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Effects of Endocrine Disruptor Compounds, Alone or in Combination, on Human Macrophage-Like THP-1 Cell Response

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Abstract

The aim of the present study was to evaluate the immunological effects on human macrophages of four endocrine disruptor compounds (EDCs) using the differentiated human THP-1 cell line as a model. We studied first the effects of these EDCs, including Bisphenol A (BPA), di-ethylhexyl-phthalate (DEHP), dibutyl phthalate (DBP) and 4-tert-octylphenol (4-OP), either alone or in combination, on cytokine secretion, and phagocytosis. We then determined whether or not these effects were mediated by estrogen receptors via MAPK pathways. It was found that all four EDCs studied reduced strongly the phagocytosis of the differentiated THP-1 cells and that several of these EDCs disturbed also TNF-α, IL-1β and IL-8 cytokine secretions. Furthermore, relative to control treatment, decreased ERK 1/2 phosphorylation was always associated with EDCs treatments—either alone or in certain combinations (at 0.1 μM for each condition). Lastly, as treatments by an estrogen receptor antagonist suppressed the negative effects on ERK 1/2 phosphorylation observed in cells treated either alone with BPA, DEHP, 4-OP or with the combined treatment of BPA and DEHP, we suggested that estrogen receptor-dependent pathway is involved in mediating the effects of EDCs on human immune system. Altogether, these results advocate that EDCs can disturb human immune response at very low concentrations.

Introduction

The endocrine system is composed of glands that secrete hormones produced in the body to regulate the activity of cells or organs. Hormones control growth, development, and reproduction as well as the electrolyte composition of body fluids and the metabolism of body [1]. According to European Union, Endocrine Disruptors (ED) are exogenous substances that cause adverse health effects in an intact organism, or its progeny, secondary to changes in
Endocrine function. Endocrine Disruptor Compounds (EDCs) regroup a large variety of substances such as those used in multiple industrial processes, including solvents/lubricants (polychlorinated biphenyls (PCBs)), plastics (Bisphenol A (BPA) and phthalates), pesticides (Dichlorodiphenyltrichloroethane (DDT)) or pharmaceuticals (such as Estradiol (E2)) [2].

EDCs exert their effects differently according to the developmental stages of the affected organisms [2, 3]. Furthermore, a possible latency in the mode of action is another feature of EDCs and exposures during critical developmental period could represent “the basis for adult diseases” [4]. In fact, effects can occur with a lag after exposure [5]. For example, an exposure during early development can induce several effects in adulthood [3]. Another major aspect of exposure to EDCs is related to transgenerational epigenetic effects. For example, Anway et al. have observed a persistence of effects along generations after exposure of female rats to several EDCs such as vinclozolin or methoxychlor [6].

Several studies have shown that EDCs directly affect innate immune system. For instance, we observed altered immune response in human macrophages after 4-nonylphenol and diisononylphthalate exposure [7]. Ohnishi et al. showed also that some agrochemicals and resin-related chemicals could potentially inhibit macrophage function [8]. In addition, Roy et al. found that maternal exposure to BPA modulates innate immunity in adult offspring but not adaptive immune responses to influenza A virus infection in mice [9]. Another work by Watanabe et al. showed that BPA enhances neutrophilic maturation of the leukocytes through estrogen receptor-independent pathway, suggesting that BPA affects the innate immunity of mammals [10].

Epidemiological studies have also uncovered the possible link between EDCs and immune disorders; for example, the exposure to phthalates is associated with increased risks to develop allergies and asthma, even though the lack of accurate exposure information limits the interpretation [11].

Since only a rather limited number of works have been devoted to study the effects of EDCs on immune system and the fact that in the human environment EDCs are present as mixtures, we initiated the present work to better understand the direct individual effects of four EDCs on human immune cells, studying these EDCs alone as well as in combination under a wide concentration range.

BPA is an organic compound produced in industrial scale principally for the production of polycarbonate plastics and epoxy resins [12]. These compounds are thus found in a wide range of products such as plastic bottles, papers, food packaging, paints, flaming-retardants compounds [13]. Many previous studies have suggested that BPA has endocrine disruptor effects. For example, in human BPA appears to impair testosterone production in fetal testis and in rodents fetal and perinatal exposures to environmentally relevant doses of BPA can adversely affect the physiological function of endocrine pancreas, mammary gland and reproductive tract [14]. BPA also disturbs the biology of immune cells and human exposure to BPA seems to play a significant role in the initiation or the enhancement of inflammatory response [15].

Phthalate esters constitute another class of EDCs used often to increase the flexibility and workability of polymers [16]. These EDCs can disturb male reproductive system and may be associated to the malformations of the epididymis and vas deferens, and to cryptorchidism) [17, 18]. Two of the five most used phthalates esters are di-(2-ethylhexyl) phthalates (DEHP) and dibutyl phthalate (DBP) [19]. DEHP and DBP are used mainly in PVC (PolyVinyl Chloride)-containing products (such as toys, food packaging, shower curtain, clothes, medical equipments, cars accessories) but are also used in inks, solvents, perfumes or nail polish [19–21].

Octylphenol is an organic compound used mainly in the synthesis of resins and detergents [22]; it is thus used for the fabrication of paper, textiles, pesticides, personal care products etc
This compound is an estrogenic disruptor and is often detectable in environments such as rivers, wastewater effluents or potable water [2, 22, 23]. Since these chemicals are widely used in industry, human exposure to these EDCs is ubiquitous, organisms are constantly exposed to complex mixtures of these EDCs which simultaneously affect different organ systems (endocrine or other systems), making it thus rather difficult to study the specific endocrine disturbances produced by individual agents [24]. The aim of this study is to determine the effects of these four EDCs, alone and in combinations, on specific immune system related functions, including phagocytosis capacity and cytokine secretion. We investigated possible differences in MAPK (Mitogen-Activated Protein Kinase) activation caused by the EDCs and determined whether such differences were mediated by their effects on estrogen receptor to address the molecular mechanisms of these chemicals on immune targets.

We used as a model for our experimentations the THP-1 monocytic cell line after differentiation with phorbol-12-myristate-13-acetate (PMA). THP-1 cells were isolated from the blood of a one-year human boy with an acute monocytic leukemia. This myeloid monocyte human cell line can be used after differentiation as a macrophage model [7, 25, 26]. THP-1 retains the capacity of IL-1 secretion and phagocytosis [26, 27].

In the present work, we showed that EDCs are able to affect some immune response parameters in macrophage-like human THP-1 cells through the involvement of Estrogen Receptor-dependent ERK1/2 phosphorylation.

Materials and Methods

Chemicals

Lipopolysaccharides (LPS) from Escherichia coli 0111:B4, phorbol 12-myristate 13-acetate (PMA), fetal bovine serum (FBS), Bisphenol A, di-ethylhexyl-phthalate, dibutylphthalate, 4-tert-octylphenol and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma (Saint Quentin en Fallavier, France). RPMI medium, L-glutamine (200 mM) and penicillin-streptomycin solution (10,000 U/ml, 10,000 µg/ml) were purchased from Invitrogen (Cergy Pontoise, France). Structures of EDCs tested in the present work are shown in S1 Fig.

Cell culture, differentiation and exposure of THP-1 cells to EDCs

THP-1 cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 50 U/mL penicillin, and 50 g/mL streptomycin (defined as culture medium afterwards) in a humidified 5% CO₂ atmosphere. Medium was changed every 3 days to ensure constant cell growth [27].

Cells were differentiated into adherent macrophage-like cells by culturing with 5 ng/mL PMA for 48 h prior to experiments as proposed by Park and colleagues [24]. Differentiated cells were exposed to EDCs alone or in combination (BPA, DBP, DEHP, 4-OP) each at 0.001, 0.1, 1 or 10 µM for 24 hours. Cells were then stimulated by LPS at 10 ng/ml for 24 hours before studying cell viability, phagocytosis capacity, cytokine secretion and MAP kinase activity.

Cell viability

Cytotoxicity of EDCs on cells were assessed by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay. MTT assay is a viability test based on the ability of only viable cells to reduce MTT (yellow in solution) into formazan (dark blue product) [28, 29]. After
incubation of differentiated THP-1 cells with EDCs, alone or in combination (0.001, 0.1, 1, 10 µM), or with DMSO vehicle (0.1% final concentration in culture medium) in 96-well plates for 24 h, cells were washed twice with medium and incubated during 4 h at 37°C with 100 µL of 5 mg MTT/mL resuspended in PBS. Resulting crystals were dissolved by adding to the wells an equal volume of SDS/DMF extraction buffer (20% Sodium Dodecyl Sulfate (w/v) and N,N-dimethylformamide; v/v). Finally, absorbance was measured at 570 nm using a microplate spectrophotometer (BioTek PowerWave XS; VWR, Strasbourg, France). The cell viability value was calculated as a percentage of the absorbance obtained from non-exposed (vehicle alone) cells.

Phagocytosis assay

THP-1 cells were differentiated into macrophages as described above and grown on polylsine-coated glass coverslips (1,000,000 cells per well in twelve-well plates). Cells were then exposed to EDCs alone or in combination (0.001, 0.1, 1, 10 µM) or to DMSO vehicle (0.1% final concentration in culture medium) for 24 h. Cells were washed with medium and incubated with 1 µm-diameter fluorescein isothiocyanate (FITC)-latex beads (L 1030; Sigma-Aldrich) dispersed in culture medium during 6 h (final ratio of 1:50 (cell: beads)). Cells were then rinsed with PBS and subsequently fixed with 4% paraformaldehyde (w/v) for 10 min. After extensive washing with PBS, coverslips were mounted in a water-soluble and non-fluorescent mounting medium (Aqua-Poly/Mount, Tebu-bio). Three hundred cells for each exposure condition were observed and phagocytosis index was calculated as follows: (number of cells ingesting at least one bead 100) /number of total cells. All cultures for each set of measurements were done in triplicate.

Confocal Laser Scanning Microscopy (CLSM)

Phagocytosis of FITC-latex beads by differentiated THP-1 cells were observed with a laser scanning microscope (LSM 510; Carl Zeiss, Thornwood, NY) equipped with a Plan-Apochromat 63 X oil immersion lens (numerical aperture 1.4) or a C-Apochromat 40 X lens (numerical aperture 1.2).

Western blotting

Before incubation under different experimental conditions, cells were treated or not with ICI-182780 (ICI) (1 µM) dissolved in culture medium for 15 minutes. Then, cells were washed with ice-cold phosphate-buffered saline (PBS) and harvested in lysis buffer [30 mM HEPES, pH 7.6, 30 mM NaCl, 1% Nonidet P-40 (vol/vol), 10% glycerol (vol/vol), 50 mM NaF, 10 mM Na pyrophosphate] supplemented with protease inhibitors (Roche Diagnostics, Indianapolis, IN) and 5 mM Na orthovanadate. Cell lysates were cleared by centrifugation at 14,000 g for 5 min at 4°C. Proteins in total lysates were assayed before SDS-PAGE, using the BCA protein assay kit (Pierce Chemical, Rockford, IL) with bovine serum albumin as a standard. Protein aliquots (20 µg) were applied to a 12% SDS-PAGE. After transfer onto nitrocellulose membrane (Whatman, Maidstone, United Kingdom), blots were blocked overnight with 4% Bovine Serum Albumin (BSA) in Tris-buffer saline, 0.1% Tween 20 and then incubated for 2 h with primary antibody: mouse anti-estrogen receptor alpha antibody (1:50 dilution, clone 6F11; Abcam), mouse anti-phosphorylated ERK1/2 antibody (1:2000 dilution, reference 9106; Cell Signaling Technology), rabbit anti-ERK total antibody (1:5000 dilution, reference 9216; Cell Signaling Technology) or mouse anti-beta actin antibodies (1:16000 dilution, reference 3700; Cell Signaling Technology). The membranes were then washed and incubated with secondary antibody for 1h: anti mouse IgG HRP-linked antibody (1:2000 dilution, reference 7076; Cell Signaling
Technology) or anti rabbit IgG HRP-linked antibody (1:2000 dilution, reference 7074, Cell Signaling Technology).

In some experiments, membranes were stripped of antibody (Restore Western blot stripping buffer; Pierce Chemical), and reprobed with a different one. The bands were visualized using a substrate kit (Supersignal West Dura; Pierce Chemical), according to the manufacturer's instructions, and visualized using ChemiDoc (Bio-Rad). Quantitative results were obtained by using Quantity One software (Bio-Rad).

Quantitative measurements of cytokines
TNF-α, IL-1β and IL-8 levels in the isolated media samples were immediately quantified using ELISA kits (BD Biosciences, Le Pont de Claix, France).

Statistical methods
Results are expressed as means ± SD. The significance of differences between groups was determined using SIGMA STAT v.3.5 by one-way ANOVA and Tukey’s test. A significant difference between two values was accepted at p < 0.05.

Results
Effect of EDCs on THP-1 cell viability
We first checked whether EDCs could affect cell viability within our experimental conditions. No significant alteration in PMA-differentiated THP-1 cell viability was observed after 24 h of exposure to selected EDCs alone or in combination whatever the conditions (BPA, DBP, DEHP, 4-OP, BPA+DEHP, BPA+DBP and 4-OP+DEHP at 0.001–10 μM) in comparison to control (DMSO vehicle, 0.1% final concentration in culture medium). THP-1 cells exposure to these molecules alone or in combination induced a decrease in cell viability always lower than 10% (data not shown).

Furthermore, when LPS solution (10 ng/ml) was provided to differentiated THP-1 cells during a subsequent 24 h-incubation, there was only a slight unsignificant decrease in cell viability (corresponding to less than 10%) whatever the previous endocrine disruptor exposure (data not shown).

Effect of EDCs on differentiated THP-1 cells phagocytosis capacity
The ability of differentiated THP-1 cells to phagocytize FITC-latex beads was evaluated by analyzing by CLSM THP-1 cells exposed or not to EDCs tested alone or in combination (respectively Fig 1A and 1B). Non-exposed cells showed a phagocytosis capacity of 82% (data not shown).

A concentration-dependent BPA-mediated inhibition of the phagocytosis capacity was observed, with 26.6, 20.1, 44.7 and 59% reduction in comparison to control (DMSO vehicle, 0.1% final concentration in culture medium) after exposures to this compound at respectively 0.001, 0.1, 1 and 10 μM (Fig 1C). These inhibitory effects were found to be significant from 0.001μM of BPA.

DEHP had no effect on phagocytosis capacity of differentiated THP-1 cells except after an exposure at 10 μM which induced a decrease of 24.9% in comparison to control.

A combination of BPA and DEHP induced significant decreases of 54.4 and 52.2% respectively at 1 and 10 μM.

THP-1 cells exposure to DBP reduced their phagocytosis capacity. These significant reductions were respectively 27.8, 33.3, 39.1 and 40.4% in comparison to control for 0.001, 0.1, 1 and
The combination of BPA and DBP (0.001, 0.1, 1 and 10 μM) induced significant decreases in phagocytosis capacity of respectively 16.8, 27.7, 53.4, 52.2% in comparison to control. DBP (0.1, 1 and 10 μM) also induced a significant increase in TNF-α secretion respectively 20.2, 29.9 and 33.7% higher than control. The other xenobiotics, alone or in combination (DEHP, 4-OP, BPA+DBP, BPA+DEHP and 4-OP+DEHP), did not significantly modify TNF-α secretion level whatever the concentrations in comparison to control.

**Effects of EDCs on TNF-α secretion by differentiated THP-1 cells**

We next evaluated the effects of EDCs on cytokine secretion in PMA-differentiated THP-1 cells. The basal level of TNF-α secretion is 1952 ± 127 pg/mL which is higher than the detection limit (2 pg/mL). Exposure of differentiated THP-1 cells to BPA (0.1, 1 and 10 μM) significantly induced a level of TNF-α secretion which was respectively 28.1, 25.4 and 38.6% higher in comparison to control (cells in culture medium treated with LPS and DMSO vehicle, 0.1% final concentration) (Fig 2). DBP (0.1, 1 and 10 μM) also induced a significant increase in TNF-α secretion respectively 20.2, 29.9 and 33.7% higher than control. The other xenobiotics, alone or in combination (DEHP, 4-OP, BPA+DBP, BPA+DEHP and 4-OP+DEHP), did not significantly modify TNF-α secretion level whatever the concentrations in comparison to control level (Fig 2).

**Effects of EDCs on IL-1β secretion by differentiated THP-1 cells**

The basal level of IL-1β secretion is 191 ± 14 pg/mL which is higher than the detection limit (0.8 pg/mL). BPA (0.1, 1 and 10 μM) induced IL-1β level in the medium which was respectively
17.6, 21.1 and 28.7% higher than the level obtained for control cells (cells in culture medium treated with LPS and DMSO vehicle, 0.1% final concentration). No significant modification of IL-1β secretion was observed with the other EDCs, whatever the concentrations and exposure conditions (EDCs alone or in combination) (Fig 3).

Effects of EDCs on IL-8 secretion by differentiated THP-1 cells

The basal level of IL-8 secretion is 393 ± 25 pg/mL which is higher than the detection limit (0.8 pg/mL). BPA (0.001, 0.1, 1 and 10 μM) induced IL-8 secretion in the medium to a level which was respectively 12.2, 27.1, 21.4 and 39.8% higher than the level obtained for control cells (cells in culture medium treated with LPS and DMSO vehicle, 0.1% final concentration). No significant modification in the secretion of IL-8 was observed with the other tested EDCs whatever the concentrations and exposure conditions (EDCs alone or in combination) (Fig 4).

Effects of EDCs on estrogen receptor expression and signaling

In order to determine if the effects of EDCs are mediated through estrogen receptor signaling, we investigated some functional aspects of these proteins expressed in differentiated THP-1 cells. For this purpose, we evaluated the effects of cell exposures to tested pollutants (BPA, DEHP, BPA+DEHP, DBP, BPA+DBP 4-OP and 4-OP+DEHP) on estrogen receptor alpha (ERα) expression and on activation level of one of the most important kinase in its signaling pathway i.e. ERK 1/2 MAPK. Protein expression level was studied by Western blot analysis of cells extracts from untreated and 0.1 μM EDC-treated THP-1 cells using antibodies directed against ERα, as well as specific antibodies directed towards the phosphorylated form of ERK 1/2 (Fig 5).

All treatments with selected EDCs (alone or in combination) but also with the ER antagonist ICI-182780 (ICI) did not affect ERα expression level in differentiated THP-1 cells in comparison to untreated cells (Fig 5A). EDCs alone or in combination induced a significant decrease in
ERK 1/2 phosphorylation in comparison to control cells (DMSO vehicle, 0.1% final concentration in culture medium) (Fig 5B). These ERK 1/2 phosphorylation levels were about 38.2, 35.3, 58.1, 56.5, 69.6, 30.8 and 27%, lower than in control cells after treatments with respectively BPA, DEHP, BPA+DEHP, DBP, BPA+DBP, 4-OP and 4-OP+DEHP. No significant...

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Fig 3. Effect of EDCs alone and in combination on the secretion of IL-1β by differentiated THP-1 cells. Differentiated THP-1 cells were exposed to different concentrations of BPA or DEHP or DBP or 4-OP or a combination of both for 24 hours. Culture medium was replaced by fresh medium containing 10 ng/mL of LPS for a subsequent 24 hours of incubation. IL-1β concentrations were determined using a sandwich ELISA test. Values are means ± SD (N = 3). Each experiment has been performed three times and in triplicate. a: vs control (Tukey’s test, p<0.05); b: vs combination (One-way ANOVA, Tukey post test, p<0.05).

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ERK 1/2 phosphorylation in comparison to control cells (DMSO vehicle, 0.1% final concentration in culture medium) (Fig 5B). These ERK 1/2 phosphorylation levels were about 38.2, 35.3, 58.1, 56.5, 69.6, 30.8 and 27%, lower than in control cells after treatments with respectively BPA, DEHP, BPA+DEHP, DBP, BPA+DBP, 4-OP and 4-OP+DEHP. No significant...

Fig 4. Effect of EDCs alone and in combination on the secretion of IL-8 by differentiated THP-1 cells. Differentiated THP-1 cells were exposed to different concentrations of BPA, DEHP, DBP, 4-OP or a combination of both for 24 hours. Culture medium was replaced by fresh medium containing 10 ng/mL of LPS for a subsequent 24 hours of incubation. IL-8 concentrations were determined using a sandwich ELISA test. Values are means ± SD (N = 3). Each experiment has been performed three times and in triplicate. a: vs control (Tukey’s test, p<0.05); b: vs combination (One-way ANOVA, Tukey post test, p<0.05).

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modification of the P-ERK/ERK ratio was observed in cells treated with the receptor antagonist 
ICI. Finally, ICI treatment with BPA, DEHP, (BPA+DEHP), (BPA+DBP) and 4-OP induced 
an increase in relative ERK 1/2 phosphorylation level in comparison with cells exposed to cor-
responding EDCs (Fig 5B). This last result suggests that this estrogen receptor antagonist is 
able to reduce the effects of BPA, DEHP and 4-OP on ERK 1/2 phosphorylation.

Discussion
The endocrine disruptors selected in the present study are xenoestrogens which can be found 
in the environment and can induce adverse effects on endocrine system of several organisms 
including human. Nevertheless, EDCs are also able to disturb other systems and induce various 
effects such as obesity, behavior change and cancer [30–32]. Since EDCs have the ability to 
affect systems other than the endocrine system, we assess in the present work direct effects of 
bisphenol A, dibutyl phthalate, di-ethylhexyl-phthalate and 4-tert-octylphenol-ubiquitous 
chemical species present in many consumers products- on immune human system, using 
THP-1 cells. To this end, we exposed differentiated THP-1 cells to these EDCs in order to gain 
further insights into the mechanism of toxicity of these chemicals on macrophages and to
better define the potential role of macrophages in the secretion of pro-inflammatory cytokines in response to EDCs. THP-1 cell line, used in the present study, is considered as a macrophage-like model [25, 33–35].

After exposure of differentiated THP-1 cells to selected EDCs alone or in combination (0, 0.001, 0.1, 1 and 10 μM) during 24 h, no significant effect on cell viability was observed whatever the conditions (BPA, DBP, DEHP, 4-OP, BPA+DEHP, BPA+DBP and 4-OP+DEHP). These results are in accordance with those described in previous studies which showed in several cell models that BPA, DBP, DEHP and 4-OP do not alter cell viability at equivalent or higher concentrations than those tested in the present work [36–38]. We thus were able to evaluate the effects of these compounds on human immune response in these experimental conditions. All four EDCs were tested in a range of concentrations between 0.001 and 10 μM, corresponding to 0.228 μg/L—2.28 mg/L for BPA, 0.39 μg/L—3.9 mg/L for DEHP, 0.278 μg/L—2.78 mg/L for DBP, and 0.206 μg/L—2.06 mg/L for 4-OP. Average plasmatic concentrations reported in the literature for these EDCs are all included within these ranges of concentrations. These values are 0.460 and 0.548 μg/L for BPA [39,40], 218 μg/L for DEHP [41], 35.1 and 135.4 μg/L for DBP [41, 42] and 13.8 to 221 μg/L for 4-OP [43]. Some tested concentrations were also higher than the average plasmatic concentrations but remained lower than those tested in previous studies for BPA [44, 45], DEHP [46, 47], DBP [48] and 4-OP [49].

Phagocytosis capacity of THP-1 cells was investigated by evaluating the ability of differentiated THP-1 cells to phagocytize fluorescent latex beads after exposure to selected EDCs (0, 0.001, 0.1, 1 and 10 μM) using CLSM.

Exposure to BPA or DBP alone induced a reduction of phagocytosis, and in a concentration-dependent manner for BPA. The combination of BPA and DBP provoked also a concentration-dependent inhibition of this parameter without any combined effects (in comparison with BPA or DBP alone). Unlike exposures to DEHP or 4-OP alone and to a combination of BPA+DEHP, which altered phagocytosis capacity but not in concentration-dependent manner, the simultaneous exposure to DEHP and 4-OP induced a concentration-dependent reduction of phagocytosis capacity with additive effect of this mixture for the highest concentration. To the best of our knowledge, only few studies have already shown the EDCs capacity to reduce phagocytosis but never with such low exposure concentrations (notably 1 nM and 100 nM in the present work). For example, in a previous work, Bennasroune et al. demonstrated an alteration of differentiated THP-1 phagocytosis after exposure to Diisononylphthalate and 4-Nonylphenol, but with higher exposure concentrations (0.2–10 μM) and without any combined effects [7]. The results of the present work confirm those of this previous study which suggested that mixtures of EDCs did not provoke any synergistic effect on this immune system parameter under these experimental conditions. Two other studies using aquatic organism models leaded by Canesi et al. and Cabas et al. showed that estrogenic chemicals are able to alter the phagocytic activity of immunocytes (hemocytes) [50, 51]. Even if the models were different, their studies are in accordance with the present work indicating that EDCs can modify the immune response. Thus, it is important to note that relatively few studies have shown that EDCs induced a diminution of phagocytosis capacity by cells from the human immune system at biological relevant concentrations. The recognition, internalization, and degradation of a pathogen are necessary for normal clearance of an infection. Dysregulation in these processes caused by these compounds can be detrimental to the host defense and human health.

Cytokines are involved in several physiological processes and are especially important for regulating inflammatory and immune (innate and adaptive) responses [52]. As cytokines have an important role in initiating responses once a pathogen penetrates the host [53–55], a few research teams have considered that it is necessary to better understand the effects of EDCs or hormones and their mixtures on the inflammatory response of several organisms. For instance,
Jin et al. have shown that the mRNA levels of different cytokines such as TNF-α, IFN, or IL-1β were affected in zebrafish when exposed to several EDCs such as nonylphenol or to hormone such as 17β-estradiol. Other previous reports showed that EDCs are able to alter the inflammatory response and host defense system against pathogen in models such as mouse macrophage cell line [56]. Therefore, we investigated potential direct effects of several EDCs alone or in combination (BPA, DBP, DEHP, 4-OP, BPA+DEHP, BPA+DBP and 4-OP+DEHP) on LPS-induced cytokine secretion by differentiated THP-1 cells in order to determine the pro-inflammatory effects of these xenobiotics.

We determined LPS-induced IL-1β, IL-8 and TNF-α cytokine secretion after cell exposures to EDCs alone or in combination. IL-1β is described as a pro-inflammatory and "alarm" cytokine which is rapidly produced by macrophages in response to inflammatory stimuli and is able to induce the expression of several genes and the synthesis of proteins that in turn, induce inflammation [57]. IL-8, also called CXL8, is a key mediator in neutrophil-mediated acute inflammation due to its chemotactic activity. Several types of cells can produce a large amount of IL-8/CXL8 in response to a wide variety of stimuli, including proinflammatory cytokines, microbes and environmental changes [58]. Regarding the effects of these xenobiotics on IL-1β secretion, exposures to BPA from 0.1 to 10 μM increased the LPS-induced stimulation of this cytokine. Moreover, BPA increased LPS-induced IL-8 secretion in a concentration-dependent manner but none of the other tested EDCs induced a modification in the secretion of this chemokine. The present study suggests that EDCs such as BPA can affect pro-inflammatory cytokine secretion by differentiated THP-1 macrophage cells. Indeed, to the best of our knowledge, no direct effect of BPA has been previously reported on IL-8 secretion by human macrophage cells. This finding should be examined more closely using biomarkers of cellular damages and complementary assay techniques in order to establish a causal link between BPA exposure and modification of IL-8 and IL-1β secretions to better understand the mechanisms involved in the direct inflammatory effects of this compound. TNF-α, a pro-inflammatory cytokine, is a key mediator of inflammation [59]. Concerning the effects of tested EDCs on TNF-α secretion, we observed that only exposures to 0.1 to 10 μM of BPA and DBP alone induced increases in the secretion of this cytokine. Our results are in accordance with previous studies which described that cytokine secretion can be modified after exposure to EDCs. Thus, Kuan et al. showed an increase of TNF-α secretion of RAW264.7 mouse macrophage cell line after exposure to bisphenol A-glycidyl-methacrylate [60]. Dysregulation of TNF-α secretion by macrophages at local disease sites is associated with development of inflammatory diseases [61]. To our knowledge, our last result suggests for the first time that BPA or DBP may affect microbes-induced inflammation by the alteration of this response, after exposures to very low and biologically relevant concentrations. Surprisingly, no significant modification of TNF-α secretion level was observed after exposures to a combination of BPA and DBP. These results suggested that BPA and DBP effects may be antagonistic in our experimental conditions. To our knowledge, no similar effects of EDCs has been shown on TNF-α secretion. However, in other cell models, it has been described that a mixture of estrogenic chemicals such as 17β-estradiol, genistein and o,p'-DDT exerted an antagonistic activity in an ERα gene reporter system in human breast cancer MCF-7 cells [62]. Futhermore, in another study, the effects of 17β-estradiol, 17α-ethinylestradiol, genistein, bisphenol A, 4-nonylphenol and 4-tert-octylphenol on the proliferation of estrogen-dependent MCF-7 cells were measured by Rajapakse et al. who showed that 4-nonylphenol and 4-tert-octylphenol appeared to be associated with an antagonistic activity which could be explained by differential activation of drug-metabolizing enzymes (e.g., cytochrome P450) or efflux pumps [63].

In order to evaluate if the effects induced by selected EDCs are mediated through estrogen receptor alpha signaling pathway, we investigated the contribution of these xenobiotics on ERα...
protein expression and on the phosphorylation level of one of the major kinases of this pathway i.e. ERK 1/2 MAPK. We observed that ERα protein expression was not altered at the tested EDC concentrations, while a decrease in ERK 1/2 phosphorylation level was observed. These effects on ERK 1/2 phosphorylation level were reduced once cells were treated with the ERα antagonist ICI-182780 before exposures to BPA, DEHP, 4-OP or to combinations of BPA and DEHP or BPA and DBP, suggesting that these EDCs mediate their effects at least in part through ERα. Our results are in accordance with previous studies suggesting the involvement of EDCs in estrogen receptor signaling pathway. For example, Yoshitake et al. showed that some EDCs (such as BPA and several alkylphenols) suppressed LPS-induced nitric oxide production in mouse macrophage cell through an ERα dependent pathway [56]. Another study indicated that exposure of LPS-stimulated THP-1 derived macrophage to 4-nonylphenol and diisonylphthalate (at 2 μM i.e. higher concentrations than those tested in the present work) altered phosphorylation level of ERK 1/2 through an ERα dependent pathway, without modification of ERα protein expression [7].

Previous studies using several cell or animal models support the involvement of EDCs in the dysregulation of immune homeostasis, but the mechanisms of action remain unclear. The novel effects observed in the present work in our experimental conditions (including notably biological relevant concentrations) could be explained by different processes such as epigenetics effects. For example, the in utero and neonatal exposure to BPA and/or phthalates (such as DBP and DEHP) can be associated with DNA hypermethylation/hypomethylation, histone modifications and expression of non-coding RNAs. These epigenetic markers can alter gene expression that may persist throughout a lifetime and these changes will result in adverse health effects such as immune disorders [64]. Another study by Hung et al. suggests that Nonylphenol and 4-OP may have functional effects on the response of circulating myeloid dendritic cells via, in part, the ER, MKK3/6-p38 MAPK signaling pathway, and histone modifications, with subsequent influence on the T-cell cytokine responses such as TNF-α secretion [65]. Thus, epigenetic regulation could partially explain the several modulations of immune response parameters observed in our study after exposures of differentiated THP-1 cells to EDCs.

In conclusion, the present study showed that the in vitro exposure of differentiated THP-1 derived macrophages to selected EDCs disturbs some immune response parameters such as phagocytosis capacity and pro-inflammatory cytokine secretion, by directly affecting the immune cell response. Moreover, these EDCs have the capacity to modulate ERK 1/2 phosphorylation level, which is mediated through estrogen receptor alpha for 4-OP, BPA, DEHP and the mixture of these two last xenobiotics. It would be interesting to confirm these results using rats as an in vivo model. It would be also of interest to identify the metabolites of the tested compounds in the present work in order to evaluate the contribution of these metabolites on cell immune response, and to investigate if the effects observed are due to epigenetics regulation.

**Supporting Information**

S1 Fig. Chemical structures of the EDCs studied. (TIF)

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Author Contributions
Conceived and designed the experiments: ABen. Performed the experiments: ABen NC MD ABei SP. Analyzed the data: ABen NC EB JF. Contributed reagents/materials/analysis tools: ABen JF PLG. Wrote the paper: ABen NC EB.

References
1. Hiller-Sturmhöfel S, Bartke A. The endocrine system: an overview. Alcohol Health Res World. 1998; 22 (3):153–64. PMID: 15706790
2. Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. Endocr Rev. 2009; 30:293–342. doi: 10.1210/er.2009-0002 PMID: 19502515
3. Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: Implications for research and public health. Environ Health. 2012; 11:42. doi: 10.1186/1476-069X-11-42 PMID: 22715989
4. Barker DJP. The developmental origins of adult disease. J Am Coll Nutr. 2004; 23(6 Suppl):588S–595S. PMID: 15640511
5. Kortenkamp A, Martin O, Faust M, Evans R, McKinlay R, Orton F, et al. State of the art assessment of endocrine disruptors. European Commission-DG environment; 2011.
6. Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science. 2005; 308(5727):1466–9. PMID: 15933200
7. Bennasroune A, Rojas L, Foucaud L, Goulaouic S, Laval-Gilly P, Fickova M, et al. Effects of 4-nonylphenol and/or diisononylphthalate on THP-1 cells: impact of endocrine disruptors on human immune system parameters. Int J Immunopathol Pharmacol. 2012; 25(2):365–76. PMID: 22697068
8. Ohnishi T, Yoshida T, Igarashi A, Muroi M, Tanamoto K. Effects of possible endocrine disruptors on MyD88-independent TLR4 signaling. FEMS Immunol Med Microbiol. 2008; 52(2):293–5. doi: 10.1111/j.1574-695X.2007.00355.x PMID: 18177342
9. Roy A, Bauer SM, Lawrence BP. Developmental exposure to bisphenol A modulates innate but not adaptive immune responses to influenza A virus infection. PloS One. 2012; 7(6):e38448. doi: 10.1371/journal.pone.0038448 PMID: 22675563
10. Watanabe H, Adachi R, Kusui K, Hirayama A, Kasahara T, Suzuki K. Bisphenol A significantly enhances the neutrophilic differentiation of promyelocytic HL-60 cells. Int Immunopharmacol. 2003 Nov; 3(12):1601–8. PMID: 14555285
11. Kimber I, Dearman RJ. An assessment of the ability of phthalates to influence immune and allergic responses. Toxicology. 2005; 271(3):73–82. doi: 10.1016/j.tox.2010.03.020 PMID: 20371260
12. Asimakopoulos AG, Thomaidis NS, Koupparis MA. Recent trends in biomonitoring of bisphenol A, 4-t-octylphenol, and 4-nonylphenol. Toxicol Lett. 2012; 210(2):141–54. doi: 10.1016/j.toxlet.2011.07.032 PMID: 2188958
13. Ballesteros-Gómez A, Rubio S, Pérez-Bendito D. Analytical methods for the determination of bisphenol A in food. J Chromatogr A. 2009; 1216(3):449–69. doi: 10.1016/j.chroma.2008.06.037 PMID: 18635192
14. Fenichel P, Chevalier N, Brucker-Davis F. Bisphenol A: an endocrine and metabolic disruptor. Ann Endocrinol. 2013; 74(3):211–20.
15. Rogers JA, Metz L, Yong VW. Review: Endocrine disrupting chemicals and immune responses: A focus on bisphenol-A and its potential mechanisms. Mol Immunol. 2013; 53(4):421–30. doi: 10.1016/j.molimm.2012.09.013 PMID: 23123408
16. Van Wezel AP, van Vlaardingen P, Posthumus R, Crommentuijn GH, Sijm DT. Environmental risk limits for two phthalates, with special emphasis on endocrine disruptive properties. Ecotoxicol Environ Saf. 2000; 46(3):305–21. PMID: 10903828
17. Foster PM, Mylchreest E, Gaido KW, Sar M. Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update. 2001; 7(3):231–5. PMID: 11392369
18. Foster PM. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int J Androl. 2006; 29(1):140–147; discussion 181–185. PMID: 16102138
19. Müller AK, Nielsen E, Ladefoged O, Fædevøredirektoratet D. Human Exposure to Selected Phthalates in Denmark. Danish Veterinary and Food Administration; 2003.
20. Saint-Laurent L, Rahins M. Les Phthalates : état des connaissances sur la toxicité et l’exposition de la population générale. Québec: Institut National de Santé Publique; 2004.
21. Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, et al. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ Health Perspect. 2010; 118 (4):565–71. doi:10.1289/ehp.0901470 PMID: 20106747

22. Miller JPV, Staples CA. Review of the Potential Environmental and Human Health–Related Hazards and Risks from Long-Term Exposure to p-tert-Octylphenol. Hum Ecol Risk Assess Int J. 2005; 11 (2):319–51.

23. Vazquez-Duhalt R, Marquez-Rocha F, Ponce E, Licea AF, Viana MT. Nonylphenol, an integrated vision of a pollutant. Appl Ecol Environ Res. 2005; 4(1):1–25.

24. Ma L. Endocrine disruptors in female reproductive tract development and carcinogenesis. Trends Endocrinol Metab. 2009; 20(7):357–63. doi:10.1016/j.tem.2009.03.009 PMID: 19709900

25. Auwerx J. The human leukemia cell line, THP-1: a multifaceted model for the study of monocyte-macrophage differentiation. Experientia. 1991; 47(1):22–31. PMID: 1999239

26. Goulaouic S, Foucaud L, Bennasroune A, Laval-Gilly P, Falla J. Effect of polycyclic aromatic hydrocarbons and carbon black particles on pro-inflammatory cytokine secretion: impact of PAH coating onto particles. J Immunotoxicol. 2008; 5(3):337–45. doi:10.1080/15476910802371016 PMID: 18830893

27. Tsuchiya S, Yamabe M, Yamaguchi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int J Cancer. 1980; 26(2):171–6. PMID: 6970727

28. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65(1–2):55–63. PMID: 6606682

29. Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol Methods. 1989; 119(2):203–10. doi:10.1016/0022-1759(89)90182-3 PMID: 2470825

30. Kaufman RH, Adam E, Hatch EE, Noller K, Herbst AL, Palmer JR, et al. Continued follow-up of pregnancy outcomes in diethylstilbestrol-exposed offspring. Obstet Gynecol. 2000; 96(4):493–9. doi:10.1016/s0029-7844(01)01082-1 PMID: 11004345

31. Valkusz Z, Nagyéri G, Radács M, Ocskó T, Hausinger P, László M, et al. Further analysis of behavioral and endocrine consequences of chronic exposure of male Wistar rats to subtoxic doses of endocrine disruptor chlorobenzenes. Physiol Behav. 2011; 103(5):421–30. doi:10.1016/j.physbeh.2011.03.016 PMID: 21419145

32. Hao C-J, Cheng X-J, Xia H-F, Ma X. The endocrine disruptor diethylstilbestrol induces adipocyte differentiation and promotes obesity in mice. Toxicol Appl Pharmacol. 2012; 263(1):102–10. doi:10.1016/j.taap.2012.06.003 PMID: 22710028

33. Abrikin M, Gobli AE, Huang R, Nilsson K, Hellman L. Human cell lines U-937, THP-1 and Mono Mac 6 represent relatively immature cells of the monocyte-macrophage cell lineage. Leukemia. 1994; 8(9):1579–84. PMID: 8090303

34. Leyva-Illades D, Cherla RP, Galindo CL, Chopra AK, Tesh VL. Global Transcriptional Response of Macrophage-Like THP-1 Cells to Shiga Toxin Type 1. Infect Immun. 2010; 78(6):2454–65. doi:10.1128/IAI.01341-09 PMID: 20351145

35. Grodzki ACG, Giulivi C, Lein PJ. Oxygen Tension Modulates Differentiation and Primary Macrophage Functions in the Human Monocytic THP-1 Cell Line. PLoS ONE. 2013; 8(1):e54926. doi:10.1371/journal.pone.0054926 PMID: 23355903

36. Benachour N, Moslemi S, Sipahutar H, Seralini G-E. Cytotoxic effects and aromatase inhibition by xenobiotic endocrine disrupters alone and in combination. Toxicol Appl Pharmacol. 2007; 222(2):129–40. PMID: 17593374

37. Shen O, Du G, Sun H, Wu W, Jiang Y, Song L, et al. Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. Toxicol Lett. 2009; 191(1):9–14. doi:10.1016/j.toxlet.2009.07.019 PMID: 19643168

38. Kim S-K, Kim B-K, Shim J-H, Gil J-E, Yoon Y-D, Kim J-H. Nonylphenol and Octylphenol-Induced Apoptosis in Human Embryonic Stem Cells Is Related to Fas-Fas Ligand Pathway. Toxicol Sci. 2006; 94(2):310–21. PMID: 16984955

39. Kosarac I, Kubwabo C, Lalonde K, Foster W. A novel method for the quantitative determination of free and conjugated bisphenol A in human maternal and umbilical cord blood serum using a two-step solid phase extraction and gas chromatography/tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2012; 898:90–4. doi:10.1016/j.jchromb.2012.04.023 PMID: 22580014

40. Kuroda N, Kinoshita Y, Sun Y, Wada M, Kishikawa N, Nakashima K, et al. Measurement of bisphenol A levels in human blood serum and ascitic fluid by HPLC using a fluorescent labeling reagent. J Pharm Biomed Anal. 2003; 30(6):1743–9. PMID: 12485715
41. Guo Z-Y, Gai P-P, Duan J, Zhai J-X, Zhao S-S, Wang S, et al. Simultaneous determination of phthalates and adipates in human serum using gas chromatography-mass spectrometry with solid-phase extraction. Biomed Chromatogr BMC. 2010; 24(10):1094–9.

42. Genuis SJ, Beesoon S, Lobo RA, Birkholz D. Human elimination of phthalate compounds: blood, urine, and sweat (BUS) study. ScientificWorldJournal. 2012; 2012:615068. doi: 10.1101/2012/615068 PMID: 23213291

43. Inoue K, Yanagisawa R, Koike E, Nishikawa M, Takano H. Repeated pulmonary exposure to single-walled carbon nanotubes exacerbates allergic inflammation of the airway: Possible role of oxidative stress. Free Radic Biol Med. 2010; 48(7):924–34. doi: 10.1016/j.freeradbiomed.2010.01.013 PMID: 20093178

44. Byun J-A, Heo Y, Kim Y-O, Pyo M-Y. Bisphenol A-induced downregulation of murine macrophage activities in vitro and ex vivo. Environ Toxicol Pharmacol. 2005 Jan; 19(1):19–24. doi: 10.1016/j.etap.2004.02.006 PMID: 21783458

45. Chen X, Xu B, Han X, Mao Z, Talbot P, Chen M, et al. Effect of bisphenol A on pluriptenogenic propensity of mouse embryonic stem cells and differentiation capacity in mouse embryoid bodies. Toxicol In Vitro. 2013 Dec; 27(8):2249–55. doi: 10.1016/j.tiv.2013.09.016 PMID: 24090929

46. Chen X, Qin Q, Zhang W, Zhang Y, Zheng H, Liu C, et al. Activation of the PI3K-akt-mTOR signaling pathway promotes DEHP-induced Hep3B cell proliferation. Food Chem Toxicol. 2013 Sep; 59:325–33. doi: 10.1016/j.fct.2013.06.016 PMID: 23793038

47. Glue C, Millner A, Bodtger U, Jinquan T, Poulsen LK. In vitro effects of monophthalates on cytokine expression in the monocytic cell line THP-1 and in peripheral blood mononuclear cells from allergic and non-allergic donors. Toxicol In Vitro. 2002 Dec; 16(6):857–62. PMID: 12423647

48. Li D, Hu Y, Shen X, Dai X, Han X. Combined effects of two environmental endocrine disruptors nonyl phenol and di-n-butyl phthalate on rat Sertoli cells in vitro. Reprod Toxicol. 2010 Nov; 30(3):438–45. doi: 10.1016/j.reprotox.2010.06.003 PMID: 20600820

49. Krüger T, Long M, Bonefeld-Jørgensen EC. Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. Toxicology. 2008 Apr 18; 246(2–3):112–23. doi: 10.1016/j.tox.2007.12.028 PMID: 18294747

50. Canesi L, Lorusso LC, Ciacci C, Bettì M, Rocchi M, Pojana G, et al. Immunomodulation of Mytilus hemocytes by individual estrogenic chemicals and environmentally relevant mixtures of estrogens: in vitro and in vivo studies. Aquat Toxicol Amst Neth. 2007; 81(1):36–44.

51. Cabas I, Liarte S, García-Alcázar A, Meseguer J, Mulero V, García-Ayala A. 17-α-Ethynylestradiol alters the immune response of the teleost gilthead seabream (Sparus aurata L.) both in vivo and in vitro. Dev Comp Immunol. 2012; 36(3):547–56. doi: 10.1016/j.dci.2011.09.011 PMID: 22035596

52. Al-Oufella H, Taleb S, Mallat Z, Tedgui A. Recent advances on the role of cytokines in atherosclerosis. Arterioscler Thromb Vasc Biol. 2011; 31(5):969–79. doi: 10.1161/ATVBAHA.110.207415 PMID: 21508343

53. Bayne CJ, Gerwick L. The acute phase response and innate immunity of fish. Dev Comp Immunol. 2001; 25(8–9):725–43. PMID: 11602193

54. Phelan PE, Pressley ME, Witten PE, Mellon MT, Blake S, Kim CH. Characterization of snakehead rhabdovirus infection in zebrafish (Danio rerio). J Virol. 2005; 79(3):1842–52. PMID: 15650208

55. Stockhammer OW, Zakrzewska A, Hegedûs Z, Stockhammer OW, Zakrzewska A, Hegedûs Z, Spaink HP, Meijer AH. Transcriptome profiling and functional analyses of the zebrafish embryonic innate immune response to Salmonella infection. J Immunol Baltim Md 1950. 2009; 182(9):4381–9. doi: 10.1111/j.1365-2958.2009.03891.x

56. Yoshitake J, Kato K, Yoshioka D, Sueishi Y, Sawa T, Akaite T, et al. Suppression of NO production and 8-nitroguanosine formation by phenol-containing endocrine-disrupting chemicals in LPS-stimulated macrophages: involvement of estrogen receptor-dependent or-independent pathways. Nitric Oxide Biol Chem Off J Nitric Oxide Soc. 2008; 18(3):223–8.

57. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood. 1991; 77(8):1627–52. PMID: 18266166

58. Mukaida N. Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. Am J Physiol Lung Cell Mol Physiol. 2003; 284(4):L566–577. PMID: 12618418

59. Gupta SC, Tyagi AK, Deshmukh-Taskar P, Hinojosa M, Prasad S, Aggarwal BB. Downregulation of tumor necrosis factor and other proinflammatory biomarkers by polyphenols. Arch Biochem Biophys. 2014; 559:91–9. doi: 10.1016/j.abb.2014.06.006 PMID: 24946050

60. Kuan Y-H, Li Y-C, Huang F-M, Chang Y-C. The upregulation of tumour necrosis factor-α and surface antigens expression on macrophages by bisphenol A-glycidyl-methacrylate. Int Endod J. 2012; 45(7):619–26. doi: 10.1111/j.1365-2958.2012.02017.x PMID: 22268514

61. Kopf M, Bachmann MF, Marsland BJ. Averting inflammation by targeting the cytokine environment. Nat Rev Drug Discov. 2010; 9(9):703–18. doi: 10.1038/nrd2805 PMID: 20811382
62. Charles GD, Gennings C, Zacharewski TR, Gollapudi BB, Carney EW. Assessment of interactions of diverse ternary mixtures in an estrogen receptor-alpha reporter assay. Toxicol Appl Pharmacol. 2002; 180(1):11–21. PMID: 11922773

63. Rajapakse N, Silva E, Scholze M, Kortenkamp A. Deviation from additivity with estrogenic mixtures containing 4-nonylphenol and 4-tert-octylphenol detected in the E-SCREEN assay. Environ Sci Technol. 2004; 38(23):6343–52. PMID: 15597891

64. Singh S, Li SS-L. Epigenetic effects of environmental chemicals bisphenol a and phthalates. Int J Mol Sci. 2012; 13(8):10143–53. doi: 10.3390/ijms130810143 PMID: 22949852

65. Hung C-H, Yang S-N, Kuo P-L, Chu Y-T, Chang H-W, Wei W-J, et al. Modulation of cytokine expression in human myeloid dendritic cells by environmental endocrine-disrupting chemicals involves epigenetic regulation. Environ Health Perspect. 2010 Jan; 118(1):67–72. doi: 10.1289/ehp.0901011 PMID: 20056579