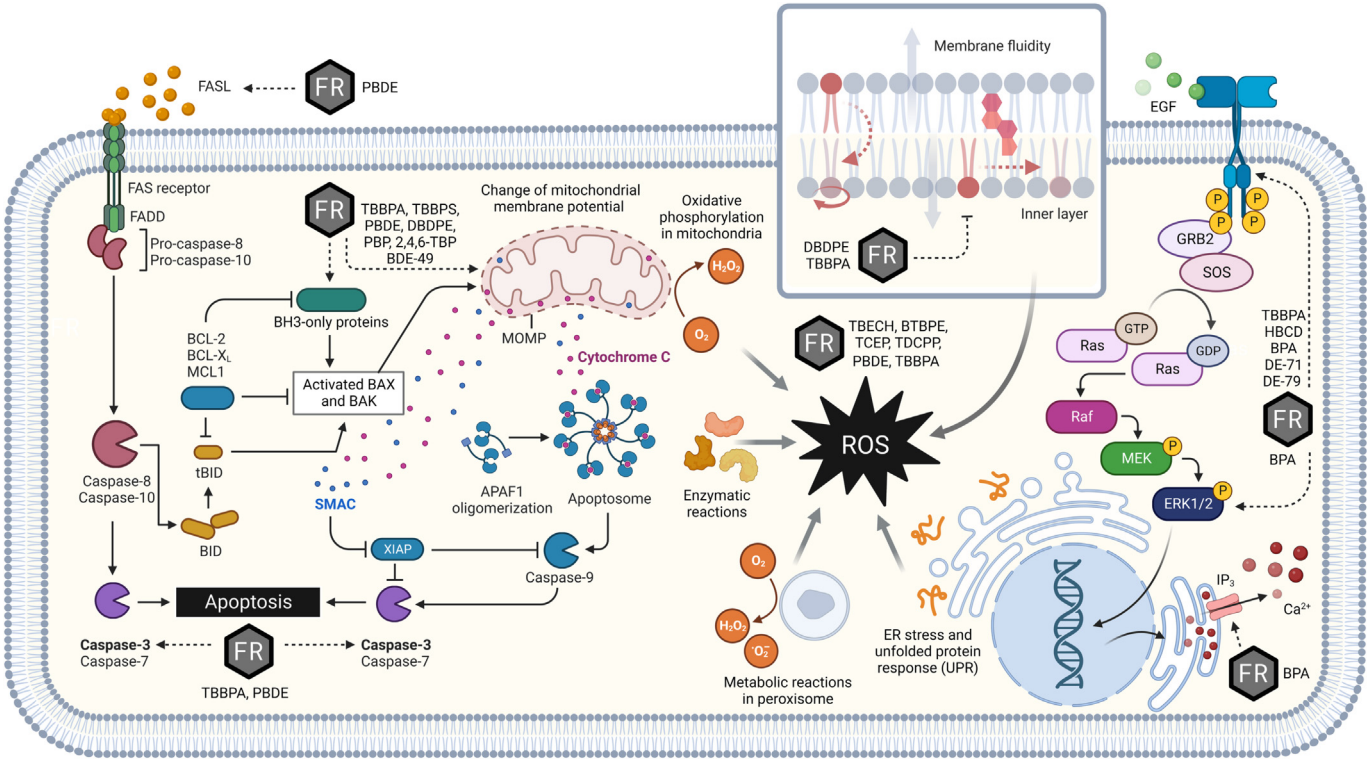

Fig. 2. Summary of toxicologically most important reported effects of FRs on subcellular structures and signaling pathways. Flame retardant (FR), polybrominated diphenyl ethers (PBDEs), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), 2,4,6-tribromophenol (2,4,6-TBP), apoptotic protease activating factor-1 (Apaf-1), bisphenol A (BPA), brominated diphenyl ethers-49 (BDE-49), decabromodiphenyl ethane (DBDPE), endoplasmic reticulum (ER), epidermal growth factor (EGF), hexabromocyclododecane (HBCD), mitochondrial outer membrane permeabilization (MOMP), pentabromophenol (PBP), polybrominated diphenyl ether mixtures (DE-71 and DE-79), reactive oxygen species (ROS), tetrabromobisphenol A (TBBPA), tetrabromobisphenol S (TBBPS), tetrabromoethylcyclohexane (TBECH), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), tris(2-chloroethyl) phosphate (TCEP).

it is crucial in response to environmental temperature which modulates membrane parameters (Aluru et al., 2021). Although membrane fluidity was not studied in the above-cited studies, it is clear that modulation of cholesterol and fatty acid metabolism by FRs may affect membrane integrity in changing environments.

The hydrophobic properties of FRs causes them to display affinity not only for lipids, but also for hydrophobic structural elements in lipophilic or amphiphilic proteins. Molecular modeling studies have suggested that TBBPA uses hydrophobic interactions to bind to the lipophilic center of human serum albumin (HSA) (Wang et al., 2014a). This was further corroborated by structural investigations in vitro which showed TBBPA-induced changes in the secondary structure of HSA, with damage toα-helical sections and increased β-sheet formation in the secondary protein structure (Jarosiewicz et al., 2020). In other proteins with lipophilic ligand-binding pockets, various FRs have been shown to efficiently compete with small-molecule hydrophobic ligands (Meerts et al., 2000; Ogunbayo and Michelangeli, 2007; Hill et al., 2018a; Hill et al., 2018b). Of high potential significance for the deleterious bioactivity of FRs is the reported interaction between several organophosphate FRs and the hydrophobic core of the crucial transcription factor p53, often called the guardian of the genome (Li et al., 2014a). Other transcription factors/nuclear receptors, such as the glucocorticoid receptor and the androgen receptor, have been reported to bind TBBPA in a hydrophobic interaction-dependent manner, leading to changes in their transcriptional regulatory efficiency (Beck et al., 2016).

The above-cited studies contribute to a major theme in FR toxicology, which is the potential disruption of receptor activity via direct and indirect interactions with signaling proteins that depend on binding low-molecular-weight ligands. Such effects are evident not only for lipid molecule receptors, and while some involve competitive binding of FRs to the ligand site, others are apparently mediated by non-competitive modulation of protein structure or indirect effects on receptor expression or localization. Koike et al. studied the effect of polybrominated diphenyl ether (PBDE) mixtures DE-71 and DE-79 and reported that some pro-inflammatory responses effected by FR treatment could be attenuated by inhibition of the epidermal growth factor receptor (EGFR), suggesting that there was a direct stimulatory interaction between FRs and EGFR (Fig. 2) (Koike et al., 2014). Sauer et al. investigated the role of EGFR activity in higher proliferation rate and tumor progression in BPA-treated cells, and they showed that in EGFR knockout cells no proliferative and anti-apoptotic activity of BPA was observed. This led to the conclusion that BPA targeted EGFR and boosted its potential function, although there is not enough evidence to show direct BPA binding to EGFR (Sauer et al., 2017). Other FRs, including HBCD and TBBPA, also have potentially significant effects on EGFR activity. These FRs could promote EGFR phosphorylation and subsequently MAPK signal transduction, which has great importance for expression of genes involved in proliferation, differentiation and immunity (Koike et al., 2016).

Plasma membrane proteins which are not bona fide receptors have also been found to be affected by FR exposure. An interesting example are ATP binding cassette (ABC) transporters expressed in the blood brain barrier (BBB). Cannon et al. observed that TBBPA treatment decreased the activity of ABCG2 transporter, without any changes in the expression level, while it had complex impact on activity of ABCB1, in this case clearly mediated by modulation of protein expression levels. Since these transporters are crucial to BBB function and any alteration in their activity could result in impairment of protective function of BBB, this type of FR bioactivity can have important consequences at the physiological level, e.g. by increasing the neurotoxicity of other environmental compounds (Cannon et al., 2019).

Intracellular membrane receptors and proteins have also been found to be affected by FR exposure. Bisphenol A (BPA) could cause Ca2+ influx and mediate activation of Nrf2 and ERK pathways by direct interaction with the IP3 receptor located in endoplasmic reticulum (ER) membranes, which is important as hyperactivation of ERK and Nrf2 might promote cancer progression (Fig. 2) (Oguro et al., 2021). A complex functional interaction has been reported for TBBPA and the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), the main calcium transporter in the ER responsible for cellular Ca^{2+} homeostasis. TBBPA could interfere with normal Ca^{2+} binding to SERCA both by directly binding the protein and by indirectly decreasing its affinity toward Ca^{2+} (Ogunbayo and Michelangeli, 2007).

Interestingly, some FRs can mediate their harmful bioactivity by binding to receptors not only within cells, but even in body fluids. Transthyretin acts as a T4 (thyroid hormone) carrier in plasma and CSF, binding to T4 and delivering it to target cells. Based on in vitro and in silico investigations it was reported that 4-OH-BDE-49, Tetradecabromo-1,4-diphenoxybenzene (TeDB-DiPhOBz), pentabromophenol (PBP) and TBBPA had even higher affinity to transthyretin than T4 (Meerts et al., 2000; Hill et al., 2018b). Remarkably, in another study some organophosphate FRs, including tris (1,3-dichloro-2-propyl) phosphate (TDCPP), tris(butoxyethyl) phosphate (TBEP), and triphenyl phosphate (TPHP), showed different and contrasting disruptive effects on normal function of T4 from the brominated congeners. These FRs increased T4 binding to transthyretin to more than optimal level, which could be deleterious and result in deliver in excessive levels of T4 to the target organ/tissue (Hill et al., 2018a).

2.2. FRs disrupt mitochondrial and redox homeostasis, leading to apoptosis

Mitochondria are cellular organelles crucial to energy homeostasis and survival signaling pathways – as such, any harmful interactions with FRs could be an important source of cell-level toxicity of these compounds. There are numerous reports of FRs targeting mitochondrial functions at different levels: structural, functional, and by indirect disruption of mitochondrial regulation of oxidative equilibrium. Morphological changes, disruption of the mitochondrial network and decrease in the number of mitochondria was observed e.g. in Leydig tumor cells treated with chlorinated organophosphorus FRs (Feng et al., 2022), in hepatocytes exposed to (2,3-dibromopropyl) phosphate (TDBPP) (Ma et al., 2021a) or a wide range of phosphoroorganic FRs (Le et al., 2021), and in multiple other models. Mitochondrial membrane potential is a sensitive marker of mitochondrial health and it is very often compromised in cells treated with FRs, leading to cytochrome *c* release, followed by caspase-9 activation, formation of Apaf-1 complex, and recruitment of cas-3 cascade. Examples include TBBPA, TBBPS, PBP and 2,4,6-tribromophenol (2,4,6-TBP), as well as PBDEs (Zhang et al., 2019a; Barańska et al., 2022a; Barańska et al., 2022b; Montalbano et al., 2020). Some more in-depth studies showed extensive modulation of mitochondrial function, for example BDE-49 exerted specific toxicity toward mitochondria, with distortion of mitochondrial morphology and polarity, inhibition of complex V and IV function, increased mtDNA copy number (a common reaction to genotoxic stress) and frequency of mtDNA mutations (Napoli et al., 2013). In another study, enzymatic activity of succinate dehydrogenase was reduced by BDE-47 treatment in zebrafish (Boxtel et al., 2008). An interesting study suggested a positive correlation between PBDE cytotoxicity and the level of its accumulation within cells, with mitochondria (a membrane-rich organelle) acting as an FR storage reservoir holding up to 30 % of FR molecules absorbed by the cell (Huang et al., 2010).

Dysregulated mitochondria are the main cellular source of reactive oxidants and deleterious reactive oxygen species (ROS), so numerous studies on mitochondrial impact of FRs mention also this negative outcome. In a cellular model of lung epithelium, TBBPA treatment caused an increase in the level of ROS production, oxidative damage to lipids, leading to mitochondrial damage and consequently a significant incidence of cell death (Wu et al., 2018). In vascular endothelial cells, exposure to 1,2,5,6-tetrabromocyclooctane (TBCO), mistakenly referred to as HBCD in the original article, tetrabromoethylcyclohexane (TBECH) and 1,2-bis (2,4,6-tribromophenoxy)ethane (BTBPE) provoked ROS formation, lipid peroxidation, and loss of mitochondrial integrity and function (Shi et al., 2021). In human hepatocytes, DBDPE caused an increase in the level of ROS and lipid peroxidation and a resulting decrease in cellular antioxidant capacity. Mitochondrial membrane integrity was disrupted and mitochondrial membrane potential was decreased in treated cells, which all contributed to increased cell apoptosis (Jing et al., 2022). In another

model of liver cells, TBBPA treatment led to mitochondrial ROS overproduction, which caused an increase of adenosine monophosphate-activated protein kinase (AMPK) expression and ultimately led to higher cell proliferation rather than apoptosis, in contrast to other cell types (Lu et al., 2021). However in the study of Zhang et al., tris(2-chloroethyl) phosphate (TCEP) worked by increasing ROS generation from liver cell mitochondria, leading to cell cycle arrest and down regulation of the crucial epigenetic enzyme SIRT3 (Zhang et al., 2016). Two studies from Saquib et al., performed in hepatocytes and endothelial cells, respectively, showed that tris(1-chloro-2-propyl) phosphate (TCPP) and tris(1,3-dichloro-2-propyl) phosphate (TDCPP) could increase not only ROS levels, but also nitric oxide production and Ca^{2+} influx into mitochondria, which ultimately decreased cell viability in both models (Saquib et al., 2021; Saquib et al., 2022).

Mitochondria are not the only site of ROS production, and other pro-oxidant enzymes and processes have been shown to be affected by FR action. In kidney cells, long-term exposure to PBDEs caused an increase in oxidative stress mediated by increased activity of the ROS-generating enzyme NOX2. In response to this oxidative stress, the Nrf2 cascade was upregulated, but chronic ROS generation due to FR treatment led ultimately to deactivation of the Nrf2 cascade, loss of superoxide dismutase and catalase activity and pro-inflammatory consequences (Shan et al., 2017). Consistent with that study, PBDEs exposure caused a significant rise in oxidative stress resulting from high level of NOX activation and ROS production in a human bronchial epithelial cell line. This study reported PBDE action as leading to a significant increase in the percentage of apoptotic cells, however, no changes were observed at the level of cell proliferation (Montalbano et al., 2020). In another study, PBDE administration in living mice led to elevated caspase-3 activation in liver and cerebellum, mediated by a high amount of ROS production and lipid peroxidation. These pro-apoptotic effects were exaggerated in glutamate cysteine ligase knock-out mice, highlighting the crucial role of intracellular glutathione as a key anti-oxidant protecting against FR toxicity (Costa et al., 2015). NADPH oxidase activation is also a result of TBBPA treatment in neutrophils, where different signaling pathways were found to be involved, including ERK1/2, tyrosine kinases and protein kinase C (Reistad et al., 2005a).

Apoptosis and survival signaling pathways are crucial for explaining the effects of FR treatment on cell viability and proliferation, not just in situations of increased oxidative stress. There are reports of FRs acting directly as well as indirectly on those signaling components in various cell types. In a comprehensive study on cerebellar granule cells, TBBPA-treated cells demonstrated high levels of ROS and ERK1/2 phosphorylation, as well as elevated levels of extracellular glutamate. Since ERK1/2 activation can be mediated by oxidative stress, calcium influx or glutamate receptor stimulation, the direct sequence of events enacted upon TBBPA treatment was not clear, but it seemed that TBBPA mediated ERK1/2 activation within the signaling cascade frame rather than by direct stimulation. Interestingly, in this model TBBPA induced cell death via a mechanism that was different from apoptosis, since dead cells lost membrane integrity (Fig. 2) (Reistad et al., 2007). However, in numerous studies on other cell types, the pro-apoptotic mechanism seemed to prevail. In lung alveolar cells, TBBPA deceased cell viability by increased caspase-3 activation (Wu et al., 2018). In hepatocytes, upon TBBPA treatment the level of anti-apoptotic Bcl-2 decreased and the level of Bax, a pro-apoptotic protein, conversely increased (Zhang et al., 2019a). The same pattern of Bcl-2 and Bax expression changes was observed in the case of PBDE treatment (Shan et al., 2017). It is worth noting that TBBPA disturbed normal cell proliferation by inducing G2/M cell cycle arrest in various mammalian cell types (Strack et al., 2007).

Interestingly, based on transcriptomic analysis the Ras signaling pathway was highly activated by TBBPA treatment of hepatocytes; this pathway is usually involved in increased cell proliferation, survival, and differentiation (Lu et al., 2021). The aforementioned ERK signaling pathway, which can be activated by Ras, was found to be upregulated in PBDE exposed human fibroblasts. Upon ERK activation, several transcription factors involved in proliferation, apoptosis, and cell cycle regulation were phosphorylated, including AP-1 subunits which also had an increased nuclear localization (Manuguerra et al., 2019). PBDE exposure of the pseudo-neuronal PC12 cell line led to increased expression of key genes involved in apoptotic pathways, both intrinsic and extrinsic: caspase-3 and caspase-9, but also FAS-L, FAS and TNF-R1 as well as caspase-8, respectively (Fig. 2) (Li et al., 2019).

2.3. FRs affect differentiation processes in specialized tissues

Among signaling pathways and cellular outcomes affected by FR exposure, a diverse group of processes linked to cellular differentiation, tissue-specific regulation of metabolism and specialized cellular functions is the subject of an increasing number of studies. Whereas the hydrophobic interaction-mediated effects as well as the action on mitochondria and survival mechanisms are both relatively generic and common to most FRs, the more specialized signal modulations are specific to structural groups of FRs, and require more intensive studies with regard to their molecular mechanism. We will cite here the most interesting and physiologically relevant of such reported bioactivities.

Halogenated FRs seem to have a capacity to interfere with protein biogenesis and folding. Since ER is as a key organelle in protein folding and its disruption by different genetic and environmental factors causes ER stress, the ability of some FRs to elevate markers of ER stress confirms this mechanism. For example, DBDPE treatment of hepatocytes caused an increase in the activity of PERK and IRE1, belonging to two separate ER stress detection pathways, confirming the occurrence of ER stress (Jing et al., 2022). TDCPP also caused ER stress and accumulation of unfolded proteins in ER lumen of human corneal epithelial cells. This led to increased activity of pro-apoptotic Bcl-2 family proteins located in the mitochondrial membrane and finally to apoptosis induction (Fig. 2) (Xiang et al., 2017). There are numerous other reports of ER stress caused by halogenated FRs, and recently also non-halogenated organophosphate FRs have been found to have similar effects. TPHP induced insulin resistance in liver cells specifically via the ER stress pathway (Yue et al., 2023), and it also inhibited the autophagy homeostasis by inducing ER stress and autophagy blockage in hepatocytes (Li et al., 2022a).

Lipid metabolism and the differentiation of dedicated lipid storage cells (adipocytes) are another commonly identified target of FR action, with potential direct (by interference with enzymes) and indirect (by regulating differentiation-related gene expression levels) mechanisms of action. While bone marrow mesenchymal stromal cells (MSCs) have potential to differentiate into osteoblasts, adipocytes, chondrocytes or myeloblasts, MSCs of rats fed with a complex mixture of organophosphorus FRs showed reprograming and a shift from normal pattern of differentiation toward more adipocytes and fewer osteoblasts. Experimental and computational evidence points to these FRs being activating ligands for the adipogenic transcription factor PPARγ, leading to a physiological shift in bone marrow function potentially leading to obesity (excessive production of adipocytes) and osteoporosis (insufficient production of osteoblasts) (Pillai et al., 2014; Macari et al., 2020). Other FRs, such as, BDE-47 were also shown to be capable of inducing adipocyte differentiation from pre-adipocytes (Sales et al., 2013). An interesting study showed an alternative mechanism for bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH) causing nonalcoholic fatty liver disease in zebrafish. TBPH-exposed zebrafish showed upregulation of genes involved in lipid metabolism regardless of the level of fat in diet. However, when on high-fat diet with TBPH, the fish also expressed a higher level of pro-inflammatory markers, compounding the deleterious phenotype (Guo et al., 2021). Glucose metabolism regulation is another adipogenic pathway that can be disrupted due to FR exposure. It was demonstrated that PBDEs upregulated the mRNA level of Igf1 and G6pc, which led to increase glucose uptake and leptin expression and ultimately facilitated adipocyte differentiation (Kamstra et al., 2014).

Neuronal differentiation and function, while not a major toxicity target for FRs, has also been found to be affected by them in several studies. Interestingly, human neural stem cells treated with low doses of TBBPA, TBBPS, TCBPA and BDE-47 showed significant changes in differentiation marker expression, although cell survival was not affected at all. Such signaling

dysregulation may lead to developmental rather than cytotoxic neurotoxicity and is a mechanism that would be hard to detect in in vivo studies. It has been reported that TBBPS, TCBPA and BDE-47 downregulated the WNT pathway, while TBBPA, TBBPS and TCBPA upregulated the NOTCH pathway – both of them are of great importance in neuronal development (Liang et al., 2019). Investigation of developmental exposure to PBDEs on mouse brain demonstrated that ionotropic glutamate receptor 1 was downregulated – this protein functions both in synaptic transmission and in regulation of central nervous system (CNS) cell differentiation. Moreover, PBDE exposure led to a decrease in the level of Ca^{2+}/calmodulin-dependent protein kinase II which may influence diverse CNS function including memory and learning (Dingemans et al., 2007). In another study, rats were exposed to long-term PBDE treatment, which led to decreased autophagy potential of the neuronal lineage cells in the offspring of exposed animals. At the cellular level, increased PPAR and caspase-3 activation was detected, which was linked to pro-apoptotic action of PBDEs, but at the same time autophagy was blocked by impaired fusion of autophagosomes and lysosomes. The molecular mechanism involved down regulation of ATG7 (a key protein for autophagosome elongation) with concurrently increased levels of autophagy markers LC3-II and p62 (confirming that the inhibitory effect of PDBEs is indirect rather than direct) (Li et al., 2019).

Studies on the neuro-ophthalmological effects of flame retardants are limited. As discussed earlier, flame retardants can affect specialized tissues at the genetic and signaling level. Some of the most significant studies have been conducted in fish models. In a recent study, TPHP toxicity to the eye at both morphological and cellular level has been reported. In this study, eye distance increased in exposed zebrafish, which was accompanied by dysregulation of key genes (*aldh1a2*, *cyp26a1*, *raraa*, *rbp5*, *rdh1*, *crabp1a* and *rbp2a*) involved in retinoid acid metabolism. The morphological changes can be ascribed to both upregulation and downregulation of these genes, depending on the TPHP concentration and treatment period (Zhang et al., 2023a). Another study in zebrafish larvae shows that acute exposure to TPHP caused a significant decrease in the expression level of retinoschisin 1a and rhodopsin and an increase in the expression of crystallin - critical proteins in visual function (Shi et al., 2019). It has also been reported that TCDD disrupts retinal structure and that BDE-47 and TBCO disrupt visual behavior in fish (Carvalho and Tillitt, 2004; Xu et al., 2017; Sun et al., 2016). Embryonic exposure of zebra fish to TBBPA also induced visual deformations (Parsons et al., 2019). In a recent study, BDE-47 was shown to interfere with the regulation of gene expression and metabolism, leading to the disruption of retinal neural development in embryonic stem cells derived from the human retina (Li et al., 2022b).

3. Genotoxic effects of FRs

Flame retardants exhibit genotoxic effects, understood as dysregulation of nucleic acid structure and/or genetic information expression, which could be mediated by direct and indirect mechanisms. There is a body of evidence implying direct interactions between DNA and FR molecules, whereas other reports highlight the indirect mechanism of FR action by: a) increase in ROS levels and oxidative damage to nucleic acids; b) FR interference with DNA repair systems (He et al., 2008a). In addition, the epigenome of FR-exposed cells can undergo some changes in terms of DNA methylation, histone modification and noncoding RNA alteration. All these mechanisms lead on one hand to DNA damage, mutagenesis or cell death, on the other hand to disruption of physiological gene expression patterns, failure of transcription or translation (Fig. 3).

3.1. FRs cause direct and indirect DNA damage

PBDEs are the FRs with highest amount of data collected on direct interactions with DNA and impact on its integrity. A comprehensive study by Huang et al. investigated the interactions of PBDEs and their metabolites (hydroxylated and quinone forms) with DNA. Quinone metabolites could attach directly to DNA with much higher efficiency that the mother compounds. These molecules underwent Michael addition to dG, dA and dC moieties, leading to formation of covalent adducts with mutagenic potential (Arif et al., 2003). Another chemical study identified depurination as the final outcome of addition of quinone metabolites of bisphenol FRs to DNA (Wu et al., 2017). On the other hand, a spectroscopic and molecular modeling study postulated major groove intercalation as a direct DNA interaction mode for TBBPA (Wang et al., 2014b). A biological study in neuroblastoma cells reported that BDE-47 could directly intercalate into DNA and cause DNA double-strand breaks (DSBs), translating into cellular genotoxic outcomes (chromosome abnormalities, nuclear structure disruption) (He et al., 2008a). Increased formation of micronuclei as a result of DSBs was also reported in live fish exposed to BDE-47 (Bolognesi et al., 2006). Direct FR binding to DNA in living cells and resulting DSBs were also shown in bladder epithelium cells exposed to 2,2-bis(bromomethyl)-1,3-propanediol (BMP) (Kong et al., 2013).

The most apparent and well-studied indirect mechanism of genotoxic damage caused by FRs are oxidative DNA lesions effected by FR-mediated ROS formation. We wrote above (Section 2.2) about the possible molecular mechanisms of reactive oxidant stimulation in vivo by FR action, we will concentrate on its genotoxic outcomes in this section. The correlation between elevated levels of ROS and DNA damage in FR-exposed cells has been reported numerous times in different cell types and organisms. Among the more toxicologically and environmentally relevant models, cultured rat neurons treated with PBDEs showed clear-cut oxidative stress with increased ROS production and declining antioxidant defense level (e.g. glutathione and superoxide dismutase), and at the same time there was a marked increase in DNA damage in the form of DSBs (He et al., 2008b). In murine macrophages, TDCPP strongly stimulated ROS generation, which led not only to decreased cell survival, but also to strong DNA damage – the authors of this study suggest a two-pronged effect on cell survival by pro-apoptotic action of ROS on one side, and cell cycle arrest caused by DNA damage signaling on the other side (Zhang et al., 2019b). A set of human cell lines representative of various tissues was exposed to a number of organophosphate FRs in the study by An et al., and all of them showed a uniform pattern of DNA damage by oxidative stress-mediated DSBs (An et al., 2016). In human blood cells, brominated FRs were shown to mediate both single-strand breaks (SSBs) and DSBs, at lower and higher concentrations, respectively. TBBPA and PBP were more genotoxic than TBBPS and 2,4,6-TBP. The same study also reported the interesting fact that these FRs did not show any covalent binding to DNA, thus ROS production is the most probable underlying mechanism of their genotoxicity (Fig. 3) (Barańska et al., 2022c).

In whole organisms exposed to FRs in vivo, evidence for DNA damage caused by ROS produced under the influence of FR treatment is also abundant. Early studies were performed by Riva et al. and Baron et al. on aquatic clams, *Dreissena polymorpha* and *Mytilus galloprovincialis*, respectively, demonstrating the DSB-inducing action of ROS produced in conditions of PDBE exposure (Riva et al., 2007; Regoli et al., 2004). Later, similar results were obtained in vivo for more complex organisms and other FR types. The most interesting and relevant studies include: a demonstration of indirect PBDE genotoxicity by ROS formation in exposed Pacific codfish (Wang et al., 2015); a comprehensive study on effect of organophosphate FRs on Chinese rare minnow which showed the requirement for oxidative stress for DNA damage detection in tissues of this fish (Chen et al., 2019a); a comparison of BDE-47 and 6-OH-BDE-47 in terms of genotoxicity to maize plants, where the higher DNA damage potency of the latter compound was linked to its stronger ability to induce ROS production (Xu et al., 2015); finally, an in vivo genotoxicity study in mice, where gene mutations and chromosomal aberrations were causally linked to ROS induced by BDE-47 treatment of live animals (You et al., 2018).

All the above-cited studies referred to DNA damage by assaying final endpoints – DSBs, chromosomal breakage etc. – rather than the molecular mechanism of initial DNA damage. This gap in knowledge was filled by a number of reports which found oxidative damage to guanine moieties and resulting formation of 8-oxoguanine as the paramount molecular outcome of FR-induced ROS. It is well established that 8-oxoguanine is both

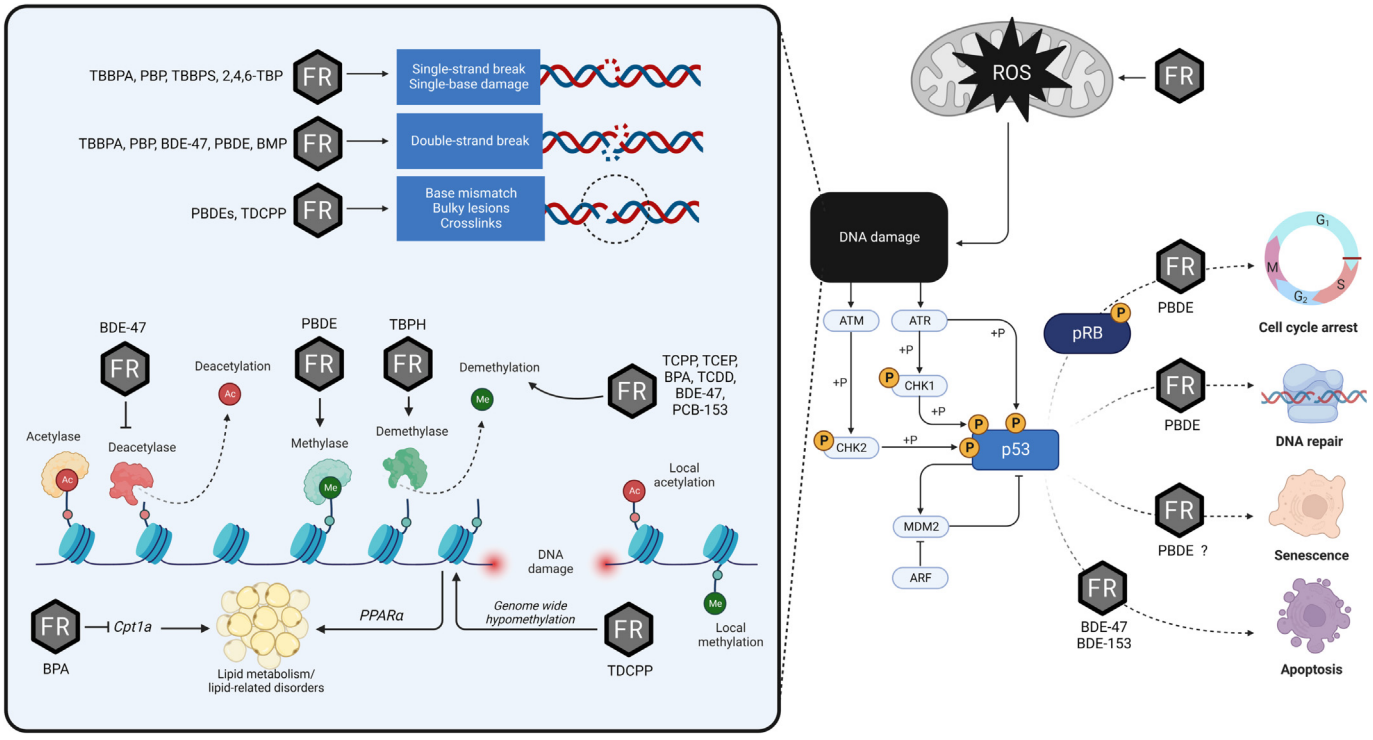

Fig. 3. Reported mechanisms of genotoxic effects of FR exposure in mammalian cells. Flame retardant (FR), 2,2-bis(bromomethyl)-1,3-propanediol (BMP), 2,4,6-tribromophenol (2,4,6-TBP), bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH), bisphenol A (BPA), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), brominated diphenyl ethers-153 (BDE-153), brominated diphenyl ethers-47 (BDE-47), pentabromophenol (PBP), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls 153 (PCB-153), reactive oxygen species (ROS), tetrabromobisphenol A (TBBPA), tetrabromobisphenol S (TBBPS), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), tris(2-chloroethyl) phosphate (TCEP), tris-2-chloroisopropyl phosphate (TCPP), acetyl (Ac), methyl (Me).

mutagenic, causing mistakes in DNA replication and transcription (mismatch pairing and G to T transversion), and undergoes further reactions that lead to depurination, SSBs and DSBs. Thus, a seminal study by Gao et al. demonstrated a positive relationship between ROS level and 8-oxoguanine formation in BDE-47 treated neuroblastoma cells (Gao et al., 2009). In a similar neuroblastoma model, several PDBEs with different potencies were used to support the direct relationship between the extent of ROS stimulation and the resulting oxidative DNA damage, further supporting the 8-oxoguanine mechanism as predominant (Pellacani et al., 2012). This effect seems to be common to structurally unrelated groups of FRs – similar results were reported for fish treated with TDCPP, with a causal relationship proposed between ROS production, 8-oxoguanine formation, DNA damage and intrinsic apoptosis induction (Fig. 3) (Chen et al., 2019b). The role of oxidatively damaged DNA and resulting DSB signaling (mainly via p53) to cell cycle arrest and apoptosis in cytostatic and cytotoxic action of FRs has been confirmed in multiple cell models. Liu et al. reported that increased ROS production from cells treated with PBDE quinone metabolite was responsible – via DNA damage detection and signaling – for p53 dependent cell cycle arrest and apoptosis by caspase-3 activation pathway. It is interesting to note that scavenging of ROS by treating exposed cells with an antioxidant agent (e.g. *N*-acetyl-L-cysteine) led to prevention of both DNA damage and apoptosis (Liu et al., 2021). The described mechanism seems to be true also in the case of zebrafish: treatment of adult fish with BDE-47 and BDE-153 showed oxidative stress which consequently led to DNA damage, upregulation of p53 and caspase-3 and downregulation of anti-apoptotic *Bcl-2* and *Bax* genes, with BDE-47 more potent of the two FRs in all of the assays (Meng et al., 2020). In human lung epithelium cellular model, the same course of events – DNA damage mediated by ROS, activating the p53 pathway and leading to apoptosis – was shown for a set of organophosphorus FRs containing an aryl group (Yuan et al., 2019). Finally, in human urothelial cells exposure to BMP was shown to cause oxidative damage to purine moieties by a direct enzymatic assay, with subsequent DSB generation and stimulation of oxidative stress responders HSP70 and Nrf2 (Kong et al., 2011). These findings support the hypothesis that oxidative stress is the main driving mechanism in DNA damage induction by FRs.

3.2. FRs impair DNA damage response and DNA repair

Since DNA damage is tightly controlled by relevant signaling pathways in animal cells, and several repair systems are upregulated in response to it, there can be two possible effects of genotoxic FR exposure: an enhancement of the DNA repair response, potentially capable of reversing the damage caused by the oxidative lesions derived from FR action, or an additional deleterious effect stemming from direct interference of FRs with the repair systems. In the latter case, the DNA damage effect would be amplified by the concurrent failure of repair mechanisms. Interestingly, there are examples of both types of effects in the literature for several different types of FRs. For example, Ji et al. reported that PDBE exposure of chicken cell lines led to higher ROS production, leading to apoptosis and DNA damage. At the same time, markers of DNA repair were upregulated, including the base excision pathway involving DNA polymerase β and histone variant γ-H2AX which is an element of the early response to DSBs. While BDE-47 treatment caused higher level of γ-H2AX formation in the repair system of treated cells, 6-OH-BDE-47 showed a stronger genotoxic effect compared to BDE-47 (Fig. 3) (Ji et al., 2011). TDCPP, TCEP, TCPP, and PBDE treatment of human blood-derived cells caused an increase in the level of DSBs, but also a stimulation of γ-H2AX binding, which can be therefore used as an indicator of DNA DSB formation (Zhang et al., 2019b; Bukowski et al., 2019). Downstream of γ-H2AX binding, non-homologous end joining (NHEJ) DNA repair system is activated to ligate DSBs, and a study in neuroblastoma cells confirmed the upregulation of NHEJ in response to acute exposure to PBDEs (Fig. 3) (Gao et al., 2009). This repair response is universal and was also observed in plants after exposure to 6-OH-BDE-47 (Xu et al., 2015).

Another group of studies reported a more complex response of DNA repair systems to FR challenge: instead of simple upregulation, the effect was concentration-dependent and involved an inhibition of DNA repair at higher FR concentrations. For example, in human breast cells exposed to low HBCD concentrations, some DNA damage was found in the form of DSBs, with concurrent induction of DNA repair response mechanism elements (ATM, OGG1 and MTH1). However, expression of the same genes was inhibited at higher HBCD concentrations (Li et al., 2017). Similarly, in zebrafish exposed to TDCPP, low concentrations activated the DNA repair system, while higher concentrations inhibited it (Chen et al., 2018). In zebrafish liver treated with TPHP, a clear concentration-dependent inhibition of NHEJ and base excision repair pathways was observed (Du et al., 2016). In another in vivo study, chronic exposure to PBDE led to accumulation of DSBs with impaired DNA repair response in mouse mammary tissue (Lamkin et al., 2022). More specifically, in neuroblastoma cells PDBEs caused induction of Xrcc1, a key protein in SSB repair, but a decline in expression of Xrcc3, a member of DSB repair system (Gao et al., 2009). Interestingly, a negative correlation between telomere length (used as a marker of DNA repair efficiency) and bis (2-chloroethyl) phosphate (BCEP) level in peripheral leukocytes of women firefighters was reported, but the underlying mechanism is unclear (Clarity et al., 2021).

3.3. FRs disrupt chromatin structure and modification, damaging the epigenome

FRs can exert genotoxic effects on cells not only by inducing direct and indirect damage to DNA and its repair systems, but also by interfering with epigenetic modifications of chromatin, leading to deleterious changes in chromatin packing, accessibility and gene expression. Studies have shown the influence of FR treatment on most types of epigenome markers, including post-translational modifications of histones and other chromatin proteins, DNA methylation as well as noncoding RNA activity. Interestingly, modulation of histone modifications by FRs is recorded relatively rarely in the literature. TBBPA was found to interfere with thyroid hormone-mediated epigenetic changes in histone acetylation and methylation levels in a set of responsive genes (Otsuka et al., 2014). PBDE prenatal exposure of Wistar rats led to suppression of protamine (Prm1, Prm2) gene expression, defects in histone-protamine exchange during spermatogenesis and consequently modified sperm head shape due to faulty chromatin packaging (Khalil et al., 2017). Histone modification analysis showed that male mice were more vulnerable to TPHP-dependent histone 3 acetylation in comparison to females, while H3K9 methylation level increased in females (Shafique et al., 2023). BDE-47 was found to downregulate the important histone deacetylase SIRT1 and consequently to increase the levels of acetylated NF-κB p65 and histone H3 – this resulted in enhancement of the inflammatory response (Fig. 3) (Zhang et al., 2015). Some studies were also based on high throughput RNA-seq analysis of human cells treated with PBDEs – while no clear-cut markers of epigenetic toxicity were identified, normal chromatin remodeling was somewhat changed, which was mediated by abnormal expression of histone gene cluster and modifying enzymes (Li et al., 2014b; Poston et al., 2018; Poston and Saha, 2019).

The literature on impact of FRs on DNA methylation is much more abundant. In many cases, several epigenetic markers were found to be modified, with the most significant effect seen on DNA methylation. For example, mice exposed to BPA experienced higher level of lipid accumulation, which was a consequence of down regulation of *Cpt1a* as the key gene of hepatic lipid metabolism. It turned out that BPA increased DNA methylation upstream of *Cpt1a* gene, but also decreased di-methylation of histone H3, lysine 4, tri methylation of histone H3, lysine 36, and histone 3 acetylation within the same promoter (Fig. 3) (Strakovsky et al., 2015; Stel and Legler, 2015). In another study, TBPH induced promotor demethylation of the PPARα gene, another important factor in lipid metabolism and lipid-related disorders (Guo et al., 2021). In a fibroblast cell line, PDBE exposure led to demethylation of PPARγ-2 promoter and consequently up regulation of the gene (Kamstra et al., 2014). FRs do not only affect methylation at individual gene promoters: PBDE exposure in Wistar rats caused sperm DNA to be hypermethylated, with sperm of younger rats having the same epigenetic modification levels in CpG islands as in older rats. Interestingly, the same pattern was seen for sncRNA expression in spermatozoa (Pilsner et al., 2021; Suvorov et al., 2020). Conversely, offspring of PBDE-exposed female mice with Rett syndrome had higher global DNA hypomethylation (Woods et al., 2012), and TDCPP induced genome-wide hypomethylation within early zebrafish embryos (Fig. 3) (Volz et al., 2016). In vitro study of TPHP effect on male and female mice demonstrated a global DNA hypomethylation in both genders (Shafique et al., 2023). TCPP, TCEP, BPA, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), BDE-47, and polychlorinated biphenyl PCB-153 also caused global DNA demethylation in neuroblastoma, fibroblasts and blood cells, which was suggested to be caused by ROS generation (Fig. 3) (Sales et al., 2013; Bukowski et al., 2019). Meanwhile, no changes in DNA methylation were observed in TPHP and TBOEP treatment of Chinese rare minnow fish (Chen et al., 2019a).

DNA methylation level is regulated by the interplay of DNA methylases and demethylases, with the methyltransferase enzyme family as the central player. There are several reports of direct interference of FRs with the activity of these crucial enzymes. BPA and similar FRs were found to upregulate EZH2, a methyltransferase important for regulating the expression of some tumor suppressor genes (Anzalone et al., 2021). Interestingly, overexpression of EZH2 by FRs seems to be mediated epigenetically as well - by increasing histone H3K4 trimethylation and histone acetylation (Bhan et al., 2014; Doherty et al., 2010). An interesting in vivo study on three generation of mice exposed to BDE-209 showed contrasting effects on the expression of two important DNA methyltransferases: while Dnmt1 expression was inhibited, Dnmt3a was induced, which may reflect the conflicting reports on hyper-or hypomethylation of DNA after FRs exposure (Hsu et al., 2021). In a generational study of human accidental exposure to polybrominated biphenyl (PBB), FRs were found to affect DNA methylation by reducing de novo as well as maintenance DNA methyltransferase activity (Greeson et al., 2020).

3.4. FR exposure leads to abnormal gene expression patterns

FRs cause deleterious modulation of gene expression also by other, not clearly described mechanisms, with deep results for cellular homeostasis. In HeLa cells exposed to quinone metabolites of PBDEs, an increase in the gene expression and protein level of PARP-1 was found. PARP-1 is not only crucial for DNA damage repair, but also an inducer of parthanatos, a caspase-independent cell death pathway. Therefore, this study introduced parthanatos as a possible underlying mechanism of cell damage due to PBDE exposure (Dong et al., 2018). Another investigation of the mechanism of PBDE action on cellular signaling in fibroblasts demonstrated upregulation of p53 and down regulation of pRb, with strong indications of indirect effects on cell cycle arrest (Fig. 3) (Manuguerra et al., 2019). In pheochromocytoma cells, PDBE quinone treatment resulted in an increase in the level of ATG5 and LC3B-II, key iron homeostasis proteins, as well as concurrent increase of ferritin heavy chain expression at the mRNA and protein level. This led to increased ferritin lysosomal localization and degradation, increased iron influx and finally ferroptotic cell death. It is worth noting that PBDE quinones also downregulated the expression of GPX4, an inhibitor of ferroptosis, which further boosted ferroptosis in exposed cells (Dong et al., 2019). In human and rat renal cells, TBBPA and HBCD treatment induced genes involved in eicosanoid and arachidonic acid metabolism (leading to potential pro-inflammatory outcomes), but also elevated level proteins involved in energy metabolism, like glyceraldehyde-3-phosphate dehydrogenase or inositol-1,4,5-trisphosphate-3-kinase (Barnett et al., 2021).

4. Alterations in reproductive function caused by FRs

Sexual reproduction, especially in higher animals, is dependent on a complex interplay of hormonal signals, cellular differentiation and coordinated activity of dedicated organs. Some of the earliest identified toxicological aspects of FR exposure are related to disruptions of this network of processes. Because reproduction (both from the point of view of

development of gametes and function of the reproductive organs, and of the post-zygotic embryonic development) is extremely complex and only outward phenomenological aspects are readily available for study, in many cases the underlying mechanism of FR action is unknown (or even not investigated at all), but it can be surmised that in most of them the previously described cellular effects, especially the capacity to disrupt membrane functions and alter the function of hydrophobic receptor proteins, are the main culprits. FR exposure can alter reproduction capacities of both genders, and directly influence the development of the growing embryo.

4.1. Effect of FR exposure on male reproductive system

The impact of FRs on male fertility is mostly negative, with reports of disrupted production and action of sex hormones (androgens), abnormal morphology of male reproductive organs (testes) and cells (sperm). Developmental exposure of Wistar rats to HBCD compromised the male reproductive system by reducing glandular weights (testis, prostate, and adrenals). An interesting disruptive effect of HBCD on the male reproductive system was an increase in anogenital distance, reflecting failure in male reproductive tract masculinization. HBCD exposure resulted in abnormal morphology of sperm cells as well (Fig. 4). These observations were explained by potential antagonistic interaction of HBCD with androgen receptors (van der Ven et al., 2009). TBBPA also has strong negative effect on the development of male reproductive system - postnatal exposure of mice to TBBPA disturbed testis development and caused abnormal structure of adult testes (Li et al., 2022c). An extensive meta-analysis of previous in vivo studies in rodents conclusively demonstrated that TBBPA decreased organ (testes and prostate) weight, sperm counts and quality, as well as hormone levels (Wu et al., 2021). Most studies ascribe these effects also to anti-androgenic activity – interestingly, one study found TBBPA to be anti-androgenic in vitro, but at the same time showed enhancement of androgen action by HBCD and PBDEs, in contrast to other studies (Christen et al., 2010). This contributes to conflicting reports about the ultimate effects of PBDEs: in one study, prenatal exposure of Wistar rats showed disruptive effects on the male reproductive system in terms of reduced testes size, abnormal sperm head morphology and even transcriptomic changes (Fig. 4) (Khalil et al., 2017). In contrast, another animal study on PBDE- and HBCD-exposed adult male Sprague Dawley rats showed no changes in terms of testes weight, testosterone levels, testicular function, and even DNA integrity of sperms (Ernest et al., 2012). To investigate if there is any correlation between cryptorchidism and exposure to PBDEs, the levels of these compounds were assessed in the breast milk and placenta of mothers of boys with cryptorchidism. The results demonstrated cryptorchid boys had higher level of PBDEs in breast milk samples, but not placenta, in comparison to control groups (Main et al., 2007). On the other hand, another study showed no significant association between urine level of several phosphoroorganic FRs and sperm count or morphology (Fig. 4) (Ingle et al., 2018).

4.2. Effect of FR exposure on female reproductive system

Female fertility is affected by FRs mostly by dysregulation of folliculogenesis. Brominated FRs such as PBDEs and HBCD were demonstrated to disturb folliculogenesis in exposed rats, with chronic treatment causing an increase in the number and size of follicles in ovaries. At the molecular level, FRs downregulated ovarian levels Cyp17a1, a crucial enzyme in gonadal steroid biosynthesis, as well as Insl3 which is required for normal follicle growth and granulosa cell proliferation (Lefèvre et al., 2016). A similar study on rats exposed to a mixture of brominated FRs reported no changes in follicle number, but abnormal follicle morphology, including multi-oocyte follicles and disordered granulosa layers. In this study, ovarian gene expression analysis confirmed the downregulation of many genes important for ovarian folliculogenesis (Allais et al., 2020). TPHP also compromised fertility of female mice by decreasing follicle numbers, with

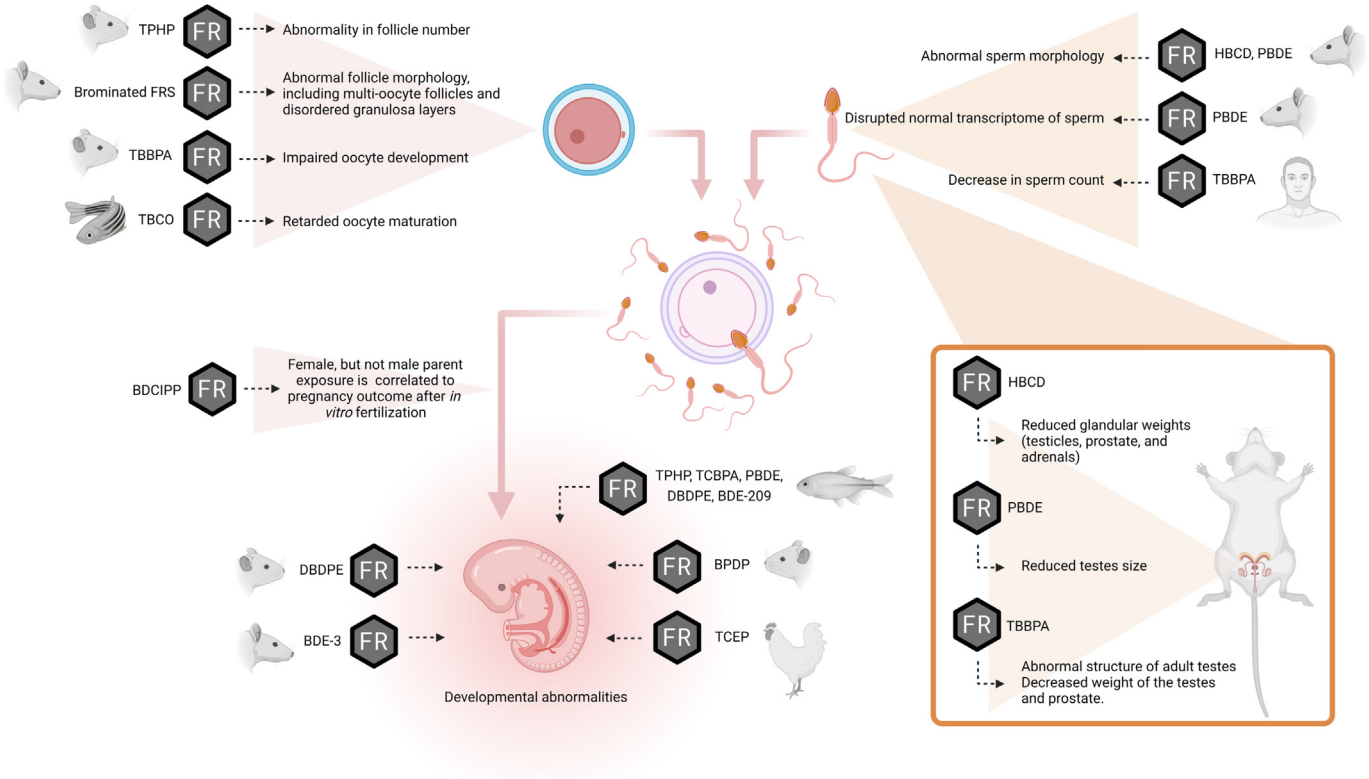

Fig. 4. Effects and mechanisms linked to reproductive toxicology of FRs in animals. Flame retardant (FR), triphenyl phosphate (TPHP), 1,2,5,6-tetrabromocyclooctane (TBCO), bis(1,3-dichloro-2-propyl)phosphate (BDCIPP), bromodiphenyl ether (BDE-3), decabromodiphenyl ethane (DBDPE), decabromodiphenyl ether (BDE-209), hexabromocyclododecane (HBCD), polybrominated diphenyl ether (PBDE), *tert*-Butylphenyl diphenyl phosphate (BPDP), tetrabromobisphenol A (TBBPA), tetrachlorobisphenol A (TCBPA), tris(2-chloroethyl) phosphate (TCEP).

downregulated estrogen signaling (Fig. 4) (Ma et al., 2021b). Some FRs disrupt gamete production itself - spindle migration and membrane protrusion in oocytes of mice exposed to DBDPE were both impaired, resulting in compromised oocyte development (Shi and Feng, 2021). TBBPA was found to have a strongly deleterious effect on bovine oocyte development, with meiotic failure, disturbed polar-body extrusion, defective spindle assembly and chromosomal alignment (Guo et al., 2022). 1,2,5,6-Tetrabromocyclooctane (TBCO) was found to cause retarded oocyte maturation in zebrafish, with dysregulated expression of a number of important genes (Van Essen et al., 2021). The female reproductive system may be negatively impacted by FRs also at the post-fertilization stage. Mice exposed to a batch of organophosphate FRs at environmentally credible concentrations showed severe placental disorders, frequent implantation failure, restricted fetal growth and stillbirths (Xu et al., 2022). A positive correlation was between the urine level of bis(1-chloro-2-propyl)phosphate (BCIPP) and spontaneous abortion in human patients, potentially linked to disruption of tryptophan and fatty acid metabolism (Li et al., 2021a). Female, but not male parent exposure to bis(1,3-dichloro-2-propyl)phosphate (BDCIPP) was found to be correlated to pregnancy outcome after in vitro fertilization (Fig. 4) (Carignan et al., 2018). Another cohort study which measured exposure to PBDEs reported much weaker associations, with some association observed only for decreased implantation and live birth (Ingle et al., 2021).

4.3. FRs dysregulate embryonic development

Most studies on the impact of FRs on embryonic development have been performed in the easiest animal model, the zebrafish, and their direct translation to mammalian physiology is dubious. However, a broad plethora of effects has been observed in this model, and many of them are probably relevant to human physiology as well, so we will list some interesting recent examples (Fig. 4). TPHP exposure led to neurodevelopmental abnormalities at various life stages of the zebrafish embryo (Zhang et al., 2023b). Embryonic toxicity of TCBPA caused permanent developmental abnormalities not only in the nervous, but also in the cardiovascular system of the fish (Liu et al., 2023). Hydroxylated PDBE congeners rewired lipid metabolism in the developing zebrafish embryo, with changes in phospholipid profile leading to tissue anomalies (Gustafsson et al., 2023). The neurodevelopmental toxicity potential of DBDPE was shown by upregulation of touch response and swimming activity in developing zebrafish larvae, with underlying neurotransmitter imbalances (Hua et al., 2022). Another study reported that zebrafish larvae had a decreased life span and body mass after exposure to BDE-209 (Han et al., 2017). Among higher vertebrates, chicken embryonic development is significantly impacted by TCEP exposure, with most quantitative traits impaired upon incubation in a shell-less egg system (Kanda et al., 2021). Also in chicken, brominated FRs were specifically concentrated in the developing embryonal brain and reached neurotoxic concentrations even with low environmental exposure ratios (Yadav et al., 2022). More interestingly, highly specific impact of *tert*-butylphenyl diphenyl phosphate (BPDP) on the hedgehog protein signaling pathway caused teratogenic disruption of limb development in murine embryos, with ossification failures and limb bud abnormalities (Yan and Hales, 2020). BDE3 inhibited fetal testicular development by selectively promoting the apoptosis of Leydig cells in the developing rat embryo (Li et al., 2021b). Developmental toxicity can cause higher-order neurobiological effects in adults, as shown by a study which demonstrated cognitive and social disorders in mice which were exposed in utero to relatively low concentrations of DBDPE – this was traced back to disruption of cell division in the developing nervous system (Shi et al., 2022). Even epidemiological exposure studies in humans confirm the potential for developmental toxicity of FRs: placental stress biomarkers in mid-pregnancy were found to be strongly correlated to organophosphate FRs and their metabolites (Varshavsky et al., 2021). Low concentrations of BDE-47 had a deleterious impact on early retinal development in a human organoid model, highlighting the synergy of neurotoxicity and developmental toxicity of PBDEs (Li et al., 2022d).

5. Immune response alteration due to FR exposure

The immune system heavily depends on network of ligands and receptors. Its cellular elements are highly accessible to xenobiotics, often being initial targets of their function. There is some evidence implying immunotoxic and immunomodulatory effects of FRs on immune cells and humoral mediators. FRs can modify immune responses by inducing/inhibiting the expression of various genes involved in cytokine secretion, antigen presenting, and other immune functions. FRs are capable of modifying innate and adaptive responses by affecting cell differentiation and inflammatory response. In addition, some FRs may cause a shift in immune T cell responses from inflammatory to allergic responses, decreased immune response toward virus infection and finally drift from activated response to non-functional immune responses. The following sections summarize evidence of effects of various FRs on the immune system (Fig. 5).

5.1. FRs disrupt the extracellular immune ligand balance

A key regulatory element of the immune system are cytokines - small secretory proteins that mediate intercellular communication by binding to specific receptors; they organize and manage the growth, differentiation and activities of immune cells. One of key cytokine subgroups are interferons (IFNs), and their production and signaling can be impacted by FR exposure. IFNs are crucial mediators in anti-viral response, and a series of studies by Watanabe et al. has shown that FRs (e.g. DBDE and TBBPA) can increase the level of IFN-γ in bodily fluids of mice (Watanabe et al., 2008a; Watanabe et al., 2010). In contrast, in human complex immune cell preparation opposite results were found for two different FRs: HBCD increased IFN-γ secretion, while TBBPA decreased it. Inappropriate elevated level of IFN-γ may lead to pathological phenomena like autoimmunity development and tumor growth, while IFN-γ reduction can facilitate viral infection (Almughamsi and Whalen, 2016). TBBPA exposure of the whole animal activated interferon signaling pathways in rats, pointing to the possibility of complex FR effects both at the level of biogenesis and bioactivity of immune ligands (Dunnick et al., 2017). A recent study on long term effects of PBDE exposure on mice showed a reduction in type I IFN signaling pathway, which was consistent with down regulation of NF-kB and ISGF3, key pro-inflammatory transcription factors active in IFN production (Lamkin et al., 2022).

Other important cytokines are also modulated by FRs. Brominated FRs can disturb the level of TNF-α, a key proinflammatory cytokine, and consequently alter the inflammatory capacity of cells. HBCD increased the level of TNF-α by activating the p38 MAPK pathway, resulting in inflammation. However, TBBPA acted inversely, decreasing TNF-α release. This put in spotlight the difficulty to predict alterations in cytokine levels caused by exposure to FRs and their potential to disrupt the inflammatory responses (Yasmin and Whalen, 2018). Similarly, different patterns of cytokine modulation were observed after TCBPA exposure in comparison to TBBPA. Indeed, TCBPA treatment of mice led to a rise in the serum level of IL-2, IL-12, TNF-α, IFN-γ, IL-4, IL-5, IL-10, and GM-CSF, leading to conclusion that TCBPA caused disturbance in both pro-inflammatory and anti-inflammatory mechanisms (Fig. 5a) (Wang et al., 2021a). Cytokine profile analysis of human peripheral blood mononuclear cells (PBMCs) treated with PBDEs revealed increase in the level of TNF-α, IL-1β, IL-6, and IL-8, implying PBDE treatment resulted mostly in induction of pro-inflammatory cytokines and ultimately in promotion of innate immune responses, which might have a driving role in development of autoimmune disorders (Mynster Kronborg et al., 2016). In mouse macrophages, both TBBPA and 1,2-dibromo-4-(1,2 dibromoethyl) cyclohexane (TBECH) had an inducing effect on the level of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α. Furthermore, macrophages pre-treated with these FRs had a weaker response to LPS stimulation both in terms of these pro-inflammatory cytokines and some anti-inflammatory ones such as IL-4, IL-10, and IL-13, reflecting how TBBPA and TBECH can modulate normal immune responses toward immune stimulation (Wang et al., 2019; Wang et al., 2020). In contrast, more complex immunomodulatory effects were

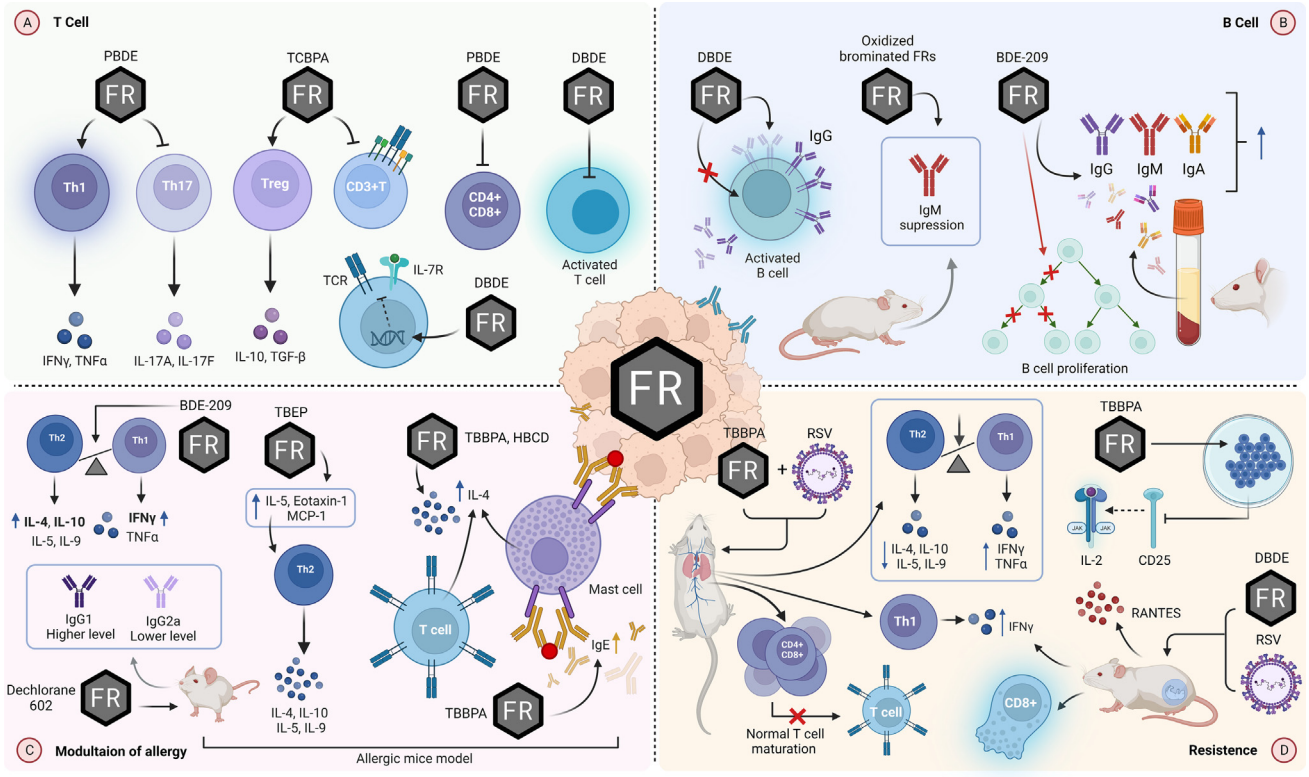

Fig. 5. Mechanisms of reported immunomodulatory and immunotoxic action of FRs on different elements of the mammalian immune system (excluding macrophages – see Fig. 6). Flame retardant (FR), polybrominated diphenyl ether (PBDE), decabromodiphenyl ether (BDE-209), decabromodiphenyl ether (DBDE), hexabromocyclododecane (HBCD), Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES), respiratory syncytial virus (RSV), tetrabromobisphenol A (TBBPA), tetrachlorobisphenol A (TCBPA), tris(2-butoxyethyl) phosphate (TBEP).

demonstrated in broiler chicks exposed to BDE-209. Downregulation of IL-18, IL-18R, IL-21, IFN-γ, and TNF superfamily members highlighted the immunomodulatory potential of this FR (Cheng et al., 2022). In human placental explants, TBBPA also had complex and paradoxical effects, stimulating the production of some pro-inflammatory cytokines (e.g. TNF-α and IL-6), but inhibiting others (e.g. IL-1β) (Arita et al., 2018).

5.2. The effect of FRs on cellular elements of the innate immune system

Since innate immunity is the first line of defense against multiple and various environmental threats and pathogens, its modulation or disruption by FRs can have highly deplorable results for human health. Macrophages (MQ) are one of the most important innate immune cell types and mediate crucial roles in early immune responses by cytokine production, inflammation, phagocytosis, and antigen presentation. A large number of studies showed direct impact of various FRs on their function, with often contradictory results, leading to the conclusion that MQs are a major cellular target of FR immunomodulation. In a study on a human cell line, TPHP and TDCPP reduced adhesion capacity of treated MQ cell model, while TNBP and TOCP stimulated this function. These results confirmed the capacity of phosphoroorganic FRs to dysregulate the pro- and anti-inflammatory profile of MQs (Li et al., 2020). BDE-47 was found to impair inflammatory responses of MQs stimulated with LPS by downregulating MMP12, IL-1β, IL-6, and TNF-α. Furthermore, BDE-47 treatment led to upregulation of E-cadherin, a membrane protein involved in cell to cell contact, which impairs LPS-induced inflammatory responses in MQs (Fig. 6) (Longo et al., 2019). However, MQs treated with BDE-47 showed an increase in the number of exosomes, which are small bioactive extracellular vesicles containing DNA, mRNA, miRNA, lipids and proteins. miRNA analysis of FR-induced exosomes showed an increase in the expression of miR-223-3p, miR-155-5p, and Let 7a-5p, which affect pathways of IL-6 and IL-1β expression, providing a mechanism of modulation of response to LPS stimulation (Longo et al., 2021). BDE-47exposure of MQs decreased the expression of HLA-DR and CD209, important elements of the antigen presenting complex called the immunological synapse. This led to modulation of MQ differentiation into pro- or anti-inflammatory phenotypes (M1 or M2), probably due to impaired MQ capacity to form immunological synapses (Longo et al., 2023). On the other hand, BDE-209 targeted MQs mainly by inducing TLR4 expression via impairment of the microRNA miR-21, a negative regulator of TLR4 expression. Excess TLR4 accumulated within MQ and facilitated lipid accumulation in MQs, foam cell formation and change in the normal differentiation pathway (Zhi et al., 2018; Zhi et al., 2019a). Another MQ cell surface receptor is CD36, a scavenger/oxidized lipoprotein receptor which also plays a role in lipid accumulation and foam cell formation. PBDE quinones could upregulate the expression of CD36 in mouse bone marrow-derived MQs. Moreover, the expression of cholesterol transporters ABCG1 and ABCA1 decreased in these cells exposed to PBDE quinones, so that these FRs prevented cholesterol exclusion from cells. This led to enhanced lipid accumulation in MQs and facilitated their differentiation into foam cells. Another outcome of PBDE quinone treatment was activation of NLRP3 and its inflammasome complex, leading to increased level of activated caspase-1 and confirming that FRs can induce inflammatory cell death called pyroptosis (Fig. 6) (Wang et al., 2021b). Consistent with this study, PBP-exposed microglia (a type of MQ from the CNS) showed higher level of NLRP3 activation, however BPA treatment caused a decrease in NLRP3 activity. Immunosuppression effects of BPA on MQs were mediated by inhibition of MAPK and NF-κB pathways (Lee et al., 2020; Bowen et al., 2020). In addition, TBBPA, TPHP, and TDCPP treatment of MQs resulted in reduction of phagocytosis, highlighting the probable membrane-related effects of these hydrophobic FRs (Wang et al., 2019; Li et al., 2020; Lee et al., 2020). Antigen presenting capacity of MQs was also targeted by FRs: TBBPA-and TBECH-treated MQs from mice expressed

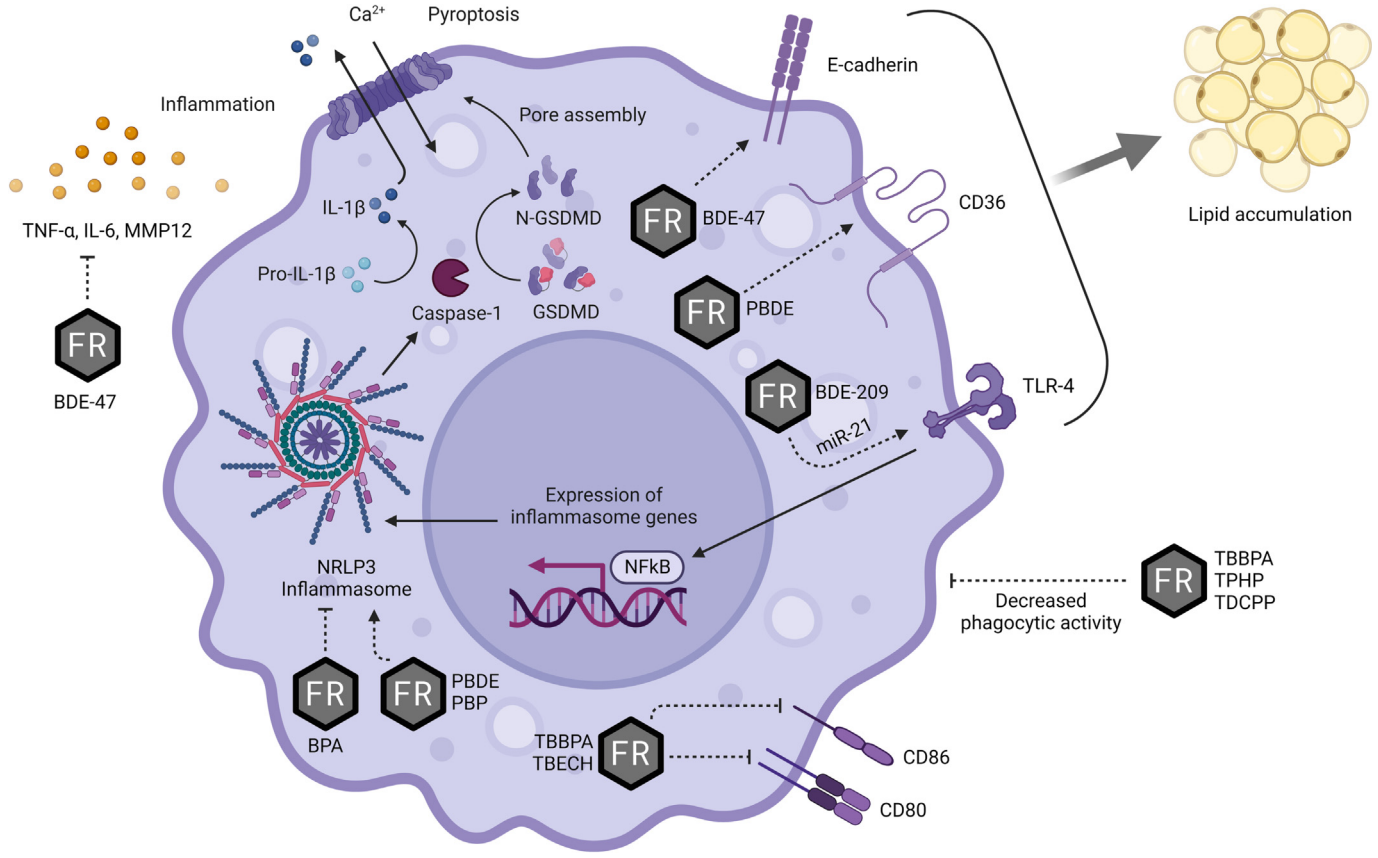

Fig. 6. Macrophages as the best-studied immune-related target of FR exposure – reported molecular targets and impacted activities. Flame retardant (FR), 2,2′,4,4′-tetrabromodiphenyl ether (BDE-4), bisphenol A (BPA), decabromodiphenyl ether (BDE-209), gasdermin D (GSDMD), *gasdermin*-D, *N*-terminal (N-GSDMD), pentabromophenol (PBP), polybrominated diphenyl ether (PBDE), tetrabromobisphenol A (TBBPA), tetrabromoethylcyclohexane (TBECH), triphenyl phosphate (TPHP), tris(1,3-dichloro-2-propyl)phosphate (TDCPP).

decreased levels of CD80 and CD86 co-stimulatory receptors, which are crucial for antigen presentation (Fig. 6) (Wang et al., 2019; Wang et al., 2020).

Other cellular components of innate immune system are also influenced by FRs, highlighting their probable role in disrupting host defense against pathogens. Overexpression of the CD63 receptor on activated basophiles decreased upon BDE-47 treatment, confirming modulatory capacity of this FR in basophile degranulation in response to allergens and parasites (Longo et al., 2019). In neutrophils, BDE-47 exposure enhanced ROS production caused by NADPH oxidase activity, leading to ERK and MAPK p38 signaling pathway activation and subsequent formation of neutrophil extracellular traps, a terminal activation mode with potentially deleterious consequences (Ye et al., 2021; Zhou et al., 2021). Another halogenated FR, dechlorane 602, led to increase in the frequency of activation of neutrophiles, eosinophile, and innate lymphocyte cells, which could promote type 2 inflammation by producing more IL-4, IL-5, and IL-13 (Zhou et al., 2020). Two studies on respiratory burst (production of ROS crucial for the initial phase of innate reaction to pathogens) reported its overactivation in neutrophils treated with TBBPA or PDBEs, with potential consequences both for spurious inflammatory reactions in the absence of pathogens as well as innate immunity depletion before actual pathogen exposure (Reistad et al., 2005b; Reistad and Mariussen, 2005). Dendritic cells (DCs) as one of the main parts of innate immune system play crucial roles in initiation and development of immune responses. TPHP treatment of DCs, a crucial cell type in initiation of immune response, increased the expression of IL-6, MHC II, CD80, CD86, and CD40 molecules in DCs, causing their overactivation (Canbaz et al., 2017). Interestingly, cellular differentiation analysis of PBDE-exposed mice showed an increase in the percentage of immature DCs, which was consistent with diminished levels of type I IFNs and attenuation of IFN signaling pathway (Lamkin et al., 2022).

5.3. Adaptive immunity-related effects of FR exposure

Adaptive immunity is mediated mostly by T cell and B cell subtypes, and their differentiation is a common target for immunomodulatory substances, including FRs. Different subsets of CD4-positive helper T cells, e.g. Th1, Th2, and Th17, have regulatory roles, while CD8-positive cells are mostly directly cytotoxic. Humoral immunity, on the other hand, consists mostly of immunoglobin production by B cells. FRs can impact both the proportion of different cell types by modulating their differentiation, and directly influence the activity of the differentiated forms. When the profile of PBMCs treated with PBDEs was assessed, the results demonstrated a dominance of Th1 cells and suppression of Th17 cells. Alteration of the normal composition of CD4+ T cells is a negative outcome from the point of view of adaptive immunity, because Th1 induction may promote autoimmune disorders and Th17 suppression may cause higher vulnerability to fungi and extracellular infections (Fig. 5a) (Mynster Kronborg et al., 2016). TCBPA-exposed mice demonstrated an immunosuppressive state with increased percentage of Treg cells and decreased frequency of CD3+ T cells. In addition, TCBPA treatment decreased lymphocyte proliferation rate, confirming its immunotoxic effect (Wang et al., 2021a). Analysis of mouse embryonic stem cells exposed to TBBPA revealed immunomodulatory effect on immune cell differentiation by downregulation of IL-7 receptor (crucial for T cell survival) and T cell receptor genes cd79a and cd79b (Tribondeau et al., 2022). Treatment of female mice with a PDBE mixture led to a significant decrease in numbers of double-positive (CD4+ CD8+) T cells in the spleen, pointing to an unbalanced T cell differentiation pathway (Fair et al., 2012). NK cells are another immune cell type involved both in innate and adaptive response and subject to FR immunomodulation. TBBPA strongly inhibited the lytic activity of exposed human NK cells, with immunosuppressive effects also on their binding capacity to other cells, with potential

deleterious effects on antiviral or anti-tumor immunity (Fair et al., 2012). A similar mode of action was later demonstrated by the same group for HBCD (Cato et al., 2014).

Humoral immunity is also affected by FRs: for example, BDE-209-treated mice had higher levels of IgG, IgM, and IgA in serum, accompanied by atrophy of thymus and spleen and decreased B cell proliferation (Liao et al., 2021; Liu et al., 2012). Exposure to DBDE decreased immune responses in developing rats, both in terms of cellular and humoral activities. DBDE decreased the frequencies of activated B, T, and NK cells and IgG antibody level (Fig. 5a, b) (Teshima et al., 2008). Developmental exposure of Wistar rats to HBCD resulted in a decrease in thymus weight and NK cell activity, however, IgG response and the percentage of circulating neutrophils increased, highlighting the complicated effects of HBCD treatment (van der Ven et al., 2009). In addition, investigation of the humoral immune system of mice exposed to dechlorane 602 showed increased levels of IgG1 and reduction in IgG2a concentration (Feng et al., 2016). In adult mice exposed to BDE-209, an imbalance in immunoglobulin levels was also seen, with potential immunotoxic effects mediated by the changes in humoral immunity (Liao et al., 2021). Exposure to oxidized brominated flame retardant forms in female mice led to suppression of the IgM antibody type formation, again leading to an immunoglobulin imbalance and potential disruption of humoral immunity (Fig. 5b) (Frawley et al., 2014).

5.4. FRs can exacerbate inflammation, allergy and related pathological reactions

Inflammation is a complex immune response that is usually a direct result of challenge to adaptive immune cells, and its incorrect and excessive activation may lead to hypersensitivity and/or allergy. Mice exposed to dechlorane 602 had lower levels of Th1-related cytokines IFN-γ and TNF-α, and higher level of Th2-related cytokines IL-4, IL-10 and IL-13, highlighting a dysregulation of Th1/Th2 balance. Since this was detected in a pronounced manner in airway epithelium, this immunomodulatory imbalance may lead to disorders including asthma and other allergic reactions (Zhou et al., 2020; Feng et al., 2016). A similar Th1/Th2 imbalance was observed in BDE-209-exposed mice. Indeed, the level of IFN-γ – a Th1-related cytokine – decreased, while the level of IL-4 and IL-10 (Th2-related) increased, which led to deviation from normal Th1/Th2 ratio (Fig. 5c) (Liao et al., 2021). Pro-inflammatory effects of PBDEs were confirmed in a human bronchial epithelial cell model: PBDE treatment led to increased level of ICAM-1, IL-6, and IL-8 via EGFR phosphorylation and activation of its signaling cascade (Koike et al., 2014). ICAM-1 upregulation was also reported in another study on BDE-209 exposure of endothelial cells. Interestingly, BDE-209 targeted miR-141 which was proven to function as a negative regulator of ICAM-1 expression (Zhi et al., 2019b). In an allergic mice model, chronic TBEP treatment led to augmentation of Th2 responses by increasing the expression of IL-5, eotaxin-1 and MCP-1. Cell proliferation in lymph nodes increased after TBEP feeding, while total cell number in bone marrow decreased, implying immune cell migration from bone marrow to secondary immune organs with resultant exacerbation of the allergic response (Fig. 5c) (Yanagisawa et al., 2020). In another allergic mouse model, isolated splenocytes were treated with HBCD and TBBPA, leading to upregulation of MHC II, CD86and T cell receptors, with no change in the expression of CD19. The resulting allergic response stimulation was also due to increasing the level of IL-4, an important element of Th2 response (Koike et al., 2013). The potential of TBBPA to enhanced allergic inflammation was confirmed by upregulated expression of IgE receptor in differentiating embryonic mouse stem cells (Tribondeau et al., 2022).

5.5. Impact of FRs on resistance to pathogens and other results of immune function

Since the immune system is a highly complex network of cells and humoral compounds that work in concert to respond to challenges, xenobiotics (including FRs) can disrupt its activity in a holistic manner, not just by acting on an individual cell type or biochemical signaling element. There are numerous reports in the literature of FRs having general immunotoxic effects by affecting the overall response to pathogens or endogenous stimuli for the immune system. A commonly used model of viral infection and the immunocompromising effect of FR exposure are mice infected with the respiratory syncytial virus (RSV). Developmental exposure of these mice to TBBPA predisposed them to more severe disease and suppressed the antiviral immune response. Indeed, infected mice had higher viral titer, and bronchoalveolar lavage fluid contained more Th1-related than Th-2 related cytokines, which can compromise antiviral immunity. Moreover, there was an increase in the level of CD4 + CD8 + immature T cells, which could reflect the potential of TBBPA to impair normal T cell maturation of RSV-infected mice (Fig. 5d) (Watanabe et al., 2010). Another study in the same model also found an increased level of IFN-γ interpreted as a marker of more severe lung disease (Takeshita et al., 2013). Finally, an earlier study on TBBPA-exposed mice found that there was a marked suppression of CD25 (an activation marker) expression in stimulated lymphocytes in conditions of RSV infection (Pullen et al., 2003). Similar effects, including an increase of IFN-γ level, upregulation of RANTES (a marker of disease severity) in lung tissue and the enhanced activity of disease-exacerbating cytotoxic lymphocytes, were also reported for another brominated FR, DBDE. Interestingly, DBDE had a stronger effect than TBBPA on cytotoxic (CD8 +) T cells, influencing their number as well as their function and cytokine production (Fig. 5d) (Takeshita et al., 2013; Watanabe et al., 2008b; Watanabe et al., 2017). Another virus with immune response to it impaired by FRs is coxsackievirus B3 (CVB3) which can cause respiratory illness, but also aseptic meningitis, myocarditis, and encephalitis. BDE-99 exposure caused higher liver uptake of virus in CVB3-infected mice and lower expression of MCP-1, reflecting the fact that BDE-99 exacerbated viral infection both by increasing viral load and by attenuating immune response (Lundgren et al., 2013). There is also a recent report of synergy between the immunosuppressive action of organophosphate FRs and cardiovascular sequelae of COVID-19 infection, although the elements of the immune system involved in this interaction remain to be identified (Rajak et al., 2022). Interestingly, the COVID-19 pandemic had another unexpected consequence for FR impact on human health – FR treatment of respiratory face masks contributed to increased exposure of the population at large to higher than usual FR concentrations (Fernández-Arribas et al., 2021).

Interestingly, the only literature about the impact of FR exposure on immune response to bacterial disease comes from research on fish models, highlighting still huge knowledge gaps in FR toxicology. Juvenile salmons exposed to PBDEs were significantly more susceptible to infection with the important bacterial pathogen *Listonella anguillarum* than non-exposed ones (Arkoosh et al., 2010). Their macrophages were found to have a much weaker response to bacterial challenge and impaired capacity to kill the bacteria by oxidative burst (Arkoosh et al., 2018). In another fish species, the fathead minnow, BDE-47 caused decreased survival after infection with *Yersinia ruckeri* and significantly weakened immune response to this pathogen, especially in male fish (Thornton et al., 2018). It can be hypothesised that similar effect could occur in the case of mammals, including humans, but the only relevant report is a negative finding of a lack of link between PBDE exposure and nasal colonization of humans with *Staphylococcus aureus* (Eggers et al., 2020). New epidemiological and experimental studies are urgently needed.

Impact of FR exposure on immunological condition can lead to even more complex outcomes, unrelated to response to pathogens. Patients affected with autism spectrum disorders (ASD) often show overactivation of pro-inflammatory cytokine production. PBDE treatment of isolated immune cells from ASD patients increased the level of pro-inflammatory IL-1β and IL-8, which may exacerbate neural cell damages by activation of microglia and astrocytes and generating neuroinflammatory conditions in the CNS (Ashwood et al., 2009). The immune system can be activated as a whole in situations unrelated to infection, e.g. after vaccination or in autoimmune disorders. Data on the impact of FRs on these processes is fragmentary and contradictory. It was reported that children with higher serum levels of PCBs had lower anti-tetanus antibody titers after vaccination,

which could confirm the adverse effect of PCB exposure on immune responses (Heilmann et al., 2006). Similarly, antibody production and other responses to the diphtheria, tetanus and pertussis vaccine were diminished in infants exposed to organophosphate esters (Hammel et al., 2022). On the other hand, evidence was described for increased production of autoimmune antibodies against thyroid peroxidase in firefighters who are routinely professionally exposed to high concentrations of various FRs (Ogunsina et al., 2022).

6. Conclusions

In the modern society, both in developed and developing countries, human exposure to FRs from household and industrial sources increases constantly. The diversity of FR congeners present in the human environment also increases due to progressive introduction of new ones and replacement of compounds restricted due to regulatory actions (e.g. the Stockholm convention). Other worrying developments with regard to the impact of FRs on public health are: concurrent exposure to other persistent organic pollutants with complex bioactivity and the potential for synergistic deleterious effects with increasingly common lifestyle diseases. For this reason, knowledge on the mechanisms of toxicity and bioactivity of FRs is more needed than ever, and our attempt to summarize elements of this knowledge serves to expose its still fragmentary character. FRs act directly on various cellular components, which leads to numerous documented physiological outcomes, especially within the reproductive and immune systems. However, new and more in-depth research is needed on actual biochemical and biophysical modes of action of FR molecules in living systems, including mechanisms of biological membrane disruption, hydrophobic interactions with cellular components as well as direct and indirect genotoxicity.

Gaps in mechanistic toxicology of FRs are apparent in this review: there is much more research available about phenomenological outcomes of FR exposure and physiological disruptions of higher order functions than on proven molecular and cellular mechanisms of interaction with biological systems. The diversity of models (cells vs. whole animals, species from different systematic groups, but also different routes and timescales of exposure to FRs) makes it difficult to interpret common modes of action from fragmentary data. However, some general conclusions can be made, more as calls to further action in terms of intensified research than final statements of fact. It can be concluded that despite the chemical diversity of FRs, many effects seen at the different levels of action, from molecular to clinical and epidemiological, are quite similar between them, which may be ascribed to their common identity as abiotic, non-reactive, hydrophobic small molecules with organic structures that include an inordinately high number of non-standard atoms (phosphorus, halogens etc.). Another important conclusion is that the immune system seems to be highly relevant to FR toxicity for humans, due to being the most complex target of their action with numerous real-life consequences of dysregulation. Consequently, extensive research effort is needed to link individual observations into a comprehensive model of immunomodulatory and immunotoxic impact of FR action.

CRediT authorship contribution statement

LK and LP collected the data and wrote the paper. LM helped in designing and preparing figures and table. LP acquired funding.

Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lukasz Pulaski reports financial support was provided by National Science Centre Poland.

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References

Allais, A., et al., 2020. In utero and lactational exposure to flame retardants disrupts rat ovarian follicular development and advances puberty. Toxicol. Sci. 175 (2), 197–209.
Almughamsi, H., Whalen, M.M., 2016. Hexabromocyclododecane and tetrabromobisphenol A alter secretion of interferon gamma (IFN-γ) from human immune cells. Arch. Toxicol. 90, 1695–1707.
Aluru, N., et al., 2021. Hepatic gene expression profiling of Atlantic cod (Gadus morhua) liver after exposure to organophosphate flame retardants revealed altered cholesterol biosynthesis and lipid metabolism. Environ. Toxicol. Chem. 40 (6), 1639–1648.
An, J., et al., 2016. The cytotoxicity of organophosphate flame retardants on HepG2, A549 and Caco-2 cells. J. Environ. Sci. Health A 51 (11), 980–988.
Anioł, B., Jankowski, D., 2018. Flame retardants of polymeric materials – calculation of the oxygen index. Techn. Trans. 115 (9), 57–66.
Anzalone, G., et al., 2021. PBDEs affect inflammatory and oncosuppressive mechanisms via the EZH2 methyltransferase in airway epithelial cells. Life Sci. 282, 119827.
Arif, J.M., et al., 2003. Interaction of benzoquinone- and hydroquinone-derivatives of lower chlorinated biphenyls with DNA and nucleotides in vitro. Chem. Biol. Interact. 142 (3), 307–316.
Arita, Y., et al., 2018. Effect of tetrabromobisphenol A on expression of biomarkers for inflammation and neurodevelopment by the placenta. Placenta 68, 33–39.
Arkoosh, M.R., et al., 2010. Disease susceptibility of salmon exposed to polybrominated diphenyl ethers (PBDEs). Aquat. Toxicol. 98 (1), 51–59.
Arkoosh, M.R., et al., 2018. Dietary exposure to a binary mixture of polybrominated diphenyl ethers alters innate immunity and disease susceptibility in juvenile Chinook salmon (Oncorhynchus tshawytscha). Ecotoxicol. Environ. Saf. 163, 96–103.
Ashwood, P., et al., 2009. Preliminary evidence of the in vitro effects of BDE-47 on innate immune responses in children with autism spectrum disorders. J. Neuroimmunol. 208 (1–2), 130–135.
Bajard, L., et al., 2021. Endocrine disrupting potential of replacement flame retardants - review of current knowledge for nuclear receptors associated with reproductive outcomes. Environ. Int. 153, 106550.
Barańska, A., Bukowska, B., Michałowicz, J., 2022a. Determination of apoptotic mechanism of action of tetrabromobisphenol A and tetrabromobisphenol S in human peripheral blood mononuclear cells: a comparative study. Molecules 27 (18), 6052.
Barańska, A., Sicińska, P., Michałowicz, J., 2022b. Apoptosis-inducing potential of selected bromophenolic flame retardants 2, 4, 6-tribromophenol and pentabromophenol in human peripheral blood mononuclear cells. Molecules 27 (16), 5056.
Barańska, A., et al., 2022c. Genotoxic mechanism of action of TBBPA, TBBPS and selected bromophenols in human peripheral blood mononuclear cells. Front. Immunol. 13, 869741.
Barnett, L.M.A., et al., 2021. Transcriptomic analysis of the differential nephrotoxicity of diverse brominated flame retardants in rat and human renal cells. Int. J. Mol. Sci. 22 (18), 10044.
Beck, K.R., et al., 2016. Evaluation of tetrabromobisphenol A effects on human glucocorticoid and androgen receptors: a comparison of results from human- with yeast-based in vitro assays. Toxicology 370, 70–77.
Bhan, A., et al., 2014. Histone methyltransferase EZH2 is transcriptionally induced by estradiol as well as estrogenic endocrine disruptors bisphenol-A and diethylstilbestrol. J. Mol. Biol. 426 (20), 3426–3441.
Bolognesi, C., et al., 2006. Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Aquat. Toxicol. 78 (Suppl. 1), S93–S98.
Bowen, C., et al., 2020. Mitochondrial-related effects of pentabromophenol, tetrabromobisphenol A, and triphenyl phosphate on murine BV-2 microglia cells. Chemosphere 255, 126919.
Boxtel, A.L.V., et al., 2008. Microarray analysis reveals a mechanism of phenolic polybrominated diphenylether toxicity in zebrafish. Environ. Sci. Technol. 42 (5), 1773–1779.
Bukowski, K., et al., 2019. DNA damage and methylation induced by organophosphate flame retardants: Tris (2-chloroethyl) phosphate and tris (1-chloro-2-propyl) phosphate in human peripheral blood mononuclear cells. Human Experim. Toxicol. 38 (6), 724–733.
Canbaz, D., et al., 2017. Immunotoxicity of organophosphate flame retardants TPHP and TDCIPP on murine dendritic cells in vitro. Chemosphere 177, 56–64.
Cannon, R.E., et al., 2019. Tetrabromobisphenol A (TBBPA) alters ABC transport at the blood-brain barrier. Toxicol. Sci. 169 (2), 475–484.
Carignan, C.C., et al., 2018. Paternal urinary concentrations of organophosphate flame retardant metabolites, fertility measures, and pregnancy outcomes among couples undergoing in vitro fertilization. Environ. Int. 111, 232–238.
Carvalho, P.S.M., Tillitt, D.E., 2004. 2,3,7,8-TCDD effects on visual structure and function in swim-up rainbow trout. Environ. Sci. Technol. 38 (23), 6300–6306.
Cato, A., et al., 2014. Brominated flame retardants, tetrabromobisphenol A and hexabromocyclododecane, activate mitogen-activated protein kinases (MAPKs) in human natural killer cells. Cell Biol. Toxicol. 30 (6), 345–360.
Chaudhry, G.R., Chapalamadugu, S., 1991. Biodegradation of halogenated organic compounds. Microbiol. Rev. 55 (1), 59–79.
Chen, D., Hale, R.C., Letcher, R.J., 2015. Photochemical and microbial transformation of emerging flame retardants: cause for concern? Environ. Toxicol. Chem. 34 (4), 687–699.
Chen, H., et al., 2018. Oxidative stress, cell cycle arrest, DNA damage and apoptosis in adult zebrafish (Danio rerio) induced by tris(1,3-dichloro-2-propyl) phosphate. Aquat. Toxicol. 194, 37–45.

Chen, R., et al., 2019a. Organophosphate flame retardants (OPFRs) induce genotoxicity in vivo: a survey on apoptosis, DNA methylation, DNA oxidative damage, liver metabolites, and transcriptomics. Environ. Int. 130, 104914.
Chen, R., et al., 2019b. Organophosphate flame retardants (OPFRs) induce genotoxicity in vivo: a survey on apoptosis, DNA methylation, DNA oxidative damage, liver metabolites, and transcriptomics. Environ. Int. 130, 104914.
Chen, Y., et al., 2020. A review on organophosphate flame retardants in indoor dust from China: implications for human exposure. Chemosphere 260, 127633.
Cheng, L., et al., 2022. The immunotoxicity of decabromodiphenyl ether (BDE-209) on broiler chicks by transcriptome profiling analysis. Ecotoxicol. Environ. Saf. 232, 113284.
Christen, V., et al., 2010. Some flame retardants and the antimicrobials triclosan and triclocarban enhance the androgenic activity in vitro. Chemosphere 81 (10), 1245–1252.
Clarity, C., et al., 2021. Associations between polyfluoroalkyl substance and organophosphate flame retardant exposures and telomere length in a cohort of women firefighters and office workers in San Francisco. Environ. Health 20, 1–14.
Costa, L.G., et al., 2015. The brominated flame retardant BDE-47 causes oxidative stress and apoptotic cell death in vitro and in vivo in mice. NeuroToxicology 48, 68–76.
Dingemans, M.M., et al., 2007. Neonatal exposure to brominated flame retardant BDE-47 reduces long-term potentiation and postsynaptic protein levels in mouse hippocampus. Environ. Health Perspect. 115 (6), 865–870.
Doherty, L.F., et al., 2010. In utero exposure to diethylstilbestrol (DES) or bisphenol-A (BPA) increases EZH2 expression in the mammary gland: an epigenetic mechanism linking endocrine disruptors to breast cancer. Hormones Cancer 1, 146–155.
Dong, W., et al., 2018. Polybrominated diphenyl ethers quinone induced parthanatos-like cell death through a reactive oxygen species-associated poly (ADP-ribose) polymerase 1 signaling. Chem. Res. Toxicol. 31 (11), 1164–1171.
Dong, W., et al., 2019. Polybrominated diphenyl ethers quinone induces NCOA4-mediated ferritinophagy through selectively autophagic degradation of ferritin. Chem. Res. Toxicol. 32 (12), 2509–2516.
Du, Z., et al., 2016. TPhP exposure disturbs carbohydrate metabolism, lipid metabolism, and the DNA damage repair system in zebrafish liver. Sci. Rep. 6, 21827.
Dunnick, J.K., et al., 2017. Tetrabromobisphenol A activates the hepatic interferon pathway in rats. Toxicol. Lett. 266, 32–41.
Eggers, S., et al., 2020. Exposure to environmental chemical mixtures is associated with nasal colonization by Staphylococcus aureus: NHANES 2001-2004. Environ. Res. 190, 109994.
Ekpe, O.D., et al., 2020. Chapter One - Introduction of emerging halogenated flame retardants in the environment. In: Oh, J.-E. (Ed.), Comprehensive Analytical Chemistry. Elsevier, pp. 1–39.
Ernest, S.R., et al., 2012. Effects of chronic exposure to an environmentally relevant mixture of brominated flame retardants on the reproductive and thyroid system in adult male rats. Toxicol. Sci. 127 (2), 496–507.
Fair, P.A., et al., 2012. Immune function in female B(6)C(3)F(1) mice is modulated by DE-71, a commercial polybrominated diphenyl ether mixture. J. Immunotoxicol. 9 (1), 96–107.
Feng, Y., et al., 2016. Effects of acute low-dose exposure to the chlorinated flame retardant dechlorane 602 and Th1 and Th2 immune responses in adult male mice. Environ. Health Perspect. 124 (9), 1406–1413.
Feng, Y., et al., 2022. Chlorinated organophosphorus flame retardants-induced mitochondrial abnormalities and the correlation with progesterone production in mLTC-1 cells. Food Chem. Toxicol. 169, 113432.
Fernández-Arribas, J., et al., 2021. COVID-19 face masks: a new source of human and environmental exposure to organophosphate esters. Environ. Int. 154, 106654.
Fishbein, L., 1974. Mutagens and potential mutagens in the biosphere. I. DDT and its metabolites, polychlorinated biphenyls, chlorodioxins, polycyclic aromatic hydrocarbons, haloethers. Sci. Total Environ. 2 (4), 305–340.
Frawley, R., et al., 2014. Relative potency for altered humoral immunity induced by polybrominated and polychlorinated dioxins/furans in female B6C3F1/N mice. Toxicol. Sci. 139 (2), 488–500.
Gao, P., et al., 2009. Influence of PCB153 on oxidative DNA damage and DNA repair–related gene expression induced by PBDE-47 in human neuroblastoma cells in vitro. Toxicol. Sci. 107 (1), 165–170.
Greeson, K.W., et al., 2020. Detrimental effects of flame retardant, PBB153, exposure on sperm and future generations. Sci. Rep. 10 (1), 8567.
Guo, W., et al., 2021. Nonalcoholic fatty liver disease development in zebrafish upon exposure to bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate, a novel brominated flame retardant. Environ. Sci. Technol. 55 (10), 6926–6935.
Guo, J., et al., 2022. Tetrabromobisphenol exposure impairs bovine oocyte maturation by inducing mitochondrial dysfunction. Molecules 27 (22), 8111.
Gustafsson, J., et al., 2023. Metabolite alterations in zebrafish embryos exposed to hydroxylated polybrominated diphenyl ethers. Sci. Total Environ. 857 (Pt 1), 159269.
Hammel, S.C., et al., 2022. Infants' diminished response to DTaP vaccine is associated with exposure to organophosphate esters. Sci. Total Environ. 837, 155782.
Han, Z., et al., 2017. Prenatal transfer of decabromodiphenyl ether (BDE-209) results in disruption of the thyroid system and developmental toxicity in zebrafish offspring. Aquat. Toxicol. 190, 46–52.
He, W., et al., 2008a. Effects of PBDE-47 on cytotoxicity and genotoxicity in human neuroblastoma cells in vitro. Mutation Res. Genet. Toxicol. Environ. Mutagenesis 649 (1–2), 62–70.
He, P., et al., 2008b. PBDE-47-induced oxidative stress, DNA damage and apoptosis in primary cultured rat hippocampal neurons. Neurotoxicology 29 (1), 124–129.
Heilmann, C., et al., 2006. Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med. 3 (8), e311.
Hill, K.L., et al., 2018a. Organophosphate triesters and selected metabolites enhance binding of thyroxine to human transthyretin in vitro. Toxicol. Lett. 285, 87–93.
Hill, K.L., et al., 2018b. In vitro and in silico competitive binding of brominated polyphenyl ether contaminants with human and gull thyroid hormone transport proteins. Environ. Sci. Technol. 52 (3), 1533–1541.
Hsu, P.-C., et al., 2021. Transgenerational effects of BDE-209 on male reproduction in F3 offspring rats. Chemosphere 272, 129829.
Hu, Z., et al., 2021. Organophosphate esters in China: fate, occurrence, and human exposure. Toxics 9 (11).
Hua, J., et al., 2022. Decabromodiphenyl ethane induced hyperactivity in developing zebrafish at environmentally relevant concentrations. Ecotoxicol. Environ. Saf. 244, 114044.
Huang, S.C., Giordano, G., Costa, L.G., 2010. Comparative cytotoxicity and intracellular accumulation of five polybrominated diphenyl ether congeners in mouse cerebellar granule neurons. Toxicol. Sci. 114 (1), 124–132.
Ingle, M.E., et al., 2018. The association between urinary concentrations of phosphorous-containing flame retardant metabolites and semen parameters among men from a fertility clinic. Int. J. Hyg. Environ. Health 221 (5), 809–815.
Ingle, M.E., et al., 2021. Reproductive outcomes associated with flame retardants among couples seeking fertility treatment: a paternal perspective. Environ. Res. 192, 110226.
Jarosiewicz, M., et al., 2020. Evaluation of the effect of selected brominated flame retardants on human serum albumin and human erythrocyte membrane proteins. Int. J. Mol. Sci. 21 (11), 3926.
Jarosiewicz, M., et al., 2021. An in vitro comparative study of the effects of tetrabromobisphenol A and tetrabromobisphenol S on human erythrocyte membranes—changes in ATP level, perturbations in membrane fluidity, alterations in conformational state and damage to proteins. Int. J. Mol. Sci. 22 (17), 9443.
Ji, K., et al., 2011. Genotoxicity of several polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDEs, and their mechanisms of toxicity. Environ. Sci. Technol. 45 (11), 5003–5008.
Jing, L., et al., 2022. Oxidative stress and endoplasmic reticulum stress contributed to hepatotoxicity of decabromodiphenyl ethane (DBDPE) in L-02 cells. Chemosphere 286, 131550.
Kamstra, J.H., et al., 2014. Transcriptional and epigenetic mechanisms underlying enhanced in vitro adipocyte differentiation by the brominated flame retardant BDE-47. Environ. Sci. Technol. 48 (7), 4110–4119.
Kanda, K., et al., 2021. Effects of tris(2-chloroethyl) phosphate exposure on chicken embryos in a shell-less incubation system. Ecotoxicol. Environ. Saf. 207, 111263.
Khalil, A., et al., 2017. Perinatal exposure to 2, 2′, 4′ 4′ – tetrabromodiphenyl ether induces testicular toxicity in adult rats. Toxicology 389, 21–30.
Koch, C., et al., 2016. Degradation of polymeric brominated flame retardants: development of an analytical approach using PolyFR and UV irradiation. Environ. Sci. Technol. 50 (23), 12912–12920.
Koike, E., et al., 2013. Brominated flame retardants stimulate mouse immune cells in vitro. J. Appl. Toxicol. 33 (12), 1451–1459.
Koike, E., et al., 2014. Penta-and octa-bromodiphenyl ethers promote proinflammatory protein expression in human bronchial epithelial cells in vitro. Toxicol. in Vitro 28 (2), 327–333.
Koike, E., Yanagisawa, R., Takano, H., 2016. Brominated flame retardants, hexabromocyclododecane and tetrabromobisphenol A, affect proinflammatory protein expression in human bronchial epithelial cells via disruption of intracellular signaling. Toxicol. in Vitro 32, 212–219.
Kong, W., et al., 2011. Induction of DNA damage in human urothelial cells by the brominated flame retardant 2, 2-bis (bromomethyl)-1, 3-propanediol: role of oxidative stress. Toxicology 290 (2–3), 271–277.
Kong, W., et al., 2013. Comparison of 2,2-bis(bromomethyl)-1,3-propanediol induced genotoxicity in UROtsa cells and primary rat hepatocytes: relevance of metabolism and oxidative stress. Toxicol. Lett. 222 (3), 273–279.
Lal, R., Saxena, D.M., 1982. Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. Microbiol. Rev. 46 (1), 95–127.
Lamkin, D.M., et al., 2022. Low-dose exposure to PBDE disrupts genomic integrity and innate immunity in mammary tissue. Front. Genet. 13, 904607.
Law, R.J., et al., 2014. Levels and trends of PBDEs and HBCDs in the global environment: status at the end of 2012. Environ. Int. 65, 147–158.
Le, Y., et al., 2021. Comprehensive analysis of organophosphorus flame retardant-induced mitochondrial abnormalities: potential role in lipid accumulation. Environ. Pollut. 274, 116541.
Lee, H.-W., Ha, S.K., Kim, Y., 2020. Bisphenol A disrupts inflammatory responses via Nod-like receptor protein 3 pathway in macrophages. Appl. Biol. Chem. 63 (1), 78.
Lefèvre, P.L., et al., 2016. Exposure of female rats to an environmentally relevant mixture of brominated flame retardants targets the ovary, affecting folliculogenesis and steroidogenesis. Biol. Reprod. 94 (1), 9 (1–11).
Li, F., et al., 2014a. The interaction between organic phosphate ester and p53: an integrated experimental and in silico approach. Mar. Pollut. Bull. 85 (2), 516–521.
Li, M., et al., 2014b. Toxic effects of decabromodiphenyl ether (BDE-209) on human embryonic kidney cells. Front. Genet. 5, 118.
Li, R.J., et al., 2017. Hexabromocyclododecane-induced genotoxicity in cultured human breast cells through DNA damage. Biomed. Environ. Sci. 30 (4), 296–300.
Li, P., et al., 2019. Autophagy impairment contributes to PBDE-47-induced developmental neurotoxicity and its relationship with apoptosis. Theranostics 9 (15), 4375.
Li, X., et al., 2020. In vitro immunotoxicity of organophosphate flame retardants in human THP-1-derived macrophages. Environ. Sci. Technol. 54 (14), 8900–8908.
Li, L., et al., 2021a. Associations between the exposure to organophosphate flame retardants during early pregnancy and the risk of spontaneous abortion based on metabolomics combined with tandem mass spectrometry. Ann. Transl. Med. 9 (16), 1305.
Li, Y., et al., 2021b. Exposure to 4-bromodiphenyl ether during pregnancy blocks testis development in male rat fetuses. Toxicol. Lett. 342, 38–49.
Li, M., et al., 2022a. Triphenyl phosphate (TPP) promotes hepatocyte toxicity via induction of endoplasmic reticulum stress and inhibition of autophagy flux. Sci. Total Environ. 840, 156461.
Li, M., et al., 2022b. Evaluation of the influences of low dose polybrominated diphenyl ethers exposure on human early retinal development. Environ. Int. 163, 107187.

Li, Y., et al., 2022c. Effects of postnatal exposure to tetrabromobisphenol A on testis development in mice and early key events. Arch. Toxicol. 96 (6), 1881–1892.
Li, M., et al., 2022d. Evaluation of the influences of low dose polybrominated diphenyl ethers exposure on human early retinal development. Environ. Int. 163, 107187.
Li, M., et al., 2023. Organophosphate esters in Chinese rice: occurrence, distribution, and human exposure risks. Sci. Total Environ. 862, 160915.
Liang, S., et al., 2019. Typical halogenated flame retardants affect human neural stem cell gene expression during proliferation and differentiation via glycogen synthase kinase 3 beta and T3 signaling. Ecotoxicol. Environ. Saf. 183, 109498.
Liao, T., et al., 2021. Short-term exposure of decabromodiphenyl ether in female adult Balb/c mice: immune toxicity and self-recovery. Toxicol. Lett. 342, 26–37.
Liu, X., et al., 2012. The PBDE-209 exposure during pregnancy and lactation impairs immune function in rats. Mediat. Inflamm. 2012, 692467.
Liu, Y., et al., 2019. Exploring the membrane toxicity of decabromodiphenyl ethane (DBDPE): based on cell membranes and lipid membranes model. Chemosphere 216, 524–532.
Liu, Z., et al., 2021. Polybrominated diphenyl ethers quinone exhibits neurotoxicity by inducing DNA damage, cell cycle arrest, apoptosis and p53-driven adaptive response in microglia BV2 cells. Toxicology 457, 152807.
Liu, W., et al., 2023. Developmental toxicity of TCBPA on the nervous and cardiovascular systems of zebrafish (Danio rerio): a combination of transcriptomic and metabolomics. J. Environ. Sci. (China) 127, 197–209.
Longo, V., et al., 2019. In vitro exposure to 2,2′,4,4′-tetrabromodiphenyl ether (PBDE-47) impairs innate inflammatory response. Chemosphere 219, 845–854.
Longo, V., et al., 2021. 2,2′4,4′-tetrabromodiphenyl ether (PBDE-47) modulates the intracellular miRNA profile, sEV biogenesis and their miRNA cargo exacerbating the LPS-induced pro-inflammatory response in THP-1 macrophages. Front. Immunol. 12, 664534.
Longo, V., et al., 2023. Impact of the flame retardant 2,2′4,4′-tetrabromodiphenyl ether (PBDE-47) in THP-1 macrophage-like cell function via small extracellular vesicles. Front. Immunol. 13.
Lu, L., et al., 2021. Low concentration Tetrabromobisphenol A (TBBPA) elevating overall metabolism by inducing activation of the Ras signaling pathway. J. Hazard. Mater. 416, 125797.
Lundgren, M., Darnerud, P.O., Ilbäck, N.-G., 2013. The flame-retardant BDE-99 dose-dependently affects viral replication in CVB3-infected mice. Chemosphere 91 (10), 1434–1438.
Ma, X., et al., 2021a. Multiplexed quantitative evaluation on mitochondrial toxicity of tris (2,3-dibromopropyl) phosphate in hepatocyte. Ecotoxicol. Environ. Saf. 221, 112425.
Ma, H., et al., 2021b. Triphenyl phosphate delayed pubertal timing and induced decline of ovarian reserve in mice as an estrogen receptor antagonist. Environ. Pollut. 290, 118096.
Macari, S., et al., 2020. Developmental exposure to the flame retardant mixture Firemaster 550 compromises adult bone integrity in male but not female rats. Int. J. Mol. Sci. 21 (7), 2553.
Main, K.M., et al., 2007. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. Environ. Health Perspect. 115 (10), 1519–1526.
Mali, H., et al., 2023. Organophosphate pesticides an emerging environmental contaminant: pollution, toxicity, bioremediation progress, and remaining challenges. J. Environ. Sci. (China) 127, 234–250.
Manuguerra, S., et al., 2019. Sub-lethal doses of polybrominated diphenyl ethers, in vitro, promote oxidative stress and modulate molecular markers related to cell cycle, antioxidant balance and cellular energy management. Int. J. Environ. Res. Public Health 16 (4), 588.
Meerts, I.A., et al., 2000. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. Toxicol. Sci. 56 (1), 95–104.
Meng, S., et al., 2020. Hepatic oxidative stress, DNA damage and apoptosis in adult zebrafish following sub-chronic exposure to BDE-47 and BDE-153. Environ. Toxicol. 35 (11), 1202–1211.
Montalbano, A.M., et al., 2020. Cytotoxic and genotoxic effects of the flame retardants (PBDE-47, PBDE-99 and PBDE-209) in human bronchial epithelial cells. Chemosphere 245, 125600.
Mynster Kronborg, T., et al., 2016. Effects of the commercial flame retardant mixture DE-71 on cytokine production by human immune cells. PLoS One 11 (4), e0154621.
Napoli, E., et al., 2013. Toxicity of the flame-retardant BDE-49 on brain mitochondria and neuronal progenitor striatal cells enhanced by a PTEN-deficient background. Toxicol. Sci. 132 (1), 196–210.
Ngoubeyou, P.S.K., et al., 2022. Toxicity of polychlorinated biphenyls in aquatic environments - a review. Aquat. Toxicol. 251, 106284.
Ogunbayo, O.A., Michelangeli, F., 2007. The widely utilized brominated flame retardant tetrabromobisphenol A (TBBPA) is a potent inhibitor of the SERCA Ca2+ pump. Biochem. J. 408 (3), 407–415.
Ogunbayo, O.A., Jensen, K.T., Michelangeli, F., 2007. The interaction of the brominated flame retardant: Tetrabromobisphenol a with phospholipid membranes. Biochim. Biophys. Acta (BBA) Biomembranes 1768 (6), 1559–1566.
Ogunsina, K., et al., 2022. Correlates of positive thyroid peroxidase antibodies among firefighters: a cross-sectional-study. J. Endocr. Soc. 6 (10), bvac125.
Oguro, A., et al., 2021. Bisphenol A stabilizes Nrf2 via Ca(2+) influx by direct activation of the IP(3) receptor. J. Toxicol. Sci. 46 (1), 1–10.
Otsuka, S., Ishihara, A., Yamauchi, K., 2014. Ioxynil and tetrabromobisphenol A suppress thyroid-hormone-induced activation of transcriptional elongation mediated by histone modifications and RNA polymerase II phosphorylation. Toxicol. Sci. 138 (2), 290–299.
Parsons, A., et al., 2019. Molecular mechanisms and tissue targets of brominated flame retardants, BDE-47 and TBBPA, in embryo-larval life stages of zebrafish (Danio rerio). Aquat. Toxicol. 209, 99–112.
Pellacani, C., et al., 2012. Evaluation of DNA damage induced by 2 polybrominated diphenyl ether flame retardants (BDE-47 and BDE-209) in SK-N-MC cells. Int. J. Toxicol. 31 (4), 372–379.
Pijnenburg, A.M., et al., 1995. Polybrominated biphenyl and diphenylether flame retardants: analysis, toxicity, and environmental occurrence. Rev. Environ. Contam. Toxicol. 141, 1–26.
Pillai, H.K., et al., 2014. Ligand binding and activation of PPAR γ by Firemaster® 550: effects on adipogenesis and osteogenesis in vitro. Environ. Health Perspect. 122 (11), 1225–1232.
Pilsner, J.R., et al., 2021. Aging-induced changes in sperm DNA methylation are modified by low dose of perinatal flame retardants. Epigenomics 13 (4), 285–297.
Poston, R.G., Saha, R.N., 2019. Epigenetic effects of polybrominated diphenyl ethers on human health. Int. J. Environ. Res. Public Health 16 (15), 2703.
Poston, R.G., et al., 2018. Persistent 6-OH-BDE-47 exposure impairs functional neuronal maturation and alters expression of neurodevelopmentally-relevant chromatin remodelers. Environ. Epigenetics 4 (1), dvx020.
Pullen, S., Boecker, R., Tiegs, G., 2003. The flame retardants tetrabromobisphenol A and tetrabromobisphenol A–bisallylether suppress the induction of interleukin-2 receptor α chain (CD25) in murine splenocytes. Toxicology 184 (1), 11–22.
Rajak, P., et al., 2022. Synergistic action of organophosphates and COVID-19 on inflammation, oxidative stress, and renin-angiotensin system can amplify the risk of cardiovascular maladies. Toxicol. Appl. Pharmacol. 456, 116267.
Reemtsma, T., et al., 2006. Polar pollutants entry into the water cycle by municipal wastewater: a European perspective. Environ. Sci. Technol. 40 (17), 5451–5458.
Regoli, F., et al., 2004. Time-course variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, Mytilus galloprovincialis, during a field translocation experiment. Aquat. Toxicol. 68 (2), 167–178.
Reistad, T., Mariussen, E., 2005. A commercial mixture of the brominated flame retardant pentabrominated diphenyl ether (DE-71) induces respiratory burst in human neutrophil granulocytes in vitro. Toxicol. Sci. 87 (1), 57–65.
Reistad, T., Mariussen, E., Fonnum, F., 2005a. The effect of a brominated flame retardant, tetrabromobisphenol-A, on free radical formation in human neutrophil granulocytes: the involvement of the MAP kinase pathway and protein kinase C. Toxicol. Sci. 83 (1), 89–100.
Reistad, T., Mariussen, E., Fonnum, F., 2005b. The effect of a brominated flame retardant, tetrabromobisphenol-A, on free radical formation in human neutrophil granulocytes: the involvement of the MAP kinase pathway and protein kinase C. Toxicol. Sci. 83 (1), 89–100.
Reistad, T., et al., 2007. In vitro toxicity of tetrabromobisphenol-A on cerebellar granule cells: cell death, free radical formation, calcium influx and extracellular glutamate. Toxicol. Sci. 96 (2), 268–278.
Riva, C., et al., 2007. Evaluation of DNA damage induced by decabromodiphenyl ether (BDE-209) in hemocytes of Dreissena polymorpha using the comet and micronucleus assays. Environ. Mol. Mutagen. 48 (9), 735–743.
Sales, L.B., et al., 2013. Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation. Toxicol. in Vitro 27 (6), 1634–1643.
Sandal, S., et al., 2004. Comparative effects of technical toxaphene, 2, 5-dichloro-3-biphenylol and octabromodiphenylether on cell viability,[Ca2+] i levels and membrane fluidity in mouse thymocytes. Toxicol. Lett. 151 (3), 417–428.
Santillo, D., Johnston, P., 2003. Playing with fire: the global threat presented by brominated flame retardants justifies urgent substitution. Environ. Int. 29 (6), 725–734.
Saquib, Q., Siddiqui, M., Al-Khedhairy, A., 2021. Organophosphorus flame-retardant tris (1-chloro-2-propyl) phosphate is genotoxic and apoptotic inducer in human umbilical vein endothelial cells. J. Appl. Toxicol. 41 (5), 861–873.
Saquib, Q., et al., 2022. Organophosphorus flame retardant TDCPP displays genotoxic and carcinogenic risks in human liver cells. Cells 11 (2), 195.
Sauer, S.J., et al., 2017. Bisphenol A activates EGFR and ERK promoting proliferation, tumor spheroid formation and resistance to EGFR pathway inhibition in estrogen receptor-negative inflammatory breast cancer cells. Carcinogenesis 38 (3), bgx003.
Shafique, S., et al., 2023. Gestational exposure to triphenyl phosphate induces epigenetic modifications in C57Bl/6 fetal liver. Birth Defects Res. 115 (3), 338–347.
Shan, Q., et al., 2017. Troxerutin reduces kidney damage against BDE-47-induced apoptosis via inhibiting NOX2 activity and increasing Nrf2 activity. Oxidative Med. Cell. Longev. 2017, 6034692.
Sharkey, M., et al., 2020. Phasing-out of legacy brominated flame retardants: the UNEP Stockholm convention and other legislative action worldwide. Environ. Int. 144, 106041.
Shi, F., Feng, X., 2021. Decabromodiphenyl ethane exposure damaged the asymmetric division of mouse oocytes by inhibiting the inactivation of cyclin-dependent kinase 1. FASEB J. 35 (4), e21449.
Shen, J., et al., 2021. The flame-retardant mechanisms and preparation of polymer composites and their potential application in construction engineering. Polymers (Basel) 14 (1), 82.
Shi, Q., et al., 2019. Acute exposure to triphenyl phosphate (TPhP) disturbs ocular development and muscular organization in zebrafish larvae. Ecotoxicol. Environ. Saf. 179, 119–126.
Shi, J., et al., 2021. HBCD, TBECH, and BTBPE exhibit cytotoxic effects in human vascular endothelial cells by regulating mitochondria function and ROS production. Environ. Toxicol. 36 (8), 1674–1682.
Shi, F., et al., 2022. Decabromodiphenyl ethane affects embryonic development by interfering with nuclear F-actin in zygotes and leads to cognitive and social disorders in offspring mice. FASEB J. 36 (8), e22445.
Stapleton, H.M., Dodder, N.G., 2008. Photodegradation of decabromodiphenyl ether in house dust by natural sunlight. Environ. Toxicol. Chem. 27 (2), 306–312.
Stel, J., Legler, J., 2015. The role of epigenetics in the latent effects of early life exposure to obesogenic endocrine disrupting chemicals. Endocrinology 156 (10), 3466–3472.
Strack, S., et al., 2007. Cytotoxicity of TBBPA and effects on proliferation, cell cycle and MAPK pathways in mammalian cells. Chemosphere 67 (9), S405–S411.
Strakovsky, R.S., et al., 2015. Developmental bisphenol A (BPA) exposure leads to sex-specific modification of hepatic gene expression and epigenome at birth that may exacerbate high-fat diet-induced hepatic steatosis. Toxicol. Appl. Pharmacol. 284 (2), 101–112.

Sun, J., et al., 2016. Combined transcriptomic and proteomic approach to identify toxicity pathways in early life stages of Japanese medaka (Oryzias latipes) exposed to 1,2,5,6-tetrabromocyclooctane (TBCO). Environ. Sci. Technol. 50 (14), 7781–7790.
Suvorov, A., et al., 2020. Aging induces profound changes in sncRNA in rat sperm and these changes are modified by perinatal exposure to environmental flame retardant. Int. J. Mol. Sci. 21 (21), 8252.
Syed, J.H., et al., 2020. Legacy and emerging flame retardants (FRs) in the urban atmosphere of Pakistan: diurnal variations, gas-particle partitioning and human health exposure. Sci. Total Environ. 743, 140874.
Takeshita, T., et al., 2013. Effect of Brazilian propolis on exacerbation of respiratory syncytial virus infection in mice exposed to tetrabromobisphenol A, a brominated flame retardant. Evid. Based Complement. Alternat. Med. 2013, 698206.
Teshima, R., et al., 2008. Effects of exposure to decabromodiphenyl ether on the development of the immune system in rats. J. Health Sci. 54 (4), 382–389.
Thornton, L.M., et al., 2018. Embryo-larval BDE-47 exposure causes decreased pathogen resistance in adult male fathead minnows (Pimephales promelas). Fish Shellf. Immunol. 80, 80–87.
Tian, J., et al., 2017. The toxic effects of tetrachlorobisphenol a in Saccharomyces cerevisiae cells via metabolic interference. Sci. Rep. 7 (1), 2655.
Tribondeau, A., Sachs, L.M., Buisine, N., 2022. Tetrabromobisphenol A effects on differentiating mouse embryonic stem cells reveals unexpected impact on immune system. Front. Genet. 13.
TriDung, N., 2020. Development of sustainable flame-retardant materials. Green Mater. 8 (3), 101–122.
van der Ven, L.T.M., et al., 2009. Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats. Toxicol. Lett. 185 (1), 51–62.
Van Essen, D., et al., 2021. The brominated flame retardant, TBCO, impairs oocyte maturation in zebrafish (Danio rerio). Aquat. Toxicol. 238, 105929.
Varshavsky, J.R., et al., 2021. Organophosphate flame retardants, highly fluorinated chemicals, and biomarkers of placental development and disease during mid-gestation. Toxicol. Sci. 181 (2), 215–228.
Volz, D.C., et al., 2016. Tris(1,3-dichloro-2-propyl)phosphate induces genome-wide hypomethylation within early zebrafish embryos. Environ. Sci. Technol. 50 (18), 10255–10263.
Wang, Y.-Q., Zhang, H.-M., Cao, J., 2014a. Exploring the interactions of decabrominateddiphenyl ether and tetrabromobisphenol A with human serum albumin. Environ. Toxicol. Pharmacol. 38 (2), 595–606.
Wang, Y.Q., Zhang, H.M., Cao, J., 2014b. Quest for the binding mode of tetrabromobisphenol A with calf thymus DNA. Spectrochim. Acta A Mol. Biomol. Spectrosc. 131, 109–113.
Wang, W., et al., 2015. Effect of polybrominated diphenyl ether exposure on Gadus macrocephalus Tilesius. Chem. Speciation Bioavail. 27 (4), 183–190.
Wang, X., et al., 2019. Environmentally relevant doses of tetrabromobisphenol A (TBBPA) cause immunotoxicity in murine macrophages. Chemosphere 236, 124413.
Wang, X., et al., 2020. Tetrabromoethylcyclohexane (TBECH) exhibits immunotoxicity in murine macrophages. Environ. Toxicol. 35 (2), 159–166.
Wang, Y., et al., 2021a. Tetrachlorobisphenol A induced immunosuppression and uterine injury in mice. Ecotoxicol. Environ. Saf. 207, 111527.
Wang, Y., et al., 2021b. Polybrominated diphenyl ether quinone exposure induces atherosclerosis progression via CD36-mediated lipid accumulation, NLRP3 inflammasome activation, and pyroptosis. Chem. Res. Toxicol. 34 (9), 2125–2134.
Watanabe, W., et al., 2008a. A new assay system for evaluation of developmental immunotoxicity of chemical compounds using respiratory syncytial virus infection to offspring mice. Environ. Toxicol. Pharmacol. 25 (1), 69–74.
Watanabe, W., et al., 2008b. Effects of decabrominated diphenyl ether (DBDE) on developmental immunotoxicity in offspring mice. Environ. Toxicol. Pharmacol. 26 (3), 315–319.
Watanabe, W., et al., 2010. Effects of tetrabromobisphenol A, a brominated flame retardant, on the immune response to respiratory syncytial virus infection in mice. Int. Immunopharmacol. 10 (4), 393–397.
Watanabe, W., et al., 2017. Perinatal exposure to tetrabromobisphenol A (TBBPA), a brominated flame retardant, exacerbated the pneumonia in respiratory syncytial virus (RSV)-infected offspring mice. J. Toxicol. Sci. 42 (6), 789–795.
Woods, R., et al., 2012. Long-lived epigenetic interactions between perinatal PBDE exposure and Mecp2 308 mutation. Hum. Mol. Genet. 21 (11), 2399–2411.
Wu, Q., et al., 2017. Interaction of bisphenol a 3,4-quinone metabolite with glutathione and ribonucleosides/deoxyribonucleosides in vitro. J. Hazard. Mater. 323 (Pt A), 195–202.
Wu, S., et al., 2018. Effects of novel brominated flame retardant TBBPA on human airway epithelial cell (A549) in vitro and proteome profiling. Environ. Toxicol. 33 (12), 1245–1253.
Wu, H., et al., 2021. Effects of tetrabromobisphenol A (TBBPA) on the reproductive health of male rodents: a systematic review and meta-analysis. Sci. Total Environ. 781, 146745.
Xiang, P., et al., 2017. Effects of organophosphorus flame retardant TDCPP on normal human corneal epithelial cells: implications for human health. Environ. Pollut. 230, 22–30.
Xu, X., et al., 2015. Phytotoxicity of brominated diphenyl ether-47 (BDE-47) and its hydroxylated and methoxylated analogues (6-OH-BDE-47 and 6-MeO-BDE-47) to maize (Zea mays L.). Chem. Res. Toxicol. 28 (3), 510–517.
Xu, T., et al., 2017. Vision, color vision, and visually guided behavior: the novel toxicological targets of 2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47). Environ. Sci. Technol. Lett. 4 (4), 132–136.
Xu, C., et al., 2022. Screening of organophosphate flame retardants with placentation-disrupting effects in human trophoblast organoid model and characterization of adverse pregnancy outcomes in mice. Environ. Health Perspect. 130 (5), 57002.
Yadav, A., et al., 2022. Peripherally administered persistent organic pollutants distribute to the brain of developing chicken embryo in concentrations relevant for human exposure. Neurotoxicology 88, 79–87.
Yan, H., Hales, B.F., 2020. Exposure to tert-butylphenyl diphenyl phosphate, an organophosphate ester flame retardant and plasticizer, alters hedgehog signaling in murine limb bud cultures. Toxicol. Sci. 178 (2), 251–263.
Yanagisawa, R., et al., 2020. The impact of oral exposure to low-dose tris (2-butoxyethyl) phosphate in allergic asthmatic mice. J. Appl. Toxicol. 40 (11), 1498–1510.
Yang, J., et al., 2019. A review of a class of emerging contaminants: the classification, distribution, intensity of consumption, synthesis routes, environmental effects and expectation of pollution abatement to organophosphate flame retardants (OPFRs). Int. J. Mol. Sci. 20 (12).
Yang, L., et al., 2022. A new and systematic review on the efficiency and mechanism of different techniques for OPFRs removal from aqueous environments. J. Hazard. Mater. 431, 128517.
Yasmin, S., Whalen, M., 2018. Flame retardants, hexabromocyclododecane (HCBD) and tetrabromobisphenol a (TBBPA), alter secretion of tumor necrosis factor alpha (TNFα) from human immune cells. Arch. Toxicol. 92, 1483–1494.
Ye, S., et al., 2021. Curcumin hinders PBDE-47-induced neutrophil extracellular traps release via Nrf2-associated ROS inhibition. Ecotoxicol. Environ. Saf. 225, 112779.
Yin, H., et al., 2020. Concentration profile, spatial distributions and temporal trends of polybrominated diphenyl ethers in sediments across China: implications for risk assessment. Ecotoxicol. Environ. Saf. 206, 111205.
You, X., et al., 2018. Gene mutation and micronucleus assays in gpt delta mice treated with 2,2′,4,4′-tetrabromodiphenyl ether. Mutagenesis 33 (2), 153–160.
Yuan, S., et al., 2019. Aryl-phosphorus-containing flame retardants induce oxidative stress, the p53-dependent DNA damage response and mitochondrial impairment in A549 cells. Environ. Pollut. 250, 58–67.
Yue, J., et al., 2023. Triphenyl phosphate proved more potent than its metabolite diphenyl phosphate in inducing hepatic insulin resistance through endoplasmic reticulum stress. Environ. Int. 172, 107749.
Zhang, Z.-F., et al., 2015. Troxerutin protects against 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47)-induced liver inflammation by attenuating oxidative stress-mediated NAD+-depletion. J. Hazard. Mater. 283, 98–109.
Zhang, W., et al., 2016. Involvement of ROS-mediated mitochondrial dysfunction and SIRT3 down-regulation in tris (2-chloroethyl) phosphate-induced cell cycle arrest. Toxicol. Res. 5 (2), 461–470.
Zhang, Y., et al., 2019a. Regulation of TBBPA-induced oxidative stress on mitochondrial apoptosis in L02 cells through the Nrf2 signaling pathway. Chemosphere 226, 463–471.
Zhang, W., et al., 2019b. Tris (1,3-dichloro-2-propyl) phosphate treatment induces DNA damage, cell cycle arrest and apoptosis in murine RAW264.7 macrophages. J. Toxicol. Sci. 44 (3), 134–144.
Zhang, Q., et al., 2023a. Neurodevelopmental toxicity of organophosphate flame retardant triphenyl phosphate (TPhP) on zebrafish (Danio rerio) at different life stages. Environ. Int. 172, 107745.
Zhang, Q., et al., 2023b. Neurodevelopmental toxicity of organophosphate flame retardant triphenyl phosphate (TPhP) on zebrafish (Danio rerio) at different life stages. Environ. Int. 172, 107745.
Zhi, H., et al., 2018. Decabromodiphenyl ether (BDE-209) enhances foam cell formation in human macrophages via augmenting Toll-like receptor 4-dependent lipid uptake. Food Chem. Toxicol. 121, 367–373.
Zhi, H., et al., 2019a. MicroRNA–21 attenuates BDE-209-induced lipid accumulation in THP-1 macrophages by downregulating Toll-like receptor 4 expression. Food Chem. Toxicol. 125, 71–77.
Zhi, H., et al., 2019b. Decarbromodiphenyl ether (BDE-209) promotes monocyte–endothelial adhesion in cultured human aortic endothelial cells through upregulating intercellular adhesion molecule-1. Environ. Res. 169, 62–71.
Zhou, P., et al., 2020. Chlorinated flame-retardant dechlorane 602 potentiates type 2 innate lymphoid cells and exacerbates airway inflammation. Environ. Sci. Technol. 55 (2), 1099–1109.
Zhou, E., et al., 2021. Histamine triggers the formation of neutrophil extracellular traps via NADPH oxidase, ERK and p38 pathways. Vet. Immunol. Immunopathol. 235, 110234.