Elucidating the toxicity of the flame retardant tris (2-butoxyethyl) phosphate (TBEP) by transcriptomics

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1. Introduction

Tris (2-butoxyethyl) phosphate (TBEP) is a compound produced at high volume that is used as both a flame retardant and a plasticizer. It is used as an additive in materials and as such, has been shown to migrate out of these materials and contaminate immediate environments. It is continuously detected as the most abundant flame retardant in house dust[1], while also being detected as far as the Arctic highlighting its persistence[2]. Worryingly, they have been detected in human serum[3], urine[4], mothers' milk[5], and placenta[6]. TBEP could therefore pose a risk to human health. Toxicological data are largely limited to model laboratory animals, with data for human models being scant and non-comprehensive. Given that the liver is a major target organ for TBEP exposure[7], we used an RNA sequencing approach to explore the toxic effects in the human liver hepatocellular carcinoma cell line, HepG2 at the molecular level. Such insight may aid risk assessment and prioritising chemicals for further study, while elucidating potential toxicological modes of action in human models.

2. Materials and methods

Non-cytotoxic concentrations were established using the resazurin cell viability assay. HepG2 cells were treated with 0.1% DMSO (control), 25 µM (low dose), and 125 µM (high dose) TBEP for 72 hrs, in triplicate and extracted RNA was sequenced using the Illumina Hiseq 1500 platform. Mapping, annotation, and counting was done using CLC Genomics Workbench. Gene set enrichment analysis was performed using the KEGG database to identify affected pathways while over-representation analysis of differentially expressed genes (DEGs) revealed enriched GO terms for effected biological processes.

3. Results and discussion

Cytotoxic range finding revealed that TBEP resulted in an increase in cellular proliferation at lower concentrations, with a sudden decrease in cell viability at 1000 µM (data not shown). Genes encoding for the KEGG pathways ribosome, oxidative phosphorylation, TCA cycle, pyrimidine metabolism, and DNA replication were found to be upregulated in response to 25 µM and 125 µM TBEP treatments (Fig. 1A). No pathways were found to be downregulated. This correlated well with over-representation analysis of DEGs (FDR<0.05) which found GO terms representative of translation and oxidative phosphorylation processes to be effected for both high- and low-dose TBEP treatments (data not shown), indicating that the increase in proliferation seen at these concentrations could be a result of increased protein translation, DNA replication, and energy capacity.

While these affected pathways are rather general and basic, they are responsible for the most vital biological processes and are therefore tightly regulated. Upregulation of such fundamental pathways have been shown to play a role a wide variety of adverse outcomes including diabetes[8], cell aging[9], and cancer[10]. While it was found that many pathways were altered in a number of cancers[10], it was specifically upregulation to protein turnover and oxidative phosphorylation that was linked to the development of leukemia in haematopoietic stem cells[11].

To identify which biological processes were most affected by the most up- or down-regulated DEGs (FDR>0.05, fold-change>2), over-representation analysis of these DEGs revealed that TBEP affected a wide variety of biological processes, most notably, GO terms representative of wound healing. Given that wound healing is a complex process involving co-ordination between haemostasis, inflammation, proliferation, and tissue remodelling, it is unsurprising that treatment with TBEP also resulted in enrichments of GO terms belonging to these biological processes. Of interest however, TBEP was found to affect steroid hormone

biosynthesis, implying endocrine disruption potential of TBEP on sex hormones by influencing sex hormone metabolism (Fig. 1B), in agreement with other studies[12].

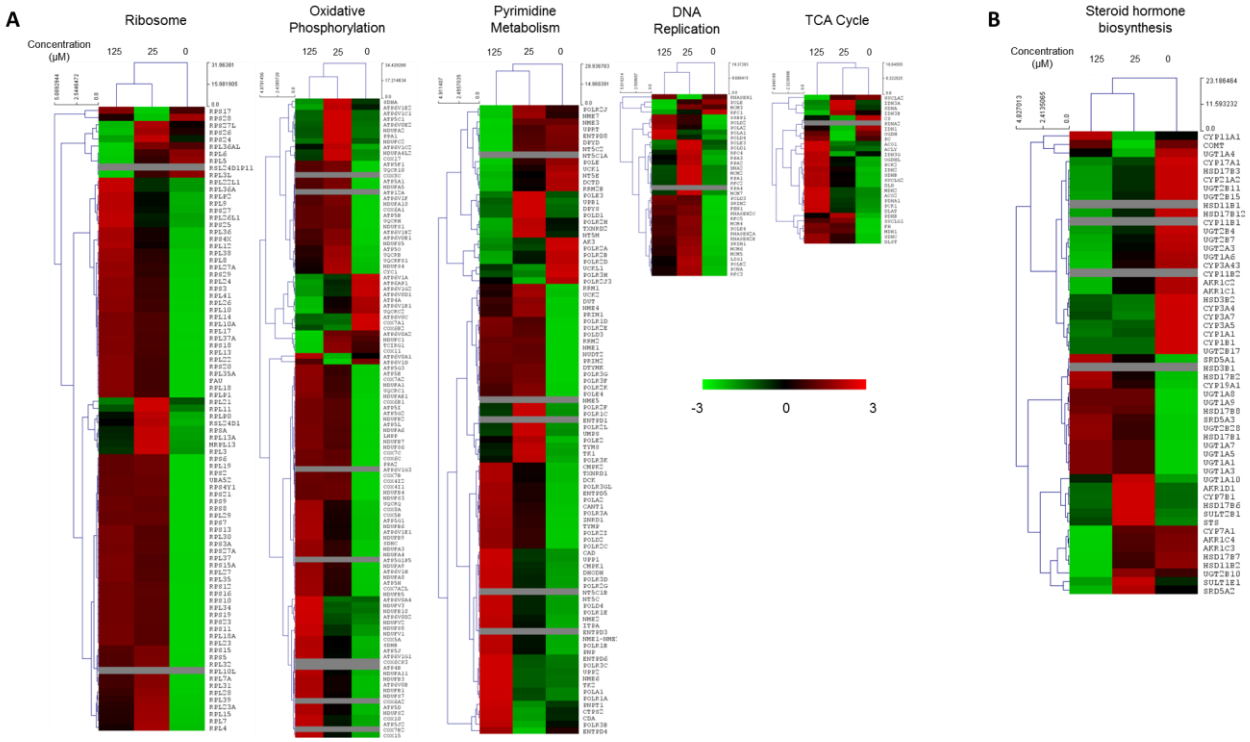

Figure 1: Heatmaps of KEGG pathways affected by TBEP. (A) Pathways found to be altered using gene set enrichment analysis. (B) Pathways of interest found to be altered using over-representation analysis

4. Conclusions

TBEP showed limited cytotoxicity, with cell proliferation increasing at lower concentrations. Results from over-representation and gene-set enrichment analysis correlated well and revealed upregulation of protein, DNA, and energy metabolism. These changes were accompanied by enrichment of GO terms for a wide variety of biological processes, but specifically for wound healing, regulation of immune function, and steroid hormone biosynthesis, which is in agreement with other studies.

5. References

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