Toxicology Reports 10 (2023) 348–356

Contents lists available at ScienceDirect

Toxicology Reports

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

Bisphenols, but not phthalate esters, modulate gene expression in activated human MAIT cells *in vitro*

J.L. Krause [a,1,2], A. Pierzchalski [a,1], H.D. Chang [b,2], A.C. Zenclussen [a], M. Bauer [a], G. Herberth [a,*]

[a] Helmholtz-Centre for Environmental Research - UFZ, Department of Environmental Immunology, Leipzig, Germany
[b] Institute for Biotechnology, Technische Universität Berlin, Germany

ARTICLE INFO

Handling Editor: Dr. L.H. Lash

Keywords:
Bisphenols
Plasticizers
In vitro model
Phthalate ester
MAIT cells
Immunomodulation

ABSTRACT

One route of human exposure to environmental chemicals is oral uptake. This is primarily true for chemicals that may leach from food packaging materials, such as bisphenols and phthalate esters. Upon ingestion, these compounds are transported along the intestinal tract, from where they can be taken up into the blood stream or distributed to mucosal sites. At mucosal sites, mucosal immune cells and in the blood stream peripheral immune cells may be exposed to these chemicals potentially modulating immune cell functions. In the present study, we investigated the impact of three common bisphenols and two phthalate esters on mucosal-associated invariant T (MAIT) cells *in vitro,* a frequent immune cell type in the intestinal mucosae and peripheral blood of humans. All compounds were non-cytotoxic at the chosen concentrations. MAIT cell activation was only slightly affected as seen by flow cytometric analysis. Phthalate esters did not affect MAIT cell gene expression, while bisphenol-exposure induced significant changes. Transcriptional changes occurred in ~ 25 % of genes for BPA, ~ 22 % for BPF and ~ 8 % for BPS. All bisphenols down-modulated expression of *CCND2, CCL20*, *GZMB* and *IRF4,* indicating an effect on MAIT cell effector function. Further, BPA and BPF showed a high overlap in modulated genes involved in cellular stress response, activation signaling and effector function suggesting that BPF may not be safe substitute for BPA.

1. Introduction

A variety of presumed "food-safe" plastics are used for wrapping of food items [1]. To improve plastic performance during the production process common plastic additives, such as bisphenols and phthalate esters (PEs) are utilized [2]. These substances can leach from the plastic material used for food packaging into our food [3]. Due to the low toxicity of bisphenol A (BPA) and PEs in historical risk assessment studies, these compounds were considered innocuous to human health [4]. Today, these compounds have been ubiquitously detected in the environment [5,6] and in human body fluids such as blood, plasma, urine and breast milk [7–9]. Within the National Health and Nutrition Examination Survey (NHANES) among US children and adults in 2013/2014, on average 1.25 μg/L BPA, 0.33 μg/L BPF and 0.34 μg/L BPS have been measured in urine [8]. A recent study used urinary bisphenol concentrations to estimate human daily intakes across the globe. The authors show that on average 2.53 μg BPA, 0.68 μg BPF and 0.60 μg BPS may be taken up per person and day [10]. Similarly, Di (2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DINP) metabolites have frequently been detected in human urine in Germany and the US [11]. The ubiquitous occurrence and the later identification of DEHP, DINP and BPA as endocrine disruptors [12–14] resulted in a more restrictive regulation. More than 90 % of BPA detected in human urine were estimated to derive from oral exposure [15]. Thus, in 2014 the

Abbreviations: BPA, bisphenol A; bpc, bacteria per cell; BPF, bisphenol F; BPS, bisphenol S; bw, body weight; CD, cluster of differentiation; DEHP, di(2-ethylhexyl) phthalate; DINP, diisononyl phthalate; DMSO, dimethyl sulfoxide; EFSA, European Food Safety Agency; EU, European Union; FCS, fetal calf serum; IFNg, interferon gamma; IMDM, Iscove Modified Dulbecco Medium; MAIT cells, mucosal-associated invariant T cells; MeOH, methanol; NHANES, National Health and Nutrition Examination Survey; PBMC, peripheral blood mononuclear cell; PE, phthalate ester; RT, room temperature; SVHC, substance of very high concern; TDI, tolerable daily intake; TNF, tumor necrosis factor.

* Correspondence to: Permoser Straße 15, 04318 Leipzig, Germany.
E-mail address: gunda.herberth@ufz.de (G. Herberth).

[1] These authors contributed equally.

[2] Present address: German Rheumatism Research Center Berlin, a Leibniz Institute – DRFZ, Schwiete laboratory for microbiota and inflammation, Berlin, Germany.

https://doi.org/10.1016/j.toxrep.2023.02.017
Received 1 November 2022; Received in revised form 15 February 2023; Accepted 28 February 2023
Available online 1 March 2023

European Food Safety Agency (EFSA) lowered the tolerable daily intake (TDI) from 50 μg/kg bodyweight (bw) to 4 μg/kg bw. Further, BPA was listed as substance of very high concern (SVHC, ED/30/2017) in 2017 within the European Union (EU). However, BPA-substitutes, like bisphenol F (BPF) and bisphenol S (BPS) are increasingly used to replace BPA [16,17], despite the structural similarity and potentially similar health risk among bisphenol compounds [18,19]. Along that line, DEHP exposure occurs *via* the oral route after ingestion of contaminated food stuff [20], slowly leaching from the material as DEHP is not bound to the plastic [21]. DEHP migration from the packaging material into the food stuff has been observed in > 80 % of cases [22]. DEHP was the most common PE, now showing a decline in usage due to rising concerns on health risk. It has been banned from product groups such as children articles and toys concentrations below 0.1 % within the EU in 2004 [23] and in 2008 within the US [20]. In 2011, DEHP has been listed as substance of very high concern and since 2021, DEHP is restricted within the EU due to its endocrine disrupting and reproductive toxic properties [24]. In parallel, an increase in DINP metabolites have been observed in human samples [25], indicating changes in production, plastic usage [25,26] or the replacement of DEHP by DINP [27]. Within the EU, DINP is not listed as substance of very high concern and is thought to be non-influential on reproduction [28]. However, regarding health outcomes, it has been demonstrated in human cohorts that BPA and various PEs associate with chronic inflammatory diseases and have immunomodulatory properties [12,29,30].

After ingestion, additives from food packaging material enter the human body [15]. From the intestinal lumen these plastic additives are absorbed [31,32] thereby potentially exposing mucosal and peripheral immune cells. Mucosa-associated invariant T (MAIT) cells are an abundant immune cell population primarily detected in the mucosae, *e.g.* of the intestine [33] and in the peripheral blood [34]. The localization of MAIT cells at barrier sites, together with their ability to recognize and respond to microbial metabolites, suggests a key role in host-microbiota-immune homeostasis [35]. It is supposed that these cells contribute to the first line of defense against invading microorganisms [36–38], but their role in immunity is not fully elucidated [39]. MAIT cells exert protective functions during homeostasis at the barrier sites of the human body suggested by the finding that lacking of MAIT cells in mice led to a reduction in intestinal epithelial integrity [40]. In skin, the activation of MAIT cells supports wound healing, as recently demonstrated [41]. Thus, both the chronic activation and inhibition of MAIT cells induced by external factors, such as exposure to environmental chemicals may have detrimental health effects by fueling inflammation *via* the secretion of pro-inflammatory cytokines or by losing the protective functionality of MAIT cells during homeostasis. In a previous *in vitro* study, we showed that bisphenols did not modulate MAIT cell activation *via* the bisphenol-exposed intestinal microbiota but impaired MAIT cell activation directly [42]. We showed that acute exposure to bisphenols at high concentrations of 354 μg/mL was cytotoxic to T cells, including MAIT cells, and impaired MAIT cell activation. There, BPA and BPF had similar detrimental effects and BPS was less harmful. In the present study, we investigated the immunomodulatory effects of bisphenols and PEs at gene expression level of MAIT cells.

2. Materials and methods

2.1. Purification of peripheral blood mononuclear cells (PBMCs)

Buffy coat of five male healthy donors were obtained from the blood donation service at the University Hospital Leipzig, Germany. The study was approved by the Ethics Committees of the University of Leipzig (079-15-09032015). Blood was mixed with the double volume of sterile PBS and layered on Ficoll-paque plus (GE Healthcare, Chicago, US). We purified PBMCs by gradient centrifugation at 400 × *g*, 30 min at 4 °C (swing bucket centrifuge, no brake). PBMCs from the interphase were collected and gradually frozen in FCS with 10 % DMSO at − 80 °C using Mr Frosty™ Freezing containers (Thermo Fisher Scientific, Waltham, US). Cells were stored at − 150 °C until use.

2.2. Calculation of chemical concentrations

We investigated the immunomodulatory effects of BPA (CAS: 80-05-7), BPF (CAS: 620-92-8) and BPS (CAS: ↱80-09-1), as well as the PEs DEHP (CAS: 117-81-7) and DINP (CAS: 20548-62-3). Since the TDI of BPA was lowered from 50 μg/kg body weight (bw) to 4 μg/kg bw, we exposed PBMCs to these two concentrations [15]. For BPA and BPF a lower concentration of 0.4 μg/kg bw was used. For DEHP the actual TDI of 50 μg/kg bw and a 12.5 × higher concentration were used. For DINP, we applied 150 μg/kg bw resembling the current TDI and a 12.5 × higher concentration. We calculated chemical exposure for an average male European with 70 kg bw deduced from chemical approval in the EU as described in a previous study. We presumed full absorption of bisphenols and PEs from the intestine to calculate the final exposure concentrations, since > 95 % BPA [32] as well as DINP and DEHP are almost completely absorbed in the intestine [31,43]. Compounds were added at a final concentration listed in Table 1.

2.3. Stimulation and exposure of MAIT cells

Immune cells (PBMCs) were stimulated with *E. coli* for the activation of MAIT cells and treated with the chemicals to determine modulatory effects on cell viability, MAIT cell activation and gene expression in sorted MAIT cells.

2.3.1. Preparation of E. coli for immune cell stimulation

As described previously, *Escherichia (E.) coli* K-12 (MG1655) was cultured in Brain-Heart Infusion liquid medium (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) for 24 h at 200 rpm to stationary growth phase [44]. Cells were harvested (5000 x *g*, 4 °C, 10 min) and fixed for 5 min in 1 % methanol-free formaldehyde (Pierce™, Thermo Fisher, Waltham, USA) in phosphate buffered saline (PBS). After washing with PBS, the cell number was determined (Multisizer 3, Beckman Coulter, Brea, USA) and adjusted in Iscove Modified Dulbecco Medium (IMDM) + 10 % FCS + 1 × Penicillin/Streptomycin (all Gibco, Thermo Fisher, Waltham, USA). Cell number adjusted *E. coli* were frozen as pellets with supernatant and stored at − 80 °C.

2.3.2. Immune cell stimulation assay

We thawed PBMCs at 37 °C and removed residual DMSO by washing in IMDM medium. 10^6/100 μL of live PBMCs in IMDM (Trypan blue staining) were seeded into 96-well plates and allowed to rest over night at 37 °C and 5 % CO_2. We stimulated PBMCs with 10 bacteria per cell (bpc) of *E. coli* base-line stimulation of MAIT cells. Simultaneously, we added chemicals at the final concentrations listed in Table 1 in a total

Table 1
Overview on utilized solvents and final concentrations of BPA, BPF, BPS, DEHP and DINP. The concentration referring to the actual TDI of BPA, DEHP and DINP within the EU is written in bold.

Compound	Solvent		Actual TDI	
		0.4 μg/kg bw	**4 μg/kg bw**	50 μg/kg bw
BPA	DMSO	0.992 μM	**9.92 μM**	124.04 μM
BPF	DMSO	0.905 μM	**9.05 μM**	113.15 μM
BPS	DMSO	1.131 μM	**11.31 μM**	141.42 μM
μg/mL	0.5 %	0.2	**2.3**	28.3
			50 μg/kg bw	650 μg/kg bw
DEHP	DMSO		**72.50 μM**	906.30 μM
μg/mL	0.5 %		**28.3**	354.0
			150 μg/kg bw	1850 μg/kg bw
DINP	MeOH		**202.93 μM**	2536.65 μM
μg/mL	0.5 %		**85.0**	1061.9

Throughout the study, we included solvent controls to assess the effect of 0.5 % DMSO or 0.5 % MeOH and to determine the effects of bisphenol- or PE-exposure.

volume of 200 µL with 0.5 % DMSO or MeOH. PBMCs were exposed for 6 h for cell sorting and transcript analysis and for 24 h for cytometric analysis. Further, we included (i) an unstimulated control (IMDM only) and (ii) 0.5 % solvent controls. For MAIT cell gene expression profiling, we included a control using anti-CD3/CD28 beads for T cell receptor-mediated activation. After 7 h (cytometric analysis), Brefeldin A was added to capture cytokines inside the cells for intracellular staining. After a total of 6 h (cell sorting and gene expression) or 24 h (cytometric analysis) of stimulation, PMBCs were harvested and stained for multi-parameter flow cytometry.

2.3.3. Flow cytometric analysis of MAIT cell activation

PBMCs were washed in 1 % FCS in PBS. We stained extracellular epitopes with antibodies against CD3, CD8a, CD161, Vα7.2 and CD69. Cells were fixed in BD Lysing solution (BD Biosciences) for 10 min at RT and permeabilized using BD Perm2 solution (BD Biosciences) for 10 min at RT. Then PBMCs were washed and intracellularly stained with antibodies against tumor necrosis factor (TNF), interferon gamma (IFNg) for flow cytometric identification of MAIT cells and MAIT cell activation analysis (Supplementary Tab. S1). Dead cells were discriminated by Fixable Viability Dye eFluor™ 506 staining. Antibodies/stains were obtained from Biolegend and eBioscience (Supplemental Tab. S1). Cytometric acquisition was performed at FACS Canto II (Becton Dickinson and Company, Franklin Lakes, USA). Data analysis was performed with FlowJo® v10 software (Becton Dickinson and Company, Franklin Lakes, USA).

2.4. MAIT cell viability analysis

We assessed MAIT cell viability using the CCK-8 viability kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Therefore, we seeded 2.5×10^4 PBMCs/well in 96well plates. After resting overnight, we exposed the PBMCs for a total of 24 h at the concentrations given in Table 1. After 22 h, 10 µL CCK-8 reagent were added followed by another 2 h of incubation. The absorbance was measured at 450 nm at an Infinite 200 microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

2.5. MAIT cell sorting, RNA extraction and gene expression profiling

2.5.1. Sorting of MAIT cells for gene expression profiling

Stimulation of PBMCs (density 1×10^6 cells) and exposure to BPA, BPF, BPS, DEHP and DINP was performed as described above. Cells were washed once in 200 µL PBS and stained for life/dead discrimination with FV520 for 20 min in the dark. After washing in FACS-WB (1 % FCS in PBS), PBMCs were stained with CD8a, CD161 and TCRVa7.2 for MAIT cell sorting (Supplemental Tab. S2). The gating strategy as well as MAIT cells purity for the PBMCs used are shown in Supplemental Fig. S1. The sorting purity of the cells was 96–98 %. Cells were filtered on 30 µm mesh filter (Miltenyi Biotec, Bergisch-Gladbach, Germany). Approximately 10,000 MAIT cells were sorted at 4 °C using a Bio-Rad S3e cell sorter (BioRad, Hercules, CA, USA) directly into 500 µL peqGOLD RNAPure (VWR International, Radnor, US) into 5 mL PS tubes, mixed vigorously and frozen at − 80 °C until RNA was extracted.

2.5.2. RNA isolation and gene expression profiling

Total RNA from sorted MAIT cells into peqGOLD RNAPure was extracted according to manufacturer's instruction. The cDNA synthesis was carried out with 200 ng of RNA by using 5 U RevertAid™ H Minus Reverse Transcriptase (Fisher Scientific, Schwerte, Germany). Intron-spanning primers were designed and UPL probes were selected by the Universal Probe Library Assay Design Center (http://qpcr.probefinder.com/organism.jsp). 93 genes of interest (Supplemental Tab. S3) were pre-amplified for 12 cycles and quantitative PCR was performed on a 96 × 96 Dynamic array with BioMark™ HD System (Fluidigm, München, Germany). The cycling program consisted of 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. PCR was performed with FastStart Universal Probe Master Mix (Roche, Mannheim, Germany).

2.6. Data visualization and statistical analyses

Quantitative data were expressed as median ± interquartile range (IQR). Non-metric multi-dimensional scaling (NMDS) dissimilarity analysis were done in R using basic functions and the *vegan* package [45]. In NMDS analysis, differences between groups were calculated by Permanova using the *adonis* function from the *vegan* package in R [46]. Pairwise group comparisons were done with the *pairwise.adonis()* function. Hierarchical clustering and calculation of heatmaps were performed with *heatmap.2()* function in R. Kruskal-Wallis test and Dunn' posthoc test with correction for multiple comparisons by Benjamini-Hochberg was performed with *kruskal_test()* and *dunn_test()* functions, respectively, from the *rstatix* package in R or using GraphPad Prism (v 9.0.1). Plots were generated in GraphPad Prism or using the *ggplot2* package in R [47] with *scico color palettes* for heatmaps [48]. Data visualization in venn diagram was done using *ggplot2* and *ggvenn* package.

3. Results

3.1. Immune cell phenotyping revealed no changes in immune cell numbers, but slight reduction of MAIT cell activation

We assessed cell viability of PBMCs after 24 h of chemical exposure *via* two orthogonal methods, *i.e.* CCK-8 viability assay and cytometric analysis of viable $CD3^+$ lymphocytes (Supplemental Fig. 2a & 2b – cytometric analysis of MAIT cells). Viability of PMBCs after 24 h did neither decrease upon exposure to the three bisphenols BPA, BPF and BPS nor for the PEs DEHP and DINP (Supplemental Fig. S2c and S2d) suggesting non-cytotoxic exposure concentrations. Next, we quantified immune cell numbers within the PBMCs after 24 h exposure to BPA, BPF, BPS, DEHP and DINP to determine whether these compounds affect immune cell populations with different selectivity (Fig. 2a). Neither the number of $CD3^+$, $CD8^-$, $CD8^+$ T cells nor the number of MAIT cells was modified (Fig. 1, Gating procedure: Supplemental Fig. S2a). Similarly, we did not observe a significant modulation of MAIT cell activation quantified by $IFNg^+$ producing, $CD69^+$ expressing MAIT cells and $CD69^+/TNF^+$ MAIT cells (Fig. 1b, Identification: Supplemental Fig. 2b).

Upon exposure to 28.3 µg/mL BPA the percentage of $CD69^+/IFNg^+$ as well as $CD69^+/TNF^+$ MAIT cells and upon exposure to 28.3 µg/mL BPF the percentage of $CD69^+/IFNg^+$ MAIT cells was reduced, but failed to reach statistical significance (Fig. 1b).

3.2. BPA and BPF reduce gene expression of MAIT cells related to activation

We were interested in the molecular mechanisms of bisphenols and Pes-mediated effects on MAIT cells. We selected genes that encoded for enzymes/proteins relevant for activation signaling, cytokine and chemokine signaling, MAIT cell effector function and cell cycle, proliferation and differentiation. In sum, we profiled 93 genes (Supplemental Tab. S3) for differential expression in sorted MAIT cells. First, we investigated potential effects of solvent exposure, *i.e.* DMSO and MeOH, on MAIT cell gene expression (Supplemental Fig. S4). Regarding gene expression in MAIT cell stimulation controls, we showed (i) a high similarity in gene expression of *E. coli*-stimulated MAIT cells independent of solvent exposure, (ii) a clearly distinct gene expression profile of unstimulated MAIT cells and anti-CD3/CD28-stimulated MAIT cells compared to *E. coli*-stimulated MAIT cells and (iii) that anti-CD3/CD28-stimulated MAIT cells clustered more closely to unstimulated MAIT cells than to *E. coli*–stimulated MAIT cells (Supplemental Figs. S3–S5, Tab. S5).

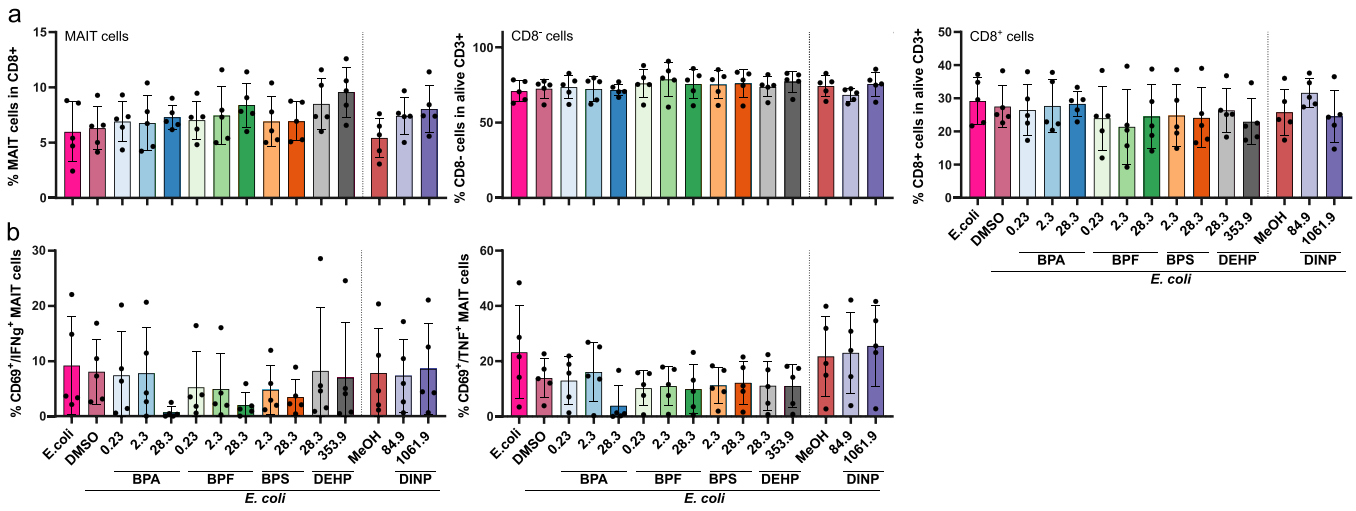

Fig. 1. Immune cell numbers and MAIT cell activation after 24 h. (a) Percentage of MAIT cells, $CD8^-$ T cells and $CD8^+$ T cells as % of parent (n = 5 biological replicates). (b) Percentage of $CD69^+/IFNg^+$ MAIT cells and $CD69^+/TNF^+$ MAIT cells as % of parent (n = 5 biological replicates). Bars represent median ± interquartile range (IQR). Pairwise comparisons were calculated by Kruskal-Wallis and Dunn's posthoc test.

To investigate the molecular effects of BPA, BPF, BPS, DEHP and DINP exposure on MAIT cells, we compared the gene expression after chemical exposure to the corresponding solvent controls, *i.e.* cells treated with 0.5 % DMSO and 0.5 % MeOH only. Using NMDS and pairwise ANOVA, we observed that only exposure to the highest concentration of BPA and BPF, *i.e.* 28.3 μg/mL, induced significant changes (Fig. 2a, Supplemental Tab. S6 and Supplemental Tab. S7).

Accordingly, clustering of mean gene expression profiles per treatment revealed the highest distance to the mean solvent control for 28.3 μg/mL BPA and 28.3 μg/mL BPF (Fig. 2b). Mean gene expression

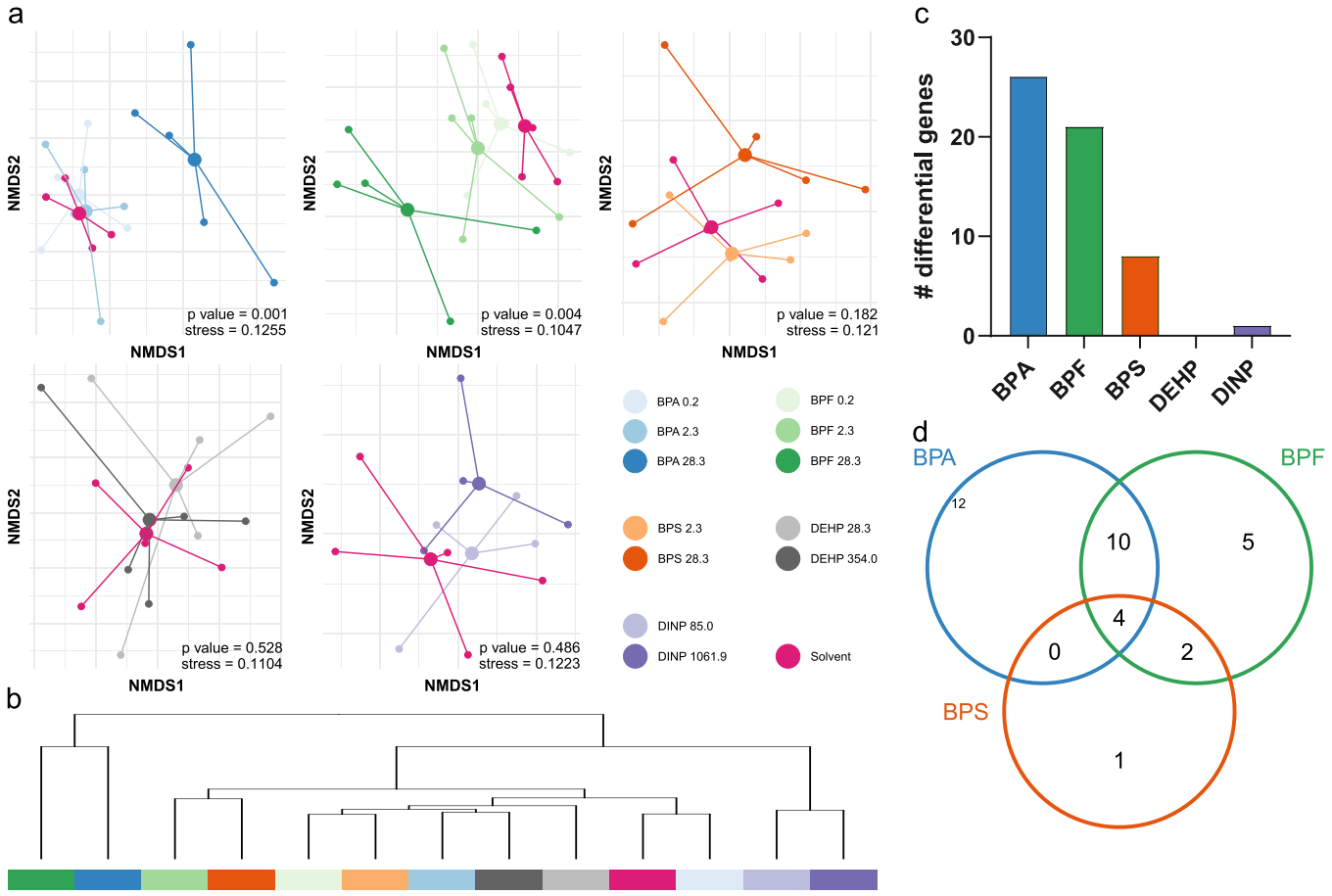

Fig. 2. Gene expression of MAIT cells exposed to BPA, BPF, BPS, DEHP and DINP compared to the mean solvent control (a) Compound-wise comparison of gene expression profiles after 24 h of exposure using NMDS with Bray-Curtis similarity. Significance was assessed using PERMANOVA. (b) Cluster dendrogram derived from hierarchical clustering of mean gene expression profiles per treatment. (c) Number of significantly modulated genes per compound after Kruskal-Wallis and Dunn's posthoc test (d) Venn diagram denoting the number of overlapping and individually modulated genes upon BPS-exposure.

profiles of MAIT cells exposed to 2.3 μg/mL BPS and 2.3 μg/mL BPF as well as exposed to DINP independent of the applied compound concentration, clustered separately, implying similar effects on MAIT cell gene expression per cluster. These findings were also observed when all samples were analyzed individually (Supplementary Fig. S7). To determine the strength of MAIT cell modulatory effects of the chosen compounds in terms of gene expression, we extracted the number of significant differentially expressed genes per compound *versus* the solvent control using Kruskal-Wallis test (Supplemental Fig. S8) with posthoc Dunn's test for pairwise comparison and *P* value adjustment. Data sets with less than three out of five replicate values per treatment were excluded from the analysis (Fig. 2c). As expected, BPA-exposure induced differential expression in the highest number of genes with 23 significant differentially expressed genes. BPF lead to significant changes in gene expression levels of eleven genes, BPS in eight genes, DINP in one gene and DEHP did not significantly impact on MAIT cell gene expression. Since DEHP and DINP only slightly affected MAIT cell gene expression, we then focussed on similarities and differences due to bisphenol-exposure. To understand whether the applied bisphenols modulated the gene expression of MAIT cells in a similar manner. We visualized the overlapping and individual differentially expressed genes in a venn diagram (Fig. 2d) and extracted the gene information (Table 2).

Of those 34 significant differentially expressed genes, four genes where modulated by all bisphenols, *i.e.* granzyme B (*GZMB*), interferon regulatory factor 4 (*IRF4*), CC chemokine ligand 20 (*CCL20*) and cyclin D2 (*CCND2*). Their expression was significantly reduced towards the expression level of unstimulated MAIT cells upon exposure to 28.3 μg/mL BPA, BPF and BPS, respectively, compared to those MAIT cells that were stimulated with *E. coli* in the presence of solvent (Fig. 3).

In addition to these genes, BPA and BPF showed the highest overlap with an additional seven overlapping genes, *i.e.* heat-shock protein 70 A1 (*HSPA1A*), inducible T cell co-stimulator (*ICOS*), interferon γ (*IFNG*), interleukin 4 receptor (*IL4R*), interleukin 17F (*IL17F*), nuclear factor of activated T cells 1 (*NFATC1*) and program cell death protein 1 (*PDCD1*) with a total overlap of 33.3 % shared significant differentially expressed genes (Supplemental Fig. S8, Tab. S8 and Tab. S9). At 28.3 μg/mL BPA and 28.3 μg/mL BPF the expression of all before-mentioned genes, except for *HSPA1A,* which was increased, was significantly reduced compared to the solvent control. Gene expression levels dropped towards the unstimulated control (Supplemental Fig. S8). BPF and BPS also shared two further genes, *i.e.* aquaporin 3 (*AQP3*) and interleukin 21 receptor (*IL21R*) resulting in a total overlap of 18.2 % of all significantly altered genes (Supplemental Fig. S9, Tab. S9 and Tab. S10). *AQP3* was significantly down-modulated by exposure to 28.3 μg/mL BPF and BPS, respectively, whereby only BPF significantly reduced the expression level of *IL21R*. In contrast, BPA and BPS did not share further differentially expressed genes (12.1 % total overlap).

Table 2
Shared and individual differentially expressed genes upon BPX-exposure.

	# Δ Genes	% Δ Genes	Gene abbreviation
Shared	4	12.1	*CCL20, CCND2, GZMB, IRF4*
BPA/ BPF	10	21.2	*HSPA1A, ICOs, IFNG, IL4R, IL17F, MAPK3, NFATC1, PDCD1, TNFSF1B*
BPA/ BPS	0	0	—
BPF/ BPS	2	6.1	*AQP3, IL21R*
BPA	12	42.4	*CCR6, IL2, CLEC7A, FASLG, SELPLG, IL22, IL10RB, PRF1, MAPK14, CTLA4, NFATC2, NFKB1B*
BPF	5	21.2	*CCR7, IL10, SOD2, IL6R, MAP2K2*
BPS	1	3.0	*CXCL10*

4. Discussion

The EFSA stated that immune system safety has to be reviewed for BPA (https://www.efsa.europa.eu/en/press/news/160426, 18.03.22). Thus, the risk of BPA-exposure on humans is currently re-evaluated with a focus on immunomodulation. In the present study, immunomodulatory properties of BPA, BPF and BPS as well as the frequently used PEs, DEHP and DINP, were investigated with a special focus on MAIT cells. These cells are of special interest due to their role in defending against invading microorganisms and mucosal homeostasis [49] and their still unclear involvement in a variety of chronic inflammatory diseases [50–52].

Focussing on the effects of bisphenols and PEs, we evaluated cell viability *via* two orthogonal methods. Both methods proved no cytotoxic effect of bisphenols and PEs on PMBCs and $CD3^+$ lymphocytes at the concentrations applied. With regard to bisphenol cytotoxicity, others have shown that bisphenols can diminish the viability of PBMCs at least at high concentrations, *i.e.* 220 μM BPA and 500 μM BPF [53]. Regarding the toxic effects of DEHP or DINP on PMBCs, data are scarce. Cytotoxicity towards other cell types has been investigated. Wenzel et al. did not observe cytotoxic effects by DEHP or DINP at concentrations of 1 μM compound in rat thyroid follicular cells [54]. Similarly, DEHP was proven non-toxic at concentrations equal to those applied in the present study, while increased concentrations > 12 mM DEHP significantly reduced viability of human bronchial epithelial 16HBE cells [55]. Long-term exposure of murine L929 adipose tissue cells to 0.1 mg/mL DINP and to 0.05 mg/mL DEHP significantly impaired cell viability [56]. In this study, already low concentrations impaired cell viability, potentially due to chronic exposure. The clear difference between acute and chronic toxicity highlights the need to also focus on low-dose chronic exposure in future studies on immunomodulation. This holds true especially for PEs and bisphenols, since exposure to both compound classes is chronic, unavoidable and unintentional, reflected by the high number of humans (> 90 %) showing detectable concentrations of bisphenols and PEs in their urine [13,57].

Regarding immunomodulation, none of the compounds altered immune cell numbers in our study. Nevertheless, the % of activated IFN^+ and TNF^+ MAIT cells was reduced at 28.3 μg/mL BPA and IFN^+ MAIT cell were reduced at 28.3 μg/mL BPF in our study. Similar effects have been observed in our previous study, where 28.3 μg/mL BPA and BPF significantly impaired MAIT cell activation [42]. The difference may derive from different assay settings *i.e.* different sex and age of blood donors and/or stimulation time between the two studies. In part, donor-to-donor variability may mask potential effects of chemicals, *e.g.* due to responding and non-responding donors [58] or simply differences in susceptibility. Regarding PEs, the recently published Norwegian EuroMix biomonitoring study detected DEHP/DINP metabolites in human urine at concentrations as high as 1 μg/mL in some participants [59]. Although exposure concentrations remain unknown, chronic exposure has been assumed [60].

Despite the non-significant effects on MAIT cells, we observed a significant modulation of MAIT cell gene expression upon exposure to BPA, BPF, BPS and DINP. Until today, data on bisphenols considering effects on gene expression are rare, especially with a focus on immune cells. The impact of BPA on the immune system has recently been summarized [61]. *E.g.*, Mesnage et al. [62] demonstrated that BPA and BPF and to a lower extent BPS modulated gene expression in human MCF7 adenocarcinoma cells and activated estrogen receptor, which is also expressed on various immune cells [63]. In line with their findings, BPA and BPF also showed the strongest impact on MAIT cell gene expression. *GZMB*, *IRF4*, *CCL20* and *CCND2* were commonly affected by bisphenol-exposure and dropped towards the gene expression level of the unstimulated control. Interestingly, the expression of *CCL20*, *GZMB* and *IRF4* were only moderately down-modulated, whereas the expression of *CCND2* dropped down to the level observed in the unstimulated control. The *CCND2* gene encodes cyclin D2, an enzyme promoting cell

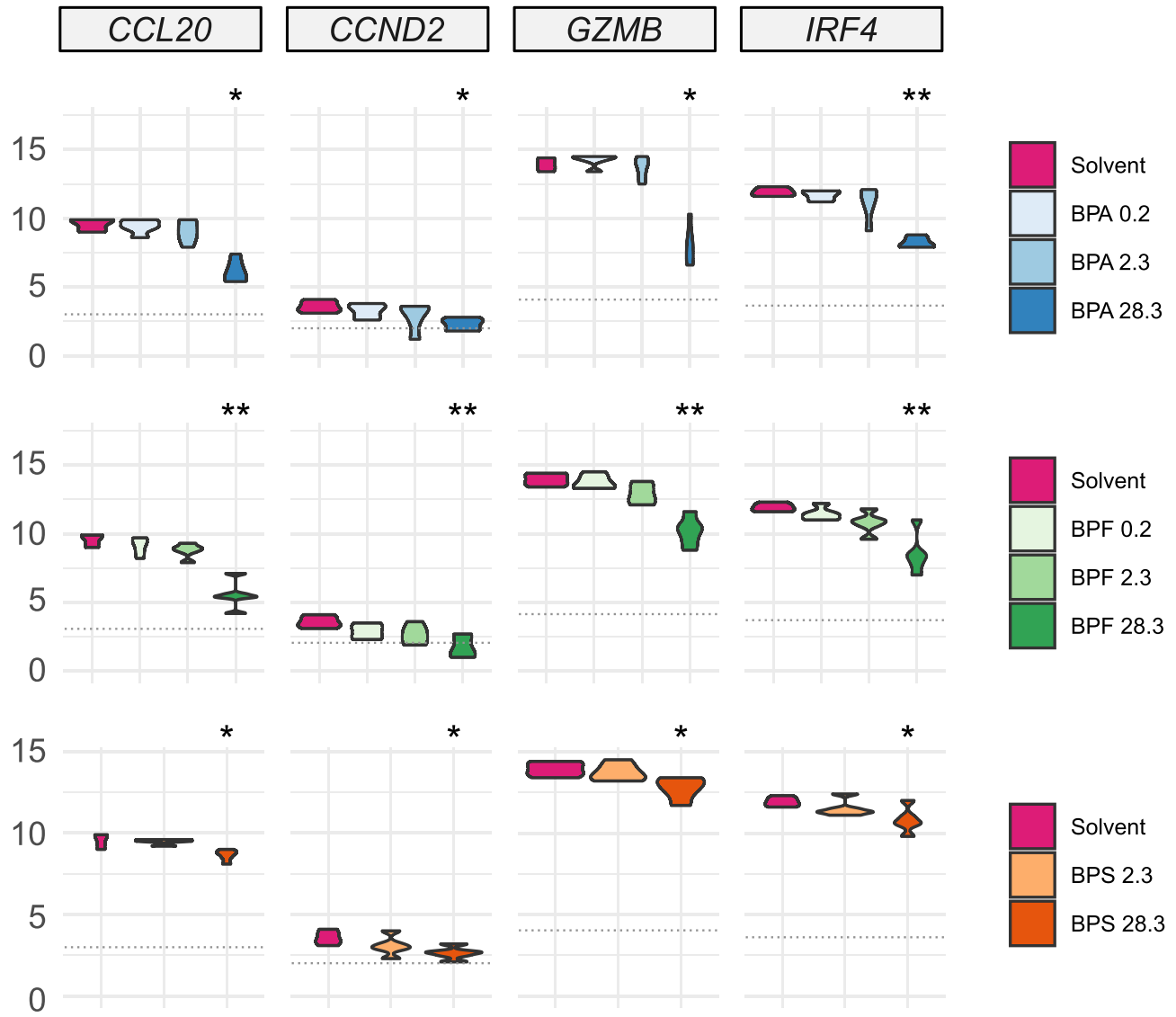

Fig. 3. Pairwise comparison of shared differentially expressed genes after bisphenol-exposure. Significance compared to the solvent control is indicated by asterisk with * < 0.05 and ** < 0.01. The mean gene expression of respective genes in unstimulated MAIT cells is indicated as grey dotted line.

cycle progression and differentiation [64]. Our data suggest that exposure to BPA and BPF at 28.3 μg/mL may hinder cell cycle progression *via CCND2*. *IRF4* represents a gene involved in cell differentiation, coding for the transcription factor interferon regulatory factor 4 and was shown to be essential for the differentiation of both $CD4^+$ and $CD8^+$ T cells to exert effector functions [65]. A negative impact of BPA and BPF on MAIT cell effector function became apparent in the simultaneous reduction of *GZMB* expression. Granzyme B is one of the effector molecules secreted by activated MAIT cells [66]. BPA and BPF are potentially able to reduce MAIT cell activation in face of microbial stimuli. A reduced responsiveness of MAIT cells may impair their functionality as first line of defense and thus endanger homeostasis in the exposed tissues, such as the intestinal mucosae. Riva et al. suggested that a dysfunctional MAIT cell compartment increases susceptibility to infections [67]. The described effects were more prominent on the transcript level than on the cellular level. This is not surprising, as transcriptional profiling is far more sensitive than protein analysis [68]. For BPA and BPF the similarity in immunomodulating properties became apparent by the high number of overlapping, significantly modulated genes. The only gene significantly upregulated upon exposure was *HSPA1A*. This gene is coding for the heat-shock protein 1A, a stress inducible chaperone necessary for cellular homeostasis [69], suggesting that BPA- and BPF-exposed MAIT cells were stressed. Some of the other genes, such as *IL4F, IFNG, ICOS, NFATC1,* code for proteins involved in activation signaling and were reduced on transcript level upon BPA and BPF exposure. Gene expression of *IL17F*, coding for the dominant IL17 molecule of activated MAIT cells [70], was clearly reduced and showed no expression in unstimulated MAIT cells. This further highlights the potential of these compounds in counteracting MAIT cell activation. Our results are in line with recent observations, where BPA-exposure inhibited $CD4^+$ T cell activation [71]. Our data suggest that acute exposure to BPA and BPF, already at moderate exposure concentrations may impair activation, signaling and effector functions of MAIT cells. BPA and its substitute BPF showed a similar immunomodulatory potency, whereas BPS showed lower effects on MAIT cell gene expression. Our study has following limitations: The low number of profiled genes represent a major limitation of the present study and might be overcome by time course analyses. Bulk RNA sequencing of sorted MAIT cells may have resolved the mode of action of bisphenols as well as identified affected signaling pathways in a broader context. To understand whether the observed effects share features of endocrine effects, since both bisphenols and PEs may impact on the endocrine system in

humans, comparison to estrogen may be advantageous. Since DEHP and DINP are metabolized quickly [72], investigating the immunomodulatory properties of primary and secondary DEHP and DINP metabolites should be included in future studies and the optimal time point for immunomodulation by these compound should be identified in time course studies. To fully exploit the potential of *in vitro* testing a broader concentration range of tested compounds should be considered to show dose-dependency of effects.

Since BPA-exposure has been linked to a variety of chronic inflammatory diseases [73] and recently BPA-induced metabolic changes were correlated with an elevated severity of IBD in mice [74], our findings may be of special interest. Especially when considering MAIT cells essential for barrier homeostasis and first line of defense in the gut [75] with potential relevance for protection from infection as recently stated [67]. In the gut, an impairment of MAIT cell responsiveness may promote chronic inflammatory diseases due to a decline of protective capability in the MAIT cell compartment.

5. Conclusion

Our data suggest that BPA together with BPF has the strongest modulating effect on MAIT cells at TDI-based concentrations and short-time exposure. As previously observed, BPF may not be a safer alternative to BPA. Although not completely without effects, BPS showed less effects on activation and gene expression of these immune cells. Furthermore, our present data point out that chemical impacts are rather visible at gene expression level, and less at cellular level. Thus, for future studies gene expression should be analyzed in more detail. According to affected genes, new cellular assays might be designed in order to visualize the effects at cellular level. Finally, lower concentrations, as found in epidemiological studies, combined to chronic exposure both *in vivo* and *in vitro*, is needed to understand the compound mode of action and causally link these findings to disease outcomes in the cohort studies.

CRediT authorship contribution statement

Conceptualization: JLK and GH. Data curation: JLK, AP, MB. Formal analysis: JLK, MB. Funding acquisition: JLK, GH, HDC, ACZ. Investigation: JLK, AP, MB. Methodology: JLK, AP, MB. Supervision: GH. Visualization: JLK. Validation: JLK, AP, MB, GH. Writing – original draft: JLK, AP, MB. Writing – review & editing: JLK, AP, ACZ, HDC, GH.

Funding

We thank the German Federal Environmental Foundation for financial support of Jannike Lea Krause. Hyun-Dong Chang is thankful for support by Dr Rolf Schwiete.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

We thank Jeremy Knespel, Beate Fink and Michaela Loschinski for their excellent technical assistance and support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2023.02.017.

References

[1] L.K. Ncube, A.U. Ude, E.N. Ogunmuyiwa, R. Zulkifli, I.N. Beas, Environmental impact of food packaging materials: a review of contemporary development from conventional plastics to polylactic acid based materials, Materials 13 (2020) 4994, https://doi.org/10.3390/ma13214994.
[2] J.N. Hahladakis, C.A. Velis, R. Weber, E. Iacovidou, P. Purnell, An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling, J. Hazard. Mater. 344 (2018) 179–199, https://doi.org/10.1016/j.jhazmat.2017.10.014.
[3] D.C. Pacyga, S. Sathyanarayana, R.S. Strakovsky, Dietary predictors of phthalate and bisphenol exposures in pregnant women, Adv. Nutr. 10 (2019) 803–815, https://doi.org/10.1093/advances/nmz029.
[4] G.R. Warner, J.A. Flaws, Bisphenol A and phthalates: how environmental chemicals are reshaping toxicology, Toxicol. Sci. 166 (2018) 246–249, https://doi.org/10.1093/toxsci/kfy232.
[5] J. Corrales, L.A. Kristofco, W.B. Steele, B.S. Yates, C.S. Breed, E.S. Williams, B. W. Brooks, Global assessment of bisphenol A in the environment: review and analysis of its occurrence and bioaccumulation, Dose-Response 13 (2015), 155932581559830, https://doi.org/10.1177/1559325815598308.
[6] I. Katsikantami, S. Sifakis, M.N. Tzatzarakis, E. Vakonaki, O.-I. Kalantzi, A. M. Tsatsakis, A.K. Rizos, A global assessment of phthalates burden and related links to health effects, Environ. Int. 97 (2016) 212–236, https://doi.org/10.1016/j.envint.2016.09.013.
[7] R. Huang, Z. Liu, H. Yin, Z. Dang, P. Wu, N. Zhu, Z. Lin, Bisphenol A concentrations in human urine, human intakes across six continents, and annual trends of average intakes in adult and child populations worldwide: a thorough literature review, Sci. Total Environ. 626 (2018) 971–981, https://doi.org/10.1016/j.scitotenv.2018.01.144.
[8] H.-J. Lehmler, B. Liu, M. Gadogbe, W. Bao, Exposure to bisphenol A, bisphenol F, and bisphenol S in U.S. adults and children: the national health and nutrition examination survey 2013–2014, ACS Omega 3 (2018) 6523–6532, https://doi.org/10.1021/acsomega.8b00824.
[9] National Research Council (US), Committee on the Health Risks of Phthalates, Phthalates and Cumulative Risk Assessment: The Task Ahead, National Academies Press, Washington, D.C., 2008, https://doi.org/10.17226/12528.
[10] H. Wang, Z. Liu, J. Zhang, R.-P. Huang, H. Yin, Z. Dang, Human exposure of bisphenol A and its analogues: understandings from human urinary excretion data and wastewater-based epidemiology, Environ. Sci. Pollut. Res. 27 (2020) 3247–3256, https://doi.org/10.1007/s11356-019-07111-9.
[11] H.M. Koch, M. Rüther, A. Schütze, A. Conrad, C. Pälmke, P. Apel, T. Brüning, M. Kolossa-Gehring, Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012, Int. J. Hyg. Environ. Health 220 (2017) 130–141, https://doi.org/10.1016/j.ijheh.2016.11.003.
[12] M.A. Burgos-Aceves, H.G. Abo-Al-Ela, C. Faggio, Physiological and metabolic approach of plastic additive effects: Immune cells responses, J. Hazard. Mater. 404 (2021), 124114, https://doi.org/10.1016/j.jhazmat.2020.124114.
[13] A. Martínez-Ibarra, L.D. Martínez-Razo, K. MacDonald-Ramos, M. Morales-Pacheco, E.R. Vázquez-Martínez, M. López-López, M. Rodríguez Dorantes, M. Cerbón, Multisystemic alterations in humans induced by bisphenol A and phthalates: experimental, epidemiological and clinical studies reveal the need to change health policies, Environ. Pollut. 271 (2021), 116380, https://doi.org/10.1016/j.envpol.2020.116380.
[14] B.R. Hannas, C.S. Lambright, J. Furr, K.L. Howdeshell, V.S. Wilson, L.E. Gray, Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following inutero exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate, Toxicol. Sci. 123 (2011) 206–216, https://doi.org/10.1093/toxsci/kfr146.
[15] European Food Safety Authority, EFSA Explains the Safety of Bisphenol A: Scientific Opinion on Bisphenol A (2015), EFSA, Parma, 2015. ⟨http://bookshop.europa.eu/uri?target=EUB:NOTICE:TM0115035:EN:HTML⟩, (Accessed 25 November 2019).
[16] D. Chen, K. Kannan, H. Tan, Z. Zheng, Y.-L. Feng, Y. Wu, M. Widelka, Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity—a review, Environ. Sci. Technol. 50 (2016) 5438–5453, https://doi.org/10.1021/acs.est.5b05387.
[17] S. Eladak, T. Grisin, D. Moison, M.-J. Guerquin, T. N'Tumba-Byn, S. Pozzi-Gaudin, A. Benachi, G. Livera, V. Rouiller-Fabre, R. Habert, A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound, Fertil. Steril. 103 (2015) 11–21, https://doi.org/10.1016/j.fertnstert.2014.11.005.
[18] S.P. den Braver-Sewradj, R. van Spronsen, E.V.S. Hessel, Substitution of bisphenol A: a review of the carcinogenicity, reproductive toxicity, and endocrine disruption potential of alternative substances, Crit. Rev. Toxicol. 50 (2020) 128–147, https://doi.org/10.1080/10408444.2019.1701986.
[19] J.R. Rochester, A.L. Bolden, Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes, Environ. Health Perspect. 123 (2015) 643–650, https://doi.org/10.1289/ehp.1408989.

[20] Agency for Toxic Substances and Disease Registry (ATSDR), Environmental Protection Agency (EPA), Toxicological Profile for Di(2-Ethylhexyl)Phthalate (DEHP), 2022.
[21] Y. Liang, C. Bi, X. Wang, Y. Xu, A general mechanistic model for predicting the fate and transport of phthalates in indoor environments, Indoor Air 29 (2019) 55–69, https://doi.org/10.1111/ina.12514.
[22] L. Edwards, N.L. McCray, B.N. VanNoy, A. Yau, R.J. Geller, G. Adamkiewicz, A. R. Zota, Phthalate and novel plasticizer concentrations in food items from U.S. fast food chains: a preliminary analysis, J. Expo. Sci. Environ. Epidemiol. (2021), https://doi.org/10.1038/s41370-021-00392-8.
[23] European Parliament and Council, DIRECTIVE 2005/84/EC, 2005.
[24] European Parliament and Council, COMMISSION REGULATION (EU) 2021/2045, 2021.
[25] M. Wittassek, G.A. Wiesmüller, H.M. Koch, R. Eckard, L. Dobler, J. Müller, J. Angerer, C. Schlüter, Internal phthalate exposure over the last two decades – a retrospective human biomonitoring study, Int. J. Hyg. Environ. Health 210 (2007) 319–333, https://doi.org/10.1016/j.ijheh.2007.01.037.
[26] A.R. Zota, A.M. Calafat, T.J. Woodruff, Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001–2010, Environ. Health Perspect. 122 (2014) 235–241, https://doi.org/10.1289/ehp.1306681.
[27] C. Chiang, J.A. Flaws, Subchronic exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate during adulthood has immediate and long-term reproductive consequences in female mice, Toxicol. Sci. 168 (2019) 620–631, https://doi.org/10.1093/toxsci/kfz013.
[28] European Food Safety Authority (EFSA), K. Volk, L. Castle, Technical report of the public consultation on the 'Draft update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2–ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials, EFS3 16 (2019), https://doi.org/10.2903/sp.efsa.2019.EN-1747.
[29] C. Jøhnk, A. Høst, S. Husby, G. Schoeters, C.A.G. Timmermann, H.B. Kyhl, I. H. Beck, A.-M. Andersson, H. Frederiksen, T.K. Jensen, Maternal phthalate exposure and asthma, rhinitis and eczema in 552 children aged 5 years; a prospective cohort study, Environ. Health 19 (2020) 32, https://doi.org/10.1186/s12940-020-00586-x.
[30] C. Franken, N. Lambrechts, E. Govarts, G. Koppen, E. Den Hond, D. Ooms, S. Voorspoels, L. Bruckers, I. Loots, V. Nelen, I. Sioen, T.S. Nawrot, W. Baeyens, N. Van Larebeke, G. Schoeters, Phthalate-induced oxidative stress and association with asthma-related airway inflammation in adolescents, Int. J. Hyg. Environ. Health 220 (2017) 468–477, https://doi.org/10.1016/j.ijheh.2017.01.006.
[31] G. Saravanabhavan, J. Murray, Human biological monitoring of diisononyl phthalate and diisodecyl phthalate: a review, J. Environ. Public Health 2012 (2012) 1–11, https://doi.org/10.1155/2012/810501.
[32] W. Völkel, T. Colnot, G.A. Csanády, J.G. Filser, W. Dekant, Metabolism and kinetics of bisphenol A in humans at low doses following oral administration, Chem. Res. Toxicol. 15 (2002) 1281–1287, https://doi.org/10.1021/tx025548t.
[33] E. Treiner, L. Duban, I.C. Moura, T. Hansen, S. Gilfillan, O. Lantz, Mucosal-associated invariant T (MAIT) cells: an evolutionarily conserved T cell subset, Microbes Infect. 7 (2005) 552–559, https://doi.org/10.1016/j.micinf.2004.12.013.
[34] L. Le Bourhis, E. Martin, I. Péguillet, A. Guihot, N. Froux, M. Coré, E. Lévy, M. Dusseaux, V. Meyssonnier, V. Premel, C. Ngo, B. Riteau, L. Duban, D. Robert, M. Rottman, C. Soudais, O. Lantz, Antimicrobial activity of mucosal-associated invariant T cells, Nat. Immunol. 11 (2010) 701–708, https://doi.org/10.1038/ni.1890.
[35] R.J. Napier, E.J. Adams, M.C. Gold, D.M. Lewinsohn, The role of mucosal associated invariant T cells in antimicrobial immunity, Front. Immunol. 6 (2015), https://doi.org/10.3389/fimmu.2015.00344.
[36] A. Kurioka, L.J. Walker, P. Klenerman, C.B. Willberg, MAIT cells: new guardians of the liver, Clin. Transl. Immunol. 5 (2016), e98, https://doi.org/10.1038/cti.2016.51.
[37] A. Toubal, I. Nel, S. Lotersztajn, A. Lehuen, Mucosal-associated invariant T cells and disease, Nat. Rev. Immunol. 19 (2019) 643–657, https://doi.org/10.1038/s41577-019-0191-y.
[38] J.D. Berkson, M. Prlic, The MAIT conundrum – how human MAIT cells distinguish bacterial colonization from infection in mucosal barrier tissues, Immunol. Lett. 192 (2017) 7–11, https://doi.org/10.1016/j.imlet.2017.09.013.
[39] D.I. Godfrey, H.-F. Koay, J. McCluskey, N.A. Gherardin, The biology and functional importance of MAIT cells, Nat. Immunol. 20 (2019) 1110–1128, https://doi.org/10.1038/s41590-019-0444-8.
[40] T.S.C. Hinks, X.-W. Zhang, MAIT cell activation and functions, Front. Immunol. 11 (2020) 1014, https://doi.org/10.3389/fimmu.2020.01014.
[41] M.G. Constantinides, V.M. Link, S. Tamoutounour, A.C. Wong, P.J. Perez-Chaparro, S.-J. Han, Y.E. Chen, K. Li, S. Farhat, A. Weckel, S.R. Krishnamurthy, I. Vujkovic-Cvijin, J.L. Linehan, N. Bouladoux, E.D. Merrill, S. Roy, D.J. Cua, E.J. Adams, A. Bhandoola, T.C. Scharschmidt, J. Aubé, M.A. Fischbach, Y. Belkaid, MAIT cells are imprinted by the microbiota in early life and promote tissue repair, Science 366 (2019), eaax6624, https://doi.org/10.1126/science.aax6624.
[42] J.L. Krause, B. Engelmann, U. Nunes da Rocha, A. Pierzchalski, H.D. Chang, A. C. Zenclussen, M. von Bergen, U. Rolle-Kampczyk, G. Herberth, MAIT cell activation is reduced by direct and microbiota-mediated exposure to bisphenols, Environ. Int. 158 (2022), 106985, https://doi.org/10.1016/j.envint.2021.106985.
[43] H.M. Koch, R. Preuss, J. Angerer, Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure – an update and latest results1, Int. J. Androl. 29 (2006) 155–165, https://doi.org/10.1111/j.1365-2605.2005.00607.x.
[44] J.L. Krause, S.S. Schäpe, F. Schattenberg, S. Müller, G. Ackermann, U.E. Rolle-Kampczyk, N. Jehmlich, A. Pierzchalski, M. von Bergen, G. Herberth, The activation of mucosal-associated invariant T (MAIT) cells is affected by microbial diversity and riboflavin utilization in vitro, Front. Microbiol. 11 (2020) 755, https://doi.org/10.3389/fmicb.2020.00755.
[45] P. Dixon, VEGAN, a package of R functions for community ecology, J. Veg. Sci. 14 (2003) 927–930, https://doi.org/10.1111/j.1654-1103.2003.tb02228.x.
[46] J. Oksanen, F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, E. Szoecs, H. Wagner, vegan: Community Ecology Package, 2020. ⟨https://CRAN.R-project.org/package=vegan⟩.
[47] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag, New York, 2016. ⟨https://ggplot2.tidyverse.org⟩.
[48] F. Crameri, Scientific Colour Maps, 2018. ⟨https://doi.org/10.5281/ZENODO.1243909⟩.
[49] I. Nel, L. Bertrand, A. Toubal, A. Lehuen, MAIT cells, guardians of skin and mucosa, Mucosal Immunol. 14 (2021) 803–814, https://doi.org/10.1038/s41385-021-00391-w.
[50] H. Koppejan, D.T.S.L. Jansen, M. Hameetman, R. Thomas, R.E.M. Toes, F.A. van Gaalen, Altered composition and phenotype of mucosal-associated invariant T cells in early untreated rheumatoid arthritis, Arthritis Res. Ther. 21 (2019) 3, https://doi.org/10.1186/s13075-018-1799-1.
[51] N.-E. Serriari, M. Eoche, L. Lamotte, J. Lion, M. Fumery, P. Marcelo, D. Chatelain, A. Barre, E. Nguyen-Khac, O. Lantz, J.-L. Dupas, E. Treiner, Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases: MAIT cells in IBD, Clin. Exp. Immunol. 176 (2014) 266–274, https://doi.org/10.1111/cei.12277.
[52] C. Sugimoto, M. Hirotani, K. Yoshikiyo, U. Koshimizu, R. Wakao, T. Horinouchi, Y. Mazaki, T. Higashi, T. Fukazawa, H. Fujita, H. Sasaki, H. Wakao, The dynamics of mucosal-associated invariant T cells in multiple sclerosis, SpringerPlus 5 (2016), https://doi.org/10.1186/s40064-016-2923-9.
[53] J. Michałowicz, K. Mokra, A. Bąk, Bisphenol A and its analogs induce morphological and biochemical alterations in human peripheral blood mononuclear cells (in vitro study), Toxicol. Vitr. 29 (2015) 1464–1472, https://doi.org/10.1016/j.tiv.2015.05.012.
[54] A. Wenzel, C. Franz, E. Breous, U. Loos, Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells, Mol. Cell. Endocrinol. 244 (2005) 63–71, https://doi.org/10.1016/j.mce.2005.02.008.
[55] Y. Ma, Y. Guo, S. Wu, Z. Lv, Q. Zhang, X. Xie, Y. Ke, Analysis of toxicity effects of di-(2-ethylhexyl) phthalate exposure on human bronchial epithelial 16HBE cells, Cytotechnology 70 (2018) 119–128, https://doi.org/10.1007/s10616-017-0111-6.
[56] T. Eljezi, P. Pinta, F. Nativel, D. Richard, J. Pinguet, O. Roy, V. Sautou, G. Grimandi, E. Moreau, In vitro cytotoxic effects of secondary metabolites of DEHP and its alternative plasticizers DINCH and DINP on a L929 cell line, Int. J. Hyg. Environ. Health 222 (2019) 583–589, https://doi.org/10.1016/j.ijheh.2019.03.005.
[57] C. Karrer, M. Andreassen, N. von Goetz, F. Sonnet, A.K. Sakhi, K. Hungerbühler, H. Dirven, T. Husøy, The EuroMix human biomonitoring study: source-to-dose modeling of cumulative and aggregate exposure for the bisphenols BPA, BPS, and BPF and comparison with measured urinary levels, Environ. Int. 136 (2020), 105397, https://doi.org/10.1016/j.envint.2019.105397.
[58] S.E. Fiedler, A.R. Kerns, C. Tsang, H.N. Love, S. Salinthone, Donor variability may mask dimethyl fumarate's effects on nuclear factor E2-related factor 2 in human peripheral blood mononuclear cells, BMC Res. Notes 10 (2017) 553, https://doi.org/10.1186/s13104-017-2862-8.
[59] U.C. Nygaard, E.S. Ulriksen, H. Hjertholm, F. Sonnet, A.K. Bølling, M. Andreassen, T. Husøy, H. Dirven, Immune cell profiles associated with measured exposure to phthalates in the Norwegian EuroMix biomonitoring study – a mass cytometry approach in toxicology, Environ. Int. 146 (2021), 106283, https://doi.org/10.1016/j.envint.2020.106283.
[60] Y. Wang, H. Zhu, K. Kannan, A review of biomonitoring of phthalate exposures, Toxics 7 (2019) 21, https://doi.org/10.3390/toxics7020021.
[61] M. Murata, J.-H. Kang, Bisphenol A (BPA) and cell signaling pathways, Biotechnol. Adv. 36 (2018) 311–327, https://doi.org/10.1016/j.biotechadv.2017.12.002.
[62] R. Mesnage, A. Phedonos, M. Arno, S. Balu, J.C. Corton, M.N. Antoniou, Editor's highlight: transcriptome profiling reveals bisphenol A alternatives activate estrogen receptor alpha in human breast cancer cells, Toxicol. Sci. 158 (2017) 431–443, https://doi.org/10.1093/toxsci/kfx101.
[63] M. Cunningham, G. Gilkeson, Estrogen receptors in immunity and autoimmunity, Clin. Rev. Allerg. Immunol. 40 (2011) 66–73, https://doi.org/10.1007/s12016-010-8203-5.
[64] D. Wang, Y. Zhang, Y.-Q. Che, CCND2 mRNA expression is correlated with R-CHOP treatment efficacy and prognosis in patients with ABC-DLBCL, Front. Oncol. 10 (2020) 1180, https://doi.org/10.3389/fonc.2020.01180.
[65] M. Huber, M. Lohoff, IRF4 at the crossroads of effector T-cell fate decision: highlights, Eur. J. Immunol. 44 (2014) 1886–1895, https://doi.org/10.1002/eji.201344279.
[66] A. Kurioka, J.E. Ussher, C. Cosgrove, C. Clough, J.R. Fergusson, K. Smith, Y.-H. Kang, L.J. Walker, T.H. Hansen, C.B. Willberg, P. Klenerman, MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets, Mucosal Immunol. 8 (2015) 429–440, https://doi.org/10.1038/mi.2014.81.
[67] A. Riva, V. Patel, A. Kurioka, H.C. Jeffery, G. Wright, S. Tarff, D. Shawcross, J. M. Ryan, A. Evans, S. Azarian, J.S. Bajaj, A. Fagan, V. Patel, K. Mehta, C. Lopez, M. Simonova, K. Katzarov, T. Hadzhiolova, S. Pavlova, J.A. Wendon, Y.H. Oo, P. Klenerman, R. Williams, S. Chokshi, Mucosa-associated invariant T cells link

intestinal immunity with antibacterial immune defects in alcoholic liver disease, Gut 67 (2018) 918–930, https://doi.org/10.1136/gutjnl-2017-314458.
[68] M.J. Gilchrist, G.J.C. Veenstra, K.W.Y. Cho, Transcriptomics and PRoteomics Methods for *Xenopus* embryos and tissues, Cold Spring Harb. Protoc. 2020 (2020), pdb.top098350, https://doi.org/10.1101/pdb.top098350.
[69] Smulders Bilog, Labanieh Oliverio, Stahelin Zapanta, Nikolaidis, membrane localization of HspA1A, a stress inducible 70-kDa heat-shock protein, depends on its interaction with intracellular phosphatidylserine, Biomolecules 9 (2019) 152, https://doi.org/10.3390/biom9040152.
[70] S. Cole, J. Murray, C. Simpson, R. Okoye, K. Tyson, M. Griffiths, D. Baeten, S. Shaw, A. Maroof, Interleukin (IL)-12 and IL-18 synergize to promote MAIT cell IL-17A and IL-17F production independently of IL-23 signaling, Front. Immunol. 11 (2020), 585134, https://doi.org/10.3389/fimmu.2020.585134.
[71] J.M. Gostner, E. Raggl, K. Becker, F. Überall, H. Schennach, J.E. Pease, D. Fuchs, Bisphenol A suppresses Th1-type immune response in human peripheral blood mononuclear cells in vitro, Immunol. Lett. 168 (2015) 285–292, https://doi.org/10.1016/j.imlet.2015.10.006.
[72] W.A.C. Anderson, L. Castle, S. Hird, J. Jeffery, M.J. Scotter, A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate, Food Chem. Toxicol. 49 (2011) 2022–2029, https://doi.org/10.1016/j.fct.2011.05.013.
[73] R. Rezg, S. El-Fazaa, N. Gharbi, B. Mornagui, Bisphenol A and human chronic diseases: current evidences, possible mechanisms, and future perspectives, Environ. Int. 64 (2014) 83–90, https://doi.org/10.1016/j.envint.2013.12.007.
[74] J.A. DeLuca, K.F. Allred, R. Menon, R. Riordan, B.R. Weeks, A. Jayaraman, C. D. Allred, Bisphenol-A alters microbiota metabolites derived from aromatic amino acids and worsens disease activity during colitis, Exp. Biol. Med. 243 (2018) 864–875, https://doi.org/10.1177/1535370218782139.
[75] A. Amini, D. Pang, C.-P. Hackstein, P. Klenerman, MAIT cells in barrier tissues: lessons from immediate neighbors, Front. Immunol. 11 (2020), 584521, https://doi.org/10.3389/fimmu.2020.584521.