Environment International 153 (2021) 106550

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Review article

Endocrine disrupting potential of replacement flame retardants – Review of current knowledge for nuclear receptors associated with reproductive outcomes

Lola Bajard [a], Chander K. Negi [a], Vicente Mustieles [b,c,d], Lisa Melymuk [a], Stéphane Jomini [e], Johanna Barthelemy-Berneron [e], Mariana F. Fernandez [b,c,d], Ludek Blaha [a,*]

[a] Masaryk University, Faculty of Science, RECETOX, Kamenice 5, CZ62500 Brno, Czechia
[b] University of Granada, Center for Biomedical Research (CIBM), Granada, Spain
[c] Ciber de Epidemiologia y Salud Publica (CIBERESP), Madrid, Spain
[d] Instituto de Investigacion Biosanitaria de Granada (ibs. GRANADA), Granada, Spain
[e] ANSES, Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du travail, Direction de l'Evaluation des Risques, Unité Evaluation des Substances Chimiques, 14 rue Pierre Marie Curie. 94701 Maisons-Alfort Cedex, France

ARTICLE INFO

Handling Editor:Heather Stapleton

Keywords:
Endocrine disruption
Nuclear receptors
Steroid hormones
Flame retardants
Emerging contaminants
ToxCast

ABSTRACT

Background and aim: Endocrine disrupting chemicals (EDCs) constitute a major public health concern because they can induce a large spectrum of adverse effects by interfering with the hormonal system. Rapid identification of potential EDCs using *in vitro* screenings is therefore critical, particularly for chemicals of emerging concerns such as replacement flame retardants (FRs). The review aimed at identifying (1) data gaps and research needs regarding endocrine disrupting (ED) properties of replacement FRs and (2) potential EDCs among these emerging chemicals.

Methods: A systematic search was performed from open literature and ToxCast/Tox21 programs, and results from *in vitro* tests on the activities of 52 replacement FRs towards five hormone nuclear receptors (NRs) associated with reproductive outcomes (estrogen, androgen, glucocorticoid, progesterone, and aryl hydrocarbon receptors) were compiled and organized into tables. Findings were complemented with information from structure-based *in silico* model predictions and *in vivo* information when relevant.

Results: For the majority of the 52 replacement FRs, experimental *in vitro* data on activities towards these five NRs were either incomplete (15 FRs) or not found (24 FRs). Within the replacement FRs for which effect data were found, some appeared as candidate EDCs, such as triphenyl phosphate (TPhP) and tris(1,3-dichloropropyl) phosphate (TDCIPP). The search also revealed shared ED profiles. For example, anti-androgenic activity was reported for 19 FRs and predicted for another 21 FRs.

Discussion: This comprehensive review points to critical gaps in knowledge on ED potential for many replacement FRs, including chemicals to which the general population is likely exposed. Although this review does not cover all possible characteristics of ED, it allowed the identification of potential EDCs associated with reproductive outcomes, calling for deeper evaluation and possibly future regulation of these chemicals. By identifying shared ED profiles, this work also raises concerns for mixture effects since the population is co-exposed to several FRs and other chemicals.

Abbreviations: ED, endocrine disrupting; EDCs, endocrine disrupting chemicals; NRs, nuclear receptors; FRs, flame retardants; EU, European Union; ER, estrogen receptor; AR, androgen receptor; GR, glucocorticoid receptor; PR, progesterone receptor; AhR, aryl hydrocarbon receptor; CAS, Chemical Abstracts Service; CoMPARA, Collaborative Modeling Project for Androgen Receptor Activity; DR, dose-response; AC50, EC50 and IC50, 50% activity (AC50), effective (EC50) or inhibitory (IC50) concentration; REC20 and RIC20, 20% relative effective (REC20) or inhibitory (RIC20) concentration; PBDEs, polybrominated diphenyl ethers; QSAR, quantitative structure-activity relationship.

* Corresponding author at: RECETOX, Kamenice 5, CZ62500 Brno, Czechia.
E-mail address: ludek.blaha@recetox.muni.cz (L. Blaha).

https://doi.org/10.1016/j.envint.2021.106550
Received 12 January 2021; Received in revised form 26 March 2021; Accepted 29 March 2021
Available online 10 April 2021

1. Introduction

The endocrine system regulates key interrelated functions in the body, including reproduction, early development, as well as metabolic and neurologic processes. In the past decades, many chemicals used in manufactured products and agriculture, for example, have accumulated in food, water, and indoor environments, interfering with the endocrine system of both humans and wildlife. Such exogenous chemicals or chemical mixtures that can cause adverse health effects by perturbing any aspect of hormone action are defined as endocrine disrupting chemicals (EDCs) (Thomas Zoeller et al., 2012; WHO and UNEP, 2012). The United Nations Environment Programme (UNEP) established a conservative list of 45 EDCs or potential EDCs by reviewing evidence from multiple sources (United Nations Environment Programme, 2017). Nevertheless, the list is likely incomplete since many more chemicals have shown endocrine disrupting (ED) activities in humans and experimental animals. Currently, more and more studies report associations between human exposure to EDCs and adverse outcomes, including male reproductive health (Sharma et al., 2020), metabolic disease (Heindel et al., 2017), and neurodevelopment (Mustieles and Fernández, 2020). The potential impact of EDCs on public health is therefore of very high concern, and increasing efforts are being made in toxicological studies to test for ED properties of compounds that are newly introduced in the market. Currently, toxicity testing is shifting towards the use of alternative approaches to animal studies such as *in vitro* assays and *in silico* based modeling, following the 3Rs (Replacement, Reduction, and Refinement) recommendation of the Article 4 and 13 of the Directive 2010/63/EU on the protection of animals used for scientific purposes (EU, 2011; Krewski et al., 2020). As part of this global movement towards "non-animal" toxicology, the United States Environmental Protection Agency (US EPA) launched the Toxicity Forecaster (ToxCast) and Toxicity Testing in the 21st Century (Tox21) programs that use high-throughput screening methods to test a large number of chemicals over a large spectrum of *in vitro* assays, including several reporter assays for ED-related activities (https://www.epa.gov/chemical-research/toxicity-forecasting; https://comptox.epa.gov/dashboard/) (Judson et al., 2010). For EDCs, the *in vitro* screening often relies on assays testing for chemical affinity and action through hormonal nuclear receptors (NRs), which are usually classified into two major categories: steroid hormone receptors and heterodimers of retinoid X receptor (Hall and Greco, 2019; Toporova and Balaguer, 2020). EDCs may act on NRs either by mimicking the endogenous hormone and activating the receptor (agonists), or by inhibiting the effect of the hormone (antagonists) (Hall and Greco, 2019; Toporova and Balaguer, 2020). Information from these *in vitro* tests is particularly important for chemicals of emerging concern to which people are potentially exposed but for which toxicological data is scarce, if not absent.

Replacement flame retardants (FRs) are a good example of such chemicals of emerging concern. Flame retardants are added to many consumer products used in our daily life, such as electronics, building insulators, textiles, furniture, to delay their eventual ignition. FRs, particularly those that are not chemically bound to the product matrix, tend to be emitted from the products and are broadly detected in indoor environments (air, dust), as well as in human matrices, suggesting that people may be chronically exposed to these chemicals (Mitro et al., 2016; Rantakokko et al., 2019; Saillenfait et al., 2018). Due to their high environmental persistence and toxicity, the long-used polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane have been added to the Stockholm Convention on Persistent Organic Pollutants, and their use has been restricted (http://www.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx) (Sharkey et al., 2020). Since then, the use of replacement FRs has markedly increased to assure compliance with flammability standards. The spectrum of chemicals has also enlarged, with hundreds of FRs now in the market showing quite diverse chemical structures. Organic FRs used as replacements include brominated (BFRs), organophosphate (OPFRs), and chlorinated (some of which are also OPFRs) flame retardants. As a result of their use, the replacement FRs are now detected in indoor air and dust, in the environment, biota, the food chain, as well as in human samples (Blum et al., 2019; Bollmann et al., 2012; Brandsma et al., 2015; Covaci et al., 2011; Demirtepe et al., 2019; Gbadamosi et al., 2021; Malarvannan et al., 2015; Mitro et al., 2016; Poma et al., 2017; Saillenfait et al., 2018; Sundkvist et al., 2010; van der Veen and de Boer, 2012; Vykoukalová et al., 2017; Wei et al., 2015; Zeng et al., 2014; Zuiderveen et al., 2020). Due to their ubiquity and ED potential, replacement FRs are among the priority compounds in the European Union (EU) assessed within the Human Biomonitoring for Europe (HBM4EU) initiative (HBM4EU - science and policy for a healthy future). Despite the clear evidence of increasing population exposure (Bastiaensen et al., 2020; Hoffman et al., 2017a), there is little or no information regarding the effects on human health for many replacement FRs, but the few studies available suggest that some of them can induce adverse outcomes (Bajard et al., 2019; Blum et al., 2019). Besides possible neurodevelopmental or metabolic disruptions, several studies indicate that some replacement FRs may have ED properties related to reproductive outcomes (Hales and Robaire, 2020). Compounds like tris-2-chloroethyl phosphate (TCEP), tris(1,3-dichloropropyl)phosphate (TDCIPP), triphenyl phosphate (TPhP), tetrabromobisphenol A (TBBPA), or tricresyl phosphate (TMPP) were suggested to have adverse effects on the male reproductive tract and/or semen quality in rodents (Chen et al., 2015; EU RAR, 2009; European Union, 2008; US-EPA, 2015; Zatecka et al., 2014, 2013). Small human cohort studies have also reported correlations between exposure to TPhP or TDCIPP and decreased sperm quality in humans (Carignan et al., 2018; Meeker et al., 2013; Meeker and Stapleton, 2010). Consistently, the effects of replacement FRs on steroid hormone levels have been observed in rodents and zebrafish (Chen et al., 2015; Liu et al., 2016, 2013, 2012; Pollock et al., 2017), and some *in vitro* studies reported estrogenic or anti-androgenic activities (Beck et al., 2016; Kojima et al., 2013; Krivoshiev et al., 2016; Reers et al., 2016; Rosenmai et al., 2021; Suzuki et al., 2013; Zhang et al., 2014).

In this paper, a systematic search of the data available regarding the effects of replacement FRs on several NRs was performed, primarily focusing on steroid receptors, i.e., estrogen receptor (ER), androgen receptor (AR), glucocorticoid receptor (GR), and progesterone receptor (PR), which are associated to reproductive outcomes. Besides, the effects on the Aryl hydrocarbon receptor (AhR), which has been shown to cross-talk with steroid receptors such as ERs and AR, were also investigated (Ohtake et al., 2008). The search was done for 52 synthetic organic replacement FRs preselected by a group of experts of the HBM4EU initiative based on their hazardous properties, exposure, societal concern, and technical feasibility (Bajard et al., 2019) The list of chemicals with full names, abbreviations and CAS numbers is provided in Table S3, and the HBM4EU scoping document can be accessed through the following link: HBM4EU_D4.9_Scoping_Documents_HBM4EU_priority_substances_v1.0-Flame-retardants.pdf.

2. Methods

2.1. The overall procedure for collecting data

The procedure is presented in Fig. 1. In brief, for each of the 52 preselected replacement FRs, results from *in vitro* studies in which agonist or antagonist activities towards ER, AR, GR, PR, or AhR have been tested were collected from both the academic literature and ToxCast database. The data collected was organized into tables, indicating whether the chemicals were tested, and, if so, in what assay(s) it was reported to be active or inactive, and what were the effective concentrations. As anti-androgenic activity appeared to be especially relevant (see results below), prediction of anti-androgenic activity from *in silico* models was also collected. The detailed procedures for data collection from the academic literature, ToxCast, and *in silico* model predictions are

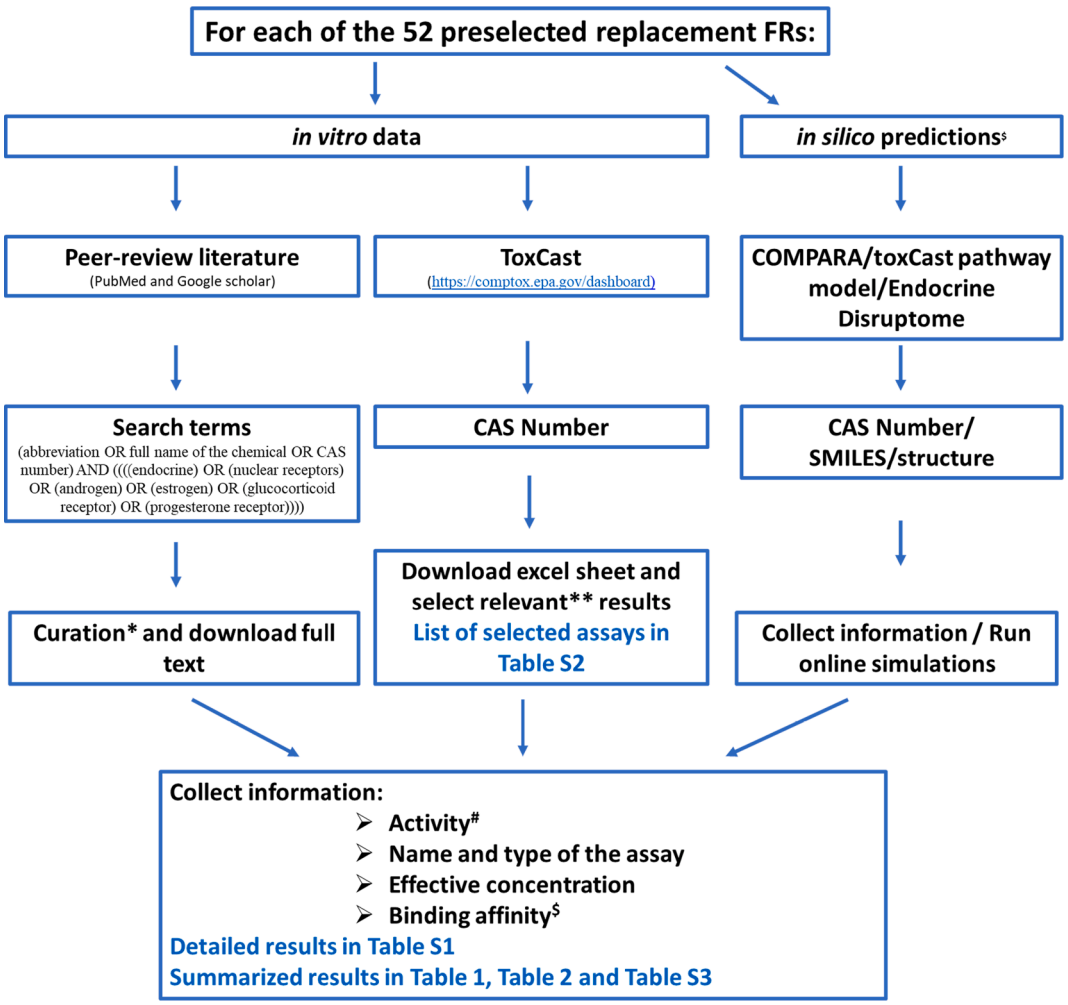

Fig. 1. Flowchart describing the procedure followed for searching and collecting *in vitro* and *in silico* data. * The paper contains tests for ER, AR, PR, GR, or AhR agonist or antagonist activities for the relevant FR. ** The assays were selected using the gene symbols: progesterone receptors (PGR), estrogen receptors α and β (ESR1 and ESR2), aryl hydrocarbon receptor (AHR), androgen receptor (AR), and glucocorticoid receptor (NR3C1). # As reported in the source. $ For anti-androgenic activity.

explained in the following paragraphs.

2.2. Academic peer-review literature search of in vitro data

PubMed and Google Scholar were used to identify publications for each of the 52 preselected replacement FRs, using the following terms: (abbreviation OR full name of the chemical OR CAS number) AND ((((endocrine) OR (nuclear receptors) OR (androgen) OR (estrogen) OR (glucocorticoid receptor) OR (progesterone receptor)))). For each relevant paper (i.e., that contained tests for ER, AR, PR, GR or AhR agonist or antagonist activities), the name and type of the assay, whether the chemical was reported as active (A) or inactive (IN), and the effective concentration (for each assay in which the chemical was active) were recorded. Effective concentrations are the concentrations (in μM) at which 50% or 20% of the maximum response is achieved. They are expressed as the 50% activity (AC50), effective (EC50) or inhibitory (IC50) concentration, or the 20% relative effective (REC20) or inhibitory (RIC20) concentration. A given chemical was considered as inactive if reported as such in the paper (e.g., not determined (ND), not effective (NE), not calculated (NC), etc.). Because criteria differ among studies and research papers, the highest concentration tested above which the chemical was considered to be inactive is indicated in Table S1.

2.3. ToxCast search of in vitro data

Results available in the ToxCast program were collected from the open dashboard of the US-EPA (https://comptox.epa.gov/dashboard), searching chemical by chemical using CAS numbers. Out of the 52 preselected replacement FRs, 21 had been tested in ToxCast for ER, AR, GR, PR, or AhR activities. The list of ToxCast assays, with the name of the assay, the number of active chemicals, the total number of tested chemicals, a brief description of the assay, and the gene symbol, were directly downloaded from the ToxCast dashboard (https://comptox.epa.gov/dashboard/assay_endpoints/). The assays related to each NR were selected using the following gene symbols: progesterone receptors (PGR), estrogen receptors α and β (ESR1 and ESR2), aryl hydrocarbon receptor (AHR), androgen receptor (AR), and glucocorticoid receptor (NR3C1). The final list of assays from ToxCast/Tox21 programs taken into consideration is detailed in Table S2. For each assay, the "Assay type" was defined based on the description provided in "Assay function type" found in the detailed description of the assay. Assays were considered as tests for agonist or antagonist activities when the "Assay function type" was either "reporter gene" (TOX21 or ATG assays) or "detection of steroid hormones" (CEETOX assays), analyzed for gain of activity (agonist test) or for loss of activity (antagonist test). Assays for which "Assay function type" was "Binding", "Signaling" or not provided were not accounted in results presented in Table 1 and Table S3.

Table 1
Summary of agonist and antagonist activities of replacement FRs against 5 NRs, as reported in the peer-review literature and ToxCast database.

Abbreviation (full name in Table S3)	CAS	Group	ER		AR		GR		PR		AhR		Tonnage (as registered in REACH)
			Agonist	Antagonist	Agonist	Antagonist	Agonist	Antagonist	Agonist	Antagonist	Agonist	Antagonist	
TDCIPP	13674-87-8	A	A2/IN12	A4/IN8	IN6	A8/IN1	IN5	A4/IN3	IN1	A2	IN3	IN1	1000-10000
TPHP	115-86-6	A	A11/IN5	A1/IN13	IN8	A6/IN4	IN9	A4/IN8	IN4	A5	A1/IN2	IN1	1000-10000
TMPP	1330-78-5	A	A5/IN9	A1/IN12	IN8	A5/IN4	IN9	A2/IN8	IN4	A2/IN3	A2/IN1	IN1	1000-10000
TBBPA	79-94-7	A	A4/IN10	A4/IN6	A1/IN9	A7/IN7	A1/IN8	A2/IN8	A2/IN2	A2/IN4	IN3	IN2	1000-10000
TBOEP	78-51-3	A	IN16	A2/IN12	IN8	A1/IN8	IN9	IN11	IN4	A2/IN3	IN2	IN1	1000-10000
TCIPP	13674-84-5	A	IN13	IN11	IN6	A2/IN6	IN5	IN7	IN1	A2	A1/IN2	IN1	0-10
EHDPP	1241-94-7	A	A2/IN5	A1/IN5	IN4	A1/IN5	IN3	IN3	IN1	A1	A2/IN1	IN1	1000-10000
TNBP	126-73-8	A	A2/IN12	IN12	IN6	A3/IN4	IN5	A2/IN5	IN1	A1/IN1	IN2	IN1	1000-10000
246-TBP	118-79-6	A	A2/IN8	A4/IN6	IN7	IN7	IN7	A1/IN6	A2/IN2	A2/IN3	A1/IN2	IN2	0-10
TEHP	78-42-2	A	A2/IN10	A3/IN8	A2/IN4	A1/IN6	IN5	A2/IN4	IN1	IN2	A1/IN2	A1	na*
BEH-TEBP	26040-51-7	A	IN7	A1/IN7	A2/IN3	A3/IN4	A2/IN1	A1/IN3	A1	IN1	IN2	IN1	100-1000
TCEP	115-96-8	A	IN16	A2/IN12	IN8	A1/IN9	IN9	IN11	IN4	IN5	A1/IN2	IN1	0-10
TEP	78-40-0	A	A1/IN9	IN9	IN6	IN6	IN4	IN5	IN1	IN2	IN2	IN1	10000-100000
DBNPG	3296-90-0	B	IN7	IN6	IN5	IN4	IN3	IN3	IN1	IN1	IN2	IN1	na*
ip-TPP	68937-41-7	B	A3/IN2	A1/IN5	IN4	A2/IN2	IN3	IN3	IN1	A1	IN2	IN1	1000-10000
CDP	26444-49-5	B	A3/IN2	A1/IN5	IN4	A3/IN2	IN3	IN3	IN1	A1	A1/IN2	IN1	na*
TIBP	126-71-6	B	A1/IN4	A1/IN5	IN4	A1/IN3	IN3	IN3	IN1	A1	A1/IN1	IN1	1000-10000
PBEB	85-22-3	B	IN3	IN4	IN2	IN2	IN2	IN2	IN1	IN1	IN1	A1	na*
HBB	87-82-1	B	IN3	IN4	A2/IN1	IN3	IN1	IN1	A1	A1	A1/IN2	NT	na*
TBNPA	1522-92-5	B	IN3	IN4	IN3	A2/IN1	IN1	IN1	IN1	A1	A1	NT	na*
PBP	608-71-9	B	IN3	A4	IN3	A2/IN1	IN1	A1	IN1	A1	IN1	NT	na*
TnPP	513-08-6	B	IN3	IN3	IN1	IN2	IN1	IN2	NT	IN1	NT	NT	1000-10000
EH-TBB	183658-27-7	B	NT	A1	NT	A2	NT	A1	NT	NT	NT	NT	na*
TBCO	3194-57-8	B	NT	A1	NT	A1	NT	NT	NT	NT	NT	NT	na*
DBE-DBCH	3322-93-8	B	NT	NT	A5**	NT	NT	NT	NT	NT	NT	NT	na*
PBT	87-83-2	B	NT	NT	NT	NT	NT	NT	NT	NT	A2	NT	na*
TDBP-TAZTO	52434-90-9	B	IN1	A1	NT	NT	NT	NT	NT	NT	NT	NT	na*
V6	38051-10-4	B	NT	NT	NT	A1	NT	NT	NT	NT	A1	NT	1000-10000
DBDPE	84852-53-9	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	10000-100000
DDC-CO	135821-03-9	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	100-1000
HEEHP-TEBP	20566-35-2	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	100-1000
TTBNPP	19186-97-1	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	100-1000
BPA-BDPP	5945-33-5, 181028-79-5	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	100-1000
EBTEBPI	32588-76-4	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	100-1000
TTBP-TAZ	25713-60-4	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
RBDPP	125997-21-9, 57583-54-7	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
DDC-DBF	31107-44-5	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
BTBPE	37853-59-1	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
Melamine polyphosphate	20208-95-1, 15541-60-3	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
DBHCTD (HCDBCO)	51936-55-1	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
OBTMPI	1084889-51-9, 1025956-65-3,893843-07-7, 155613-93-7	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
PBB-Acr	59947-55-1	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
TBX	23488-38-2	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
4'-PeBPO-BDE208	58965-66-5	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
HBCYD	25495-98-1	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
DBS	31780-26-4	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
BDBP-TAZTO	75795-16-3	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
DBP-TAZTO	57829-89-7	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
DBP	615-58-7	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
DDC-Ant (Dec 603)	13560-92-4	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
HCTBPH/Dec 604	34571-16-9	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*
Diethylphosphinic acid	813-76-3	C	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	na*

Groups: **A**, all ten activities have been tested, information from several sources; **B,** information incomplete - not all activities tested and/or only one source (e.g., ToxCast); **C,** no information found for any of the ten activities.
Ax/INx: number of assays (x) in which the chemical was indicated as active (A) or inactive (IN).
Heat map: color coding relates to the number of assays in which the chemical was active, and the proportion of active *vs.* inactive assays: Yellow: ($A < 3$ AND $IN \geq A$) OR $A = 1$ - Weak evidence of activity; Orange: ($A \geq 3$ AND $IN \geq A$) OR ($1 < A \leq 4$ AND $IN < A$) - Moderate evidence of activity; Red: $A > 4$ AND $IN < A$ - Strong evidence of activity.
* na - information on tonnage NOT AVAILABLE (not registered in REACH, for example).
** all five papers from the same laboratory.
ER, estrogen receptor; AR, androgen receptor; GR, glucocorticoid receptor; PR, progesterone receptor; AhR, Aryl hydrocarbon receptor; NT, not tested.

However, these results are provided in Table S1 and are commented in the text as complementary information to results from agonist and antagonist assays, when relevant.

2.4. Computational and in silico predictive models

Information from computational and *in silico* models predicting the anti-AR activity or binding affinity of ligands (replacement FRs) with the AR was collected either from ToxCast (where results from the "toxcast pathway model" and the "Collaborative Modeling Project for Androgen Receptor Activity - CoMPARA" model are provided) or using the open-source platform Endocrine Disruptome®. The "toxCast pathway model" for AR activity is a mathematical AR pathway-based model that integrates the results from 11 high-throughput screening *in vitro* assays from the US EPA's ToxCast and Tox21 data into the network model for AR pathway (Kleinstreuer et al., 2017). The result of the model is expressed as an area under the curve (AUC) normalized to the positive control, hydroxyflutamide, for AR antagonism. CoMPARA is a consensus model which is based on *in silico* methods, such as quantitative structure–activity relationship (QSAR) and read-across, and predicts the binding, agonist, and antagonist AR activities (Mansouri et al., 2020). The results are presented as active/inactive binders, agonists, and

antagonists. Endocrine Disruptome® is an open-source, web-based prediction tool that runs on an open-source platform called Docking interface for Target Systems (DoTS) and uses molecular docking to predict the binding of chemicals to different human NRs (Kolšek et al., 2014). The input system is the chemical structure that can be drawn, or its simplified molecular-input line-entry system (SMILES). Results are provided as binding free energies (in kcal mol^{-1}) and the probability of binding represented by color-coding. The binding probability is calculated from the true positive rate (sensitivity, SE) and the validation experiments presented as red color indicating a high probability of binding, ($SE < 0.25$), orange and yellow representing medium probability, ($0.25 < SE < 0.50$) and ($0.50 < SE < 0.75$) respectively, and green representing the low probability of binding ($SE > 0.75$) (Kolšek et al., 2014). In the current review, medium to high probability for receptor binding was considered as active for AR antagonism.

3. Results and discussion

3.1. General observations from available data

To facilitate the discussion, the 52 replacement FRs were divided into three different groups based on data availability for the investigated ED activities (see Table 1 and Fig. 2A). For Group A (13 FRs), some information was found on their interactions with all ten studied ED activities, i.e., agonist- or antagonist effects towards ER, AR, GR, PR, and AhR. For Group B (15 FRs), some but incomplete information was found (either data were not found for all the activities, or they were found only from one source). For Group C (the remaining 24 FRs), no information on ED activities towards studied NRs was found.

Two replacement FRs, TCEP and triethyl phosphate (TEP), appeared to be overall inactive towards the studied NRs. For TCEP, this finding, however, does not correspond to its reported adverse effects related to male fertility in rodents (Chen et al., 2015; US-EPA, 2015). This could be explained by interference of TCEP with other receptors or enzymes not reviewed here, but it may also indicate a more general inconsistency between *in vitro* and *in vivo* toxicological data for TCEP. It was already noticed in a recent publication that TCEP is mostly inactive in *in vitro* tests, while *in vivo* data tends to indicate high hazard (Bajard et al., 2019). This may be related to, e.g., different *in vivo* bioavailability or toxicokinetics that cannot be fully captured by *in vitro* screens for prioritization of chemicals. Differences between species might also account for these inconsistencies since most *in vivo* assays are performed in rodents or fishes, while *in vitro* assays often use human cell lines, for example. Such inconsistencies between *in vitro* and *in vivo* data may exist for other chemicals as well and might be missed in case the data are insufficient. The example of TCEP highlights the limitation of relying exclusively on one or the other type of data to evaluate the hazard of substances.

One general observation was also the inconsistency of data from different information sources. Interestingly, different studies that used the same assays for testing the same activity might report that the chemical was active, while others indicated no activity. In most cases, the variability in the results presumably originates from substantial differences in the experimental setup, including the type of the *in vitro* assay or cells, the sensitivity of the assay, the time- and concentration ranges tested, etc. However, in some cases, this might even reflect the differences in the criteria used for classifying a chemical as active or inactive. For example, in several research papers, chemicals are considered inactive if no effect is detected below a given concentration of the chemical (e.g., 10 μM in (Suzuki et al., 2013)). On the other hand, in ToxCast, a chemical is considered inactive if none of the three regression parameters, which are automatically derived for the obtained assay (i.e., constant, hill and gain-loss), sufficiently fits the dose–response curve (Ryan, 2017). For example, regarding the anti-GR activity of TDCIPP, two studies reported that the chemical was "inactive" (i.e., not active below 10 μM), which is however consistent with four other sources that reported that TDCIPP was "active" with effective concentrations ranging from 16 to 132 μM (Table S1).

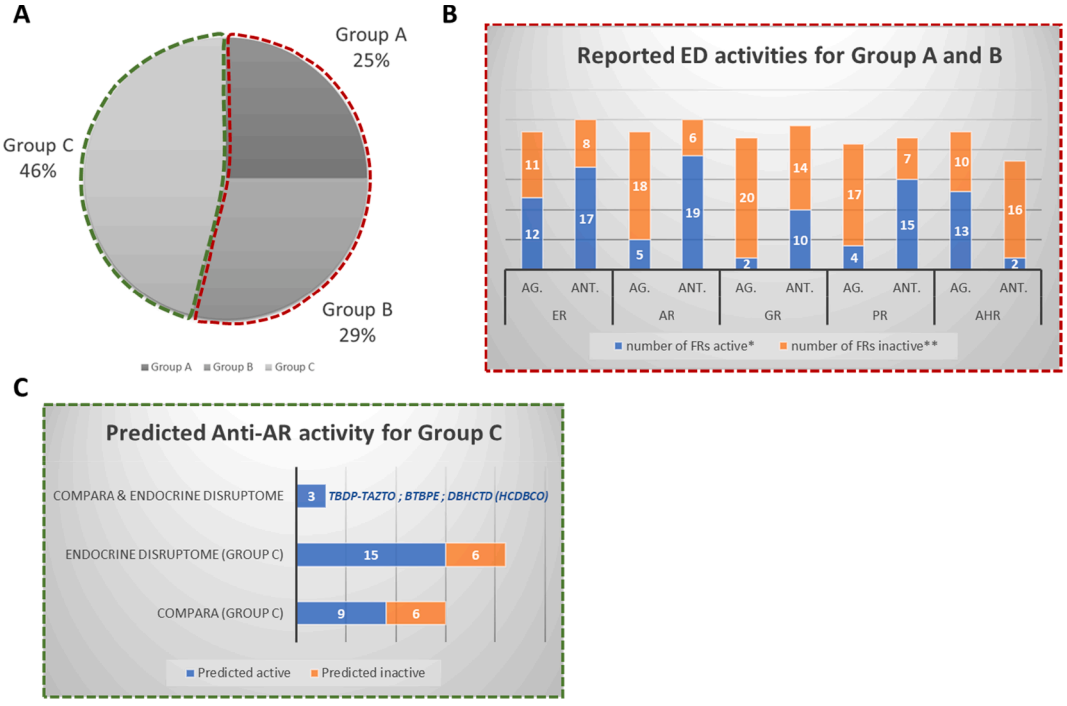

Fig. 2. – Summary of *in vitro* data availability and reported activity of 52 replacement FRs for five NRs. A. Data availability for *in vitro* tests for the 10 ED activities. Percentage of the 52 reviewed replacement FRs in each group A, B, and C. For 46% of the 52 replacement FRs, no data were found, and for 29%, it was incomplete. B. Number of replacement FRs from group A and group B reported active (blue) *vs.* inactive (orange) for each of the ED activities. *Chemical was reported active in at least one study. ** Chemical was reported inactive in all the available studies. C. Anti-androgenic activity of Group C replacement FRs predicted from two computational models. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Replacement FRs with solid evidence showing a high potential to modulate NRs

Within Group A and Group B FRs, the chemicals that were more frequently reported to be active than inactive and that showed activity in at least two assays were examined in more detail. We investigated the effective concentrations to identify replacement FRs that are likely to present the strongest ED potential via NRs.

For TPhP, there was solid evidence for estrogenic activity supported by the results from 11 assays both from ToxCast and from four research papers (Table 1, Tables S1 and S3) (Kojima et al., 2016, 2013; Suzuki et al., 2013; Zhang et al., 2014). ER agonistic activity for TPhP was consistently observed at low effective concentrations ranging from 0.27 to 10.9 μM, which are below the cytotoxicity limit of 11.7 μM reported in ToxCast (Kojima et al., 2016, 2013; Suzuki et al., 2013; Zhang et al., 2014). Also, TPhP was active in seven additional assays testing for ER binding (Table S1). The data collected also revealed that TPhP antagonizes the PR. The high anti-PR activity was reported in all the five assays tested, with effective concentrations ranging from 1.14 to 19 μM. There is also good evidence of antagonist activity against AR for TPhP. It was active in six assays, with effective concentrations ranging from 5.8 to 78.6 μM (Table S1). It was inactive in the other four assays, but for two ToxCast assays, it was flagged as "borderline inactive" because it showed a clear dose–response (DR) and almost reached the cut-off threshold (Figure S1A and B). Some evidence also supports antagonist activity against GR: TPhP was active in four assays (effective concentrations ranging from 2.6 to 15 μM) and inactive in another eight assays. Overall, results show that there is solid *in vitro* evidence supporting the ED potential of TPhP. This is in line with *in vivo* animal studies in rodents and zebrafish and small epidemiologic studies that reported adverse effects on fertility and pregnancy outcomes (Carignan et al., 2017; Chen et al., 2015; Liu et al., 2013; Meeker et al., 2013; Meeker and Stapleton, 2010; US-EPA, 2015). Consistently, TPhP is listed in the UNEP list of (potential) EDCs, and both the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the European Chemicals Agency (ECHA) noted in their recent reports a potential concern for endocrine disruption and a need for additional data (ANSES, 2018; ECHA, 2019). TPhP is also in the list of chemicals going through ECHA's ED assessment, although the status is still under development (https://echa.europa.eu/fr/ed-assessment). The data collected in this review strongly support its classification as an EDC.

The review also revealed solid evidence of potential *in vitro* ED effects of TDCIPP. Anti-androgenic activity (AR antagonist) seems well established: eight assays reported activity with effective concentrations ranging from 1.9 to 75.5 μM (Table S1). This is further supported by activity in four additional ToxCast assays testing for binding to AR or assessing AR-dependent cell growth. Anti-androgenic activity and binding to AR are also predicted by two models in ToxCast (Table 2). The antagonist activity of TDCIPP against PR was reported in two assays with an IC50 as low as 0.85 μM (Suzuki et al., 2013), which was further supported by activity in two ToxCast assays for binding towards PR (Table S1). The collected evidence also points to moderate antagonist GR activity GR: activity was reported in four assays (effective concentrations from 16 to 132 μM), one ToxCast assay flagged as "borderline inactive" (see dose–response (DR) curve in Figure S1C), and two assays with no activity below 10 μM (Table S1). Finally, although one study reported estrogenic activity with a REC20 of 6.4 μM (Zhang et al., 2014), this was not confirmed in any of the remaining 12 assays in which TDCIPP was tested for ER-agonist activity, although one of them classified it as "borderline inactive" in ToxCast (see DR curve in Figure S1D). Overall, the data collected here point to anti-androgenic and anti-PR activities of TDCIPP *in vitro*, which is in line with the few *in vivo* rodent and human studies available (WHO, 1998; Meeker and Stapleton, 2010; Meeker et al., 2013; US-EPA, 2015; Carignan et al., 2017; see also Bajard et al., 2019 for review). Altogether, these data show the ED potential of TDCIPP and call for a thorough assessment.

Finally, a large amount of data was found for TBBPA. Seven assays reported anti-androgenic activity (anti-AR), and two additional assays reported binding to AR but with highly variable effective concentrations ranging from 0.022 to 91.1 μM. It should also be noted that TBBPA was reported inactive in the other seven assays testing for antagonist activity. Nevertheless, three of these studies set a 10 μM threshold (Hamers et al., 2006; Harju et al., 2007; Molina-Molina et al., 2013), and the results may still be consistent with other studies reporting effective concentrations above this value. The activity of TBBPA was also reported in some assays for ER, GR, and PR, with some effective concentrations in the μM range for PR agonist activity, for example. However, many assays testing for the same outcome reported inactivity (Table S1). Collected evidence indicates some ED potential for TBBPA, but firm conclusions regarding ED assessment are hindered by data inconsistencies.

The activities for three additional replacement FRs should also be highlighted, although there is currently less evidence. First, 4-(1,2-dibromoethyl)-1,2-dibromocyclohexane (DBE-DBCH) seems to be a strong AR agonist with low EC50s within the nM range (depending on the DBE-DBCH isomer) (Table S1). These low effective concentrations were reported in five studies coming from the same research team using different reporter assays (Asnake et al., 2014; Khalaf et al., 2009; Kharlyngdoh et al., 2016; Larsson et al., 2006; Pradhan et al., 2013). This androgenic activity may underly the strong effects of DBE-DBCH on gonadal differentiation observed in amphibians at low exposure levels (Liu et al., 2017) and deserves further examination and confirmation by independent studies. Second, isopropyl triphenyl phosphate (ip-TPP) seems to be an ER agonist, and its estrogenicity was reported in three ToxCast assays showing low AC50s ranging from 1.71 to 5 μM (Table S1). Another ToxCast assay where cell growth is used as an indirect reporter for estrogenic activity (ACEA-ER-80hr) also supports the estrogenic potential of ip-TPP. These *in vitro* data support the reproductive and developmental toxicity of ip-TPP previously reported in rats and potentially in humans (Carignan et al., 2018; US-EPA, 2015). Altogether, this calls for further studies and thorough risk assessment because high exposures cannot be excluded as ip-TPP is a component of the FM 550 flame retardant mixture and is registered with high tonnage use in the EU REACH. Finally, evidence suggests anti-ER activity of pentabromophenol (PBP). The chemical was reported to be active in all four ToxCast assays in which it was tested, with AC50s ranging from 6.15 to 67 μM (Table S1). To our knowledge, information on ED effects *in vivo* for this chemical is missing and should be generated.

3.3. Shared activity profiles of several replacement FRs - antagonist activities against AR and PR

For the 28 FRs from Groups A and B, some similarities in their ED profiles were observed (see Table 1 and Fig. 2B). First, the large majority of the tested replacement FRs did not display direct agonist activities towards AR, GR, and PR, neither antagonist activity against AhR, although AhR data was too scarce to draw firm conclusions. Second, antagonistic activities towards AR and PR were frequently observed, and 12 replacement FRs (namely, TDCIPP, TPHP, TMPP, TBBPA, tri(2-butoxyethyl) phosphate (TBOEP), tris(1-chloro-2-propyl) phosphate (TCIPP), tri-n-butyl phosphate (TNBP), ip-TPP, cresyl diphenyl phosphate (CDP), tri-*iso*-butyl phosphate (TIBP), tribromoneopentyl alcohol (TBNPA) and PBP) were reported active at least once for both anti-AR and anti-PR. For eight of these FRs, there are also reports of estrogenic activity. This generic pattern of shared anti-AR, anti-PR, and estrogenic activities was also described by Suzuki and colleagues for TDCIPP, TPhP, TMPP, and some PBDEs, using hierarchical clustering (Suzuki et al., 2013). Seventeen FRs from Groups A and B have also been reported to have antiestrogenic activity in at least one assay, but, for 14 of them, there are more reports of inactivity, raising some doubts on the anti-estrogenic effect. Similarly, ten replacement FRs were shown to have anti-GR activity in at least one assay, but for seven of them, there are

Table 2
Anti-androgenic properties of replacement FRs: detailed information from *in vitro* tests and three predictive models.

Abbreviation	Anti-androgenic activity							Tonnage
	in vitro data from paper & ToxCast				Model predictions			
	Antagonist			Binding/ signaling	CoMPARA[#]	ToxCast Pathway Model (AUC)[##]	Endocrine Disruptome	
	summary	Lowest EfD	Highest EfD					
TDCIPP	A7/IN1	1,9	75,5	A4/IN2	A/A	0.29	-5,7	1000-10000
TPHP	A5/IN4	5,8	78,6	A1/IN1	IN/IN	0.038	-8,4	1000-10000
TMPP	A4/IN4	4,1	253	A2/IN2	IN/IN	--	-8,4	1000-10000
TBBPA	A7/IN7	0,022	91,1	A3/IN1	A/A	0.1	-4,3	1000-10000
TBOEP	A1/IN8	0,9		IN3	IN/IN	0	-5,2	1000-10000
TCIPP	A1/IN6	10		IN3	IN/IN	0	-5,9	0-10
EHDPP	IN5			A2/IN1	IN/IN	1.3E-4	-7,6	1000-10000
TNBP	A3/IN4	10	24	IN3	IN/IN	0	-5,5	1000-10000
2,4,6-TBP	IN7			IN3	IN/IN	0	-5,2	0-10
TEHP	IN6			A3/IN2	IN/IN	0	-4,7	na*
BEH-TEBP	A3/IN4	*doubts on values*		IN3	A/IN	0		100-1000
TCEP	IN9			IN2	IN/IN	0	-4,8	0-10
TEP	IN6			IN3	IN/IN	0	-4,8	10000-100000
DBNPG	IN4			IN3	IN/IN	0	-4,9	na*
ip-TPP	A2/IN2	31,7	49,9	--	--	--		1000-10000
CDP	A2/IN2	15,1	48,5	--	--	--	-8,5	na*
TIBP	A1/IN3	2.51e-4		--	IN/IN	--	-6	1000-10000
PBEB	IN2			--	IN/IN	--	-5,1	na*
HBB	IN3			--	IN/IN	--	-4,4	na*
TBNPA	A2/IN1	43,8	114	--	IN/IN	--	-4,5	na*
PBP	A2/IN1	29,2	73,9	--	IN/IN	--	-5,2	na*
TnPP	IN2			--	IN/IN	--	-5,3	1000-10000
EH-TBB	A2	7.10-10	43,5	--	--	--	-5,6	na*
TBCO	A1	0,007		--	IN/IN	--	-6,5	na*
DBE-DBCH	NT			--	IN/IN	--	-5,8	na*
PBT	NT			--	IN/IN	--	-4,9	na*
TDBP-TAZTO	NT			--	A/A	--	-4,4	na*
V6	NT			--	A/A	--	-4,5	1000-10000
DBDPE	NT			--	A/A	--	**	10000-100000
DDC-CO	NT			--	A/A	--		100-1000
HEEHP-TEBP	NT			--	IN/IN	--	-4,9	100-1000
TTBNPP	NT			--	--	--	**	100-1000
BPA-BDPP	NT			--	--	--		100-1000
EBTEBPI	NT			--	A/A	--	**	100-1000
TTBP-TAZ	NT			--	--	--	**	na*
RBDPP	NT			--	A/A	--		na*
DDC-DBF	NT			--	A/A	--		na*
BTBPE	NT			--	A/A	--	-4,4	na*
Melamine polyphosphate	NT			--	--	--		na*
DBHCTD (HCDBCO)	NT			--	A/A	--	-5,7	na*
OBTMPI	NT			--	--	--	**	na*
PBB-Acr	NT			--	--	--	-5,9	na*
TBX	NT			--	IN/IN	--	-5,4	na*
4'-PeBPO-BDE208	NT			--	A/A	--	**	na*
HBCYD	NT			--	--	--	-5,4	na*
DBS	NT			--	IN/IN	--	-6	na*
BDBP-TAZTO	NT			--	--	--	-6,6	na*
DBP-TAZTO	NT			--	--	--	-6,6	na*
DBP	NT			--	IN/IN	--	-5,6	na*
DDC-Ant (Dec 603)	NT			--	--	--		na*
HCTBPH/Dec 604	NT			--	--	--	-4,8	na*
Diethylphosphinic acid	NT			--	--	--	-4,1	na*

Ax/INx: number of assays (x) in which the chemical was indicated as active (A) or inactive (IN).
Heat map: color coding relates to the number of assays in which the chemical was active, and the proportion of active *vs.* inactive assays: Yellow: ($A < 3$ AND $IN \geq A$) OR $A = 1$ - Weak evidence of activity; Orange: ($A \geq 3$ AND $IN \geq A$) OR ($1 < A \leq 4$ AND $IN < A$) - Good evidence of activity; Red: $A > 4$ AND $IN < A$ - Strong evidence of activity.

* na - information on tonnage NOT AVAILABLE (not registered in REACH, for example) # Antagonist/Binding.
For the Endocrine disruptome tool: The binding probability is represented with the following color coding; red color indicates a high probability of binding (SE < 0.25), orange and yellow represent medium probability, (0.25 < SE < 0.50) and (0.50 < SE < 0.75) respectively, and green represent the low probability of binding (SE > 0.75) to anti-AR. ** Dark grey indicates chemicals for which the models fail to predict due to high molecular weight (>600 g/mol).
https://pubs.acs.org/doi/10.1021/acs.chemrestox.6b00347 - ≥ 0.1 = Active ; 0.1 > x ≥ 0.001 = very weak, considered inconclusive ; < 0.001 = inactive.
ER, estrogen receptor; AR, androgen receptor; GR, glucocorticoid receptor; PR, progesterone receptor; AhR, Aryl hydrocarbon receptor; NT, not tested..

data from more anti-GR assays in which the same compounds were found inactive.

Because the anti-androgenic activity was consistently reported for most Group A and B FRs, results of computational and *in silico* models were also examined for all replacement FRs from the list, including the 27 other FRs for which no *in vitro* data on anti-androgenic activity was found. A QSAR model from the international collaboration CoMPARA (Mansouri et al., 2020) predicted AR antagonist activity for 9 of these 27 compounds with no *in vitro* data (namely, N,N'-ethylenebis(tetrabromophthalimide) (EBTEBPI), hexachlorocyclopentenyldibromocyclooctane (DBHCTD), decabromodiphenylethane (DBDPE), tris(2,3-dibromopropyl)isocyanurate (TDBP-TAZTO), resorcinol bis(diphenylphosphate) (RDBPP), Dechlorane 602 (DDC-DBF), Dechlorane Plus (DDC-CO), (1,2-bis(2,4,6-tribromophenoxy)ethane) (BTBPE) and pentabromophenoxy-nonabromodiphenyl ether (4′-PeBPO-BDE208)) (Table 2 and Fig. 2C). The Endocrine Disruptome model predicted medium probability of binding to the antagonist conformation of the AR for 15 of these 27 replacement FRs, namely, DBE-DBCH, pentabromotoluene (PBT), TDBP-TAZTO, 2-(2-hydroxyethoxy)ethyl 2-hydroxypropyl 3,4,5,6-tetrabromophthalate (HEEHP-TEBP), BTBPE, DBHCTD, pentabromobenzyl acrylate (PBB-Acr), 2,3,5,6-tetrabromo-p-xylene (TBX), hexabromocyclodecane (HBCYD), dibromostyrene (DBS), 1,3-bis(2,3-dibromopropyl)-5-(2-propen-1-yl)-1,3,5-triazine-2,4,5(1H,3H,5H)-trione (BDBP-TAZTO), 1-(2,3-dibromopropyl)-3,5-diallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (DBP-TAZTO), 2,4-dibromophenol (DBP), Dechlorane 604 (HCTBPH/Dec 604), and diethylphosphinic acid (Table 2 and Fig. 2C). For six compounds, no prediction was obtained because the platform does not support the docking of ligands with molecular weights exceeding 600 g/mol. The *in silico* predicted binding of replacement FRs to anti-AR is presented in Table 2, where the affinity is represented by color coding based on the probability of binding with the receptor from higher to lower order; red > orange > yellow > green. The results are consistent with the conclusion reached by Wang and colleagues in a recent study using a computational tool to predict the binding affinity of 25 organophosphate esters (of which several were replacement FRs) with 12 NRs (Wang et al., 2020). The authors also validated the accuracy of Endocrine Disruptome tools by comparing the predictions with experimental data from the literature and observed that the predictions were relatively reliable with an accuracy, sensitivity, and specificity of 78.8, 60.0, and 80.9%, respectively. The ToxCast pathway model could not predict activity for these chemicals as it works/or integrates data from the already existing ToxCast/TOX21 research program where these chemicals have not been tested yet. Considering the anti-AR activity predicted by the *in silico* models (CoMPARA or Endocrine Disruptome), 21 replacement FRs may therefore be added to the 19 FRs from Group A and B that showed anti-AR activity in one or more assays. Further evaluation is needed for the compounds for which no predictions were obtained in either of the models due to certain limitations (for instance, tris(tribromoneopentyl)phosphate (TTBNPP), 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine (TTBP-TAZ) and octabromotrimethyphenyl indane (OBTMPI)).

Overall, these shared toxicological profiles highlight the possibility of additive or synergic ED-like effects (anti-androgenic in particular) among these replacement FRs. Such mixture effects may increase the toxicological potency of individual chemicals when acting in mixtures, as shown, for example, in male reproductive tracts of rats exposed to a mixture of 18 anti-androgenic compounds (Conley et al., 2018). Mixture effects of replacement FRs are of real concern, considering repeatedly reported co-occurrence of replacement FRs in dust, fish, wildlife, and human samples (Demirtepe et al., 2019; Malarvannan et al., 2015; Mitro et al., 2016; Saillenfait et al., 2018; Shi et al., 2016; Sundkvist et al., 2010).

3.4. Critical gaps in knowledge

For 24 out of the 52 replacement FRs screened, no experimental *in vitro* data related to interactions with the selected NRs were found. For several of these Group C FRs, there are indications of broad use, and human exposure is thus likely. Five out of these Group C FRs (DBDPE, DDC-CO, TTBNPP, bisphenol A bis(diphenylphosphate) (BPA-BDPP) and EBTEBPI) are registered in the EU REACH legislation with high or very high tonnage (up to the 10,000–100,000 tonnes per year for DBDPE). Many of these Group C FRs have been detected in indoor dust or air, such as DBDPE, DDC-CO, Dechlorane 603 (DDC-Ant), TTBP-TAZ, BTBPE, BPA-BDPP, DBHCTD, RBDPP, and PBB-Acr (Ballesteros-Gómez et al., 2014; Brandsma et al., 2013; Demirtepe et al., 2019; Guo et al., 2018a; Khairy and Lohmann, 2018; Kurt-Karakus et al., 2017; Li et al., 2015; Nguyen et al., 2019; Rantakokko et al., 2019; Vojta et al., 2017), or in human matrices, such as BTBPE, DBDPE, DBHCTD, DBP, DDC-Ant, DDC-CO, DDC-DBF/Dechlorane 602, OBTMPI, and TBX (Čechová et al., 2017; Fromme et al., 2015; Haglund et al., 2016; Shi et al., 2016; Zhou et al., 2014b, 2014a). Therefore, generating comprehensive toxicological data for these compounds should be a research priority.

For some of the 15 replacement FRs included in Group B (i.e., only scarce experimental data available), low effective concentrations below 10 μM have been reported, which may indicate a higher toxicological concern. These include, for example, estrogenic activity (for ip-TPP and CDP), anti-estrogenic activity (for PBP), anti-androgenic activity (for 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB)), agonist activity towards PR (for HBB), androgenic activity (for HBB and DBE-DBCH, also known TBECH), and agonist AhR activity (for and 2,2-bis(chloromethyl) trimethylenebis[bis(2-chloroethyl) phosphate] (V6)) (Table 1 and Table S1). Further studies are needed to confirm these preliminary observations, especially for compounds like ip-TPP, which is registered under EU REACH with high tonnage, for EH-TBB, used in commercial FR mixtures FM550 and FM BZ54, and often detected in home dust (Demirtepe et al., 2019; Nguyen et al., 2019; Stapleton et al., 2014; Yang et al., 2020), or for V6 which was detected in baby products and dust (Fang et al., 2013).

It also appears that, in general, limited AhR data were available for the 52 replacement FRs screened, although this is an important mediator of various chronic toxicities, such as immunotoxicity, developmental and reproductive toxicity (Petersen et al., 2006; Rothhammer and Quintana, 2019). Indeed, agonist and antagonist AhR data were only available for 23 and 18 replacement FRs, respectively, and came almost exclusively from ToxCast. The exceptions were three studies with 2,4,6-tribromophenol (246-TBP), TBBPA, HBB, and/or PBT, of which one showed AhR-agonist activity for HBB and PBT (Brown et al., 2004; Hamers et al., 2006; Harju et al., 2007), and one study reporting AhR-agonist activity at relatively low effective concentrations for eight out of nine replacement FRs included in our initial list (Rosenmai et al., 2021). As shown in Table 1, the majority of the tested replacement FRs were inactive for AhR-antagonist activity in this limited set of tests (16 out of 18).

In summary, for the 39 replacement FRs included in Groups B and C (limited or no information was available on the activities associated with the studied NRs), priority should be given to those produced in the highest amounts and/or which have been detected at significant levels

in the environment or human matrices, such as DBDPE, DDC-CO or V6 (Fang et al., 2013; Stapleton et al., 2011). In addition, a recent human study has reported significant statistical associations between levels of EH-TBB, BTBPE, DBE-DBCH, DDC-CO, or DBDPE and the sex steroids follicle-stimulating hormone (FSH) and/or testosterone in blood samples from local residents of an e-waste dismantling region in China (Guo et al., 2018b). These replacement FRs may have ED potential, and more tests on these ED properties and additional biomonitoring exposure data in human populations are needed.

3.5. Other ED activities

This review focuses on *in vitro* and *in silico* data related to interactions with 5 NRs, which represent a subset of potential targets of EDCs. Indeed, receptor agonism and antagonism examined in this review constitute two of the ten key characteristics of EDCs defined in an expert consensus statement, which include, for example, signal transduction, hormone synthesis, or hormone transport (La Merrill et al., 2020). Other ED properties of replacement FRs may not be fully reflected. For example, TPhP showed activity in an enzyme-based assay in a high throughput screening for EDCs (Morisseau et al., 2009). Another frequent target of EDCs is the thyroid hormone pathway/homeostasis, and some replacement FRs were reported to interfere with it. For instance, PBP, 246-TBP or TBBPA are very potent competitive inhibitors of the thyroxine (T4) binding to transthyretin (TTR) *in vitro* (Hamers et al., 2006; Meerts et al., 2000), and TNBP, TMPP, TDCIPP, and TCIPP were reported to inhibit the binding of the triiodothyronine (T3) hormone to thyroid receptor β (Zhang et al., 2016). *In vitro* interactions with TTR were also reported for a few more replacement FRs, with two studies describing either an increase in T4-TTR binding after exposure to TPhP, TDCIPP, TEP, and TBOEP (Hill et al., 2018), or a TTR displacement induced by TPhP, CDP, TMPP and EHDPP (Rosenmai et al., 2021). Besides, several lines of evidence also link TDCIPP and TPhP to the thyroid hormone pathway in humans and fishes. Both compounds were shown to affect thyroid hormone levels (Kim et al., 2015; Meeker et al., 2013; Meeker and Stapleton, 2010; Preston et al., 2017; Wang et al., 2015), and some adverse effects that may result from thyroid disruption, such as neurodevelopmental defects (Castorina et al., 2017; Jarema et al., 2015; Lipscomb et al., 2017; Noyes et al., 2015). Exposure to TPhP may also be associated with increased risks of papillary thyroid cancer in humans (Hoffman et al., 2017b). These other aspects of ED add to the evidence collected in this paper as arguments for classifying TPhP, TDCIPP, and possibly TBBPA and PBP as potential EDCs, and would deserve deeper and more systematic analysis. In agreement with these conclusions, all four are in the list of potential endocrine disruptors elaborated by the endocrine disruption exchange (TEDX, Search the TEDX List — The Endocrine Disruption Exchange).

4. Conclusions

This comprehensive data mining and its structured presentation (1) allowed to assess the consistency of data and identified replacement FRs with ED potential, (2) revealed that number of replacement FRs share similar profiles of activities against NRs, raising concerns regarding possible mixture effects, and (3) highlighted the striking lack of experimental *in vitro* data for 24 of the 52 studied replacement FRs, including high tonnage chemicals or compounds frequently detected in human biomonitoring studies.

The collection of results from *in vitro* tests on agonist and antagonist activities toward five NRs (ER, AR, GR, PR, and AhR) highlighted the ED potential of several replacement FRs. It particularly points to TPhP and TDCIPP as probable EDCs: estrogenic and anti-PR activity of TPhP, and anti-androgenic activity of both TPhP and TDCIPP have repeatedly been reported in different assays at relatively low concentrations. This is further supported by observations of other ED activities, such as disruption of the thyroid hormone pathway. For three other compounds, less information was available, but it consistently showed anti-estrogenicity (PBP), estrogenicity (ip-TPP), and androgenicity (DBE-DBCH). These observations are also supported by a few *in vivo* studies for ip-TPP and DBE-DBCH. TBBPA and TMPP are other replacement FRs that were reported active in some assays for estrogenicity, anti-estrogenicity, or anti-androgenicity, but because of other conflicting data, firm conclusions cannot be drawn. Although this systematic review constitutes a point of departure for the identification and prioritization of replacement flame retardants with endocrine disruption potential, it should be kept in mind that endocrine disruption goes beyond the receptor level, including other key characteristics (La Merrill et al., 2020) that remain to be characterized.

This work also reveals that tests for activity on these five key NRs are critically lacking for 24 replacement FRs, and testing is incomplete for the other 15 compounds. Among these 39 replacement FRs, several are ubiquitous, and there is a concern for widespread human exposure, considering their high production volume and/or confirmed human exposure, such as for DBDPE or DDC-CO.

Finally, the data collected suggest that many replacement FRs might share profiles of their ED activities, where 12 replacement FRs were reported to be active in both anti-AR and anti-PR assays, of which eight also showed estrogenicity. In particular, the anti-androgenic potential is of concern and has also been predicted by *in silico* model(s) for 21 additional replacement FRs with no *in vitro* data available. This raises high concerns for mixture effects and urges investigation of the ED activities of the "non-tested" replacement FRs. Specifically, their anti-androgenic potential should be addressed as a priority.

Declaration of Competing Interest

The authors declare they have no actual or potential competing financial interests.

Acknowledgements

The research was supported by funding from the European Unionś Horizon 2020 Research and Innovation Programme under grant agreements No 733032 (HBM4EU), No. 859891 (PRORISK), and No. 857560 (CETOCOEN EXCELLENCE Teaming 2 project). The RECETOX Research infrastructure was further supported by the Czech Ministry of Education, Youth and Sports (LM2018121; CZ02.1.01/0.0/0.0/18_046/0015975).

Author contributions

LoB collected, organized, and interpreted the data (open literature, reports, ToxCast dashboard, and AOP-wiki) and drafted the manuscript; CN provided and commented results obtained with endocrine disruptome; VM and MF critically reviewed, edited, and provided expertise on endocrine disruption; LM critically reviewed, edited and provided expertise on human exposure; SJ and JBB critically reviewed, edited and provided expertise on risk assessment issues; LuB supervised the work and contributed to the paper concept and writing. All authors reviewed the manuscript before submission and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106550.

References

ANSES, 2018. Analysis of the most appropriate risk management option (RMOA) - triphenyl phosphate (TPP).

Asnake, S., Pradhan, A., Banjop-Kharlyngdoh, J., Modig, C., Olsson, P.E., 2014. 1,2-dibromo-4-(1,2 dibromoethyl) cyclohexane (TBECH)-mediated steroid hormone receptor activation and gene regulation in chicken LMH cells. Environ. Toxicol. Chem. 33, 891–899. https://doi.org/10.1002/etc.2509.

Bajard, L., Melymuk, L., Blaha, L., 2019. Prioritization of hazards of novel flame retardants using the mechanistic toxicology information from ToxCast and Adverse Outcome Pathways. Environ. Sci. Eur. 31, 14. https://doi.org/10.1186/s12302-019-0195-z.

Ballesteros-Gómez, A., De Boer, J., Leonards, P.E.G., 2014. A novel brominated triazine-based flame retardant (TTBP-TAZ) in plastic consumer products and indoor dust. Environ. Sci. Technol. 48, 4468–4474. https://doi.org/10.1021/es4057032.

Bastiaensen, M., Ait Bamai, Y., Araki, A., Goudarzi, H., Konno, S., Ito, S., Miyashita, C., Yao, Y., Kishi, R., Covaci, A., 2020. Temporal trends and determinants of PFR exposure in the Hokkaido Study. Int. J. Hyg. Environ. Health 228, 113523. https://doi.org/10.1016/j.ijheh.2020.113523.

Beck, K.R., Sommer, T.J., Schuster, D., Odermatt, A., 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. https://doi.org/10.1016/j.tox.2016.09.014.

Blum, A., Behl, M., Birnbaum, L.S., Diamond, M.L., Phillips, A., Singla, V., Sipes, N.S., Stapleton, H.M., Venier, M., 2019. Organophosphate Ester Flame Retardants: Are They a Regrettable Substitution for Polybrominated Diphenyl Ethers? Environ. Sci. Technol. Lett. 6, 638–649. https://doi.org/10.1021/acs.estlett.9b00582.

Bollmann, U.E., Möller, A., Xie, Z., Ebinghaus, R., Einax, J.W., 2012. Occurrence and fate of organophosphorus flame retardants and plasticizers in coastal and marine surface waters. Water Res. 46, 531–538. https://doi.org/10.1016/j.watres.2011.11.028.

Brandsma, S.H., Leonards, P.E.G., Leslie, H.A., de Boer, J., 2015. Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web. Sci. Total Environ. 505, 22–31. https://doi.org/10.1016/j.scitotenv.2014.08.072.

Brandsma, S.H., Sellström, U., de Wit, C.A., de Boer, J., Leonards, P.E.G., 2013. Dust Measurement of Two Organophosphorus Flame Retardants, Resorcinol Bis (diphenylphosphate) (RBDPP) and Bisphenol A Bis(diphenylphosphate) (BPA-BDPP), Used as Alternatives for BDE-209. Environ. Sci. Technol. 47, 14434–14441. https://doi.org/10.1021/es404123q.

Brown, D.J., Van Overmeire, I., Goeyens, L., Denison, M.S., De Vito, M.J., Clark, G.C., 2004. Analysis of Ah receptor pathway activation by brominated flame retardants. Chemosphere 55, 1509–1518. https://doi.org/10.1016/j.chemosphere.2003.10.019.

Carignan, C.C., Minguez-Alarcón, L., Butt, C.M., Williams, P.L., Meeker, J.D., Stapleton, H.M., Toth, T.L., Ford, J.B., Hauser, R., 2017. Urinary concentrations of organophosphate flame retardant metabolites and pregnancy outcomes among women undergoing in vitro fertilization. Environ. Health Perspect. 125, 1–8. https://doi.org/10.1289/EHP1021.

Carignan, C.C., Mínguez-Alarcón, L., Williams, P.L., Meeker, J.D., Stapleton, H.M., Butt, C.M., Toth, T.L., Ford, J.B., Hauser, R., 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. https://doi.org/10.1016/j.envint.2017.12.005.

Castorina, R., Bradman, A., Stapleton, H.M., Butt, C., Avery, D., Harley, K.G., Gunier, R. B., Holland, N., Eskenazi, B., 2017. Current-use flame retardants: Maternal exposure and neurodevelopment in children of the CHAMACOS cohort. Chemosphere 189, 574–580. https://doi.org/10.1016/j.chemosphere.2017.09.037.

Čechová, E., Vojta, Š., Kukučka, P., Kočan, A., Trnovec, T., Murínová, Ľ.P., de Cock, M., van de Bor, M., Askevold, J., Eggesbø, M., Scheringer, M., 2017. Legacy and alternative halogenated flame retardants in human milk in Europe: Implications for children's health. Environ. Int. 108, 137–145. https://doi.org/10.1016/j.envint.2017.08.008.

Chen, G., Jin, Y., Wu, Y., Liu, L., Fu, Z., 2015. Exposure of male mice to two kinds of organophosphate flame retardants (OPFRs) induced oxidative stress and endocrine disruption. Environ. Toxicol. Pharmacol. 40, 310–318. https://doi.org/10.1016/j.etap.2015.06.021.

Conley, J.M., Lambright, C.S., Evans, N., Cardon, M., Furr, J., Wilson, V.S., Gray, L.E., 2018. Mixed "Antiandrogenic" Chemicals at Low Individual Doses Produce Reproductive Tract Malformations in the Male Rat. Toxicol. Sci. 164, 166–178. https://doi.org/10.1093/toxsci/kfy069.

Covaci, A., Harrad, S., Abdallah, M.A.E., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour. Environ. Int. 37, 532–556. https://doi.org/10.1016/j.envint.2010.11.007.

Demirtepe, H., Melymuk, L., Diamond, M.L., Bajard, L., Vojta, Š., Prokeš, R., Sáňka, O., Klánová, J., Palkovičová Murínová, Ľ., Richterová, D., Rašplová, V., Trnovec, T., 2019. Linking past uses of legacy SVOCs with today's indoor levels and human exposure. Environ. Int. 127, 653–663. https://doi.org/10.1016/j.envint.2019.04.001.

ECHA, 2019. Decision on substance evaluation - Triphenyl phosphate.

EU, 2011. DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union 23, 75–82.

EU RAR, 2009. European Union Risk Assessment Report Tris(2-chloroethyl) phosphate (TCEP). Eur. communities. https://echa.europa.eu/documents/10162/2663989d-1795-44a1-8f50-153a81133258.

European Union, 2008. European Union Risk Assessment Report: TDCPP.

Fang, M., Webster, T.F., Gooden, D., Cooper, E.M., McClean, M.D., Carignan, C., Makey, C., Stapleton, H.M., 2013. Investigating a novel flame retardant known as V6: Measurements in baby products, house dust, and car dust. Environ. Sci. Technol. 47, 4449–4454. https://doi.org/10.1021/es400032v.

Fromme, H., Cequier, E., Kim, J.-T., Hanssen, L., Hilger, B., Thomsen, C., Chang, Y.-S., Völkel, W., 2015. Persistent and emerging pollutants in the blood of German adults: Occurrence of dechloranes, polychlorinated naphthalenes, and siloxanes. Environ. Int. 85, 292–298. https://doi.org/10.1016/j.envint.2015.09.002.

Gbadamosi, M.R., Abdallah, M.A.E., Harrad, S., 2021. A critical review of human exposure to organophosphate esters with a focus on dietary intake. Sci. Total Environ. 771, 144752 https://doi.org/10.1016/j.scitotenv.2020.144752.

Guo, J., Stubbings, W.A., Romanak, K., Nguyen, L.V., Jantunen, L., Melymuk, L., Arrandale, V., Diamond, M.L., Venier, M., 2018a. Alternative Flame Retardant, 2,4,6-Tris(2,4,6-tribromophenoxy)-1,3,5-triazine, in an E-waste Recycling Facility and House Dust in North America. Environ. Sci. Technol. 52, 3599–3607. https://doi.org/10.1021/acs.est.7b06139.

Guo, L.C., Pan, S., Yu, S., Liu, T., Xiao, J., Zhu, B., Qu, Y., Huang, W., Li, M., Li, X., Zeng, W., Rutherford, S., Lin, L., Zhang, Y., Ma, W., 2018b. Human Sex Hormone Disrupting Effects of New Flame Retardants and Their Interactions with Polychlorinated Biphenyls, Polybrominated Diphenyl Ethers, a Case Study in South China. Environ. Sci. Technol. 52, 13935–13941. https://doi.org/10.1021/acs.est.8b01540.

Haglund, P., Kaj, L., Brorström Lundén, E., 2016. Analysis of new brominated flame retardants in human serum and background air. Stockholm.

Hales, B.F., Robaire, B., 2020. Effects of brominated and organophosphate ester flame retardants on male reproduction. Andrology 8, 915–923. https://doi.org/10.1111/andr.12789.

Hall, J.M., Greco, C.W., 2019. Perturbation of Nuclear Hormone Receptors by Endocrine Disrupting Chemicals: Mechanisms and Pathological Consequences of Exposure. Cells 9, 13. https://doi.org/10.3390/cells9010013.

Hamers, T., Kamstra, J.H., Sonneveld, E., Murk, A.J., Kester, M.H.A., Andersson, P.L., Legler, J., Brouwer, A., 2006. In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicol. Sci. 92, 157–173. https://doi.org/10.1093/toxsci/kfj187.

Harju, M., Hamers, T., Kamstra, J.H., Sonneveld, E., Boon, J.P., Tysklind, M., Andersson, P.L., 2007. Quantitative structure-activity relationship modeling on in vitro endocrine effects and metabolic stability involving 26 selected brominated flame retardants. Environ. Toxicol. Chem. 26, 816. https://doi.org/10.1897/06-308R.1.

Heindel, J.J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M.A., Nadal, A., Palanza, P., Panzica, G., Sargis, R., Vandenberg, L.N., vom Saal, F., 2017. Metabolism disrupting chemicals and metabolic disorders. Reprod. Toxicol. 68, 3–33. https://doi.org/10.1016/j.reprotox.2016.10.001.

Hill, K.L., Hamers, T., Kamstra, J.H., Willmore, W.G., Letcher, R.J., 2018. Organophosphate triesters and selected metabolites enhance binding of thyroxine to human transthyretin in vitro. Toxicol. Lett. 285, 87–93. https://doi.org/10.1016/j.toxlet.2017.12.030.

Hoffman, K., Butt, C.M., Webster, T.F., Preston, E.V., Hammel, S.C., Makey, C., Lorenzo, A.M., Cooper, E.M., Carignan, C., Meeker, J.D., Hauser, R., Soubry, A., Murphy, S.K., Price, T.M., Hoyo, C., Mendelsohn, E., Congleton, J., Daniels, J.L., Stapleton, H.M., 2017a. Temporal Trends in Exposure to Organophosphate Flame Retardants in the United States. Environ. Sci. Technol. Lett. 4, 112–118. https://doi.org/10.1021/acs.estlett.6b00475.

Hoffman, K., Lorenzo, A., Butt, C.M., Hammel, S.C., Henderson, B.B., Roman, S.A., Scheri, R.P., Stapleton, H.M., Sosa, J.A., 2017b. Exposure to flame retardant chemicals and occurrence and severity of papillary thyroid cancer: A case-control study. Environ. Int. 107, 235–242. https://doi.org/10.1016/j.envint.2017.06.021.

Jarema, K.A., Hunter, D.L., Shaffer, R.M., Behl, M., Padilla, S., 2015. Acute and developmental behavioral effects of flame retardants and related chemicals in zebrafish. Neurotoxicol. Teratol. 52, 194–209. https://doi.org/10.1016/j.ntt.2015.08.010.

Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen, H.M., Reif, D.M., Rotroff, D.M., Shah, I., Richard, A.M., Dix, D.J., 2010. In vitro screening of environmental chemicals for targeted testing prioritization: The toxcast project. Environ. Health Perspect. 118, 485–492. https://doi.org/10.1289/ehp.0901392.

Khairy, M.A., Lohmann, R., 2018. Selected organohalogenated flame retardants in Egyptian indoor and outdoor environments: Levels, sources and implications for human exposure. Sci. Total Environ. 633, 1536–1548. https://doi.org/10.1016/j.scitotenv.2018.03.243.

Khalaf, H., Larsson, A., Berg, H., McCrindle, R., Arsenault, G., Olsson, P.E., 2009. Diastereomers of the brominated flame retardant 1,2-dibromo-4-(1,2 dibromoethyl) cyclohexane induce androgen receptor activation in the HepG2 hepatocellular carcinoma cell line and the LNCaP prostate cancer cell line. Environ. Health Perspect. 117, 1853–1859. https://doi.org/10.1289/ehp.0901065.

Kharlyngdoh, J.B., Asnake, S., Pradhan, A., Olsson, P.E., 2016. TBECH, 1,2-dibromo-4-(1,2 dibromoethyl) cyclohexane, alters androgen receptor regulation in response to mutations associated with prostate cancer. Toxicol. Appl. Pharmacol. 307, 91–101. https://doi.org/10.1016/j.taap.2016.07.018.

Kim, S., Jung, J., Lee, I., Jung, D., Youn, H., Choi, K., 2015. Thyroid disruption by triphenyl phosphate, an organophosphate flame retardant, in zebrafish (Danio rerio) embryos/larvae, and in GH3 and FRTL-5 cell lines. Aquat. Toxicol. 160, 188–196. https://doi.org/10.1016/j.aquatox.2015.01.016.

Kleinstreuer, N.C., Ceger, P., Watt, E.D., Martin, M., Houck, K., Browne, P., Thomas, R.S., Casey, W.M., Dix, D.J., Allen, D., Sakamuru, S., Xia, M., Huang, R., Judson, R., 2017. Development and Validation of a Computational Model for Androgen Receptor Activity. Chem. Res. Toxicol. 30, 946–964. https://doi.org/10.1021/acs.chemrestox.6b00347.

Kojima, H., Takeuchi, S., Itoh, T., Iida, M., Kobayashi, S., Yoshida, T., 2013. In vitro endocrine disruption potential of organophosphate flame retardants via human nuclear receptors. Toxicology 314, 76–83. https://doi.org/10.1016/j.tox.2013.09.004.

Kojima, H., Takeuchi, S., Van den Eede, N., Covaci, A., 2016. Effects of primary metabolites of organophosphate flame retardants on transcriptional activity via human nuclear receptors. Toxicol. Lett. 245, 31–39. https://doi.org/10.1016/j.toxlet.2016.01.004.

Kolšek, K., Mavri, J., Sollner Dolenc, M., Gobec, S., Turk, S., 2014. Endocrine disruptome - An open source prediction tool for assessing endocrine disruption potential through nuclear receptor binding. J. Chem. Inf. Model. 54, 1254–1267. https://doi.org/10.1021/ci400649p.

Krewski, D., Andersen, M.E., Tyshenko, M.G., Krishnan, K., Hartung, T., Boekelheide, K., Wambaugh, J.F., Jones, D., Whelan, M., Thomas, R., Yauk, C., Barton-Maclaren, T., Cote, I., 2020. Toxicity testing in the 21st century: progress in the past decade and future perspectives, Archives of Toxicology. Springer Berlin Heidelberg. https://doi.org/10.1007/s00204-019-02613-4.

Krivoshiev, B.V., Dardenne, F., Covaci, A., Blust, R., Husson, S.J., 2016. Assessing in-vitro estrogenic effects of currently-used flame retardants. Toxicol. Vitr. 33, 153–162. https://doi.org/10.1016/j.tiv.2016.03.006.

Kurt-Karakus, P.B., Alegria, H., Jantunen, L., Birgul, A., Topcu, A., Jones, K.C., Turgut, C., 2017. Polybrominated diphenyl ethers (PBDEs) and alternative flame retardants (NFRs) in indoor and outdoor air and indoor dust from Istanbul-Turkey: Levels and an assessment of human exposure. Atmos. Pollut. Res. 8, 801–815. https://doi.org/10.1016/j.apr.2017.01.010.

La Merrill, M.A., Vandenberg, L.N., Smith, M.T., Goodson, W., Browne, P., Patisaul, H.B., Guyton, K.Z., Kortenkamp, A., Cogliano, V.J., Woodruff, T.J., Rieswijk, L., Sone, H., Korach, K.S., Gore, A.C., Zeise, L., Zoeller, R.T., 2020. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nat. Rev. Endocrinol. 16, 45–57. https://doi.org/10.1038/s41574-019-0273-8.

Larsson, A., Eriksson, L.A., Andersson, P.L., Ivarson, P., Olsson, P.E., 2006. Identification of the brominated flame retardant 1,2-dibromo-4-(1,2- dibromoethyl)cyclohexane as an androgen agonist. J. Med. Chem. 49, 7366–7372. https://doi.org/10.1021/jm060713d.

Li, W.-L., Qi, H., Ma, W.-L., Liu, L.Y., Zhang, Z., Zhu, N.-Z., Mohammed, M.O.A., Li, Y.-F., 2015. Occurrence, behavior and human health risk assessment of dechlorane plus and related compounds in indoor dust of China. Chemosphere 134, 166–171. https://doi.org/10.1016/j.chemosphere.2015.04.024.

Lipscomb, S.T., McClelland, M.M., MacDonald, M., Cardenas, A., Anderson, K.A., Kile, M. L., 2017. Cross-sectional study of social behaviors in preschool children and exposure to flame retardants. Environ. Heal. A Glob. Access Sci. Source 16, 1–10. https://doi.org/10.1186/s12940-017-0224-6.

Liu, P.Y., Meng, T., Li, Y.Y., Cai, M., Li, X.H., Chen, J., Qin, Z.F., 2017. Tetrabromoethylcyclohexane affects gonadal differentiation and development in the frog Pelophylax nigromaculatus. Aquat. Toxicol. 192, 40–47. https://doi.org/10.1016/j.aquatox.2017.09.009.

Liu, X., Ji, K., Choi, K., 2012. Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. Aquat. Toxicol. 114–115, 173–181. https://doi.org/10.1016/j.aquatox.2012.02.019.

Liu, X., Ji, K., Jo, A., Moon, H.B., Choi, K., 2013. Effects of TDCPP or TPP on gene transcriptions and hormones of HPG axis, and their consequences on reproduction in adult zebrafish (Danio rerio). Aquat. Toxicol. 134–135, 104–111. https://doi.org/10.1016/j.aquatox.2013.03.013.

Liu, X., Jung, D., Jo, A., Ji, K., Moon, H.B., Choi, K., 2016. Long-term exposure to triphenylphosphate alters hormone balance and HPG, HPI, and HPT gene expression in zebrafish (Danio rerio). Environ. Toxicol. Chem. 35, 2288–2296. https://doi.org/10.1002/etc.3395.

Malarvannan, G., Belpaire, C., Geeraerts, C., Eulaers, I., Neels, H., Covaci, A., 2015. Organophosphorus flame retardants in the European eel in Flanders, Belgium: Occurrence, fate and human health risk. Environ. Res. 140, 604–610. https://doi.org/10.1016/j.envres.2015.05.021.

Mansouri, K., Kleinstreuer, N., Abdelaziz, A.M., Alberga, D., Alves, V.M., Andersson, P.L., Andrade, C.H., Bai, F., Balabin, I., Ballabio, D., Benfenati, E., Bhhatarai, B., Boyer, S., Chen, J., Consonni, V., Farag, S., Fourches, D., García-Sosa, A.T., Gramatica, P., Grisoni, F., Grulke, C.M., Hong, H., Horvath, D., Hu, X., Huang, R., Jeliazkova, N., Li, J., Li, X., Liu, H., Manganelli, S., Mangiatordi, G.F., Maran, U., Marcou, G., Martin, T., Muratov, E., Nguyen, D.T., Nicolotti, O., Nikolov, N.G., Norinder, U., Papa, E., Petitjean, M., Piir, G., Pogodin, P., Poroikov, V., Qiao, X., Richard, A.M., Roncaglioni, A., Ruiz, P., Rupakheti, C., Sakkiah, S., Sangion, A., Schramm, K.W., Selvaraj, C., Shah, I., Sild, S., Sun, L., Taboureau, O., Tang, Y., Tetko, I.V., Todeschini, R., Tong, W., Trisciuzzi, D., Tropsha, A., Van Den Driessche, G., Varnek, A., Wang, Z., Wedebye, E.B., Williams, A.J., Xie, H., Zakharov, A.V., Zheng, Z., Judson, R.S., 2020. Compara: Collaborative modeling project for androgen receptor activity. Environ. Health Perspect. 128, 1–17. https://doi.org/10.1289/EHP5580.

Meeker, J.D., Cooper, E.M., Stapleton, H.M., Hauser, R., 2013. Exploratory analysis of urinary metabolites of phosphorus-containing flame retardants in relation to markers of male reproductive health. Endocr. Disruptors 1, e26306. https://doi.org/10.4161/endo.26306.

Meeker, J.D., Stapleton, H.M., 2010. House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environ. Health Perspect. 118, 318–323. https://doi.org/10.1289/ehp.0901332.

Meerts, I.A.T.M., Van Zanden, J.J., Luijks, E.A.C., Van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, Å., Brouwer, A., 2000. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in Vitro. Toxicol. Sci. 56, 95–104. https://doi.org/10.1093/toxsci/56.1.95.

Mitro, S.D., Dodson, R.E., Singla, V., Adamkiewicz, G., Elmi, A.F., Tilly, M.K., Zota, A.R., 2016. Consumer Product Chemicals in Indoor Dust: A Quantitative Meta-analysis of U.S. Studies. Environ. Sci. Technol. 50, 10661–10672. https://doi.org/10.1021/acs.est.6b02023.

Molina-Molina, J.M., Amaya, E., Grimaldi, M., Sáenz, J.M., Real, M., Fernández, M.F., Balaguer, P., Olea, N., 2013. In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. Toxicol. Appl. Pharmacol. 272, 127–136. https://doi.org/10.1016/j.taap.2013.05.015.

Morisseau, C., Merzlikin, O., Lin, A., He, G., Feng, W., Padilla, I., Denison, M.S., Pessah, I. N., Hammock, B.D., 2009. Toxicology in the fast lane: Application of high-throughput bioassays to detect modulation of key enzymes and receptors. Environ. Health Perspect. 117, 1867–1872. https://doi.org/10.1289/ehp.0900834.

Mustieles, V., Fernández, M.F., 2020. Bisphenol A shapes children's brain and behavior: towards an integrated neurotoxicity assessment including human data. Environ. Health 19, 66. https://doi.org/10.1186/s12940-020-00620-y.

Nguyen, L.V., Diamond, M.L., Venier, M., Stubbings, W.A., Romanak, K., Bajard, L., Melymuk, L., Jantunen, L.M., Arrandale, V.H., 2019. Exposure of Canadian electronic waste dismantlers to flame retardants. Environ. Int. 129, 95–104. https://doi.org/10.1016/j.envint.2019.04.056.

Noyes, P.D., Haggard, D.E., Gonnerman, G.D., Tanguay, R.L., 2015. Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicol. Sci. 145, 177–195. https://doi.org/10.1093/toxsci/kfv044.

Ohtake, F., Baba, A., Fujii-Kuriyama, Y., Kato, S., 2008. Intrinsic AhR function underlies cross-talk of dioxins with sex hormone signalings. Biochem. Biophys. Res. Commun. 370, 541–546. https://doi.org/10.1016/j.bbrc.2008.03.054.

Petersen, S.L., Krishnan, S., Hudgens, E.D., 2006. The aryl hydrocarbon receptor pathway and sexual differentiation of neuroendocrine functions. Endocrinology 147, 41–44. https://doi.org/10.1210/en.2005-1157.

Pollock, T., Mantella, L., Reali, V., de Catanzaro, D., 2017. Influence of tetrabromobisphenol a, with or without concurrent triclosan, upon bisphenol a and estradiol concentrations in mice. Environ. Health Perspect. 125, 1–10. https://doi.org/10.1289/EHP1329.

Poma, G., Glynn, A., Malarvannan, G., Covaci, A., Darnerud, P.O., 2017. Dietary intake of phosphorus flame retardants (PFRs) using Swedish food market basket estimations. Food Chem. Toxicol. 100, 1–7. https://doi.org/10.1016/j.fct.2016.12.011.

Pradhan, A., Kharlyngdoh, J.B., Asnake, S., Olsson, P.E., 2013. The brominated flame retardant TBECH activates the zebrafish (Danio rerio) androgen receptor, alters gene transcription and causes developmental disturbances. Aquat. Toxicol. 142–143, 63–72. https://doi.org/10.1016/j.aquatox.2013.07.018.

Preston, E.V., McClean, M.D., Claus Henn, B., Stapleton, H.M., Braverman, L.E., Pearce, E.N., Makey, C.M., Webster, T.F., 2017. Associations between urinary diphenyl phosphate and thyroid function. Environ. Int. 101, 158–164. https://doi.org/10.1016/j.envint.2017.01.020.

Rantakokko, P., Kumar, E., Braber, J., Huang, T., Kiviranta, H., Cequier, E., Thomsen, C., 2019. Concentrations of brominated and phosphorous fl ame retardants in Finnish house dust and insights into children's exposure. Chemosphere 223, 99–107. https://doi.org/10.1016/j.chemosphere.2019.02.027.

Reers, A.R., Eng, M.L., Williams, T.D., Elliott, J.E., Cox, M.E., Beischlag, T.V., 2016. The Flame-Retardant Tris(1,3-dichloro-2-propyl) Phosphate Represses Androgen Signaling in Human Prostate Cancer Cell Lines. J. Biochem. Mol. Toxicol. 30, 249–257. https://doi.org/10.1002/jbt.21786.

Rosenmai, A.K., Winge, S.B., Möller, M., Lundqvist, J., Wedebye, E.B., Nikolov, N.G., Lilith Johansson, H.K., Vinggaard, A.M., 2021. Organophosphate ester flame retardants have antiandrogenic potential and affect other endocrine related endpoints in vitro and in silico. Chemosphere 263, 127703. https://doi.org/10.1016/j.chemosphere.2020.127703.

Rothhammer, V., Quintana, F.J., 2019. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. Nat. Rev. Immunol. 19, 184–197. https://doi.org/10.1038/s41577-019-0125-8.

Ryan, N., 2017. A User's Guide for Accessing and Interpreting ToxCast Data.

Saillenfait, A.M., Ndaw, S., Robert, A., Sabaté, J.P., 2018. Recent biomonitoring reports on phosphate ester flame retardants: a short review. Arch. Toxicol. 92, 2749–2778. https://doi.org/10.1007/s00204-018-2275-z.

Sharkey, M., Harrad, S., Abou-Elwafa Abdallah, M., Drage, D.S., Berresheim, H., 2020. Phasing-out of legacy brominated flame retardants: The UNEP Stockholm Convention and other legislative action worldwide. Environ. Int. 144, 106041 https://doi.org/10.1016/j.envint.2020.106041.

Sharma, A., Mollier, J., Brocklesby, R.W.K., Caves, C., Jayasena, C.N., Minhas, S., 2020. Endocrine-disrupting chemicals and male reproductive health. Reprod. Med. Biol. 19, 243–253. https://doi.org/10.1002/rmb2.12326.

Shi, Z., Zhang, L., Li, J., Zhao, Y., Sun, Z., Zhou, X., Wu, Y., 2016. Novel brominated flame retardants in food composites and human milk from the Chinese Total Diet Study in 2011: Concentrations and a dietary exposure assessment. Environ. Int. 96, 82–90. https://doi.org/10.1016/j.envint.2016.09.005.

Stapleton, H.M., Klosterhaus, S., Keller, A., Ferguson, P.L., Van Bergen, S., Cooper, E., Webster, T.F., Blum, A., 2011. Identification of flame retardants in polyurethane foam collected from baby products. Environ. Sci. Technol. 45, 5323–5331. https://doi.org/10.1021/es2007462.

Stapleton, H.M., Misenheimer, J., Hoffman, K., Webster, T.F., 2014. Flame retardant associations between children's handwipes and house dust. Chemosphere 116, 54–60. https://doi.org/10.1016/j.chemosphere.2013.12.100.

Sundkvist, A.M., Olofsson, U., Haglund, P., 2010. Organophosphorus flame retardants and plasticizers in marine and fresh water biota and in human milk. J. Environ. Monit. 12, 943–951. https://doi.org/10.1039/b921910b.

Suzuki, G., Tue, N.M., Malarvannan, G., Sudaryanto, A., Takahashi, S., Tanabe, S., Sakai, S.I., Brouwer, A., Uramaru, N., Kitamura, S., Takigami, H., 2013. Similarities in the endocrine-disrupting potencies of indoor dust and flame retardants by using human osteosarcoma (U2OS) cell-based reporter gene assays. Environ. Sci. Technol. 47, 2898–2908. https://doi.org/10.1021/es304691a.

Thomas Zoeller, R., Brown, T.R., Doan, L.L., Gore, A.C., Skakkebaek, N.E., Soto, A.M., Woodruff, T.J., Vom Saal, F.S., 2012. Endocrine-disrupting chemicals and public health protection: A statement of principles from the Endocrine Society. Endocrinology 153, 4097–4110. https://doi.org/10.1210/en.2012-1422.

Toporova, L., Balaguer, P., 2020. Nuclear receptors are the major targets of endocrine disrupting chemicals. Mol. Cell. Endocrinol. 502, 110665 https://doi.org/10.1016/j.mce.2019.110665.

United Nations Environment Programme, 2017. Overview Report I: Worldwide initiatives to identify endocrine disrupting chemicals (EDCs) and potential EDCs The International Panel on Chemical Pollution (IPCP) 1–40.

US-EPA, 2015. Flame Retardants Used in Flexible Polyurethane Foam: An Alternatives Assessment. https://www.epa.gov/sites/production/files/2015-08/documents/ffr_final.pdf.

van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119–1153. https://doi.org/10.1016/j.chemosphere.2012.03.067.

Vojta, Š., Melymuk, L., Klánová, J., 2017. Changes in Flame Retardant and Legacy Contaminant Concentrations in Indoor Air during Building Construction, Furnishing, and Use. Environ. Sci. Technol. 51, 11891–11899. https://doi.org/10.1021/acs.est.7b03245.

Vykoukalová, M., Venier, M., Vojta, Š., Melymuk, L., Bečanová, J., Romanak, K., Prokeš, R., Okeme, J.O., Saini, A., Diamond, M.L., Klánová, J., 2017. Organophosphate esters flame retardants in the indoor environment. Environ. Int. 106, 97–104. https://doi.org/10.1016/j.envint.2017.05.020.

Wang, Q., Lai, N.L.S., Wang, X., Guo, Y., Lam, P.K.S., Lam, J.C.W., Zhou, B., 2015. Bioconcentration and transfer of the organophorous flame retardant 1,3-dichloro-2-propyl phosphate causes thyroid endocrine disruption and developmental neurotoxicity in zebrafish larvae. Environ. Sci. Technol. 49, 5123–5132. https://doi.org/10.1021/acs.est.5b00558.

Wang, X., Zhang, R., Song, C., Crump, D., 2020. Computational evaluation of interactions between organophosphate esters and nuclear hormone receptors. Environ. Res. 182, 108982 https://doi.org/10.1016/j.envres.2019.108982.

Wei, G.L., Li, D.Q., Zhuo, M.N., Liao, Y.S., Xie, Z.Y., Guo, T.L., Li, J.J., Zhang, S.Y., Liang, Z.Q., 2015. Organophosphorus flame retardants and plasticizers: Sources, occurrence, toxicity and human exposure. Environ. Pollut. 196, 29–46. https://doi.org/10.1016/j.envpol.2014.09.012.

WHO, 1998. Flame Retardants: Tris (chloropropyl) phosphate and tris (2-chloroethyl) phosphate. Environ. Heal. Criteria 209, 129.

WHO, UNEP, 2012. State of the science of Endocrine Disrupting Chemicals - 2012.

Yang, C., Rozárka Jílková, S., Melymuk, L., Anne Harris, S., M. Jantunen, L., Pertili, J., Winn, L., L. Diamond, M., 2020. Are We Exposed to Halogenated Flame Retardants from both Primary and Secondary Sources? Environ. Sci. & Technol. Lett. 7, 585–593. https://doi.org/10.1021/acs.estlett.0c00268.

Zatecka, E., Castillo, J., Elzeinova, F., Kubatova, A., Ded, L., Peknicova, J., Oliva, R., 2014. The effect of tetrabromobisphenol A on protamine content and DNA integrity in mouse spermatozoa. Andrology 2, 910–917. https://doi.org/10.1111/j.2047-2927.2014.00257.x.

Zatecka, E., Ded, L., Elzeinova, F., Kubatova, A., Dorosh, A., Margaryan, H., Dostalova, P., Peknicova, J., 2013. Effect of tetrabrombisphenol A on induction of apoptosis in the testes and changes in expression of selected testicular genes in CD1 mice. Reprod. Toxicol. 35, 32–39. https://doi.org/10.1016/j.reprotox.2012.05.095.

Zeng, X., He, L., Cao, S., Ma, S., Yu, Z., Gui, H., Sheng, G., Fu, J., 2014. Occurrence and distribution of organophosphate flame retardants/plasticizers in wastewater treatment plant sludges from the Pearl River Delta. China. Environ. Toxicol. Chem. 33, 1720–1725. https://doi.org/10.1002/etc.2604.

Zhang, Q., Ji, C., Yin, X., Yan, L., Lu, M., Zhao, M., 2016. Thyroid hormone-disrupting activity and ecological risk assessment of phosphorus-containing flame retardants by in vitro, in vivo and in silico approaches. Environ. Pollut. 210, 27–33. https://doi.org/10.1016/j.envpol.2015.11.051.

Zhang, Q., Lu, M., Dong, X., Wang, C., Zhang, C., Liu, W., Zhao, M., 2014. Potential estrogenic effects of phosphorus-containing flame retardants. Environ. Sci. Technol. 48, 6995–7001. https://doi.org/10.1021/es5007862.

Zhou, S.N., Buchar, A., Siddique, S., Takser, L., Abdelouahab, N., Zhu, J., 2014a. Measurements of Selected Brominated Flame Retardants in Nursing Women: Implications for Human Exposure. Environ. Sci. Technol. 48, 8873–8880. https://doi.org/10.1021/es5016839.

Zhou, S.N., Siddique, S., Lavoie, L., Takser, L., Abdelouahab, N., Zhu, J., 2014b. Hexachloronorbornene-based flame retardants in humans: Levels in maternal serum and milk. Environ. Int. 66, 11–17. https://doi.org/10.1016/j.envint.2014.01.010.

Zuiderveen, E.A.R., Slootweg, J.C., de Boer, J., 2020. Novel brominated flame retardants - A review of their occurrence in indoor air, dust, consumer goods and food. Chemosphere 255, 126816. https://doi.org/10.1016/j.chemosphere.2020.126816.