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Research Article

Deciphering Adverse Outcome Pathway Network Linked to Bisphenol F Using Text Mining and Systems Toxicology Approaches

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ABSTRACT

Bisphenol F (BPF) is one of several Bisphenol A (BPA) substituents that is increasingly used in manufacturing industry leading to detectable human exposure. Whereas a large number of studies have been devoted to decipher BPA effects, much less is known about its substituents. To support decision making on BPF's safety, we have developed a new computational approach to rapidly explore the available data on its toxicological effects, combining text mining and integrative systems biology, and aiming at connecting BPF to adverse outcome pathways (AOPs). We first extracted from different databases BPF-protein associations that were expanded to protein complexes using protein-protein interaction datasets. Over-representation analysis of the protein complexes allowed to identify the most relevant biological pathways putatively targeted by BPF. Then, automatic screening of scientific abstracts from literature using the text mining tool, AOP-helpFinder, combined with data integration from various sources (AOP-wiki, CompTox, etc.) and manual curation allowed us to link BPF to AOP events. Finally, we combined all the information gathered through those analyses and built a comprehensive complex framework linking BPF to an AOP network including, as adverse outcomes, various types of cancers such as breast and thyroid malignancies. These results which integrate different types of data can support regulatory assessment of the BPA substituent, BPF, and trigger new epidemiological and experimental studies.

Key words: artificial intelligence; AOP-helpFinder; integrative systems toxicology; adverse outcome pathway network; HBM4EU.

Bisphenol A (BPA) is a presumed endocrine disruptor due to its chemical similarity to natural estrogens and also a metabolic disruptor and neurotoxicant. Several studies have been carried out to understand its modes of action (MoA). Some of these studies revealed effects at lower doses than the no-observed-adverse-effect levels, mostly corresponding to the regulation of non-genomic pathways whereas the activation of nuclear receptors were involved at higher doses (FitzGerald and Wilks, 2014). Bisphenol A has been banned in some countries (Canada, EU) for some specific uses (baby bottles, coating of infant formula). This led to replacement of BPA for the production of epoxy resins and polycarbonate to reduce putative adverse effects. As a consequence, there is an increasing use of substituents such as bisphenol S (BPS) in thermal paper, bisphenol B, and bisphenol F (BPF) and its isomers in canned foods and soft drinks. However, the MoA and potential toxicities of BPA analogs are still poorly characterized. Whether these substituents are safer than BPA remains a

matter of debate. Linking BPF and other BPS to adverse outcome pathways (AOPs) and therefore enhancing our knowledge on their putative toxicities is a major challenge for regulatory needs.

Evidence for environmental and health effects of chemical substances has increased considerably over the last years (certain hormone-dependent cancers, neurocognitive disorders, reproductive perturbations). Therefore, regulatory measures need to be taken in the EU and in member states for those chemicals. Thus, there is a need for assessment of hazards and risks of the thousands of existing substances we are exposed to; however, to avoid animal testing which has also its limitations, both *in vitro* and *in silico* methods were developed and recommended by Toxicity testing in the 21th Century in 2007 and Economic Co-operation and Development Organization (OECD) guidelines. Together with the U.S. Environmental Protection Agency (U.S. EPA), the National Toxicology Program has recommended to include, among others, *in silico* approaches in future assessments of toxicity as an inexpensive and efficient tool for screening purposes. Recently, the concept of new approach methodologies has been put forward; it refers to nonanimal technologies that can be used to provide information on chemical hazard and risk assessment (ICCVAM, 2018).

To carry out these assessments, the OECD has proposed 2 frameworks (Sakuratani *et al.*, 2018). The first 1 is a science-based approach, the Integrated Approaches to Testing and Assessment (IATA) that combines various types of existing and new data (*in vitro*, *in vivo*, and computational) to study a specific question. The second 1 is the AOP, that can be used in the development of IATA (Tollefsen *et al.*, 2014). Adverse outcome pathway consists in capturing and organizing key events (KEs), at different levels of biological organization (molecular, cellular, tissue, organ, organism, and population), that lead to toxic effects. Adverse outcome pathway starts with a molecular initiating event (MIE), which can be triggered by a stressor (eg: chemical). These MIEs are connected to a sequence of KEs linked together by KE relationships (KERs), which lead to an adverse outcome (AO) (Ankley *et al.*, 2010; Villeneuve *et al.*, 2014). Such AOP frameworks provide a scheme describing mechanistic knowledge from existing tests (Knapen *et al.*, 2015). They are particularly relevant for putative endocrine disruptors (defined by their MoA).

To successfully incorporate AOPs into risk assessment multiple interacting pathways or networks of AOPs should be accounted for (Garcia-Reyero, 2015). Individual AOP could be considered as a linear description of biological events, and can be merged via their shared events (MIE or KE). Consequently, AOP networks can be constructed by connecting 2 or more individual AOPs, if they have at least 1 common KE or KER, to provide a better description of the biological complexity (Knapen *et al.*, 2018) with considerable additional value for emerging toxicological knowledge (Pollesch *et al.*, 2019). Adverse outcome pathway networks can be initiated by 1 or more stressors that can be environmental chemicals.

Computational approaches allow to explore and identify key information, and therefore can be used to develop AOP networks. In their study, Oki and Edwards, generated computationally predicted AOPs by integrating multiple data sources from HTS studies by using Frequent Itemset Mining (Oki and Edwards, 2016).

In this study, we describe a computational approach to establish linkages between environmental stressors and health effects, using available information from the literature and databases. Different sources of information were considered such as PubMed, ToxCast, CompTox, and AOP-wiki, and integrated to develop individual AOP and AOP networks. We took advantage of existing tools, WebGestaltR and the recently hybrid method called AOP-helpFinder (Carvaillo *et al.*, 2019; Liao *et al.*, 2019), an artificial intelligence (AI) method that automatically screens and analyses abstracts from published articles to decipher relevant links between chemical substances (ie, stressors) and AOPs. The presented strategy demonstrates the ability to identify links between BPF, KEs, and toxic effects using existing sparse information. The integrative approach revealed a plausible complex AOP induced by BPF, leading to thyroid cancer, as well as an AOP network related to various types of malignancies.

MATERIALS AND METHODS

Overall strategy. Linkage between BPF (see §2), biological events and toxic effects were investigated using a systems toxicology approach (Figure 1). This multistep approach is based on integration of existing knowledge from various sources of information. In the first step, specific data on BPF-protein associations were extracted from chemical biology databases. Second, these BPF-protein associations were expanded to protein complexes. By using a high confidence interactome (Li *et al.*, 2017), we were able to decipher protein complexes associated with BPF (§3). Then, protein-pathway information was integrated into these protein complexes to statistically order linkage between the BPF and biological pathways (§4). Finally, to have a better understanding of the mode of action of BPF leading to the identified pathways, literature searches were performed, and relevant information were integrated (§5 and 6). Therefore, these steps allowed to suggest a plausible AOP induced by BPF leading to thyroid cancer, that has been extended to an AOP network (§7).

Bisphenol F. To get the most complete biological picture of BPF, the 3 isomers for BPF were analyzed (Figure 2): the 2,2′-isomer of BPF (CAS rn 2467-02-9) (2,2-BPF), the 2,4′-isomer of BPF (CASRN 2467-03-0) (2,4-BPF), and 4,4′-isomer of BPF (CAS rn 620-92-8) (4,4-BPF). The commercially available BPF mixed isomers was also taken into consideration (CAS rn 1333-16-0). Information from the different sources were compiled using the common name "BPF," and various synonyms (Supplementary Table 1). These synonyms were extracted from the CompTox database, and only the valid ones were retained (19 for 4,4-BPF, 12 for 2,2-BPF, 11 for 2,4-BPF, and 13 for BPF mixture).

Linking BPF to protein complexes. To identify proteins known to be associated with BPF, we compiled information from 2 publicly available databases. First, we extracted information from the ToxCast database (https://actor.epa.gov/dashboard; dashboard accessed on December 2018) (Judson *et al.*, 2010). The ToxCast database is based on high-throughput technologies, and aggregate information for thousands of chemical substances. This U.S. Environmental Protection Agency infrastructure contains information for 9076 chemicals that have been tested on 359 assays with a total of 1192 endpoints (Judson *et al.*, 2010; Kavlock *et al.*, 2012).

Then to collect more proteins associated with BPF, the Comparative Toxicogenomics database (CTD) (as of December 2018) (Davis *et al.*, 2018) was used. CTD contained 1 898 228 chemical-protein curated associations mined from peer-

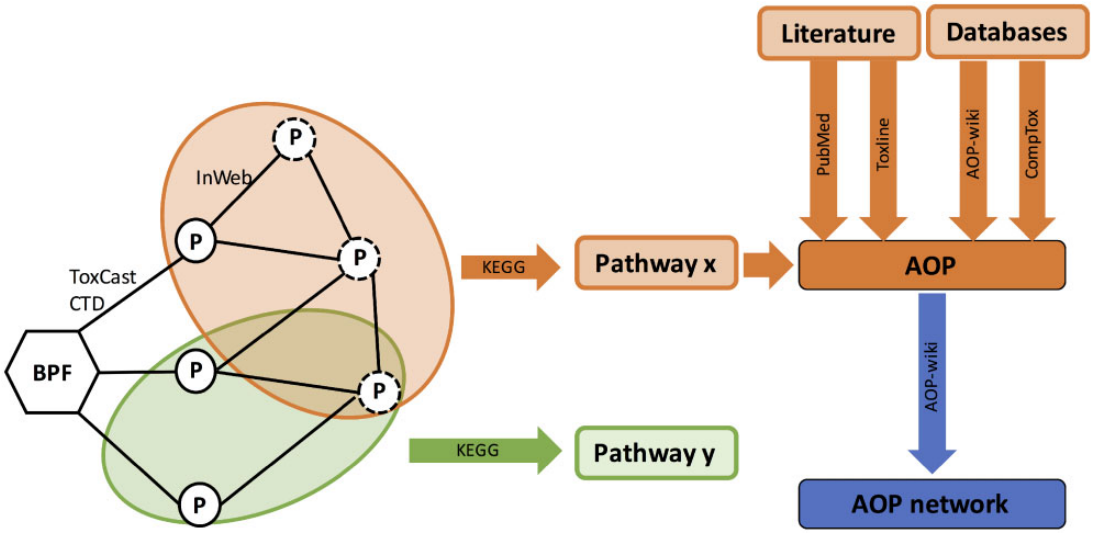

Figure 1. Overview of the systems toxicology strategy for developing adverse outcome pathway (AOP) networks for bisphenol F (BPF). In a first step, BPF-protein associations were extracted from the Comparative Toxicogenomics database and the ToxCast database. Then creation of protein complexes by integration of the first-order protein partners (P, dashed line) to the extracted proteins (P, full line) using a high confidence interactome based on experimental evidences (InWeb data source). The next step consisted of performing a biological enrichment of the protein complexes to statistically rank pathways linked to them. Finally, different data types were integrated from various sources (literature, databases) using AOP-helpFinder and by manual curation, to build comprehensive mechanisms between BPF and toxic effects, to develop an individual AOP and AOP network for which BPF may be a stressor.

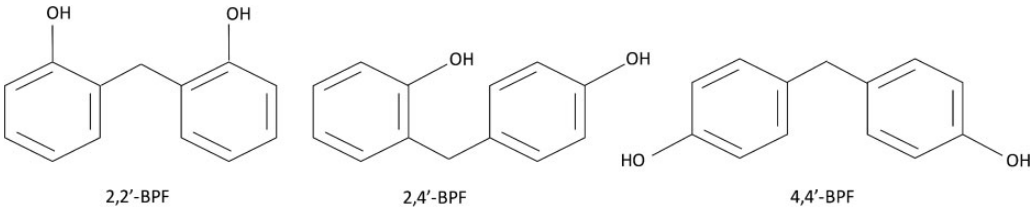

Figure 2. The 3 isoforms of bisphenol F.

reviewed scientific literature, between 13 108 chemicals and 47 581 proteins for 593 organisms. All proteins were mapped to HUGO name identifiers and Entrez gene id to facilitate further data integration.

Because in a biological system, proteins tend to function in groups or complexes, an important step was to enrich the list of compiled proteins using information from a high confidence human protein interactome that is protein-protein interactions (PPIs) based on experiments and inferred model organism data (Li *et al.*, 2017). The InWeb 3.0 tool (www.cbs.dtu.dk/services/VirtualPulldown-1.1b/web/) was used to identify the first-order interacting proteins. Such strategy is based on a neighbor's pull down approach (Lage *et al.*, 2007). The version that we used, contained a total of 507 142 unique PPIs involving 14 441 human proteins, as of December 2018. As a result, the list of relevant proteins associated with BPF was extended by inclusion of their first-order PPI partners, considering a significant pull down score threshold of 0.25 (Lage *et al.*, 2007).

Biological enrichment of the protein complexes. To identify pathways and toxic effects related to BPF, information on biological pathways were integrated into the protein complex linked to BPF. To catch as much as possible information, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways-based database was used as a source of information (Kanehisa *et al.*, 2019).

To perform the over-representation analysis (ORA), the R package WebGestaltR (version 3), was used. The protein complex was therefore tested for significant pathways associations using a test based on a hypergeometric distribution. A significance level of 0.05 after Benjamini-Hochberg correction for multiple testing of *p* values was used to select the most relevant associations.

Exploration of the literature to contribute to the development of AOP. The literature search was done in 2 ways with the aim to capture as much as possible information to link BPF to AOPs. As AOPs are in development, and limited number has been validated, we decided to combine our approach with a manual exploration of the PubMed database.

First, an automatic search was performed using the recently developed AOP-helpFinder tool (Carvaillo *et al.*, 2019), that is a hybrid approach that combined text mining procedure and graph theory, to identify linkage between BPF and biological events present in already defined AOPs. The TOXLINE database was screened to compile scientific publications mentioning BPF in a toxicological context (https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen? TOXLINE) (as of July 2017). To capture as much as possible of the existing information, several synonyms for BPF where used (Supplementary Table 1) that were extracted from the CompTox database (https://comptox.epa.gov/dashboard) (Williams *et al.*, 2017). Then, to prepare the biological data, AOPs were considered. Based on the AOP-wiki database (as of August 2017) (https://aopwiki.org), a dictionary was developed. The AOP-wiki database contains information related to AOPs for which mechanistic representations of toxicological effects over various

level of the biological organization were reported. The developed dictionary contains information related to MIE/KE and AO. Both MIE and KE were integrated in the same "list" that contains 1318 events. The AO list contains 61 events. These 2 categories were analyzed separately using the text mining tool, mainly due to their wording composition. Biological events associated with MIE and KE were more complex sentences compared with AO that are more likely to be defined by 1 or few words. Finally, the literature screening approach was performed using the AOP-helpFinder tool. The text mining part is used to identify co-mentioned words (eg, a BPF isomer and a biological event) in an abstract from the scientific literature. The graph theory allowed to order the findings by calculating scores. Based on this tool, 2 different scores were calculated: a position score that determine the position of the co-occurred terms in an abstract. The more the AOP-related words are placed toward the end of the abstract, the more they can be considered as a result and not a working hypothesis. The second score, the weighted score takes into consideration the complexity of the AOP-related terms (from 1 word to 21 words) (Carvaillo *et al.*, 2019).

Then, the PubMed database (as of February 2019) was screened manually to decipher targeted information between identified proteins (through the biological enrichment of the protein complexes, as described above) involved in a disease of interest. More precisely, the research on PubMed was made using the keyword "thyroid cancer" associated with the name of each protein. The search results were analyzed by searching for the keywords in the title and abstracts of the articles. If links were mentioned in the summary, the article was read to find the relevant information and retrieve it. A second research was then conducted to find a link between proteins with a link with thyroid cancer and BPF. The results of this research were analyzed in the same way as the previous one. The results obtained from these 2 bibliographic researches also made it possible to reveal links between BPF or thyroid cancer and proteins that are not part of the initial protein complex. As a result, these links were further screened with a final bibliographical research.

Integrating biological data. To get a more comprehensive and complete mechanistic view of BPF associated with the identified AOPs, we included as much as possible information (molecular targets, biological events, toxic effects) at different levels of the biological organization by using several databases. The U.S. EPA's ToxCast program has screened thousands of chemicals for biological activity, primarily using high-throughput *in vitro* bioassays. The ToxCast dashboard (https://actor.epa.gov/dashboard/) (as of March 2019) was used. Relevant data were also extracted from other sources of information such as the CompTox database (https://comptox.epa.gov/dashboard/) (as of March 2019) and the PubChem bioassays database (https://pubchem.ncbi.nlm.nih.gov/) (as of March 2019). This step allows us to build an individual AOP induced by BPF.

Generation of AOP network. To characterize an AOP network induced by BPF, we assembled shared events (MIE/KE/AO) between the putative AOP developed by the systems biology approach, and available information using the AOP-wiki database (as of April 2019). As a result, the structure of the AOP network is described by at least 2 or more AOPs that share at least 1 event. The development of the AOP network was done by screening the AOP-wiki database to extract events shared with the proposed individual AOP for BPF.

RESULTS

Generation of Protein Complexes for BPF Compounds

Using the ToxCast and CTDs, we extracted proteins for which the chemicals show biological activities. Therefore, we were able to compile information regarding 9 proteins for 2,4′-BPF, 12 proteins for 2,2′-BPF, and 111 proteins for 4,4′-BPF (Figure 3). As a result, 114 unique proteins were identified, and among them 6 proteins were common to the 3 compounds (AR, ESR1, POU2F1, VRD, NR1I2, and NFE2L2). No information was obtained for the BPF mixed isomer. Therefore, the data analyses were concentrated on these 114 unique proteins that are connected to at least 1 of 3 BPF isomers.

Using the list of identified proteins, BPF isomers were linked to protein complexes by determining first-order PPI partners for each of the 114 proteins. In the generated network, 307 proteins (including the 114 input proteins) were connected to their respective partners, with a total of 496 edges (see Supplementary Table 2).

Pathways-based Analysis of the Protein Complexes

To identify pathways associated with the 3 studied BPF, the protein complexes were enriched by ORA using the KEGG database. Among the 307 proteins present in the network, 288 were mapped to unique entrezGeneID, and therefore used for the ORA (Supplementary Table 3). Among these 288 proteins, 158 have annotations in the KEGG database. Consequently, the ORA of these 158 proteins revealed several statistically significant pathways (see Supplementary Table 4) and were mapped to a number of categories such as the function of the synapses or cell signaling. Some of these appear to be more specific and potentially connected in terms of mechanisms of action.

Several lipid metabolism-related pathways were retrieved with the KEGG analysis. For example, pathways such as "linoleic acid metabolism" (with a corrected p value of 8.51^{e-13}, via 13 proteins) and "arachidonic acid metabolism" (with a corrected p value of 4.35^{e-10}, through 13 proteins), were significantly connected to BPF. Interestingly, those 2 omega-6 fatty acids have been associated with inflammation, a process which is also linked to the development of several pathologies (including cancer).

Another finding was linked to endocrine-related pathways including signaling by cortisol (together with Cushing syndrome), estrogen, aldosterone, progesterone (together with 2 global steroidogenesis pathways: steroid hormone biosynthesis, ovarian steroidogenesis) and also parathyroid hormone and thyroid hormone pathways suggesting that BPF may act as an endocrine disruptor. Interestingly, changes in the expression of several adenylyl cyclases (involved in the production of cAMP and subsequently in signaling by the transcriptional factor, cAMP Responsive Element Binding protein) and of metabolizing enzymes (including cytochromes P450 and transferases) were observed suggesting that BPF could act on several processes related to these hormones (signaling and metabolism).

Moreover, among the identified pathways, 4 were associated with endocrine-related cancers (prostate, endometrial, breast, thyroid) (Table 1). According to the CompTox database (access as of March 2019), no cancer information was associated yet to the BPF compounds. Therefore, we decided to explore the most significant link (BPF-thyroid cancer) using an integrative approach including text mining, literature searches, and additional databases exploration.

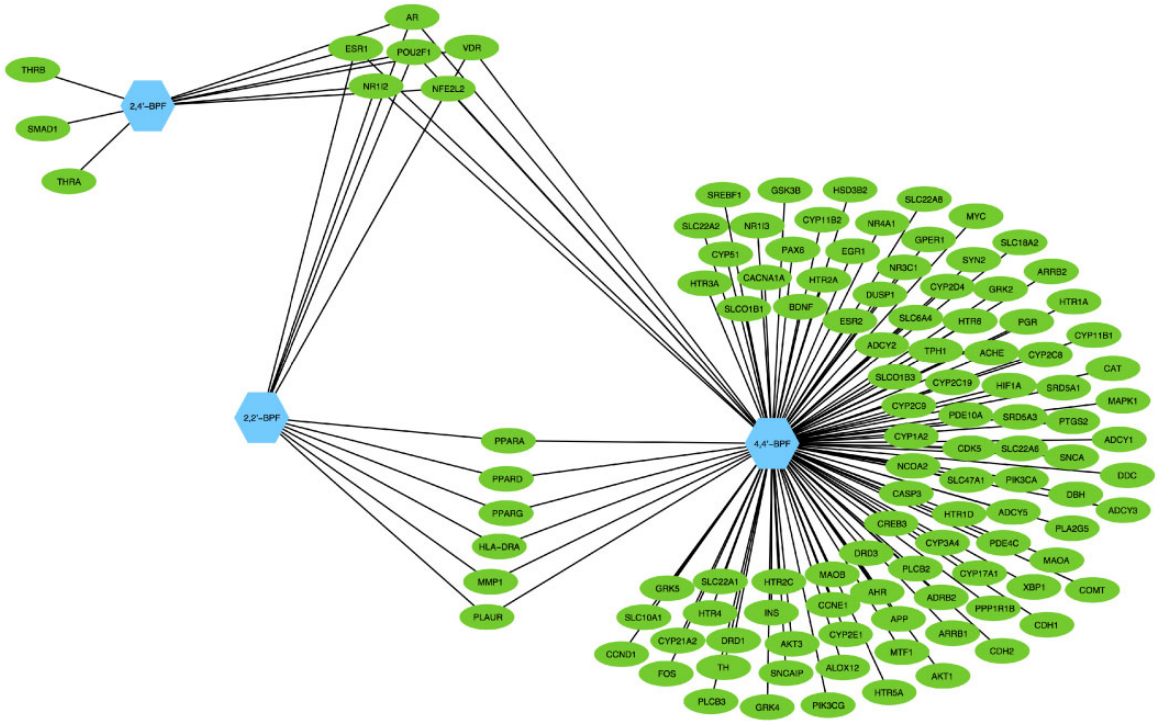

Figure 3. Bisphenol F -protein associations network. View of the proteins (green ovals) associated with the 3 bisphenol F isomers (blue hexagons). Data were extracted from the ToxCast and Comparative Toxicogenomics databases. Proteins are denoted by HUGO gene symbols to facilitate further analysis.

Table 1. List of the Statistically Significant Endocrine-Related Cancers Associated With the 3 Studied BPF Isomers

Pathways Name	*p* Value	FDR[a]	Gene Name
Thyroid cancer	1.00E-05	8.38E-05	CCND1; CDH1; MAPK1; MYC; NCOA4; PPARG; RXRG
Endometrial cancer	2.62E-05	1.90E-04	AKT1; AKT3; CCND1; CDH1; GSK3B; MAPK1; MYC; PIK3CA
Prostate cancer	3.49E-05	2.37E-04	AKT1; AKT3; AR; CCND1; CCNE1; CREB3; CREB3L4; GSK3B; MAPK1; PIK3CA
Breast cancer	1.25E-05	9.92E-05	AKT1; AKT3; CCND1; ESR1; ESR2; FOS; FRAT1; FRAT2; GSK3B; MAPK1; MYC; PGR; PIK3CA
Pathways in cancer	1.26E-07	2.29E-06	ADCY1; ADCY2; ADCY3; ADCY5; ADCY8; AKT1; AKT3; AR; CASP3; CCND1; CCNE1; CDH1; EGLN2; ESR1; ESR2; FOS; FRAT1; FRAT2; GSK3B; HIF1A; MAPK1; MYC; NCOA4; NFE2L2; PIK3CA; PLCB2; PLCB3; PPARD; PPARG; PTGS2; RXRG

[a]FDR, corrected *p* value with Benjamini-Hochberg method.

Knowledge-Driven Analysis to Link BPF to Biological Events

As a next step, to be able to further support the association of BPF with AOPs, an unsupervised analysis of the existing literature was performed using the tool AOP-helpFinder, followed by a manual targeted literature analysis, and data integration. The main idea was to decipher potential linkages, based on existing knowledge, between BPF and the previously identified endocrine-related cancers by systems biology (Table 1).

First, an automatic screening of the literature was carried out using the newly developed AOP-helpFinder tool (Carvaillo *et al.*, 2019). This tool was run on the 190 publications mentioning BPF extracted from the TOXLINE database, using the developed AOP dictionary (Carvaillo *et al.*, 2019). This search led to the identification of 1 AO that is cancer, and 8 KEs (Supplementary Table 5). Allergic contact dermatitis challenge was the most common KE (KE 312), retrieved in 7 of 15 articles that mentioned a term related to AOPs, out of the 190 screened publications. Interestingly, BPA is associated with 63 dermatitis-related proteins in the CTD and BPF is also linked to dermatitis via 5 proteins (ASRGL1, BMP6, CXCL2, IFI30, also in CTD).

Among the other findings, the KEs (AOP-wiki KE 870 "Increase, Cell proliferation" and KE 1555 "Increase cell proliferation") were linked to BPF (Perez *et al.*, 1998), which is relevant since due to their potential endocrine-disrupting effects (eg, binding to estrogen receptors), bisphenols including BPF have been described as promoters of cell proliferation (Perez *et al.*, 1998). Such a connection between BPF and cell proliferation events is in line with the known effect of estrogens on benign and malignant thyrocyte proliferation through transcriptional activation of various oncogenes including Bcl-2 or c-fos and stimulation of non-genomic pathways (including the ERK and Akt pathways which stimulate cell division) (Kumar *et al.*, 2010; Manole *et al.*, 2001).

Based on these text mining-based results and the ones from the systems biology analysis, we decided to further explore thyroid cancer, as this outcome was the most significant 1 identified by ORA. Therefore in a second literature-based step, associations between the stressor BPF, the biological events identified previously (cell proliferation and thyroid cancer), and the proteins found by ORA (CCND1; CDH1; MAPK1; MYC; NCOA4; PPARG; RXRG) (Table 1) were explored by manual

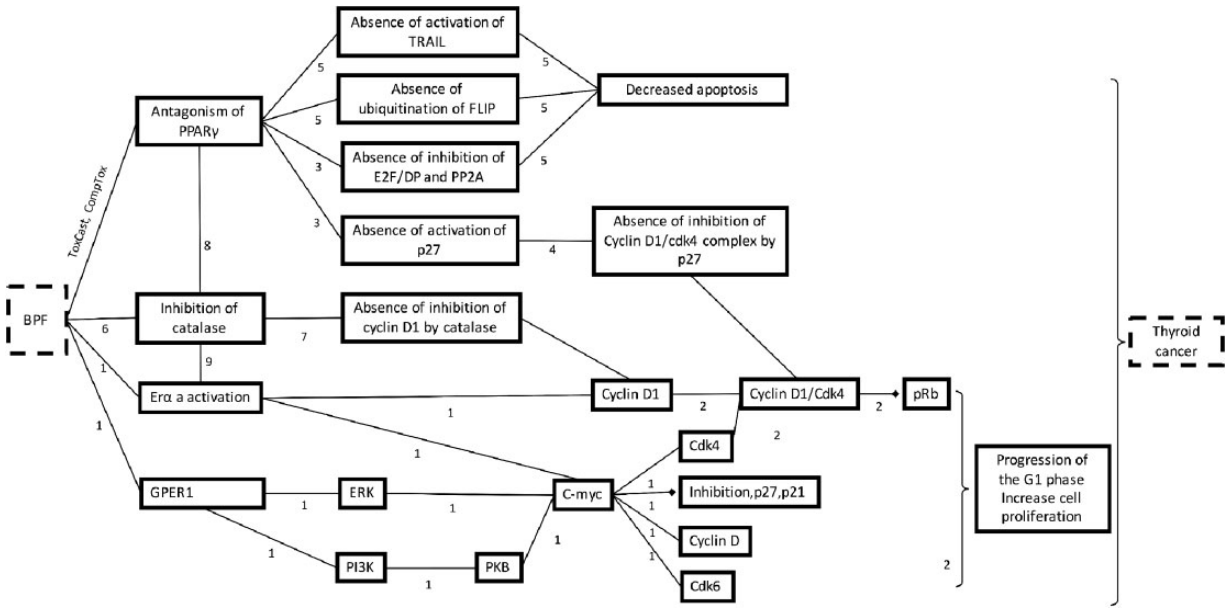

Figure 4. Representation of a putative individual adverse outcome pathway (AOP) linking bisphenol F to thyroid cancer. The AOP was constructed by integrating information from the ToxCast and CompTox databases, and from the literature (numbers on the arrows correspond to the scientific literature—see Supplementary Table 6).

curation of available scientific publications using the PubMed database. This targeted analysis of the literature allowed to identify 9 publications (Supplementary Table 6) that were read by scientific experts to characterize a putative individual AOP (Figure 4). Among the possible MIE linked to BPF, we identified estrogen-activated pathways (such as estrogen receptor alpha [ER-α] activation), inhibition of catalase and antagonism of PPARγ (ie, one of the proteins found by ORA). To strengthen the links between BPF and PPARγ, and to validate the potential relationship between BPF and cell proliferation, several databases were explored (ToxCast, CompTox, and PubChem bioassays). A human fluorescence assay from the ToxCast database (as of March 2019), revealed BPF binding to PPARγ with an apparent affinity (AC50) of 38.5 μM. Therefore, BPF may modulate PPARγ signaling pathways involved in various biological functions including cell proliferation and differentiation. Among BPA derivatives, bisphenol-A-diglycidyl-ether is a known PPARγ antagonist (Sato *et al.*, 2016). From the PubChem bioactivities database, the 3 BPF compounds were defined as human PPARγ antagonists (BioAssay 743199) (as of March 2019). In the CompTox database, the 3 BPF compounds were associated with cell proliferation (Tox21 cell-based assay on human) below their cytotoxicity limit (AC50 of 18.33 μM for 4,4-BPF, AC50 of 37.35 μM for 2,2-BPF, and AC50 of 16.46 μM for 2,4-BPF). These findings were added in the developed individual AOP (Figure 4).

AOP Network Characterization Related to BPF

As a final step, a putative AOP network was developed using the proposed individual AOP (Figure 4) and additional information from the AOP-wiki database (Figure 5). Based on available information from the AOP-wiki database and comparison with the events in the proposed AOP (Figure 4), 2 MIEs, 4 KEs, and 4 AOs were retrieved. These links are very coherent; for example, among the AO, endometrial, and breast cancers were also retrieved among the 5 identified pathways (endocrine-related cancers) using the KEGG database (Table 1). Promotion (proliferation of a tumoral clone) of endometrial and breast cancer cells has also been associated with the specific activation of ER-α (MIE1181/KE1065 and one of the MIE of the AOP in Figure 4) and with KEs that are indirectly linked to cell proliferation (KE870: progression of the G1 phase, increased cell proliferation; KE1182: increase, cell proliferation, epithelial cell; and KE1189: increase, proliferation, endothelial cells) through at least 2 signaling pathways (the transcriptional activation of cyclin D1 by ER-α (Sutherland *et al.*, 1998) and the non-genomic triggering of MAP kinases (Klinge *et al.*, 2005). Progression through the G1 checkpoint, allows the tumor cell to replicate its DNA before mitosis (also linked to AOP136, AO872). In addition to proliferation, inhibition of control processes was also retrieved, in particular decreased apoptosis (KE1183) which contributes to tumor survival, is a hallmark of many types of cancers (including breast cancer, AOP200, AO1193) and is nowadays targeted in chemotherapy (Kisková *et al.*, 2019; Ko *et al.*, 2019).

DISCUSSION

Different approaches have been taken in the past to connect chemical substances to biological events that may be involved in AOPs. In some cases, literature search was carried out and curated manually (Bajard *et al.*, 2019). In other cases, systems toxicology approaches were used, for example by linking a substance or chemical mixture to proteins, then to protein complexes by including PPIs (Audouze and Grandjean, 2011; Audouze *et al.*, 2018; Kongsbak *et al.*, 2014). Recently, we have developed a strategy starting from a novel hybrid tool to identify linkage of a substance to AOP events through an AI text mining and scoring approach of published abstracts that we called AOP-helpFinder; This tool together with systems biology allowed us to link BPS to adipogenesis and obesity (Carvaillo *et al.*, 2019). In this study, we have attempted to combine the different strategies described above to characterize AOPs which could be linked to BPF isomers. Indeed, biological enrichment of a protein complex (using PPIs) associated with the BPF isomers, literature mining (with AOP-helpFinder and manual curation) and data integration from various sources, allowed to improve our assessment of biological pathways and biological events

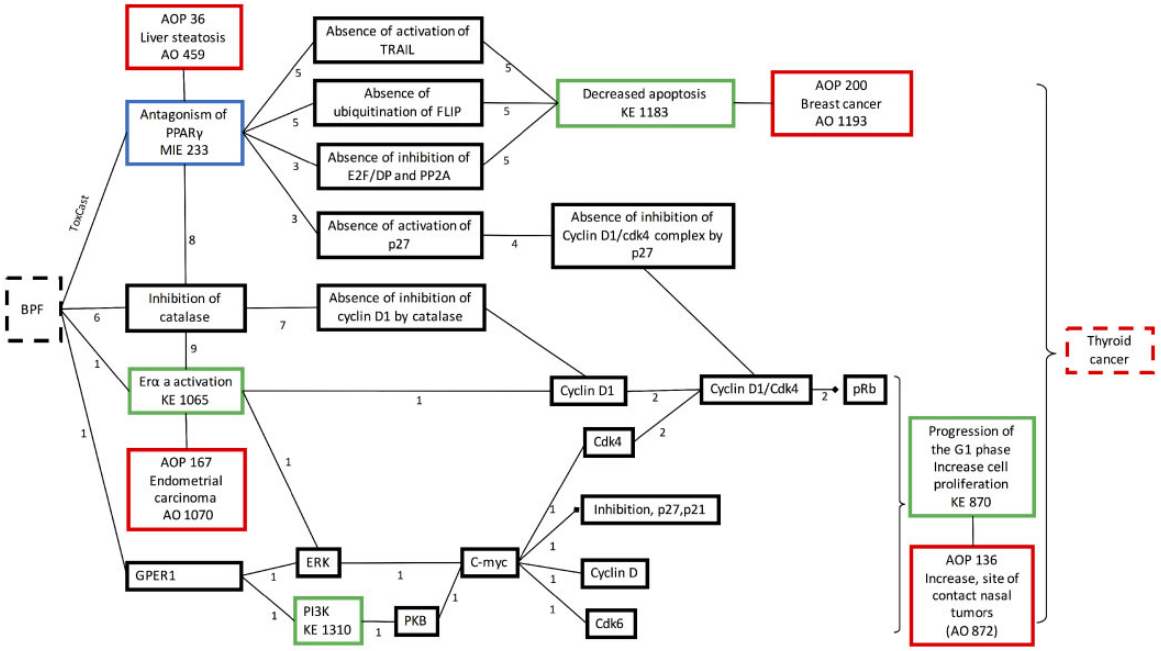

Figure 5. Representation of the adverse outcome pathway (AOP) network involving BPF. The primary AOP linked to thyroid cancer (dashed line) developed in Fig. 4 was enriched by querying the AOP-wiki database. Various events (MIEs, KEs and AOPs) were then added.

connected to BPF. By using such a higher-level combination of methods, we were not only able to link BPF to an individual AOP but we were also able to associate these isomers to a complex AOP network. These networks are particularly relevant to represent multiple outcomes of a substance and also of a mixture of substances. By combining different modules of text mining/scoring and of systems biology approaches, we provide a strategy to accelerate connection of a substance to AOs and identify potential critical steps of its putative mode of action.

One advantage of such approaches is the ability to gather disparate types of information (chemical-protein associations, PPIs, protein-signaling pathway annotations) from various sources (literatures, databases), and to integrate them with the aim to identify previously uncharacterized links (Audouze and Grandjean, 2011). The development of such models is now feasible due to recent advances in both experimental (via high-throughput technologies) and computational areas, eg, though advanced developed methods to identify association between chemicals and health disorders (Audouze *et al.*, 2010). A recent study showed that AOP networks allowed to develop new AOPs, reflecting the power of generating AOP networks to better understand mechanistic pathways (Knapen *et al.*, 2018). Adverse outcome pathway networks can also be used for assay development and refinement, as shown in a study that created an AOP network for reproductive and developmental toxicity in fish, based on 5 relevant existing AOPs (Knapen *et al.*, 2015). A recently developed stressor-AOP network webserver, integrating information from the ToxCast and AOP-wiki databases, revealed that many chemical stressors can putatively interfere with 1 or several AOPs (Aguayo-Orozco *et al.*, 2019). Due to the rapidly expanding available toxicology data (including omics), data-driven and computer-based tools are now believed to be a realistic option for the development of nonanimal-based hazard and risk assessment. Pipelines for generating and enriching AOP descriptions including literature mining and integration of diverse data sources have been proposed recently (Carvaillo *et al.*, 2019; Nymark *et al.*, 2018).

Such integrative approach is essentially qualitative, and will be improved in the future to generate more quantitative models, that will take into consideration dose and time effects. A next step would be to include, for example, the dose-dependent activation of MIEs or detailed pathways of toxicity. Another aspect will be to establish quantitative AOP networks. A recent study examined how AOPs can be used to develop computational pathway-based quantitative models that will be useful for regulatory chemical safety assessment (Perkins *et al.*, 2019). All such improvements could be envisaged by considering other data sources (eg, joint pathways analysis from cross-omics studies and databases such as Effectopedia and the human toxome knowledgebase).

Compared with BPA, the effects of BPF on human health are poorly characterized. Most studies focused on its physiological and endocrine activities. Few models have been used to characterize such effects in humans; for example, a fetal testis assay developed in 3 different species (mouse, rat, human) showed that nanomolar concentrations of BPA, BPS, and BPF are able to reduce basal testosterone secretion in the human *ex vivo* model (Eladak *et al.*, 2015). This *ex vivo* study suggested that as for BPA, BPF acts as an endocrine disruptor in humans. Such results are supported by *in vitro* studies using human cell lines which indicate estrogenic and antiandrogenic effects of BPF (Cabaton *et al.*, 2009; Molina-Molina *et al.*, 2013; Satoh *et al.*, 2004). In addition to such endocrine-disrupting effects, our approach has suggested a link between BPF and cell proliferation. The putative effect on thyroid cancer appeared to be the most significant 1 and this was highlighted in the AOP model that is presented. Relevant initiating events were identified including inhibition of PPARγ and activation of ER-α. Overall, these results could be used as the basis for further epidemiological and experimental studies, thus providing additional evidence for causal links between BPF

and tumor development. However, in addition to the potential influence of BPF on tumorigenesis, the linkage of 1 MIE (antagonism of PPARγ) with liver steatosis (AOP36, A0459) is coherent with one of our recent findings suggesting that bisphenols could be associated with obesity and metabolic disruptions (Carvaillo *et al.*, 2019). Decreased PPARγ activity leads to decreased expression of Hydroxysteroid 17-Beta Dehydrogenase 10 (or 3-hydroxyacyl-CoA dehydrogenase type-2) (KER260), leading to impaired ß-oxidation and mitochondrial dysfunction (Yang *et al.*, 2011), and therefore to lipid accumulation. Interestingly, a direct relationship between obesity and thyroid diseases has been suggested (Santini *et al.*, 2014).

New and innovative computational strategies are needed to help in the identification of health effects of chemical substituents and mixture, and to link them to AOPs that could be used for regulatory risk assessment. We believe that the development of systems toxicology approaches that relies on existing data, as the one proposed here, is extremely relevant in the area of IATA that contribute to the reduction of animal testing. These approaches also contribute to associate exposure to chemicals of concern with actual hazards and health outcomes.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

DECLARATION OF CONFLICTING INTERESTS

The author/authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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AUTHOR CONTRIBUTIONS

M.R. performed the text mining experiments. J.C.C. developed the AOP-helpFinder tool. M.R., X.C., and K.A. developed the AOP and AOP network. K.A. designed the study and performed the integrative systems biology experiments. All authors have contributed in discussing the study results and writing the manuscript.

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