Bisphenol A modulates the metabolic regulator oestrogen-related receptor- α in T-cells

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Abstract

Bisphenol A (BPA) is a widely used plastics constituent that has been associated with endocrine, immune and metabolic effects. Evidence for how BPA exerts significant biological effects at chronic low levels of exposure has remained elusive. In adult men, exposure to BPA has been associated with higher expression of two nuclear receptors, oestrogen receptor- β (ER β) and oestrogen-related-receptor- α (ERR α), in peripheral white blood cells *in vivo*. In this study, we explore the expression of *ESR2* (ER β) and *ESRRA* (ERR α) in human leukaemic T-cell lymphoblasts (Jurkat cells) exposed to BPA *in vitro*. We show that exposure to BPA led to enhanced expression of *ESRRA* within 6 h of exposure (mean $\pm$ S.E.M.: 1.43 ± 0.08 -fold increase compared with the control, $P < 0.05$). After 72 h, expression of *ESRRA* remained significantly enhanced at concentrations of BPA ≥ 1 nM. Oxidative metabolism of BPA by rat liver S9 fractions yields the potent oestrogenic metabolite, 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP). Exposure of cells to 1–100 nM MBP increased the expression of both *ESRRA* (significantly induced, $P < 0.05$, at 1, 10, 100 nM) and *ESR2* (1.32 ± 0.07 -fold increase at 100 nM exposure, $P < 0.01$). ERR α is a major control point for oxidative metabolism in many cell types, including T-cells. Following exposure to both BPA and MBP, we found that cells showed a decrease in cell proliferation rate. Taken together, these results confirm the bioactivity of BPA against putative T-cell targets *in vitro* at concentrations relevant to general human exposure.

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Introduction

Bisphenol A (BPA) is a synthetic compound originally synthesised as an alternative to oestrogen (Dodds & Lawson 1936). It is used widely as a monomer in polycarbonate plastics and in the epoxy resins that line food and drinks containers and is one of the world's

highest production volume chemicals (Ritter 2011). BPA is leached from food packaging into food and ingestion through food is a major route of human exposure, with additional exposures from dental sealants, inhalation of household dusts and dermal routes (Bailey & Hoekstra 2011). This has led to ubiquitous exposure of the general population, with >95% of people showing detectable levels of BPA in their urine (Calafat *et al.* 2008, Galloway *et al.* 2010, Vandenberg *et al.* 2010).

There has been a level of controversy over the adverse health consequences of exposure to BPA. Recent studies have demonstrated that human and wildlife populations are exposed to levels of BPA capable of negatively impacting on reproductive, developmental and metabolic endpoints in different wildlife species and laboratory animal studies (Calafat *et al.* 2008, Vandenberg *et al.* 2010). In addition, an increasing number of epidemiological studies have now shown that general population exposure to BPA is associated with adverse health outcomes. A recent literature review has found

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more than 90 studies linking BPA to human health (Rochester 2013). BPA exposures were positively associated with endocrine changes in men (Galloway *et al.* 2010, Meeker *et al.* 2010) and with negative effects on immune function (Clayton *et al.* 2011). Positive prospective associations have been reported between BPA exposure and a range of reproductive and developmental conditions including recurrent miscarriage (Sugiura-Ogasawara *et al.* 2005) and externalising behaviour scores in the offspring of exposed mothers (Braun *et al.* 2011). Urinary BPA concentrations were associated with cardiovascular disease diagnosis in large scale cross-sectional (Lang *et al.* 2008, Melzer *et al.* 2010, 2012a) and longitudinal studies (Melzer *et al.* 2012b).

The plausibility of these associations and a comprehensive understanding of the risks posed by exposure to BPA are complicated by a lack of understanding of the mechanisms underlying its varied endocrine disruptive actions. Many of the physiological effects of BPA have been studied in relation to its widely reported activity as an oestrogen agonist (Lee *et al.* 2003, Chapin *et al.* 2008). A number of additional receptor-mediated effects have been reported including anti-androgen activity (Bonefeld-Jørgensen *et al.* 2007), thyroid hormone disruption (Moriyama *et al.* 2002), altered pancreatic β -cell function (Roper *et al.* 2008, Soriano *et al.* 2012) and obesity-promoting effects (Newbold *et al.* 2008, Marmugi *et al.* 2012), binding to oestrogen-related receptor gamma (ERR γ ; Takayanagi *et al.* 2006, Okada *et al.* 2008) and activation of peroxisome proliferator-activated receptor- γ (PPAR γ)-mediated pathways (Kwintkiewicz *et al.* 2010). A confirmation of BPA interaction with a large number of different pathways was obtained from computational analysis of the extensive *in vitro* tests carried out within the ToxCast program (Reif *et al.* 2010). Comprehensive reviews of the available data highlight both the complexity of BPA's biochemical and molecular interactions, and that these varied receptor systems and molecular pathways may be differentially affected in different target tissues and species (Wetherill *et al.* 2007, Thayer & Belcher 2011).

In order to gain a better understanding of the molecular mechanisms underlying the health effects of BPA, we have recently studied the *in vivo* expression of a panel of oestrogen receptor (ER)-ERR and androgen receptor genes in the peripheral blood leukocytes taken from 96 adult men (Melzer *et al.* 2011). We found that there were positive associations between higher BPA concentrations and higher expression of two genes, *ESR2* (oestrogen receptor 2, ER β) and *ESRRA* (oestrogen-related receptor alpha, ERR α). This finding is of interest because it suggests that BPA and/or its metabolites are bioactive in the human body and that associations with hormone signalling and related disorders are biologically plausible. ER β is important in mediating the physiological actions of oestrogens in target tissues including prostate, colon, cardiovascular and CNS

(Imamov *et al.* 2005), with a tissue distribution and ligand specificity that is distinct from that of ER α . ERR α belongs to the NR3 β orphan nuclear receptor family subgroup, consisting of ERR α , β and γ . Although responsive to some synthetic oestrogens, there are no known naturally occurring ligands for ERR α (Willy *et al.* 2004). Genetic studies on mice showed that ERR subtypes are involved in mitochondrial biogenesis, oxidative phosphorylation and lipid metabolism (Tremblay & Giguere 2007). Both ERR α and γ are essential for proper cardiac function (Huss *et al.* 2007) and may play a broader role in metabolic homeostasis (Tennesen *et al.* 2011). The functional relevance of changes in ER β and ERR α in blood leukocytes has not been comprehensively determined, although ERR α is reported to be a selective transcriptional regulator of certain T-cell effector functions, facilitating gene expression and glucose and mitochondrial metabolism during T-cell growth and proliferation (Michalek *et al.* 2011).

The objectives of the current study were to explore the expression of the *ESR2* and *ESRRA* genes in human leukaemic T-cell lymphoblasts (Jurkat cells) exposed to BPA and one of its metabolites, *in vitro*. We used Jurkat cells because they are an amenable *in vitro* lymphocyte model and our *in vivo* expression studies highlighted the potential sensitivity of lymphocytes to the effects of BPA; BPA exposure is known to have effects on cytokine production in T-cells *in vitro* (Yan *et al.* 2008) and ERR α has many functions, including modulation of the immune response (Ranhota 2012). In addition, we investigated the effect of a putative metabolite of BPA: oxidative metabolism of BPA by rat or human liver S9 fractions yields the potent oestrogenic metabolite 4-methyl-2,4-bis(4-hydroxyphenyl)pent-1-ene (MBP), which has around 1000-fold higher oestrogenic activity than BPA (Okuda *et al.* 2010). 3D models of MBP suggest that its structure allows for interaction with amino acid residues in ER α and ER β that are important for binding of oestradiol by these receptors (Baker & Chandsawanghuwana 2012), but which are absent from BPA. We compared the expression of ER β and ERR α following exposure to 17 α -ethinylestradiol (EE $_2$), MBP and BPA at environmentally relevant concentrations and over different time intervals. As ERR α is a major control point for oxidative metabolism in many cell types, including T-cells, we determined the effects of BPA and MBP exposure on downstream end-points such as cell proliferation and ATP content.

Materials and methods

Synthesis of MBP

MBP was synthesised at Hiroshima International University as described previously (Yoshihara *et al.* 2004). MBP was in the form of a white powder with a melting point at 130.5–132 °C; purity was certified from the narrow melting point range

obtained and the single peak obtained by HPLC. The ^1H NMR data (400 MHz, CDCl_3) of authentic MBP obtained using a JEOL, ALPHA-FT NMR Spectrometer were δ 1.20 (s, 6H), 2.74 (s, 2H), 4.64 (s, 1H), 4.71 (d, 1H, $J=2.0$ Hz), 4.74 (s, 1H), 5.07 (d, 1H, $J=2.0$ Hz), 6.67 (d, 2H, $J=8.8$ Hz), 6.67 (d, 2H, $J=8.4$ Hz), 7.09 (d, 2H, $J=8.8$ Hz), 7.12 (d, 2H, $J=8.8$ Hz). Chemical shifts are reported in ppm downfield from the peak of tetramethylsilane used as an internal standard. Splitting patterns are designated as 's and d' indicating 'singlet and doublet' respectively.

Cell culture, dosing and exposure

All further experiments were carried out at the University of Exeter. Jurkat cells, human leukaemic T-cell lymphoblasts, were purchased (catalogue number: 88042803) and cultured in suspension in RPMI-1640 without phenol red (Sigma–Aldrich Company Ltd.), supplemented with 10% fetal bovine serum, stripped with charcoal-dextran (Gibco) and 200 mM L-glutamine (Sigma–Aldrich). The cells were grown in the suspensions at the temperature of 37 °C in 5% CO_2 and the culture was maintained between 0.5 and $5 \times 100,000$ cells/ml. Before the experiments, cells were grown in a single tissue culture flask angled neck and vented cap. Medium was changed ahead of the start of each experiment and it was not changed during the treatments. Cell viability was assayed with Trypan blue (Sigma–Aldrich) staining (dilution: 1/10th of 100 μl culture volume). Cell viability was always above 80%. The cells were exposed to EE_2 ($\geq 98\%$, Sigma–Aldrich), BPA ($\geq 99\%$, Sigma–Aldrich) and to the oxidative metabolite of BPA, MBP, synthesised as described earlier. Exposure experiments were repeated at least twice and included between three and eight replicates for each treatment.

Exposure to EE_2

Exposures were carried out in 25 cm^2 flasks ($n=3$); 1 μM EE_2 and the solvent control (0.001% ethanol) were added to the cell culture medium and total RNA extraction was performed at the time point 0, 6, 24, 36, 48, 72 h, chosen to provide an overview of the time-dependent activation of the target genes across a time interval of 3 days. The selection of a time window of 72 h was based on previous studies present in the literature (Richter *et al.* 2007). During each time point, three flasks from the control and the exposed group were randomly selected, sampled for RNA extraction and then discarded.

Exposure to BPA and MBP

Jurkat cells were divided into treatment and control flasks, 1 μM BPA and solvent control (0.001% ethanol) were added accordingly. The cells were then seeded onto 24-well plates. Total RNA extraction was performed at time point 0, 6, 24, 36, 48, 72 h. When exposures of 1, 10 and 100 nM BPA concentrations were then tested, mRNA expression was measured at a single time-point (72 h). The time-point was selected based on the results of the previous exposures to 1 μM EE_2 and 1 μM BPA. Treatments and solvent controls were added according to the different concentrations as described

previously. Treatments were replicated in eight wells per experiment and the control in six. Two parallel experiments were conducted; results were checked for statistical differences and then tabulated and merged. Similarly, a set of experiments was performed exposing cells to MBP that was tested at 1, 10, 100 nM concentrations. Study design, replication and sampling time were as the ones described earlier.

Real-time RT-PCR measurement of gene expression in target genes

Total RNA was extracted after sampling using the RNeasy Mini Kit from Qiagen according to the manufacturer's instructions. RNA yield was quantified using a NanoDrop spectrophotometer. Total RNA (500 ng) was reversed transcribed in 20 μl reactions using the Superscript III VILO kit (Invitrogen). Real-time PCR amplifications were performed on the ABI 7900HT platform using 96-well plates (Applied Biosystems). Each well contained 5 μl TaqMan 2 $\times$ universal master mix (no AMPerase) (Applied Biosystems), 3.5 μl dH_2O , 1 μl cDNA template and 0.5 μl probe. Cycling conditions were 50 °C for 2 min, 95 °C for 10 min followed by 50 cycles at 95 °C for 15 s and 60 °C for 1 min. The expression levels of the genes *ESR2* (*ERβ*) and *ESRRA* (*ERRα*) were assessed through relative quantification using the $2^{-\Delta\Delta\text{Ct}}$ method (Livak & Schmittgen 2001). Data are presented as the fold-change in gene expression relative to three endogenous control transcripts and normalised to the levels of the test transcripts in the untreated control. PCR signal of the target transcripts was standardised using three housekeeping (HK) genes: $\beta 2$ microglobulin (*B2M*), hypoxanthine phosphoribosyl-transferase 1 (*HPRT1*) and family with sequence similarity 102 (*FAM102A*). This was done to account for differences in the amount of cDNA that is loaded into each PCR wells and differences between plates. The expression of each of the five genes was measured in triplicate for each sample. A minimum of three HK genes is recommended for a reliable standardisation factor and to reduce errors in real-time quantitative RT-PCR data (Vandesompele *et al.* 2002). *B2M* and *HPRT1* are widely used HK genes (Vandesompele *et al.* 2002) while *FAM102A* expression was shown empirically not to be influenced by BPA exposure in our previous experiments. Probes were inventoried Applied Biosystems assays, with the exception of the *ESRRA* probe, which was a custom assay (probe and primer sequences available on request).

ATP measurements and cell count

Levels of intracellular ATP were measured using an ATP Assay kit (Enliten, Promega). Assays were performed on the Infinite F200 microplate luminometer (Tecan and Beckman, Männedorf, Switzerland) combined with 96-well white microplates. Cell count was carried out using the particle counting Multisizer Coulter Counter (Beckman, USA).

Statistical analysis

Values in tables and graphs are expressed as mean $\pm$ S.E.M. (unless otherwise specified). Two-way factorial ANOVA was

used to test the interaction between treatment and time of exposure when multiple time-points were considered. Data were checked for the normality of distribution and variance homogeneity. Where the overall ANOVA showed significance, *post-hoc* comparisons between treatment and its control were performed. When only the effect of different treatments was tested, one-way ANOVA was used followed by Bonferroni *post-hoc* test. *P* value <0.05 was considered significant.

Results

Time and dose responsiveness of *ESR2* and *ESRRA* in Jurkat cells

All target genes (*ESR2*, *ESRRA*) and HK genes (*B2M*, *HPRT1*, *FAM102*) were empirically checked and consistently detected in Jurkat cells. The oestrogen responsiveness of cells was initially tested using EE₂ (Naciff *et al.* 2010). A time-dependent increase in expression of both *ESR2* and *ESRRA* target gene transcripts was seen (Fig. 1A and B) following exposure to 1 µM EE₂. For *ESR2*, a monotonic increase was seen, with maximum intensity of expression at 72 h and indication of an increasing trend (Fig. 1A). On the other hand, *ESRRA* showed a non-monotonic trend with a non-significant increase in gene expression after 6 h, a subsequent drop and a significant increase at 48 and 72 h (mean ± s.e.m., up to twofold increase ± 0.09 compared with the control, *P* < 0.05). Exposure to 1 µM BPA under the same conditions produced a similar level of increase (Fig. 1C and D). The time profile of BPA exposure indicates that *ESR2* expression was significantly induced compared with the control after 36 h (1.36 ± 0.10-fold increase, *P* < 0.05). Expression of *ESRRA* again showed a non-monotonic response: a statistically significant increase was registered after only 6 h of exposure to BPA followed by a drop at 24 h and a second significant increase at 48 h (1.58 ± 0.08-fold increase, *P* < 0.01), remaining elevated for the duration

Figure 1 Gene expression intensity as a function of time following exposure of Jurkat cells to 1 µM EE₂ (A and B) or 1 µM BPA (C and D). Values are expressed as fold-change increase compared with each time-point control (mean ± s.e.m.). **P* < 0.05.

of the experiment. Overall, exposure to a high concentration of BPA showed a stronger and quicker increase in mRNA expression levels of *ESRRA* than *ESR2*.

The cell were exposed to environmentally relevant concentrations (1–100 nm) of BPA and to MBP, its oxidative metabolite (Fig. 2). The *ESR2* mRNA expression level did not change in the treatment groups exposed to increasing concentrations of BPA (Fig. 2A). There was, however, a significant increase in *ESR2* expression following 72 h exposure to 100 nM MBP: the average value in the treatment group (mean ± s.e.m., 1.32 ± 0.075, *n* = 16) was significantly higher than the control group (1.01 ± 0.046, *n* = 12, Fig. 2C). For *ESRRA*, significantly increased expression was seen following exposure to both BPA and MBP at all concentrations from 1 nM upwards (Fig. 2B and D). Jurkat cells exposed to increasing concentrations of BPA showed levels of *ESRRA* expression increased by up to 82% (±13%, *n* = 16). Similar results were registered after exposure to MBP (Fig. 2C and D).

Effects of BPA and MBP on cell proliferation and ATP

Jurkat cells exposed to 100 nM BPA showed a decreased proliferation after 48 h compared with the control (Fig. 3). After 72 h, the difference in cell count between exposed and control groups was greater (*P* < 0.001). There was considerable variability in the ATP content of cells which were generally lower following exposure to either compound (Fig. 3B). However, the levels of ATP measured in control and exposed group and adjusted for cell numbers were not significantly different at either time point (*P* = 0.07).

Discussion

We have previously reported that human exposure to BPA is associated with the activation of the oestrogen-responsive genes *ESR2* and *ESRRA* measured in the peripheral blood leukocytes in the general adult population (Melzer *et al.* 2011). The ERs and ERRs share common targets and control overlapping transcriptional regulatory networks. This makes them of high interest for investigating the effects of BPA and related compounds showing diverse oestrogen-like effects. The aim of this study was to further explore the expression of *ESR2* and *ESRRA* in human leukaemic T-cell lymphoblasts exposed to BPA and of a metabolite under laboratory conditions where potentially confounding variables could be controlled. We show here that BPA at environmentally relevant concentrations can cause changes to hormone sensitive gene transcripts and that this has physiological consequences in altering cell growth rate in T-cells. This is important because these results taken together with our previous study suggest that *ESRRA* gene expression can be significantly modulated by BPA and its

Figure 2 Gene expression intensity after 72 h exposure of Jurkat cells to 1–10–100 nM BPA (A and B) or 1–10–100 nM MBP (C and D). Values are expressed as fold-change increase compared with control (Ctrl). Box plots follow Tukey's rules and outliers are represented by dots (see D). * $P < 0.05$.

metabolites in human blood cells both in laboratory and *in vivo* conditions and, hence, imply that BPA is capable of plausible bioactivity in the human body.

Of particular interest is what we believe to be the first report of a significant increase in expression of *ESRRA* following exposure to both EE_2 and BPA in T-cells. *ERRα* is a regulator of cell metabolism across many different cell types (Giguère 2008, Villena & Kralli 2008) and has a central role in the regulation of energy homeostasis and adaptive oxygen capacity. Activity of *ERRα*, which has no known endogenous ligands, is known to be regulated at the protein level; activity is highly sensitive to co-regulatory proteins involved in energy homeostasis such as the steroid receptor co-activator, peroxisome proliferation activation receptor gamma co-activators (PGC)-1 α/β and the co-repressor receptor-interacting protein 140 (RIP140). Our data show additional regulation at the level of the mRNA, indicating both the importance of transcriptional regulation (Giguère 2008) and highlighting a novel target for the action of endocrine-disrupting chemicals in the body.

The activity of *ERRα* as a transcriptional regulator in T-cells has recently been described in detail (Michalek *et al.* 2011). T-cell activation and differentiation are energetically demanding processes and considerable metabolic reprogramming is required to enable the rapid proliferation and functional responsiveness of T effector (Teff) and regulator (Treg) cell subsets. Accordingly, *ERRα* appears to play a key role in Teff cell function through the induction of metabolic gene expression to programme mitochondria for aerobic glycolysis and to regulate glycolytic metabolism more generally. Interference in this process by BPA could in theory alter the balance of Teff and Treg cells, leading to immunotoxic effects. Although the full significance of *ERRα* expression in the

Jurkat lymphoblastic cell line that we have used here is not yet known, we were able to show a significant inhibition of cell proliferation following exposure to 100 nM BPA, consistent with this theory.

It would be of considerable interest to determine the effects of BPA on *ESRRA* expression and metabolic activity in a wider range of different cell types, especially in light of the associations previously reported between exposure to BPA and metabolic diseases (Melzer *et al.* 2012a, 2012b). *ERRα* is present in a wide variety of cell types and is highly expressed in cells with elevated metabolic demand such as heart, skeletal muscle and brown adipose tissue (Eichner & Giguère 2011). It is suggestive to note that BPA has shown affinity for the metabolic regulator *PPARγ* in a previous study (Kwintkiewicz *et al.* 2010) and in addition there is evidence of agonist interaction between *PPARγ* and *ERRα* (Willy *et al.* 2004) and *PPARγ* and *ERβ* (Foryst-Ludwig *et al.* 2008). More recently, Marmugi *et al.* (2012) have used a transcriptomic approach to show that exposure of male CD1 mice to low doses of BPA was able to influence *de novo* fatty acid synthesis through alterations in lipogenic genes. These results suggest that the role of BPA in influencing metabolic processes is certainly deserving of further attention.

BPA metabolism and excretion are believed to take place largely in the first 24 h after exposure, although there are studies suggesting this could take longer (Stahlhut *et al.* 2009, Sieli *et al.* 2011) or may be stored in human adipose tissue (Rubin & Soto 2009). After liver metabolism, glucuronide-conjugated BPA shows little or no affinity for nuclear receptors (Matthews *et al.* 2001), although a recent study has shown that BPA glucuronidated compound can be active *in vitro* through a rapid membrane-initiated signalling pathway (Viñas *et al.* 2013). In human biomonitoring studies, analyses carried out with blood, serum or plasma samples

Figure 3 Growth rate decrease (A and B) in Jurkat cells exposed to 100 nM BPA and 100 nM MBP after 48 and 72 h compared with control. Values are expressed as mean $\pm$ S.E.M. P values are reported above the bars when $P < 0.05$.

measured unconjugated BPA which is considered the portion of BPA not yet metabolised by the liver and so capable of being bioactivated. A recent review of 16 of these studies indicates that the range of blood/serum BPA concentrations spans between 0.5 and 2.5 ng/ml (Vandenberg *et al.* 2010). Given the range of concentrations chosen for our experiments (1–10 nM; 1 nM BPA=0.228 ng/ml), we could argue that this reflects very well the population internal exposure to unconjugated BPA. The same range of concentration has been taken as reference in other major experimental studies (Richter *et al.* 2007, Hugo *et al.* 2008), whilst a recent review of human toxicokinetic studies suggests that the circulating concentration may be lower than this (Doerge & Fisher 2011).

Oxidative cleavage of BPA to form the oestrogenically active metabolite MBP was first reported in rat liver and Okuda *et al.* (2010) reported that MBP was 500-fold more potent than BPA in inducing dose-dependent changes in the expression of *ESR1* and *ESR2* mRNA. In this study, we have shown a small but significant upregulation of ER β following exposure of cells to 100 nM MBP, which would agree with an enhanced oestrogenic activity of this compound. In addition, MBP showed a near identical pattern of upregulation of *ESRRA*, with a significant increase in expression seen at concentrations as low as 1 nM. The full significance of this metabolite in humans is not yet known. MBP is formed by recombination of the radical fragment of BPA, and its formation requires both microsomal and cytosolic fractions. A similar mechanism of metabolism has been reported in sewage sludge bacterial isolates (Spivack *et al.* 1994). Although the glucuronate/sulphate conjugates are the main metabolites excreted in urine, a comparison of phase 1 metabolism in human and rat liver microsomes has identified an additional oxidation product, BPA-catechol, as a minor metabolite (~10%) in both species, and BPA-catechol is certainly measurable in human urine (Ye *et al.* 2011). As BPA-catechol is also reported to possess oestrogenic activity, further investigation of the pathways of metabolism of BPA and related compounds in humans would seem to be a priority for future research.

Conclusions

We provide here a first report of the enhanced expression of two oestrogen responsive genes, *ESR2* (ER β) and *ESRRA* (ERR α) in human leukaemic T-cell lymphoblasts exposed to BPA at environmentally relevant concentrations. Significantly enhanced expression of both of these transcription factors *in vivo* has previously been associated with exposure to BPA in peripheral white blood cells in adult men (Melzer *et al.* 2011). As ERR α is a major control point for oxidative metabolism in many cell types, including T-cells, we also determined the effects of BPA and MBP exposure on cell growth and ATP content and showed a significant inhibition of cell proliferation at

nanomolar concentrations of both compounds. These results add to the weight of evidence for how BPA is able to exert significant biological effects at chronic low levels of exposure and identify a novel metabolic target for the action of endocrine disrupting chemicals.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

T Galloway, L Harries and D Melzer were responsible for study concept, design, interpretation of data and preparation of manuscript. R Cipelli contributed to study design, practical work, interpretation of data and preparation of manuscript. K Okuda and S Yoshihara prepared and supplied chemical metabolites and contributed towards preparation of the manuscript.

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