12-20-2013

Secretion of interferon gamma from human immune cells is altered by exposure to tributyltin and dibutyltin

Shanieek Lawrence
Tennessee State University

Jacqueline Reid
Tennessee State University

Margaret Whalen
Tennessee State University

Recommended Citation

Lawrence, S., Reid, J. and Whalen, M. (2015), Secretion of interferon gamma from human immune cells is altered by exposure to tributyltin and dibutyltin. Environ. Toxicol., 30: 559-571. https://doi.org/10.1002/tox.21932

HHS Public Access

Author manuscript

Environ Toxicol. Author manuscript; available in PMC 2016 May 01.

Published in final edited form as:

Environ Toxicol. 2015 May ; 30(5): 559–571. doi:10.1002/tox.21932.

Secretion of Interferon gamma (IFNγ) from Human Immune Cells is Altered by Exposure to Tributyltin (TBT) and Dibutyltin (DBT)

Shanieek Lawrence[*], **Jacqueline Reid**[*], and **Margaret Whalen**

Department of Biological Sciences, Tennessee State University, Nashville, TN, 37209

[*]Department of Chemistry, Tennessee State University, Nashville, TN, 37209

Abstract

Tributyltin (TBT) and dibutyltin (DBT) are widespread environmental contaminants found in food, beverages, and human blood samples. Both of these butyltins (BTs) interfere with the ability of human natural killer (NK) cells to lyse target cells and also alter secretion of the pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) from human immune cells *in vitro*. The capacity of BTs to interfere with secretion of other pro-inflammatory cytokines has not been examined. Interferon gamma (IFNγ) is a modulator of adaptive and innate immune responses, playing an important role in overall immune competence. This study shows that both TBT and DBT alter secretion of IFNγ from human immune cells. Peripheral blood cell preparations that were increasingly reconstituted were used to determine if exposures to either TBT or DBT affected IFNγ secretion and how the makeup of the cell preparation influenced that effect. IFNγ secretion was examined after 24 h, 48 h and 6 day exposures to TBT (200- 2.5 nM) and DBT (5-0.05 μM) in highly enriched human NK cells, a monocyte-depleted preparation of PBMCs, and monocyte-containing PBMCs. Both BTs altered IFNγ secretion from NK cells at most of the conditions tested (either increasing or decreasing secretion). However, there was significant variability among donors as to the concentrations and time points that showed changes as well as the baseline secretion of IFNγ. The majority of donors showed an increase in IFNγ secretion in response to at least one concentration of TBT or DBT at a minimum of one length of exposure.

Keywords

NK cells; PBMCs; Tributyltin; Dibutyltin; Interferon gamma

INTRODUCTION

The use of butyltins (BTs) in various applications has led to very significant contamination of the environment by these compounds (Kimbrough, 1976; Tanabe et al. 1998; Loganathan et al., 2000; Roper, 1992; Gipperth, 2009; Takahashi et al., 1999; Kannan et al., 1995; Forsyth et al., 1992; 1997; Sadiki et al., 1996). Tributyltin (TBT) has been used as an ingredient in antifouling paints as well as an antifungal agent and biocide in textiles, paper production, industrial cooling waters, and wood preservation (Kimbrough, 1976; Laughlin

Correspondence: Margaret M. Whalen, Department of Chemistry, Tennessee State University, 3500 John A. Merritt Blvd., Nashville, TN 37209, mwhalen@tnstate.edu, Phone: 615-963-5247, Fax: 615-963-5326.

and Linden, 1985; Roper, 1992). Its use in antifouling paints has been banned since 2008 but it will continue to contaminate the environment for many years due to its chemical stability and other uses (Gipperth, 2009). TBT is found in food, especially fish, (Kannan et al., 1995; Takahashi et al., 1999) and also in human blood. Blood levels range as high as 261 nM (85 ng/mL) (Whalen et al., 1999; Kannan et al., 1999). Studies show that the exposure of mammals to TBT causes increased incidences of tumors (Wester et al., 1990), decreased NK cell function (Ghoneum et al., 1990), thymic atrophy and thymus dependent immunosuppression (Snoeij *et al.*, 1987, 1989; Vos et al. 1990). Dibutyltin (DBT) is used as a stabilizer in plastics (Roper, 1976) and as an anti-worming agent in some poultry (Epstein et al. 1991). DBT is also found in drinking water and other beverages due to leaching from PVC plastics used in their production and/or storage (Forsyth et al., 1992, 1997; Sadiki et al., 1996). Levels of DBT in human blood are as high as 300 nM (94 ng/mL) (Whalen et al., 1999; Kannan et al., 1999). Both TBT and DBT decrease lytic function, target-binding function, cell-surface protein expression, and cytolytic protein expression in human natural killer (NK) cells at levels that are in the range found in human blood (Whalen et al., 1999; Dudimah et al., 2007a, b; Whalen et al., 2002; Odman-Ghazi et al., 2003; Thomas et al., 2004; Catlin et al., 2005). These changes are accompanied by a rapid activation of a portion of the signaling pathway(s) that regulates NK lytic function (beginning with protein kinase C through mitogen-activated protein kinases (MAPKs) (Aluoch and Whalen, 2005; Aluoch et al., 2006; Aluoch et al., 2007; Odman-Ghazi et al., 2010; Abraha et al., 2010). Recently, we have shown that exposure to these BTs alters the secretion of the cytokine tumor necrosis factor alpha (TNFα) from human immune cells including NK cells (Hurt et al. 2013).

The functions of both innate and adaptive immune cells are regulated by the cytokine interferon gamma (IFNγ). It causes increased antigen presentation on macrophages by increasing their expression of MHC class I molecules and regulates T cell immune response through Th1 cells. It is also involved in the recruitment of innate immune cells to sites of infection or tumor (Zaidi and Merlino, 2011). IFNγ is an approximately 17kD protein, which is dimerized in its physiologically active form (Billau and Matthys, 2009). It is secreted by T cells, NK cells, and to a lesser extent by myeloid lineage cells such as macrophages (Billiau and Matthys, 2009; Darwich et al., 2008). IFNγ is a pro-inflammatory cytokine which has the ability to cause chronic inflammation, which has been shown to facilitate the development of certain cancers such as gastrointestinal cancers (Macarthur et al., 2004). Careful regulation of IFNγ is crucial in order to avoid loss of immune competency or the increased risks associated with chronic inflammation. IFNγ may behave both in a pro-tumorigenic as well as in an antitumor manner depending on the specific circumstances. Under circumstances where IFNγ increases tumor cell death by macrophages, T cells, and NK cells it will prevent cancer development. However, the stimulation of myeloid derived suppressor cell development by IFNγ may lead to cancer (Zaidi and Merlino, 2011).

Natural killer (NK) cells are capable of secreting IFNγ (Andoniou et al., 2008; Girart et al., 2007) and we have found that both TBT and DBT have dramatic effects on the ability of human NK cells to destroy tumor cells (Dudimah et el., 2007a, b) as well as their ability to secrete the pro-inflammatory cytokine TNFα (Hurt et al., 2013). In addition we showed that both BTs interfered with the secretion of TNFα from a preparation of peripheral blood

mononuclear cells (PBMCs) that had been depleted of monocytes (Hurt et al., 2013). Thus, there is interest in investigating whether either TBT or DBT has the ability to alter immune cell secretion of other pro-inflammatory cytokines including IFNγ.

The current study examined the effects of exposures to TBT or DBT on secretion of IFNγ from highly purified NK cells, monocyte-depleted PBMCs, and PBMCs. Examination of the effects of these compound on IFNγ secretion from increasingly reconstituted systems allowed us to determine whether there were differences in the effects of the BTs between the purified NK system and the more complex systems of immune cells, thus more closely approximating the physiological setting.

MATERIALS AND METHODS

Preparation of NK cells

NK cells were prepared from buffy coats (source leukocytes from healthy adult donors) purchased from Key Biologics, LLC (Memphis, TN). Highly purified NK cells were prepared using a rosetting procedure. RosetteSep human NK cell enrichment antibody cocktail (0.6–0.8 mL) (StemCell Technologies, Vancouver, British Columbia, Canada) was added to 45 mL of buffy coat. The mixture was incubated for 25 min at room temperature (~ 25º C). Seven (7)-8 mL of the mixture was layered onto 4 mL of Ficoll-Hypaque (1.077 g/mL) (MP Biomedicals, Irvine, CA) and centrifuged at 1200 g for 30–50 min. NK cells were collected and washed twice with phosphate buffered saline (PBS) pH 7.2 and stored in complete media (RPMI-1640 supplemented with 10% heat-inactivated bovine calf serum (BCS), 2 m*M L*-glutamine and 50 U penicillin G with 50 μg streptomycin/ml) at 1 million cells/mL at 37 °C and air/CO_2 , 19:1.

Preparation of monocyte- depleted PBMCs and PBMCs

Preparations of PBMCs were isolated from Leukocyte filters (PALL- RC2D) obtained from the Red Cross Blood Bank Facility (Nashville, TN) as described in Meyer et al., 2005. Leukocytes were retrieved from the filters by back-flushing them with elution medium (PBS containing 5 mM disodium EDTA and 2.5% [w/v] sucrose) and collecting the eluent. Eluent contained the leukocytes with red cell contamination. Eluent was layered onto Ficoll-Hypaque (1.077g/mL) and centrifuged as described above. Granulocytes and red cells pelleted at the bottom of the tube while the PBMCs floated on the Ficoll-Hypaque. PBMCs were collected and washed (250 g, 10 min.) with PBS. Cells were then suspended in complete medium which consisted of RPMI-1640 supplemented with 10% heat-inactivated BCS, 2 m*M L*-glutamine and 50 U penicillin G with 50 μg streptomycin/mL. This preparation constituted PBMCs. Monocyte-depleted PBMCs (10–20% $CD16^+$, 10–20 % $CD56^+$, 70–80% $CD3^+$, 3–5% $CD19^+$, 2–20% $CD14^+$) were prepared by incubating the cells in glass Petri dishes (150 X 15 mm) at 37 °C and air/CO_2 , 19:1 for 1 h .

Chemical preparation

TBT and DBT were purchased from (Sigma-Aldrich, St. Louis, MO). TBT was a neat standard, dissolved initially in deionized water to give a 1 mM solution. A DBT stock solution was prepared by dissolving DBT in dimethylsulfoxide (DMSO). Desired

concentrations of either TBT or DBT were prepared by dilution of the stock into complete media. The final concentration of DMSO for DBT exposures did not exceed 0.01%. Appropriate DMSO controls were run.

Cell treatments

NK cells, monocyte-depleted PBMCs, or PBMCs (at a concentration of 1.5 million cells/mL) were treated with TBT with appropriate control at concentrations of 2.5–200 nM for 24 h, 48 h, or 6 days. Cells were treated with DBT with appropriate control at concentrations of 0.05–5 μM for the same lengths of incubation as used with TBT. Following the incubations the cells were pelleted and the supernatants were collected and frozen at −70°C until assay.

Cell viability

Cell viability was assessed at the beginning and end of each exposure period. Viability was determined using the trypan blue exclusion method. Briefly, cells were mixed with trypan blue and counted using a hemocytometer. The total number of cells and the total number of live cells were determined for both control and treated cells to determine the percent viable cells. Results are shown in Tables 1 and 2.

IFNγ secretion assay

IFNγ levels were assessed using the OptEIA™ enzyme-linked immunosorbent assay (ELISA) human IFNγ kit (BD-Pharmingen, San Diego, CA). Capture antibody diluted in coating buffer was applied to the wells of a 96 well flat-bottom microwell plate specifically designed for ELISA (Fisher, St.Louis MO). The plate was incubated overnight at 4°C. Following the incubation, capture antibody was removed by washing the plate three times with wash buffer (PBS with 0.05% Tween-20). The wells were then treated with blocking buffer to prevent non-specific binding and the plate was sealed and incubated at room temperature for 1 h. Blocking buffer was removed with three washes, and cell supernatants and IFNγ standards were added to the plate, which was sealed and incubated at room temperature for 2 h. The plate was washed five times following the incubation with samples and standards and detection antibody was added then incubated for 1 h at room temperature. Detection antibody was removed by washing 7 times and a substrate solution was added to each well and incubated for 30 min at room temperature. The incubation with substrate was ended by addition of acid and the absorbance was measured at 450 nm on a Thermo Labsystems Multiskan MCC/340 plate reader (Fisher Scientific).

Statistical analysis

Statistical analysis of the data was carried out utilizing ANOVA and Student's t test. Data were initially compared within a given experimental setup by ANOVA. A significant ANOVA was followed by pair wise analysis of control versus exposed data using Student’s t test, a p value of less than 0.05 was considered significant.

RESULTS

Viability of NK cells, monocyte-depleted PBMCs, and PBMCs exposed to TBT

Table 1 shows the effects of TBT exposures (2.5–200 nM) on the viability of highly purified NK cells, monocyte-depleted PBMCs, and PBMCs. Exposure of NK cells to 2.5–200 nM TBT for 24 h had no effect on their viability as compared to the control. Exposure to these same concentrations for 48 h diminished viability by a small amount at the highest concentration of TBT (200 nM). NK cells exposed to 2.5–200 nM TBT for 6 days showed no change in viability as compared to controls. Like NK cells, monocyte-depleted PBMCs and PBMCs both showed no negative effects of a 24 h exposure to TBT at any concentration, but were slightly affected by the 200 nM TBT after 48 h and more significantly after 6 days. This indicated that there was not a generalized toxicity of the compound on cells due to TBT exposures.

Viability of NK cells, monocyte-depleted PBMCs, and PBMCs exposed to DBT

The effects of DBT exposures (0.05–5 μM) on the viability of highly purified NK cells, monocyte-depleted PBMCs, and PBMCs are shown in Table 2. NK cells exposed to 2.5 and 5 μM DBT for 24 h showed small but significant decreases in viability as compared to the control. All other DBT concentrations had no effect on viability at the 24 h exposure. 48 h exposures to 1, 2.5 and 5 μM DBT decreased NK viability by 18%, 30%, and 32%, respectively. Monocyte-depleted PBMCs and PBMCs also showed significant decreases in viability with exposures to the highest concentrations of DBT. But as with TBT, there were decreases in viability only at the highest concentrations, which increased slightly with length of exposure.

IFNγ secretion from NK cells exposed to TBT

Table 3 shows the effects of exposing highly purified NK cells to 0, 2.5, 5, 10, 25, 50, 100 and 200 nM TBT for 24 h, 48 h, and 6 days on IFNγ secretion from each of the donors tested (KB=Key Biologic buffy coat). There was very wide variation in the baseline secretion of IFNγ from one donor to the next at each of the time points tested. 24 h exposures were carried out with cells prepared from 7 individual donors and 6 of the 7 donors showed significant decreases in IFNγ when exposed to 200 nM TBT. Cells from 5 of the 7 donors showed an increase in IFNγ secretion with exposure to at least one TBT concentration. The concentrations that caused increases varied from one donor to the next. For instance, the cells from donor KB114 treated with 5, 10, 25,50 and 100 nM TBT showed significant increases of 2.8, 2.6, 3.9, 4.3, and 1.4 fold respectively, while the cells from donor KB113 showed an increase of 5.5 fold only at the 25 nM exposure. When NK cells from 4 individual donors were exposed to TBT for 48 h there was a significant decrease in IFNγ, as compared to control in cells from 3 of the 4 donors at the 200 and 100 nM concentrations. Increases in IFNγ secretion were seen in 2 of the 4 donors, while the other 2 donors only showed decreases (or no change) when exposed to TBT. NK cells exposed to TBT for 6 days all showed a significant decrease in IFNγ secretion at the 200 nM concentration. Cells from each of the 4 donors secreted higher levels of IFNγ when exposed to a minimum of one TBT concentration. The concentrations at which increases were seen again varied depending on the donor. Likewise the intensity of the increase varied from one

donor to the next as it did at 24 h and 48 h. Comparing donor KB146 to donor KB147, the 5, 10, 25, 50, and 100 nM concentrations of TBT each caused very significant increases (4, 8.5, 6.2, 13.8, and 4.5 fold, respectively) in secretion in cells from KB146, while only the 50 nM exposure to TBT caused an increase in cells from KB147 (4 fold). Figure 1A shows the effects at each of the time points for an individual donor (KB147).

IFNγ secretion from monocyte-depleted PBMCs exposed to TBT

The effects of exposures to TBT on secretion of IFNγ from monocyte-depleted PBMCs after 24 h, 48 h, and 6 d from each donor (F=filter obtained from the Red Cross) are shown in Table 4. As with the enriched NK cell preparation, there was wide variation in the baseline secretion of IFNγ from monocyte-depleted PBMCs. This preparation is largely NK cells and T cells. When monocyte-depleted PBMCs were exposed to TBT for 24 h there were statistically significant increases in IFNγ secretion induced by TBT for every donor at the 25 and 50 nM concentrations. Exposure to 200 nM TBT caused nearly complete inhibition of secretion in every donor. Figure 1B shows the effects of TBT exposures at each of the lengths of exposure for an individual donor (F142). The same pattern of increases in IFNγ secretion at 25 and 50 nM and a nearly complete block of secretion at the 200 nM exposure was seen at each of the other lengths of exposure for all donors.

IFNγ secretion from PBMCs exposed to TBT

The effects of exposing PBMCs from 4 individual donors to 0–200 nM TBT for 24 h, 48 h, and 6 d on the secretion of IFNγ are shown in Table 5 (F=filter obtained from the Red Cross). Again, there was variation among donors in the baseline secretion of IFNγ (donor F116 was at the high end and donor F120 at the low end). PBMCs from all 4 donors showed significant decreases in secretion after exposure to 200 nM TBT for 24 h. Cells from 2 of the 4 donors (F116 and F117) were either unchanged or showed decreased secretion of IFNγ when exposed to TBT at any length of exposure. PBMCs from donors F119 and F120 produced increased amount of IFNγ when exposed to certain concentrations of TBT at every length of exposure. For example, F120 PBMCs produced higher levels of IFNγ than control cells after each length of exposure, but the concentration at which the increases occurred varied: 5–100 nM TBT caused increased secretion after 24h (ranging as high as 2.6 fold at 100 nM); only 50 and 100 nM TBT increased secretion after 48 h (with 100 nM causing an 8 fold increase); after 6 d of exposure to 100 nM TBT the increase in secretion was 19 fold. The effects of TBT exposures on PBMCs from donor F120 at each of the time points are shown in Figure 1C.

IFNγ secretion from NK cells exposed to DBT

Effects of exposing highly purified NK cells to 0, 0.05, 0.1, 0.25, 0.5, 1, 2.5 and 5 μM DBT for 24 h, 48 h, and 6 days on IFNγ secretion from several individual donors are shown in Table 6. As with the TBT exposed cells there is notable variation in the baseline secretion of IFNγ from individual donors. NK cells prepared from 6 donors were tested for the effect of 24 h exposures to DBT. Secretion of IFNγ by NK cells from all 6 donors was essentially completely blocked by exposures to 0.5, 1, 2.5, and 5 μM DBT for 24 h. However, 4 of the 6 donors showed increased secretion with exposures to 0.1 μM DBT for 24 h. Increases were also seen at the 0.05 μM in 3 of the 6 donors. 48 h exposures to DBT caused essentially a

complete block of IFNγ secretion at the same concentrations that blocked secretion after 24 h. However, NK cells from all donors had increased secretion of IFNγ at the lowest level of DBT exposure (0.05 μM). Cells that did not show any increase in IFNγ after 24 h exposures to DBT exhibited increased secretion after 48 h (KB130 and KB139). Following a 6 d exposure to 1, 2.5, and 5 μM DBT, there was decreased secretion from cells from all 3 donors tested. Cells from KB130 and KB155 showed increased secretion in response to 6 day exposures to several DBT concentrations (peak increases of 1.5 fold and 2.8 fold at 0.05 μM for KB130 and KB155, respectively). Data from donor KB155 is plotted for all time points in Figure 1D.

IFNγ secretion from monocyte-depleted PBMCs exposed to DBT

Results of exposing monoctye-depleted PBMCs isolated from different donors to 0–5 μM DBT for 24 h, 48 h, and 6 days on IFNγ secretion are shown in Table 7. When cells isolated from 6 donors were exposed to DBT for 24 h, secretion of IFNγ was significantly decreased at the 0.5, 1, 2.5, and 5 μM concentrations in 5 of the 6 donors. Cells from 5 of the 6 donors also showed increased secretion with exposures to DBT for 24 h. The concentrations at which DBT caused increased secretion varied among donors. Exposures to 1, 2.5, and 5 μM DBT for 48 h almost completely blocked the secretion of IFNγ from PBMCs regardless of donor. Likewise, PBMCs from all donors had increased secretion of IFNγ at exposure to DBT at lower concentrations (which varied among donors). The intensity of the increase also varied depending on the donor. This same pattern persisted out to 6 days of exposure. Figure 1E shows the effects of 0–200 nM exposure for 24 h, 48 h, and 6d for cells from donor F142.

IFNγ secretion from PBMCs exposed to DBT

Alterations in IFNγ secretion caused by exposing PBMCs isolated from different donors to 0–5 μM DBT for 24 h, 48 h, and 6 days are shown in Table 8. As was seen with NK cells and monocyte-depleted PBMCs, there is a consistent decrease in IFNγ secretion at the highest DBT concentrations (0.5–5 μM) after 24 h. In contrast to the less reconstituted cell preparations, PBMCs did not show a strong pattern of increased secretion at the lower concentration of DBT with a 24 h exposure. While the highest concentrations of DBT (5 and 2.5) continued to cause decreased IFNγ secretion after 48 h and 6d, there were significant increases in IFNγ secretion in 4 of 6 donors after 48 h and 3 of 4 donors after 6 d. All but one donor (F117) showed an increase in IFNγ after at least one exposure length (concentration at which this occurred varied among the donors). Data for 24 h, 48 h, and 6 d exposure of PBMCs from donor F120 are plotted in Figure 1F.

DISCUSSION

IFNγ is an important regulator of immune responsiveness that is produced primarily by T and NK lymphocytes and to some extent by myeloid cells (Zaidi and Merlino, 2011, Billiau and Matthys, 2009; Darwich et al., 2008). It is important to determine the effects of environmental contaminants such as the BTs (which have been found in human blood) (Whalen et al., 1999; Kannan et al., 1999) on the secretion of this potent regulator. Compound-induced decreases in IFNγ secretion could lead to loss of appropriate Th-1 cell

development and macrophage stimulation, both of which are crucial in establishing an immune response to pathogens (Schroder et al., 2004). Alternatively, if a compound were to increase IFNγ levels, this could lead to out of control inflammatory response, which is known to be a factor in a number of diseases including cancer (Macarthur et al., 2004). In this study we examined the effects of both TBT and DBT on secretion of IFNγ by immune system cells.

The effects of TBT and DBT on IFNγ secretion were examined in three distinct cell preparations (highly enriched NK cells, monocyte-depleted PBMCs and PBMCs). This type of study is important in order to determine if increasing the complexity of the cell population altered the effects of exposures to the compounds. It is not possible to carry out studies of these compounds in humans. Thus, studies carried out in more reconstituted systems such as the ones examined here will more closely approximate the effects that might be seen *in vivo*.

When highly purified NK cells were exposed to 200 nM TBT there was a substantial decrease in secretion of IFNγ. This effect remained as the cell preparation became more complex, with both monocyte-depleted PBMCs and PBMCs having the same patterns of response to TBT exposures as did the highly enriched NK cells. A notable difference was that monocyte-depleted PBMCs (which are primarily T cells and NK cells) had a consistent increase at the lower TBT concentrations at more time points than were seen with either NK cells alone or with PBMCs. Additionally, stimulation of IFNγ secretion with TBT was less common in the PBMCs than in NK cells or monocyte-depleted NK cells. This suggests that the presence of monocytes diminished the increases in secretion seen at low TBT concentrations (usually in the 50 nM to 2.5 nM range) that were quite consistently seen in the non-monocyte containing preparation. As monocytes are not considered to be significant secretors of IFNγ, their presence may simply be dampening the ability to see the TBT-induced stimulations. Alternatively, it may be that monocytes are secreting a factor(s) that interferes with TBT-induced secretion of IFNγ. The fact that increases are seen but not as consistently or intensely could be explained by either scenario. TBT-induced decreases in IFNγ secretion at the 200 and 100 nM TBT concentrations are quite reproducible among the 3 different cell preparations. Thus, TBT is altering secretion of IFNγ by diminishing its secretion at the highest levels of exposure while stimulating secretion at concentrations of 50 nM and below. These changes are seen with even the most reconstituted cell system studied, indicating that the effect has significant potential to be occurring in a physiologically relevant setting.

Decreases induced by the higher TBT concentrations would prevent IFNγ from carrying out its important role in regulating T-cell development and macrophage effector functions (Schroder et al. 2004). Conversely, TBT-induced increases in a pro-inflammatory cytokine such as IFNγ can lead to a number of diseases that are caused or exacerbated by increased inflammation (Macarthur et al., 2004). Secretion of IFNγ by human NK cells has been shown to require mitogen activated protein kinase (MAPK) activation (Girart et al., 2007). The effects of TBT on IFNγ secretion are occurring at levels that are found in human blood and because of the important role of this cytokine have significant implications for human health. TBT at these levels also decreases the lytic function of human NK cells (Dudimah et al., 2007a, Whalen et al., 2002). We have previously shown that TBT is able to activate

MAPKs in NK cells (Aluoch and Whalen, 2005; Aluoch et al., 2006; Aluoch et al., 2007). Future studies will investigate the role of MAPK activation in the TBT-induced changes in IFNγ secretion (both decreases and increases).

DBT caused very significant alterations of IFNγ secretion from each of the 3 types of cell preparations examined. In general, DBT at concentrations of 0.5–5 μM significantly decreased IFNγ secretion, with concentrations of 1–5 μM causing a complete block of secretion in nearly every donor of each type of cell preparation. In highly purified NK cells of donor KB155 there was a 12 fold increase in IFNγ secretion when the cells were exposed to 0.1μM DBT (100 nM) for 48 h, which is well below the highest levels that have been found in some human blood samples (300 nM) (Whalen et al., 1999; Kannan et al., 1999). In monocyte-depleted PBMCs, this same concentration of DBT after 48 h was able to cause a similar (8.5 fold) very large increase in IFNγ secretion in cells from donor F105. The peak increase for that donor after 48 h was at the 0.25 μM concentration and was 16.7 fold. PBMCs treated for 48 h at these same concentrations of DBT also had one donor (F120) that showed a very large stimulation of IFNγ secretion. The majority of increases seen with DBT were more modest than those mentioned above but range in the 2–3 fold level. Thus, in contrast to what was seen with TBT, the effects of DBT did not seem to be different as the complexity of the cell population increased. Like TBT, DBT exposures also caused very large decreases in IFNγ secretions at the highest concentrations and increased secretion at lower concentrations. The decreases of secretion cannot be accounted for simply by compound-induced killing of the cells, although concentrations of 1–5 μM do cause some cell death as length of exposure increases. Stimulation of secretion occurs at concentrations that have no negative impact on cell viability. Thus, indicating that the compound is in some manner activating IFNγ secretion and/or synthesis. As with TBT, DBT has been shown to decrease NK lytic function (Dudimah et al., 2007b, Whalen et al., 2002) and activate MAPKs (Odman-Ghazi et al., 2010) at some of these same concentrations that are affecting IFNγ secretion.

It is clear from this study that there is significant variability among donors both in their response to compound exposures as well as the baseline levels of IFNγ secretion. It is not clear what causes the variation in baseline IFNγ secretion, but clearly individuals vary quite widely in their baseline secretion and in the exact concentrations and lengths of exposure to a compound that elicit a stimulatory effect on IFNγ secretion. Interestingly, while there is some variation in baseline lytic function of highly purified NK cells among different donors it is less than what is seen with IFNγ secretion. Additionally, the effects of TBT and DBT exposures on NK lytic function were also not as variable (Dudimah et al. 2007a, 2007b). The monocyte-depleted PBMCs and PBMCs also show some variability in lytic function from donor to donor and are far less susceptible to exposure to BTs and other contaminants than the highly purified NK cells (Whalen et al., 1999; Whalen et al., 2003; Reed et al., 2004; Taylor et al., 2005). While the lytic function of highly purified NK cells is blocked by exposures as low as 25 nM after 6 days of exposure only the highest concentrations of TBT (200 nM) caused decreases in lytic function of the monocyte-depleted PBMCs and PBMCs at any length of incubation including 6 days (Whalen et al., 1999; Dudimah et al., 2007a). Again, the response of lytic function to compound exposures was far less variable in any of the cell preparations than is the response of IFNγ secretion. Additionally, the capacity of

both BTs to stimulate secretion of IFNγ was seen at lower concentrations of BT exposure where no effects on lytic function were seen (especially in the more reconstituted cell systems).

A stimulatory effect on secretion is common to nearly every donor with both TBT and DBT exposures. This is true from the least to the most reconstituted cell preparations. A previous study examined effects of both TBT and DBT on secretion of TNFα from human white blood cells. That study showed that TBT caused decreased secretion of TNFα in NK cells. There was no TBT-induced stimulation of secretion at any concentration in purified NK cells. However TBT (at lower concentrations) did stimulate secretion of TNFα in a cell preparation that contained both T and NK cells (monocyte-depleted PBMCs). DBT, at higher concentrations, decreased TNFα secretion and increased secretion at lower concentrations in both NK cell and the monocyte-depleted cell preparations (Hurt et al., 2013). This is in contrast to the effects of these compounds on IFNγ, where the composition of the cell preparation did not influence the effectiveness of DBT and had only a moderating influence with TBT.

In summary, the current study shows that secretion of IFNγ in three distinct immune cell preparations is altered by BT exposures. Exposures to both TBT and DBT result in significant decreases in IFNγ secretion at the highest exposure levels. Each compound is able to stimulate secretion of IFNγ from each of the three cell preparations, but the magnitude of the increase as well as the concentration and length of exposure at which the stimulation occurred, varied from one donor to the next.

Acknowledgments

Grant U54CA163066-02 and 2T34GM007663-32 from the National Institutes of Health

References

Abraha A, Rana K, Whalen MM. Role of protein kinase C in the TBT-induced inhibition of lytic function and MAPK activation in human natural killer cells. Arch Environ Cont Toxicol. 2010; 59:661–669.

Aluoch A, Whalen MM. Tributyltin-induced effects on MAP kinases p38 and p44/42 in human natural killer cells. Toxicology. 2005; 209:263–277. [PubMed: 15795062]

Aluoch AO, Odman-Ghazi SO, Whalen MM. Alteration of an essential NK cell signaling pathway by low doses of tributyltin in human natural killer cells. Toxicology. 2006; 224:229–237. [PubMed: 16781040]

Aluoch AO, Odman-Ghazi SO, Whalen MM. Pattern of MAP kinases p44/42 and JNK activation by non-lethal doses of tributyltin in human natural killer cells. Arch Toxicol. 2007; 81:271–277. [PubMed: 17019560]

Andoniou CE, Coudert JD, Degli-Esposti MA. Cross talk between NK cells and adaptive immune cells. Killers and beyond: NK-cell-mediated control of immune responses. Eur J Immunol. 2008; 38:2927–2968. [PubMed: 18979520]

Billiau A, Matthys P. Interferon-γ: A historical perspective. Cytokine & Growth Factor Reviews. 2009; 20:97–113. [PubMed: 19268625]

Catlin R, Shah H, Bankhurst AD, Whalen MM. Dibutyltin exposure decreases granzyme B and perforin in human natural killer cells. Environmental Toxicology and Pharmacology. 2005; 20:395–403. [PubMed: 21783618]

Darwich L, Coma G, Pena R, Bellido R, et al. Secretion of interferon-γ by human macrophages demonstrated at the single-cell level after co-stimulation with interleukin (IL)-12 plus IL-18. Immunology. 2008; 126:386–393. [PubMed: 18759749]

Dudimah FD, Odman-Ghazi SO, Hatcher F, Whalen MM. Effect of Tributyltin (TBT) on the ATP levels in human natural killer cells: Relationship to TBT- induced decreases in NK function. J Appl Toxicol. 2007a; 27:86–94. [PubMed: 17149696]

Dudimah FD, Gibson C, Whalen MM. Effect of Dibutyltin (DBT) on ATP levels in human natural killer cells. Environ Toxicol. 2007b; 22:117–123. [PubMed: 17366562]

Epstein RL, Phillippo ET, Harr R, Koscinski W, Vosco G. Organotin residue determination in poultry and turkey sample survey in the United States. Journal of Agricultural and Food Chemistry. 1991; 39:917–921.

Forsyth DS, Weber D, Cldroux C. Determination of butyltin, cyclohexyltin and phenyltin compounds in beers and wines. Food Additives and Contaminants. 1992; 9:161–169. [PubMed: 1499773]

Forsyth DS, Jay B. Organotin leachates in drinking water from chlorinated poly (vinyl chloride) (CPVC) pipe. Applied Organometallic Chemistry. 1997; 11:551–558.

Ghoneum M, Hussein AE, Gill G, Alfred LJ. Suppression of murine natural killer cell activity by tributyltin: *In vivo* and *in vitro* assessment. Environ Res. 1990; 52:178–186. [PubMed: 2394205]

Gipperth L. The legal design of the international and European Union ban on tributyltin antifouling paint: Direct and indirect effects. J Environ Management. 2009; 90:S86–S95.

Girart MV, Fuertes MB, Domaica CI, Rossi LE, Zwirner NW. Engagement of TLR3, TLR7, and NKG2D regulate IFN-γ secretion but not NKG2D-mediated cytotoxicity by human NK cells stimulated with suboptimal doses of IL-12. J Immunol. 2007; 179:3472–3479. [PubMed: 17804388]

Hurt K, Hurd-Brown T, Whalen MM. Tributyltin and dibutyltin alter secretion of tumor necrosis factor alpha from human natural killer (NK) cells and a mixture of T cells and NK cells. Journal of Appl Toxicol. 2013; 33:503–510. [PubMed: 23047847]

Kannan K, Senthilkumar K, Giesy JP. Occurrence of butyltin compounds in human blood. Environ Sci Technol. 1999; 33:1776–1779.

Kannan K, Tanabe S, Tatsukawa R. Occurrence of butyltin residues in certain foodstuffs. Bull Environ Contam Toxicol. 1995; 55:510–516. [PubMed: 8555674]

Kimbrough RD. Toxicity and health effects of selected organotins compounds: A Review. Environ Health Perspect. 1976; 14:51–56. [PubMed: 789069]

Laughlin RB, Linden O. Fate and effects of organotin compounds. Ambio. 1985; 14:88– 94.

Loganathan, BG.; Kannan, K.; Owen, DA.; Sajwan, KS. Butyltin compounds in freshwater ecosystems. In: Lipnick, RL.; Hermens, J.; Jones, K.; Muir, D., editors. Persistent, Bioaccumulative, and Toxic Chemicals I: Fate and Exposure. Am. Chem. Soc. Pub. Oxford Univ. Press; London: 2000.

Macarthur M, Hold GL, El-Omar EM. Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal maliganancy. Am J Physiol Gastrointest Liver Physiol. 2004; 286:G515–G520. [PubMed: 15010360]

Meyer TPH, Zehnter I, Hofmann B, Zaisserer J, Burkhart J, Rapp S, Weinauer F, Schmitz J, Illert WE. Filter buffy coats (FBC): A source of peripheral blood leukocytes recovered from leukocyte depletion filters. J Immunol Meth. 2005; 307:150–166.

Odman-Ghazi SO, Hatcher F, Whalen MM. Expression of Functionally Relevant Cell Surface Markers in Dibutyltin-exposed Human Natural Killer Cells. Chemico-Biological Interactions. 2003; 146:1–18. [PubMed: 12902148]

Odman-Ghazi SO, Abraha A, Isom ET, Whalen MM. Dibutyltin activates MAP kinases in human natural killer cells, in vitro. Cell Biology and Toxicology. 2010; 26:469–479. [PubMed: 20333459]

Reed A, Dzon L, Loganathan BG, Whalen MM. Immunomodulation of human natural killer cell cytotoxic function by organochlorine pesticides. Human & Experimental Toxicology. 2004; 23:463–471. [PubMed: 15553171]

Roper, WL. Toxicological profile for tin. U.S department of health and human services, agency for toxic substances and disease registry; 1992.

Sadiki AI, Williams DT, Carrier R, Thomas B. Pilot study on the contamination of drinking water by organotin compounds from PVC materials. Chemosphere. 1996; 32:2389–2398. [PubMed: 8653382]

Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-γ: an overview of signals, mechanisms and functions. J Leuk Biol. 2004; 75:163–189.

Snoeij NJ, Penninks AH, Seinen W. Thymus atrophy and immunosuppression induced by organotins compounds. Arch Toxicol Suppl. 1989; 13:171–174. [PubMed: 2774925]

Snoeij NJ, Penninks AH, Seinen W. Biological activity or organotins compounds- An overview. Environ Res. 1987; 44:335–353. [PubMed: 3319574]

Takahashi S, Mukai H, Tanabe S, Sakayama K, Miyazaki T, Masuno H. Butyltin residues in the liver of humans and wild terrestrial mammals and in the plastic products. Environ Pollut. 1999; 106:213–218. [PubMed: 15093048]

Tanabe S, Prudente M, Mizuno T, Hasegawa J, Iwata H, Miyazaki N. Butyltin contamination in marine mammals from north Pacific and Asian coastal waters. Environ Sci Technol. 1998; 32:193–198.

Taylor TR, Tucker T, Whalen MM. Persistent inhibition of human natural killer cell function by ziram and pentachlorophenol. Environ Toxicol. 2005; 20:418–424. [PubMed: 16007644]

Thomas LD, Shah H, Green SA, Bankhurst AD, Whalen MM. Tributyltin exposure causes decreased granzyme B and perforin levels in human natural killer cells. Toxicology. 2004; 200:221–233. [PubMed: 15212818]

Vos JG, DeKlerk A, Krajnc EI, Van Loveren V, Rozing J. Immunotoxicity of bis(tri-n-butyltin)oxide in the rat: Effects on thymus- dependent immunity and on nonspecific resistance following long-term exposure in young versus aged rats. Toxicol Appl Pharmacol. 1990; 105:144–155. [PubMed: 2118283]

Wester PW, Krajnc EI, van Leeuwen FX, Loeber JG, van der Heijden CA, Vaessen HA, Helleman PW. Chronic toxicity and carcinogenicity of bis(tri-n-butyltin)oxide (TBTO) in the rat. Food Chem Toxicol. 1990; 28:179–196. [PubMed: 2344992]

Whalen MM, Loganathan BG, Kannan K. Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer (NK) cells in vitro. Environ Res. 1999; 81:108–116. [PubMed: 10433842]

Whalen MM, Ghazi S, Loganathan BG, Hatcher F. Expression of CD16, CD18 and CD56 in Tributyltin-exposed human natural killer cells. Chemico-Biol Interact. 2002; 139:159–176.

Whalen MM, Loganathan BG, Yamashita N, Saito T. Immunomodulation of human natural killer cell cytotoxic function by triazine and carbamate pesticides. Chemico-Biological Interactions. 2003; 145:311–319. [PubMed: 12732457]

Zaidi MR, Merlino G. The two faces of interferon-γ in cancer. Clin Cancer Res. 2011; 17:1–7.

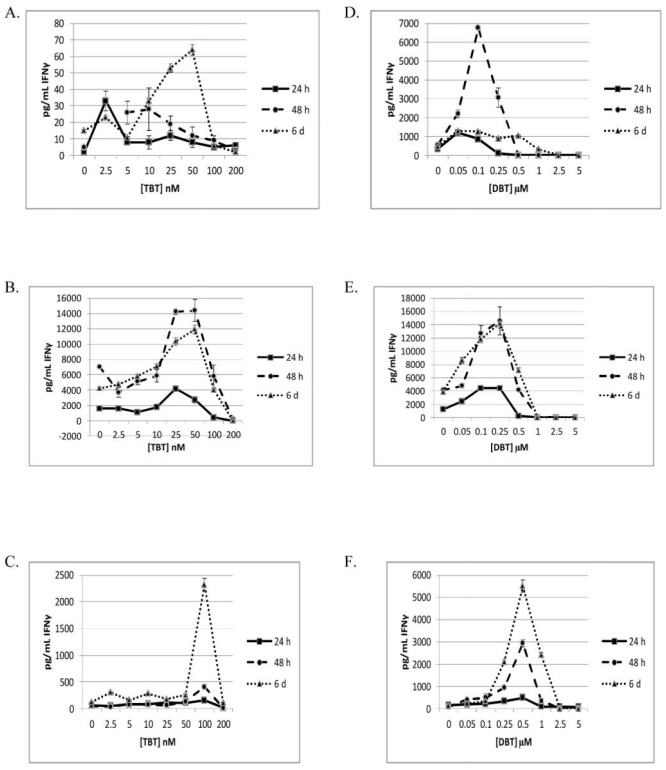

Figure 1.

Effects of 24 h, 48 h and 6 day exposures to TBT and DBT on IFNγ secretion from highly purified human NK cells, monocyte-depleted PBMCs, and PBMCs in an individual donor. A) NK cells exposed to 0–200 nM TBT (donor KB147). B) Monocyte-depleted PBMCs exposed to 0–200 nM TBT (donor F142). C) PBMCs exposed to 0–200 nM TBT (donor F120). D) NK cells exposed to 0–5 μM DBT (donor KB155). E) Monocyte-depleted PBMCs exposed to 0–5 μM DBT (donor F142). F) PBMCs exposed to 0–5 μM DBT (donor F120). The data point for a 48 h exposure of NK cells to 2.5 nM TBT (177±120 pg/mL) was omitted in (A) as it was not significant and made it difficult to see the effects at other concentrations.

Table 1

Percent viability of NK cells, Monocyte-depleted PBMCs and PBMCs exposed to TBT for 24 h, 48 h and 6 days.

		Percent Viable Cells		
24 h exposure	**[TBT] nM**	**NK**	**Monocyte-depleted PBMCs**	**PBMCs**
	0	96±3	97±3	96±3
	2.5	96±3	98±1	98±2
	5	97±2	99±1	97±3
	10	97±2	98±2	96±2
	25	98±1	98±2	98±2
	50	96±2	96±6	97±2
	100	96±1	98±2	97±2
	200	92±6	92±4	95±2
48 h exposure	0	96±1	95±3	96±3
	2.5	96±2	98±1	94±5
	5	97±1	99±1	96±3
	10	97±1	97±1	97±7
	25	95±3	96±1	95±4
	50	96±2	94±3	96±4
	100	94±3	89±9	98±1
	200	82±5*	77±5*	84±4*
6 day exposure	0	78±4	84±11	93±2
	2.5	78±4	87±4	92±1
	5	81±3	88±10	95±2
	10	80±7	90±10	94±2
	25	80±5	85±13	94±2
	50	74±8	74±17	92±6
	100	72±6	64±12	86±8
	200	66±11	54±13*	74±5*

Values are mean±S.D. of triplicate determinations.

*Indicates a significant decrease in viability compared to control cells, $p<0.05$

Table 2

Percent viability of NK cells, Monocyte-depleted PBMCs and PBMCs exposed to DBT for 24 h, 48 h and 6 days.

		Percent Viable Cells		
24 h exposure	**[DBT] μM**	**NK**	**Monocyte-depleted PBMCs**	**PBMCs**
	0	95±2	96±2	95±3
	0.05	96±3	97±2	98±2
	0.1	95±4	98±3	97±2
	0.25	94±6	96±2	96±2
	0.5	94±2	97±3	95±4
	1	87±9	95±4	93±7
	2.5	88±6*	84±9*	90±14
	5	81±10*	83±6*	87±7*
48 h exposure	0	93±4	96±2	96±2
	0.05	96±4	98±2	96±1
	0.1	95±3	99±1	96±3
	0.25	93±2	97±1	96±4
	0.5	89±2	94±6	95±7
	1	75±7*	86±3*	84±13
	2.5	63±3*	71±16*	76±11*
	5	61±11*	69±9*	73±4*
6 day exposure	0	73±15	87±9	90±7
	0.05	74±7	88±7	89±6
	0.1	77±8	89±8	91±8
	0.25	75±8	79±11	85±14
	0.5	72±2	67±13*	80±17
	1	67±6	57±10*	73±11*
	2.5	57±10	53±14*	69±10*
	5	57±14	64±5*	67±1*

Values are mean±S.D. of triplicate determinations.

* Indicates a significant decrease in viability compared to control cells, $p<0.05$

Table 3

Effects of 24 h, 48 h and 6 day exposures to TBT on IFNγ secretion from highly purified human NK cells.

24 h	Interferon gamma secreted in pg/mL (mean±S.D.)						
[TBT] nM	**KB113**	**KB114**	**KB115**	**KB121**	**KB147**	**KB149**	**KB155**
0	34±10	5,213±140	40±14	9,453±419	2±1	658±22	292±8
2.5	20±32	8,097±2529	221±52*	7,129±956	33±6*	605±49	534±28*
5	0±20	14,656±125*	98±13*	,6517±1499	8±1*	605±49	425±10*
10	62±21	13,375±835*	102±12*	4,530±1318*	8±4	640±30	321±1*
25	186±56*	20,158±845*	292±16*	6,747±315*	12±3*	261±21*	329±15
50	74±21	22,519±133*	58±4	5,653±1852	8±3	124±11*	181±4*
100	0±0*	7,467±456*	100±6*	3,289±248*	5±2	14±37*	54±2*
200	0±2*	25±35*	21±12	0±4*	6±2	0±4*	36±14*

48 h	Interferon gamma secreted in pg/mL (mean±S.D.)			
[TBT] nM	**KB114**	**KB120**	**KB147**	**KB148**
0	12,605±990	39,468±6507	5±1	60±8
2.5	21,752±1631*	46,178±1008	177±120	70±4
5	12,412±435	40,426±5335	26±7*	16±1*
10	16,765±542*	42,306±2340	28±13	22±9*
25	26,972±759*	41,545±7501	19±5*	11±4*
50	31,592±947*	37,357±3866	12±5	8±5*
100	499±211*	622±167*	9±3	0±1*
200	0±0*	0±0*	3±2	0±0*

6 day	Interferon gamma secreted in pg/mL (mean±S.D.)			
[TBT] nM	**KB146**	**KB147**	**KB148**	**KB155**
0	4±3	15±1	23±5	864±15
2.5	49±32	23±9	266±168	2,145±20*

6 day	Interferon gamma secreted in pg/mL (mean±S.D.)			
[TBT] nM	**KB146**	**KB147**	**KB148**	**KB155**
5	16±5*	11±3	208±50*	1,709±28*
10	34±5*	33±11	71±10*	1,320±20*
25	25±8*	53±51	72±18*	1,498±61*
50	55±7*	64±4*	36±3*	642±7*
100	18±1*	6±2*	9±4*	103±10*
200	1±1*	2±2*	5±6*	76±6*

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells, $p<0.05$

Table 4

Effects of 24 h, 48 h and 6 day exposures to TBT on IFNγ secretion from monocyte-depleted PBMCs.

24 h	Interferon gamma secreted in pg/mL (mean±S.D.)				
[TBT] nM	**F93**	**F96**	**F97**	**F126**	**F142**
0	1,037±75	245±18	13±1	4,667±130	1,585±119
2.5	1,492±193*	1,445±100*	55±16*	4,473±103	1,576±53*
5	1,339±184	811±145*	84±19*	3,247±104*	1,100±32*
10	3,430±71*	1,773±193*	94±20*	5,548±427	1,761±62
25	5,698±473*	2,260±278*	178±33*	20,473±1520*	4,182±249*
50	6,779±420*	2,216±328*	177±62*	7,484±573*	2,745±388*
100	12,340±659*	138±115	0±3*	774±116*	423±48*
200	0±18*	0±22*	0±2*	882±452*	0±20*

48 h	Interferon gamma secreted in pg/mL (mean±S.D.)			
[TBT] nM	**F96**	**F97**	**F126**	**F142**
0	3,250±325	152±3	9,160±935	7,045±52
2.5	6,555±1859	135±15	10,616±287	3,707±655*
5	5,186±359*	180±1*	10,327±393	5,113±426*
10	6,836±650*	308±33*	13,138±592*	5,872±849
25	9,293±263*	364±40*	21,938±494*	14,251±383*
50	9,319±953*	531±94*	31,804±428*	14,402±1415*
100	2,652±203	76±11*	549±271*	5,784±1497
200	0±66*	0±1*	127±33*	184±15*

6 day	Interferon gamma secreted in pg/mL (mean±S.D.)		
[TBT] nM	**F96**	**F126**	**F142**
0	2,057±81	5,752±264	4,234±229
2.5	3,586±213*	3,903±151*	4,772±225*

6 day	Interferon gamma secreted in pg/mL (mean±S.D.)		
[TBT] nM	**F96**	**F126**	**F142**
5	3,382±120*	10,721±157*	5,821±112*
10	3,873±127*	21,479±262*	7,095±128*
25	4,171±171*	41,550±852*	10,351±19*
50	3,875±239*	39,620±1000*	11,933±270*
100	2,458±79*	358±132*	4,011±78
200	0±0*	1,236±1759*	0±13*

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells, $p<0.05$

Environ Toxicol. Author manuscript; available in PMC 2016 May 01.

Table 5

Effects of 24 h, 48 h and 6 day exposures to TBT on IFNγ secretion from PBMCs.

24 h	Interferon gamma secreted in pg/mL (mean±S.D.)			
[TBT] nM	**F116**	**F117**	**F119**	**F120**
0	2,209±154	658±32	1,052±44	60±2
2.5	1,556±359	454±39*	1,443±32*	53±10
5	1,698±107*	190±16*	1,958±5*	79±6*
10	1,373±130*	149±41*	1,158±118	84±2*
25	1,103±51*	162±20*	823±12*	113±12*
50	789±50*	173±26*	692±7*	105±3*
100	159±25*	0±11*	662±15*	156±14*
200	31±22*	0±8*	0±14*	13±1*

48 h	Interferon gamma secreted in pg/mL (mean±S.D.)			
[TBT] nM	**F116**	**F117**	**F119**	**F120**
0	10,317±827	2,193±9	1,367±14	51±13
2.5	6,571±423*	1,949±400	2,765±276*	38±4
5	5,419±13*	1,501±50*	2,847±24*	78±17
10	7,803±564*	1,659±71*	1,958±81*	73±33
25	8,462±1,911	1,033±53*	1,395±66	61±38
50	10,658±259	975±36*	1,854±24*	132±2*
100	1,672±1,010*	182±5*	1,461±23*	410±48*
200	2,259±724*	62±34*	76±5*	11±3*

6 day	Interferon gamma secreted in pg/mL (mean±S.D.)			
[TBT] nM	**F116**	**F117**	**F119**	**F120**
0	9,362±538	4,189±378	1,631±63	122±2
2.5	5,926±942*	3,841±248	1,811±4*	306±25*

6 day	Interferon gamma secreted in pg/mL (mean±S.D.)			
[TBT] nM	**F116**	**F117**	**F119**	**F120**
5	5,285±924*	4,617±59	2,308±87*	162±19
10	11,772±5,415	3,424±185	2,358±76*	283±38*
25	8,656±1,252	3,546±174	2,014±56*	178±5*
50	13,887±6,679	1,926±80*	1,591±51	256±7*
100	1,156±714*	396±95*	886±61*	2325±76*
200	0±67*	72±40*	0±4*	110±22

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells, $p<0.05$

Environ Toxicol. Author manuscript; available in PMC 2016 May 01.

Table 6

Effects of 24 h, 48 h and 6 day exposures to DBT on IFNγ secretion from highly purified human NK cells.

24 h	Interferon gamma secreted in pg/mL (mean±S.D.)					
[DBT] μM	**KB113**	**KB114**	**KB125**	**KB130**	**KB139**	**KB155**
0	172±2	19,094±545	15±1	6,148±60	638±12	341±17
0.05	121±25*	36,510±1,193*	99±18*	4,474±111*	658±72	1,196±29*
0.1	408±22*	27,635±525*	72±13*	2,296±62*	664±29	872±27*
0.25	125±2*	6,052±776*	7±1*	78±3*	104±14*	122±18*
0.5	4±4*	0±0*	0±0*	0±0*	0±23*	0±13*
1	7±12*	0±0*	0±0*	0±0*	0±0*	0±6*
2.5	3±3*	0±0*	0±0*	0±0*	0±0*	0±6*
5	11±9*	0±0*	0±0*	0±0*	0±0*	0±36*

48 h	Interferon gamma secreted in pg/mL (mean±S.D.)			
[DBT] μM	**KB125**	**KB130**	**KB139**	**KB155**
0	58±4	30,765±934	4,035±916	554±118
0.05	450±13*	37,971±1,579*	5,469±453*	2,214±222*
0.1	825±18*	23,575±940*	9,343±1,630*	6,766±67*
0.25	877±26*	6,559±1,104*	11,796±2,500*	3,065±509*
0.5	4±1*	0±0*	1,538±823*	0±20*
1	0±0*	0±0*	0±0*	0±0*
2.5	0±0*	0±0*	0±0*	0±0*
5	0±0*	0±0*	0±0*	0±0*

6 day	Interferon gamma secreted (mean±S.D.) in pg/mL		
[DBT] μM	**KB130**	**KB139**	**KB155**
0	26,050±1,626	2,101±390	472±42
0.05	40,183±3,125*	1,052±220*	1,302±8*

6 day	Interferon gamma secreted (mean±S.D.) in pg/mL		
[DBT] µM	**KB130**	**KB139**	**KB155**
0.1	35,283±12,021	2078±467	1,267±169*
0.25	6,317±333*	416±122*	904±47*
0.5	0±0*	0±0*	1,044±106*
1	0±0*	0±0*	325±11*
2.5	0±0*	0±0*	0±0*
5	0±0*	0±0*	0±0*

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells, $p<0.05$

Table 7

Effects of 24 h, 48 h, 6 day exposures to DBT on IFNγ secretion from monocyte-depleted PBMCs.

24 h	Interferon gamma secreted in pg/mL (mean±S.D.)						
[DBT] μM	**F93**	**F99**	**F105**	**F126**	**F139**	**F142**	**F145**
0	80±4	650±17	41±2	1,247±50	22±2	1,259±57	3,104±64
0.05	272±88	665±185	42±4	3,055±77*	44±19	2,462±349*	3,869±140*
0.1	309±114	3,270±1263	59±4*	7,448±234*	85±1*	4,426±163*	4,827±175*
0.25	353±20*	623±104	69±8*	1,888±44*	18±5	4,426±241*	2,447±85*
0.5	542±25*	185±31*	11±3*	27±18*	0±1*	248±46*	211±73*
1	1,840±30*	201±21*	3±1*	0±15*	0±1*	0±8*	60±7*
2.5	58±101	145±0*	5±2*	0±4*	0±8*	0±42*	80±64*
5	47±60	192±59*	1±1*	4±26*	0±2*	0±58*	144±67*

48 h	Interferon gamma secreted in pg/mL (mean±S.D.)					
[DBT] μM	**F99**	**F105**	**F126**	**F139**	**F142**	**F145**
0	652±25	41±2	3,914±506	1,147±46	4,157±220	2,660±81
0.05	2,054±30*	122±9*	5,559±433*	1,500±390	4,770±382	4,017±118*
0.1	2,233±106*	350±6*	9,381±335*	3,253±185*	12,667±1210*	5,685±85*
0.25	1,915±28*	685±63*	8,309±1212*	16,667±150*	14,590±2154*	3,509±210*
0.5	162±35*	369±3*	1,104±186*	347±58*	4,210±165	141±30*
1	0±12*	24±2*	0±8*	0±81*	90±17*	0±26*
2.5	0±7*	1±1*	0±18*	0±64*	37±30*	0±24*
5	0±15*	0±1*	0±18*	0±114*	67±111*	0±10*

6 day	Interferon gamma secreted in pg/mL (mean±S.D.)				
[DBT] μM	**F105**	**F126**	**F139**	**F142**	**F145**
0	67±2	4,029±108	2,057±198	3,893±594	2828±386
0.05	155±2*	12,468±902*	1,780±110	8,648±354*	3791±226*

6 day	Interferon gamma secreted in pg/mL (mean±S.D.)				
[DBT] µM	**F105**	**F126**	**F139**	**F142**	**F145**
0.1	336±21*	15,390±524*	2,027±90	11,836±497*	4778±17*
0.25	688±8*	14,590±104*	4,223±555*	14,203±322*	3348±336
0.5	522±38*	1,301±121*	2,243±100	7,128±187*	356±86*
1	42±2*	0±88*	480±89*	0±51*	0±23*
2.5	4±1*	0±151*	447±185*	0±6*	0±138*
5	7±1*	0±750*	523±157*	0±61*	0±222*

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells, $p<0.05$

Environ Toxicol. Author manuscript; available in PMC 2016 May 01.

Environ Toxicol. Author manuscript; available in PMC 2016 May 01.

Table 8

Effects of 24 h, 48 h and 6 day exposures to DBT on IFNγ secretion from PBMCs.

24 h	Interferon gamma secreted in pg/mL (mean±S.D.)				
[DBT] μM	**F116**	**F117**	**F119**	**F120**	**F150**
0	2,009±69	899±11	2,948±7	153±23	2,470±82
0.05	2,679±92*	555±35*	1,645±1*	192±23	1,390±100*
0.1	2,492±42*	354±12*	2,129±47*	218±25*	2,260±104
0.25	2,532±35*	195±5*	2,597±29*	338±15	473±15*
0.5	588±11*	17±4*	1,503±132*	492±84*	187±132*
1	1±11*	0±8*	61±5*	99±26	126±58*
2.5	0±15*	0±4*	14±29*	88±28*	77±38*
5	0±17*	0±2*	0±6*	76±3*	120±105*

48 h	Interferon gamma secreted in pg/mL (mean±S.D.)					
[DBT] μM	**F116**	**F117**	**F119**	**F120**	**F125**	**F150**
0	7,257±1,184	2,420±91	3,244±86	84±64	840±30	3,368±48
0.05	11,604±106*	1,673±59*	2,915±171	403±42*	1,691±54*	2,321±137*
0.1	18,491±966*	1,435±8*	3,850±399	489±159*	4,373±139*	3,361±255
0.25	30,877±1,051*	810±32*	3,928±63*	942±60*	6,359±140*	1,681±205*
0.5	6,650±1,082	233±3*	2,815±239	2,943±158*	801±40	38±15*
1	0±270*	0±9*	724±32*	343±129	0±25*	23±30*
2.5	0±197*	0±0*	54±38*	0±0*	0±3*	31±45*
5	0±170*	0±3*	31±13*	0±4*	0±20*	28±18*

6 d	Interferon gamma secreted in pg/mL (mean±S.D.)			
[DBT] μM	**F117**	**F119**	**F120**	**F125**
0	2,643±90	3,258±187	125±2	423±18
0.05	2,084±34*	2,647±69*	233±4*	1,951±133*

6 d	Interferon gamma secreted in pg/mL (mean±S.D.)			
[DBT] µM	**F117**	**F119**	**F120**	**F125**
0.1	2,107±88*	4,270±143*	337±26*	5,432±60*
0.25	2,108±31*	6,075±103*	2,096±119*	7,162±303*
0.5	1,026±17*	2,616±149*	5,510±143*	1,625±100*
1	0±10*	712±10*	2,406±94*	0±4*
2.5	0±3*	59±7*	9±6*	0±0*
5	0±1*	0±7*	0±9*	0±1*

Values are mean±S.D. of triplicate determinations.

* Indicates a significant change in secretion compared to control cells, $p<0.05$