In silico prediction of potential mixture toxicity mechanisms underlying endocrine disorders as a result of e-waste recycling activities: Exposure to organophosphate flame retardants and toxic metal(oid)s

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Introduction

E-waste or Waste from Electrical and Electronic Equipment (WEEE) represents one of the world's rapidly growing waste streams. In developed countries, it typically constitutes approximately 1% of the total solid waste (UNEP, 2007). E-waste dismantling activities can release various hazardous compounds such as persistent organic pollutants (POPs) or toxic metal(oid)s, to which workers or residents living near e-waste recycling facilities could be constantly exposed. Due to the ban of commonly used flame retardants (such as polybrominated diphenyl ethers (PBDEs)) by the Stockholm Convention on POPs, the use of their alternatives is in rise. In recent years, organophosphate flame retardants (OPFRs) have found their application in different industries, including but not limited to plastics, electronics, construction, furniture and textiles (Balasch et al., 2022). Both OPFRs and toxic metal(oid)s are known to be endocrine disruptors (EDs), exerting their adverse effects by interfering with hormonal activity. Given that these substances are simultaneously present in the living environment, additional investigation is needed to assess human exposure to their mixture (Bajard et al., 2021). Thus, the current *in silico* toxicogenomic analysis aims to: (i) demonstrate the linkage between the exposure to toxic fumes from e-waste recycling activities consisting of environmentally relevant toxic mixture (OPFRs and toxic metal(oid)s), and endocrine system diseases (ESD); and (ii) predict the possible molecular mechanisms underlying endocrine toxicity of the specific mixture.

Material and methods

In this study, for *in silico* data mining and gene ontology analysis, the Comparative Toxicogenomic Database (http://CTD.mdibl.org), GeneMANIA prediction server (https://genemania.org) and ToppGene suit portal (https://toppgene.cchmc.org/) were used. Also, to obtain functional information about the predicted genes, GeneCards database (https://www.genecards.org/) was searched. Among the organic compounds, triphenyl phosphate (TPP) and tris(1,3-dichloro-2-propyl)phosphate (TDCPP) were chosen, as one of the most commonly found OPFRs contaminants near e-waste recycling areas. Along with four toxic metal(oid)s (lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg)), TPP and TDCPP were analyzed using various CTD tools in order to determine the gene set common to all six chemicals. Afterward, the Set Analyzer tool was used to retrieve the genes mutual to environmentally relevant toxic mixture and development of ESD. Furthermore, in order to obtain the exact manner of interaction between each of the investigated chemicals and genes annotated to endocrine pathology, the CTD data cards were manually explored. A network of genes connected to endocrine diseases development affected by the exposure to our mixture of OPFRs and toxic metal(oid) was generated by the GeneMANIA online server (https://genemania.org), while gene ontology (GO) enrichment analysis was performed by using ToppGene Suite's Topp Fun tool (https://toppgene.cchmc.org/enrichment.jsp). The list of top 5 endocrine-related biological processes, molecular functions and molecular pathways affected by the investigated toxic mixture was obtained. Finally, to elucidate the endocrine-specific toxicity mechanisms and gain a deeper insight into the endocrine pathology caused by the exposure to the investigated toxic substances, chemical-phenotype analysis was performed through the anatomy section in CTD. All results reported here were based on the data downloaded in December 2023.

Results and discussion

Data obtained from CTD analysis indicated that all six substances present in the investigated mixture (TPP, TDCPP, Pb, Cd, As, Hg) act on 24 mutual genes. Moreover, we aimed to determine the gene set enriched to endocrine diseases development. According to our research, investigated toxic chemicals interacted with 15 genes related to endocrine diseases, as follows: BAX, BCL2, CASP3, CAT, CYP1A1, FAS, GSR, GSTT1,

HMOX1, IGF1, IL6, MT2A, NQO1, RELA, SOD1. Based on their primary functions, these genes can be categorized as follows: apoptosis regulation (BAX, BCL2, CASP3, and FAS); antioxidant defense and detoxification (CAT, GSR, GSTT1, HMOX1, NQO1, MT2A and SOD1); metabolism (CYP1A1); roles in growth and inflammation: (IGF1 and IL6); inflammatory responses (RELA). 49.48% of the interactions among these genes potentially affected by the investigated mixture, along with 20 related genes, were predicted by the server, 28.21% were in co-expression, 9.28% were in physical interaction, 8.22% belonged to the same pathway, 3.31% were in co-localization and 1.49% were with shared protein domains. From the provided gene ontology (molecular functions and biological processes) and pathway terms related to endocrine disorders and the investigated mixture of toxic substances, oxidative stress, apoptosis, and inflammation were found to be the most prominent and interconnected mechanisms. Oxidative stress, characterized by functions like antioxidant activities and oxidoreductase activities, is intricately linked to both apoptosis, highlighted by functions such as BH3 domain binding and death domain binding, and inflammation, as evidenced by pathways like IL18 signaling. This intertwined relationship suggests that disturbances in oxidative equilibrium can initiate a chain reaction, culminating in cellular damage, programmed cell death, and ensuing inflammatory cascades (Ojo et al., 2023). This also aligns with existing literature confirming the link between oxidative stress and possible adverse effects on endocrine tissues (Mancini & Silvestrini, 2022). Finally, to gain a more profound understanding of the toxicity mechanisms underlying endocrine disorders, we specifically examined phenotypes connected with oxidative stress response, recognizing its importance as a pivotal pathway in endocrine disease development, with the capability to induce apoptosis and incite inflammatory cascades. Retrieved results suggested the possible development of oxidative stress in endocrine tissues after exposure to investigated toxic substances, with the most outstanding effects on male and female reproductive tissue and thyroid gland.

Conclusion

Considering all obtained results, it is clear that exposure to a mixture of OPFRs and toxic metal(oid)s could be linked to ESD development through interconnected mechanisms of oxidative stress, apoptosis, and inflammation. Furthermore, identified genes, linked to environmentally relevant toxic mixtures and ESD, align with distinct primary functions: apoptosis regulation, antioxidant defense and detoxification, metabolism, roles in growth and inflammation, and inflammatory responses. These genes hold promise as potential genomic biomarkers for evaluating the toxic impact of mixtures on the endocrine system. Moreover, following exposure to our investigated toxic substances, our phenotype analysis suggested the most pronounced effects on the reproductive tissues in both male and female, as well as on the thyroid. Considering all of this, the current research has proven to be valuable for enhancing the comprehension of molecular mechanisms associated with exposure to a specific mixture of environmental chemicals and direct further toxicological investigation.

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