

## Direct effect of *p,p'*-DDT on mice liver

Bárbara Arroyo-Salgado, Jesús Olivero-Verbel\*, Angélica Guerrero-Castilla

*Environmental and Computational Chemistry Group, School of Pharmaceutical Sciences, Zaragocilla Campus, University of Cartagena, 130015, Cartagena, Colombia*

Contact with the pesticide dichlorodiphenyltrichloroethane (*p,p'*-DDT) can be the cause of various harmful effects in humans, wildlife, and the environment. This pesticide is known to be persistent, lipophilic, resistant to degradation, and bioaccumulative in the environment and to be slowly released into bloodstream. Growing evidence shows that exposure to DDT is linked to type 2 diabetes mellitus. Individuals exposed to elevated levels of DDT and its metabolite have an increased prevalence of diabetes and insulin resistance. To evaluate these possible relationships, experiments were performed on eight-week-old female mice, divided into three groups (n = 10 per group): Group 1 received a vehicle-control intraperitoneal (i.p.) injection of sesame oil; Groups 2 and 3 received an i.p. dose of 50 and 100 µg/g *p,p'*-DDT respectively, dissolved in sesame oil. All groups were treated once daily for four days. Real-time PCR analysis of several genes was undertaken. Additionally, biochemical parameters and histopathological changes were measured. NQO1, HMOX1, NR113 and NR3C1 were up-regulated in DDT-exposed animals compared to the vehicle control group, while only SREBP1 was down-regulated in the 100 µg/g group. MTTP and FABP5, not previously reported for DDT exposure, but involved in regulation of fatty acid fluxes, could also function as biomarkers cross-talking between these signaling pathways. These results suggest that beyond epidemiological data, there is increasing molecular evidence that DDT may mimic different processes involved in diabetes and insulin resistance pathways.

**Uniterms:** Pesticides. Persistent organic pollutant. Dichlorodiphenyltrichloroethane/exposure. Type 2 diabetes mellitus/exposure to dichlorodiphenyltrichloroethane/experimental study. Insulin/resistance/exposure to dichlorodiphenyltrichloroethane. Gene expression.

O contato com o praguicida diclorodifeniltricloroetano (*p, p'*-DDT) pode ser a causa de vários efeitos nocivos sobre os seres humanos, animais silvestres e o meio ambiente. Sabe-se de sua característica de bioacumulação, ser altamente persistente no meio ambiente, lipofílico, resistente à degradação e lentamente liberado na corrente sanguínea. Existe uma evidência crescente de que a exposição ao DDT pode ser ligada a Diabetes mellitus tipo 2. Os indivíduos expostos a níveis elevados de DDT e seu metabólito apresentam maior prevalência de diabetes e resistência à insulina. A fim de obter informações sobre essas possíveis relações, camundongos fêmeas de oito semanas de idade foram divididos em três grupos (n = 10 por grupo): Grupo 1 recebeu um veículo de óleo de gergelim via i.p.; os Grupos 2 e 3 receberam, via i.p., 50 e 100 µg/g de *p, p'*-DDT, respectivamente, dissolvidos em óleo de gergelim. Todos os grupos foram tratados uma vez ao dia durante quatro dias. Além da análise de PCR em Tempo Real de vários genes, os parâmetros bioquímicos e alterações histopatológicas também foram medidos. A expressão gênica do mRNA dos genes NQO1, HMOX1, NR113 e NR3C1 foi maior nos animais expostos ao DDT, em comparação ao grupo controle, enquanto a expressão gênica do SREBP1 diminuiu na concentração de 100 µg/g. Os genes MTTP e FABP5 envolvidos na regulação do fluxo de ácidos graxos, embora não estudados quanto à exposição ao DDT, também podem funcionar como biomarcadores de resposta cruzada entre essas vias de sinalização. Esses resultados sugerem que, além de dados epidemiológicos, há cada vez mais evidências moleculares de que o DDT poderia, de fato, imitar diferentes processos que envolvem as rotas de diabetes e de resistência à insulina.

**Unitermos:** Praguicidas. Poluente orgânico persistente. Diclorodifeniltricloroetano/exposição. Diabetes mellitus tipo 2/exposição ao diclorodifeniltricloroetano/estudo experimental. Insulina/resistência/exposição ao diclorodifeniltricloroetano. Expressão gênica.

\*Correspondence: J. Olivero-Verbel. Environmental and Computational Chemistry Group. School of Pharmaceutical Sciences. University of Cartagena. Zaragocilla Campus. Cartagena - 130015 – Colombia. E-mail: [jesusolivero@yahoo.com](mailto:jesusolivero@yahoo.com)

## INTRODUCTION

Organochlorine pesticides (OC) were widely used in agriculture and pest control, but their use was banned during the 1970s and 1980s because of their high toxicity and environmental persistence. However, Asia, Latin America, and Africa are still producing and using OC because of their high effectiveness as pesticides and their low cost (Lucena *et al.*, 2007).

Actually, abundant OC exists in the environment, with DDT (1,1,1-trichloro-2,2-bis[4-chlorophenyl]ethane), methoxychlor[1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane, MXC], tris(4-chlorophenyl)methanol (TCPM), hexachlorocyclohexanes (HCHs) and related compounds taking priority. Dichlorodiphenyltrichloroethane (DDT) is a synthetic chemical that includes *p,p'*-DDT, *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), and *p,p'*-dichlorodiphenyldichloroethane (*p,p'*-DDD or *p,p'*-TDE). Technical grade DDT is a mixture of three isomers of DDT, the main components of which are *p,p'*-DDT (85%), *o,p'*-DDT (15%), and *o,o'*-DDT (trace amounts).

DDT has been extensively used worldwide to control malaria, typhus, body lice, and bubonic plague (ATSDR, 2002), but was banned in the United States by the Environmental Protection Agency in 1972, as a consequence of its potentially harmful effects on humans, wildlife, and the environment (Porta *et al.*, 1999). However, it continues to be found frequently at high concentrations in human serum.

The half-life of DDT in the environment usually ranges from 2 to 15 years. It is known to bioaccumulate and to be highly persistent in the environment as well as resistant to degradation. High levels of DDT are still present in the environment and in the food chain. Even *p,p'*-DDE, its main metabolite, is frequently found all over the world in food, adipose tissues, and breast milk, among others (Arrebola *et al.*, 2012).

Some studies suggest that DDT-induced toxicity is associated with estrogen receptor (ER). DDT metabolites *p,p'*-DDT, *o,p'*-DDT and DDE promote estrogenic and anti-androgenic properties (Sohoni, Sumpter, 1998), the production of reactive oxygen species (ROS) (Jin *et al.*, 2014), toxicity and carcinogenicity (Everett, Frithsen, Player, 2011; La Merrill *et al.*, 2014). Moreover, perinatal DDT exposure contributes to the development of insulin resistance and metabolic syndrome in adult female mice (La Merrill *et al.*, 2014).

Data from several studies suggest a possible association between DDT and type 2 diabetes mellitus (T2DM) (Everett, Frithsen, Player, 2011). DDT has been found to be associated with increased odds of T2DM (Cox

*et al.*, 2007). However, there is no conclusive epidemiological evidence for such relationship (Bloomgarden, 2000). Some existing epidemiological studies have demonstrated associations between DDT and DDE exposures and diabetes in humans (Rylander, Rignell-Hydbom, Hagmar, 2005; Cox *et al.*, 2007; Patel, Bhattacharya, Butte, 2009; Lee *et al.*, 2010a; Lee *et al.*, 2010b).

Prevalence of obesity and T2DM has increased at alarming rates in countries where DDT is still in use (Danaei *et al.*, 2011). T2DM is classified as a complex disease, where interactions between genetic and environmental factors may underlie the etiology of diabetes. T2DM has become a global epidemic in recent decades. Some 382 million individuals worldwide are estimated to have diabetes, with this number projected to increase to 592 million by 2035. The vast majority of those affected will develop T2DM (Sargis, 2014).

Adult rodents exposed to DDT had impaired insulin secretion, glucose intolerance, and elevated gluconeogenesis (Kacew, Singhal, 1974; Yau, Mennear, 1976). Rats and monkeys exposed to acute DDT also presented increased hepatic cholesterol circulation and triglyceride synthesis (Sanyal *et al.*, 1982). There is evidence that DDT may alter adipogenesis and adipokine-cytokine production *in vitro* (Moreno-Aliaga, Matsumura, 2002) and that persistent organic pollutants (POPs) may induce abdominal obesity, impair insulin sensitivity, reduce glucose uptake, and cause dyslipidemia and hepatosteatosis (Ruzzin *et al.*, 2010; Ibrahim *et al.*, 2011). This pesticide could also contribute to other risk factors of diabetes, such as obesity, by distressing neural circuits that regulate feeding behavior or by altering differentiation of adipocytes (Thayer *et al.*, 2012). It is possible that DDT and a high-fat diet may induce hepatic *de novo* lipogenesis (Rolo, Palmeira, Wallace, 2002), although the exact role of DDT/DDE on these processes requires additional studies (Lee *et al.*, 2010a).

The liver is the tissue most sensitive to xenobiotic exposure and constitutes the main target of DDT toxicity. Currently, there is little information on the intracellular mechanisms related to the effects of DDT on health and its relationship to diabetes. However, DDT and related OC exert estrogenic effects through a variety of molecular mechanisms involving ER-alpha (ER $\alpha$ ), cellular-signaling systems such as activator protein (AP-1), p38, and extracellular signal-regulated kinase 1 and 2 (ERK1/2), activation of the p38 mitogen-activated protein kinase (MAPK) signaling cascade (Frigo *et al.*, 2005), and apoptosis.

Recently, Collotta, Bertazzi, Bollati (2013) argued that DDT could impact DNA methylation and microRNA expression, thus altering gene regulation. Moreover,

*p,p'*-DDT was found to alter adipocyte differentiation and induce the expression of CCAAT/enhancer binding protein- $\alpha$  (C/EBP $\alpha$ ) and peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) through the modification of transcription factors regulating these events (Moreno-Aliaga, Matsumura, 2002).

In addition, exposure to the pesticide DDT have been reported to cause changes in the expression of genes such as heme oxygenase decycling 1 (HMOX1), epoxide hydrolase 1 (EPHX1), gamma-glutamylcysteine synthetase (GCLM), and glutathione peroxidase 2 (GPX2), among others. Exposure to this pesticide is also associated with oxidative stress, one of the intracellular imbalances that is associated with mitochondrial dysfunction and insulin resistance (Kiyosawa *et al.*, 2008a). Recently Jin *et al.* (2014) showed that *p,p'*-DDT increased ROS content, and this was accompanied by the activation of the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway. In addition, Shen and Novak (1997) showed that DDT at physiological concentrations of 10 nM elevated signal transducer and activator of transcription 1 (STAT1) phosphorylation.

The aim of the present research was to establish the effect of an acute dose of DDT in female BalBc mice, specifically in terms of tissue damage and gene expression profile. We measured markers of oxidative stress, insulin signaling, apoptosis and changes in lipid metabolism. This study suggests that, beyond epidemiological data, there is increasing molecular evidence that DDT could in fact mimic different processes leading to diabetes. The present research hypothesizes that persistent pesticides such as DDT contribute to pathological mechanisms associated with the development of diabetes.

## MATERIAL AND METHODS

### Experimental animals

Female BALBc mice were purchased from the Colombian National Institute of Health. Handling of animals was in compliance with the guidelines for the care and use of animals for scientific purposes. All mice were housed in conventional plastic cages at 23 °C  $\pm$  2 °C, 60 $\pm$ 10% humidity, 12 h light/12 h dark photoperiod, and *ad libitum* drinking water and standard diet. Body weight was measured with a scale to the nearest 0.01 g (OxauS, Navigator<sup>TM</sup>, USA) during the entire experiment.

### Experimental Groups and Protocol

Eight-week-old female mice were utilized in

the experiments. Animals were divided into three groups (n = 10 per group) (Figure 1). Group 1 received a vehicle-control intraperitoneal (i.p.) injection consisting of sesame oil for four days; Groups 2 and 3 received 50 mg/kg *p,p'*-DDT and 100 mg/kg *p,p'*-DDT respectively dissolved in sesame oil i.p. This treatment was given during four consecutive days. *p,p'*-DDT (*p,p'*-dichlorodiphenyltrichloroethane, CAS N<sup>o</sup> 50-29-3) was purchased from Supelco Analytical (Bellefonte, PA, USA). The doses and time used for the present study were derived from the results of our preliminary experiments.

Animals were observed daily for manifestations of adverse effects and clinical symptoms. At the end of the experiment, the animals were anesthetized with sodium pentobarbital and euthanized by cervical dislocation. Necropsy was carried out and portal blood was obtained in sodium citrate. Serum was isolated by centrifugation (2000 $\times$ g for 10 min) and kept at -80 °C until use. After blood collection, the livers were rapidly excised, and sections were immediately stored in RNAlater<sup>®</sup> (Sigma-Aldrich Co. USA) at -80 °C for gene expression analysis. A portion of the liver was fixed with 10% buffered formalin.

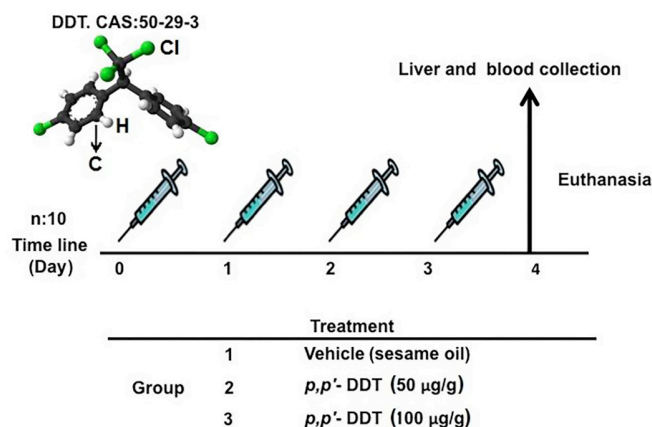


FIGURE 1 - Experimental protocol for mice exposure to DDT.

### Biochemical Assays

Aspartate aminotransferase (AST) and alanine transferase (ALT) activities were measured in plasma employing commercial kits (Biosystems, Barcelona, Spain). The activities of enzymes were expressed as U/L.

### Changes in hepatic mRNA expression by real-time polymerase chain reaction (PCR)

Total RNA was extracted from liver tissue using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA,

USA). The concentration of RNA was determined by spectrophotometry (A260), and its purity was assessed by measuring the A260/A280 ratio (1.9-2.0) with a Nanodrop spectrophotometer® (Thermo Scientific, Wilmington, DE, USA). The integrity of RNA was checked by visual inspection of 28S and 18S ribosomal RNA on an agarose gel. Aliquots of RNA samples were stored at  $-80^{\circ}\text{C}$ . First-strand cDNA was synthesized from 1  $\mu\text{g}$  of total RNA by reverse transcription with a QuantiTect Reverse Transcription Kit (Qiagen Inc., Valencia, CA, USA), according to the manufacturer's instructions. The resultant cDNA was used as a template in a 20  $\mu\text{L}$  PCR reaction

containing 10 pmol, each with forward and reverse gene specific primers. The analysis of real-time PCR was performed utilizing SYBR® Green PCR Master Mix (Qiagen Inc., Valencia, CA, USA) in a Step One® 7000 thermocycler from Applied Biosystems, employing a PCR initial heat activation step by 10 min at  $95^{\circ}\text{C}$ , followed by 40 cycles of  $95^{\circ}\text{C}$  for 15 s and  $60^{\circ}\text{C}$  for 1 min. In total, 12 genes were analyzed, including one housekeeping gene. Gene names, accession numbers, forward and reverse primer sequences, as well as amplicon sizes, are listed in Table I.

The selection of gene biomarkers was aimed to

**TABLE I** - Oligonucleotide sequences for real-time PCR

	Gene name	Gene symbol	Entrez Gene ID	Forward (5' → 3')	Reverse (5' → 3')	Amplicon Size (pb)
Oxidative stress	Peroxisome proliferator activated receptor alpha	PPAR $\alpha$	19013	CAACGGCGTCGAAGACAAA	CAACGGCGTCGAAGACAAA	73
	NAD(P)H dehydrogenase, quinone 1	NQO1	18104	CAATCAGCGTTCCGGAATTACGA	GAAGAGCCCTGATTGTACTGGC	76
	Heme oxygenase (decycling) 1	HMOX1	15368	GGAGATAGAGCGCAACAAGC	GACATGGCCTTCTGGTATGG	105
Signal transduction	Nuclear receptor subfamily 1, group I, member 3	NR1I3	12355	GGAGCGGCTGTGGAAATATTGCAT	TGGGCCTCTTTGCTACAAGATGGA	95
Apoptosis	Nuclear receptor subfamily 3, group C, member 1	NR3C1	14815	GACAGCTCTGTTCCAGACTC	CCTGGATGACCAAATGACCC	139
	Cyclin-dependent kinase inhibitor 1A (P21)	CDKN1A	12575	TTGTGCGCTGTCTTGCCTCT	TATCACTCCAAGCGCAGAT	124
Insulin receptor	Insulin receptor	INSR	16337	GAGAGGATGTGAGACGACG	GGACATCCGGAACAACCTG	112
	Insulin receptor substrate 2	IRS2	384783	TAGCCACAGGAGCAACACAC	TTACTCCCTAACCACGCCTG	180
Fatty acid metabolism	Microsomal triglyceride transfer protein	MTPP	17777	ATGATCCTCTTGGCAGTGCTT	GTCACACAACCTGGCCTCTCA	82
	Fatty acid binding protein 5, epidermal	FABP5	16592	GGAAGGAGAGCAGATAACAAGA	TGTGTCATGAACAATGCCACC	73
	Sterol regulatory element binding transcription factor 1	SREBP1	20787	GATCAAAGAGGAGCCAGTGC	CACTCAGCAGCCACCATCTA	190



include those that may be involved in cross-talking between insulin signaling and DDT exposure. These comprised signal transduction, apoptosis, insulin receptor, and fatty acid metabolism, all supported by data from epidemiological studies linking DDT exposure and prevalence of diabetes, cardiovascular diseases, and elevated markers of liver toxicity.

Changes in gene expression were normalized against the reference endogenous control gene,  $\beta$ -actin (ACTB). The comparative CT method ( $2^{-\Delta\Delta Ct}$ ) was used for the relative quantification of each gene. Following amplification, melting curve analysis was performed to verify the correct product according to its specific melting temperature. All experiments were run in duplicate and negative controls contained no cDNA.

## Histopathology

Fixed tissues were embedded in paraffin, and five  $\mu$ m sections were cut and mounted on glass slides, followed by staining with hematoxylin and eosin (H&E). Sections were examined by a pathologist without knowledge of the treatment group to which each animal belonged. The histopathology of each tissue section was scored according to reported guidelines.

## Statistical Analysis

Morphometric and biochemical parameters are presented as mean  $\pm$  standard error (SE). ANOVA was used to compare means for variables between control and treated groups using Tukey as the post-test. The significance for all cases was set at  $P < 0.05$ . The real-time PCR data are presented as mean  $\pm$  SE for ten samples. Statistical analysis was performed using GraphPad Prism software (v4.0; GraphPad Software Inc., San Diego, CA, USA).

## RESULTS

### General characteristics observed in animals from different groups

During treatment animals did not show signs of any demonstrative behavioral changes or adverse effects, and no deaths were recorded during the entire experiment.

### Biochemical Assays

No significant differences were noted for the hepatosomatic index (Figure 2A). Enzymatic activities

ALT and AST are presented in Figure 2 (B-C). DDT-treated mice did not show significant signs of liver toxicity under working conditions.

### Expression of mRNA for genes involved in liver function

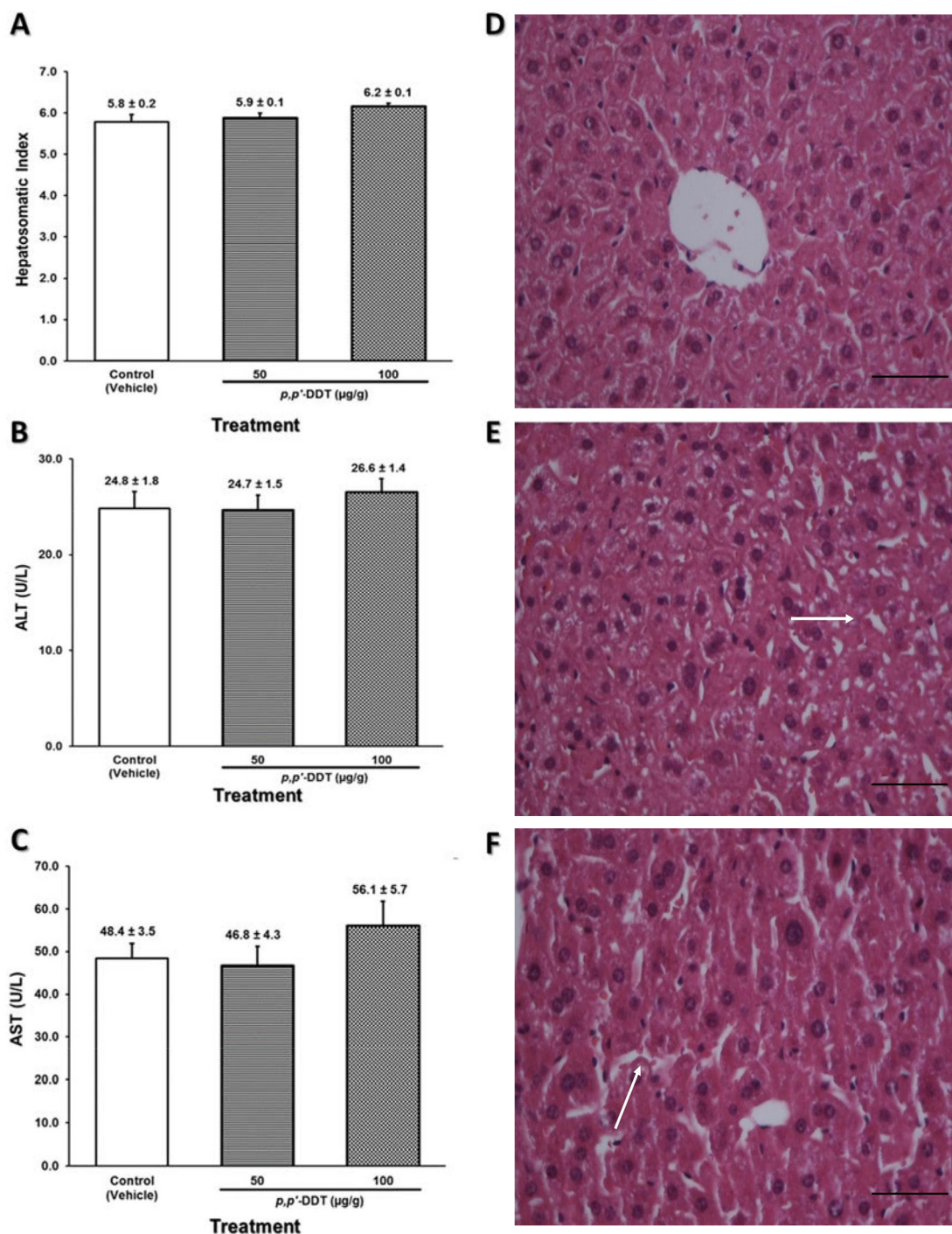
The mRNA expression profile of selected genes is shown in Table II. Compared to vehicle-control, significant up-regulation of mRNA expression was observed in the DDT-treated group (100 mg/kg) for oxidative stress responsive genes such as NAD(P) H dehydrogenase, quinone 1 (NQO1) ( $10.05 \pm 3.00$ ), peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ) ( $2.38 \pm 0.57$ ) and HMOX1 ( $2.21 \pm 0.43$ ), as well as in genes involved in fatty acid metabolism, including microsomal triglyceride transfer protein (MTTP) ( $1.95 \pm 0.30$ ) and fatty acid binding protein 5, epidermal (FABP5) ( $1.95 \pm 0.28$ ). Statistically significant differences were not noted for PPAR $\alpha$ . On the other hand, sterol regulatory element binding transcription factor 1 (SREBP1) ( $0.50 \pm 0.07$ ) was previously shown to be down-regulated at the largest tested concentration. Nuclear receptor subfamily 1, group I, member 3 or constitutive androstane receptor (CAR), a gene related to signal transduction/transcription, was induced during both treatments ( $2.69 \pm 0.57$  and  $3.11 \pm 0.64$ ). Regarding insulin receptor (INSR) and insulin receptor substrate 2 (IRS2), no changes were observed ( $1.19 \pm 0.16$ ;  $1.17 \pm 0.20$  and  $0.88 \pm 0.14$ , respectively). Finally, for nuclear receptor subfamily 3, group C, member 1 (NR3C1) ( $1.71 \pm 0.21$ ) significant up-regulation of mRNA expression was observed in the DDT-treated group (100 mg/kg). Animals treated with DDT at 50 mg/kg did not present significant changes in gene expression.

## Histopathology

Histopathological findings from liver sections are presented in Figure 2. Liver histopathology (H&E) shows normal parenchymal architecture in the control group (Figure 2 D), with the hepatic central vein surrounded by hepatocytes organized in rows in a circumferential manner, separated by liver capillary sinusoids showing a mild dilatation. These presented minor changes in treated mice (E-F), mostly represented by mild and moderate dilatation of liver capillary sinusoids.

## DISCUSSION

The purpose of this study was to evaluate the possible role of DDT in the expression of genes belonging



**FIGURE 2** - DDT-treated mice did not show significant signs of liver toxicity under working conditions. Control-vehicle and DDT-treated mice had similar hepatosomatic indices and plasma transaminase activities (ALT and AST) (A–C). Liver histopathology (H&E) shows the hepatic central vein surrounded by hepatocytes organized in rows in a circumferential manner and separated by liver capillary sinusoids which show a mild dilatation (D). E and F show a mild and moderate dilatation of liver capillary sinusoids, respectively. Scale bar: 50 µm.

to several pathways associated with oxidative stress, insulin signaling, apoptosis, and changes in lipid metabolism.

In this study, female BalBc adult mice were acutely

treated with 50 and 100 mg/kg *p,p'*-DDT. None of the treated groups displayed significant changes in body weight when compared to the control group. Results from the present study did not show changes in the levels of

**TABLE II** - Hepatic expression of genes involved in insulin signaling measured in hepatic tissue of DDT-exposed mice

	Gene name	Gene symbol	Control	DDT 50 µg/g	DDT 100 µg/g
Oxidative stress	Peroxisome proliferator activated receptor alpha	PPAR $\alpha$	1.06 $\pm$ 0.11	1.18 $\pm$ 0.18	2.38 $\pm$ 0.57
	NAD(P)H dehydrogenase, quinone 1	NQO1	1.24 $\pm$ 0.26	2.29 $\pm$ 0.53	10.05 $\pm$ 3.00*
Signal transduction	Heme oxygenase (decycling) 1	HMOX1	1.12 $\pm$ 0.18	1.24 $\pm$ 0.21	2.21 $\pm$ 0.43*
	Nuclear receptor subfamily 1, group I, member 3	NR1I3	1.39 $\pm$ 0.33	2.69 $\pm$ 0.57	3.11 $\pm$ 0.64*
Apoptosis	Nuclear receptor subfamily 3, group C, member 1	NR3C1	1.06 $\pm$ 0.11	1.12 $\pm$ 0.15	1.71 $\pm$ 0.21*
	Cyclin-dependent kinase inhibitor 1A (P21)	CDKN1A	1.43 $\pm$ 0.34	0.89 $\pm$ 0.22	1.12 $\pm$ 0.36
Insulin receptor	Insulin receptor	INSR	1.03 $\pm$ 0.08	1.07 $\pm$ 0.12	1.19 $\pm$ 0.16
	Insulin receptor substrate 2	IRS2	1.11 $\pm$ 0.17	1.17 $\pm$ 0.20	0.88 $\pm$ 0.14
Fatty acid metabolism	Fatty acid binding protein 5, epidermal	FABP5	1.18 $\pm$ 0.22	1.20 $\pm$ 0.18	1.95 $\pm$ 0.30
	Microsomal triglyceride transfer protein	MTTP	1.05 $\pm$ 0.11	1.05 $\pm$ 0.15	1.95 $\pm$ 0.28*
	Sterol regulatory element binding transcription factor 1	SREBP1	1.10 $\pm$ 0.14	0.95 $\pm$ 0.11	0.50 $\pm$ 0.07*

\*. Significant difference ( $P < 0.05$ ) compared to the control group.

liver enzymes when compared to the control group.

Gene expression data showed that DDT increased hepatic NQO1 expression compared to the control group. NQO1 is a gene induced by a wide variety of chemicals, including oxidants, xenobiotics, electrophiles, and phenolic compounds (Zhu, Li, 2012). This gene is involved in the body's defense system against oxidative stress (Gaikwad *et al.*, 2001) and its up-regulation may be an adaptive mechanism to protect against oxidative and inflammatory stress. NQO1 may also play a role in lipid metabolism and insulin resistance (Zhu, Li, 2012), since its expression levels have been positively correlated with adiposity, glucose tolerance, and markers of liver dysfunction, suggesting a possible involvement of NQO1 in the metabolic complications of human obesity (Palming *et al.*, 2007).

In the model presented here, hepatic mRNA levels of HMOX1 were significantly induced by *p,p'*-DDT, similar to what was observed with *o,p'*-DDT (Kiyosawa *et al.*, 2008b). HMOX1 is also elevated in response to oxidative stress, and its up-regulation mediates signal transduction networks involving AP-1, nuclear factor-kappa B (NF- $\kappa$ B) and nuclear factor E2-related factor-2 (Nrf2), as well as upstream kinases related to the maintenance of cellular redox homeostasis (Farombi, Surh, 2006).

Results demonstrated hepatic expression of CAR was markedly increased for both treatments. Pesticide exposure can lead to alterations in the endogenous levels of hormones, and subsequently compromise their hormone signaling. For example, DDT and its metabolite DDE activate nuclear receptor subfamily 1, group I, member 2 or pregnane X receptor (PXR) and



CAR in rodents (Tebourbi, Sakly, Rhouma, 2011). On the contrary, (Kiyosawa *et al.*, 2008a), using C57BL/6 mice treated with 300  $\mu\text{g/g}$  *o,p'*-DDT and comparing the results of their study to those of Sprague-Dawley rat data, reported that mouse CAR mRNA levels decreased. This may be a result of the activation of mouse-PXR, while ER-mediated effects were insignificant in rats, probably due to the inhibitory effects of CAR on ER activities.

PPAR $\alpha$  and MTTP had a weak increase in gene expression. Common fatty acids have been shown to activate PPAR $\alpha$  and to regulate the expression of genes of various lipid oxidation pathways and transport (Hsu, Huang, 2007), and its activation leads to the concomitant activation of genes encoding for classical peroxisomal straight chain fatty acid-oxidation system, microsomal cytochrome P450 CYP4A isoforms CYP4A1 and CYP4A3, and some of the genes involved in the mitochondrial-oxidation, among others (Reddy, Hashimoto, 2001). Several studies have suggested an association between MTTP and diabetic dyslipidemia (Rubin *et al.*, 2008). In particular, high-fat diets as well as diabetic conditions are associated with elevated hepatic and intestinal MTTP expressions (Lin *et al.*, 1994). DDT also induced a down-regulation of hepatic expression of SREBP-1, opposite to what has been reported for this gene in DMT2 (Knip *et al.*, 2005). Studies have shown that SREBP-1 overexpression may lead to lipid metabolism disorder and cause lipid accumulation and a fatty liver. However, there are many studies on high and low expression of SREBP-1c in rats and humans. It may be due to SREBP down-regulation of beta cells, affecting signaling pathways for insulin secretion stimulated by glucose and glucolipototoxicity during T2DM (Shao *et al.*, 2010).

Findings presented here showed transcriptional changes are clearly evidenced in the absence of histopathological damage in mice exposed to moderate doses of DDT. These results are in agreement with those of other authors, who showed that rodents exhibited negligible histopathology with rapid *o,p'*-DDT metabolism (Kiyosawa *et al.*, 2008a; Kiyosawa *et al.*, 2008b). Since toxicogenomic profiling may precede clinical chemistry, histopathology, clinical, or even ultrastructural changes (Heinloth *et al.*, 2007; Miyawaki *et al.*, 2011; Wang, Papoutsi, Wiesmann, 2011), gene expression measurements are useful to gain insight into the early signaling perturbations that presage toxicologic effects (Ruepp *et al.*, 2002).

Numerous epidemiological studies have suggested that DDT exposure is likely to contribute to the increase of T2DM (Lee *et al.*, 2010a; Everett, Frithsen, Player, 2011)

Due to the lipophilic nature of DDT, it is trapped and stored within the adipose tissue. Therefore, the concentration of these compounds in the adipose tissue may exceed that found in blood. Interestingly, there is the possibility that patients with diabetes may retain more of these pollutants than healthy individuals (Everett, Frithsen, Player, 2011), although there is no conclusive epidemiological evidence for such a relationship. Thus, future studies of effects of DDT on adipose tissues will be necessary. However, it is now clear that legacy OC insecticides affect multiple pathways that involve glucose homeostasis (Swaminathan, 2013) and collectively contribute to hyperglycemia (Rahimi, Abdollahi, 2007).

Diabetes may be produced by an immunotoxic effect of POPs via their binding with ER. This mechanism would induce a chronic low-grade inflammation process, decreased mitochondrial function, fatty acid oxidation, and increased lipolysis, which are related to the insulin resistance syndrome (Guilherme *et al.*, 2008), as well as another mechanism involving tissue specific up- or down-regulation of gene expression, which might promote glucose intolerance and induce diabetes-independent oxidative stress and mitochondrial dysfunction (Wallace, 2005). In fact, recent studies have shown that oxidative stress has a role in the development of insulin resistance, which is characterized by hyperinsulinemia (Henriksen, Diamond-Stanic, Marchionne, 2011). One of the major sources of ROS is xenobiotics, and as presented here, oxidative stress is an important mechanism that may link DDT to diabetes, affecting cell functions, metabolism, gene expression profile, and other pathological conditions (Young, Woodside, 2001). The research on oxidative stress and its connection with other signal transduction pathways could also disclose new scenarios to better evaluate the toxicological response to xenobiotics (Miyawaki *et al.*, 2011), in particular, the role of DDT on regulation of glucose metabolism.

## CONCLUSIONS

The findings of this study suggests that acute exposure to DDT in mice did not promote adverse effects on weight and on hepatic markers of toxicity or liver histopathology. However, there were changes at the molecular level represented in the activation of several genes known to participate in insulin resistance pathways, such as CAR, SREBP, NQO1, NR3C1, HMOX1 and MTTP. This conclusion could be particularly relevant in view of the recently emerging evidence showing that DDT may mimic different processes involved in diabetes.



## ACKNOWLEDGEMENTS

The authors express their gratitude to Colciencias and the University of Cartagena (Grant 110745921616, 2008).

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## REFERENCES

- ARREBOLA, J.P.; CUELLAR, M.; CLAURE, E.; QUEVEDO, M.; ANTELO, S.R.; MUTCH, E.; RAMIREZ, E.; FERNANDEZ, M.F.; OLEA, N.; MERCADO, L.A. Concentrations of organochlorine pesticides and polychlorinated biphenyls in human serum and adipose tissue from Bolivia. *Environ. Res.*, v.112, p.40-47, 2012.
- AGENCY FOR TOXIC SUBSTANCES & DISEASES REGISTRY. ATSDR. Public health statement for DDT, DDE, AND DDD, Sep., 2002. Retrieved from Available at <http://www.atsdr.cdc.gov/phs/phs.asp?id=79&tid=20>. Accessed on: June 17, 2014.
- BLOOMGARDEN, Z.T. European Association for the study of diabetes Annual Meeting, 1999: treatment modalities. *Diabetes Care*, v.23, n.7, p.1012-1017, 2000.
- COLLOTTA, M.; BERTAZZI, P.A.; BOLLATI, V. Epigenetics and pesticides. *Toxicology*, v.307, p.35-41, 2013.
- COX, S.; NISKAR, A.S.; NARAYAN, K.M.; MARCUS, M. Prevalence of self-reported diabetes and exposure to organochlorine pesticides among Mexican Americans: Hispanic health and nutrition examination survey, 1982-1984. *Environ. Health Perspect.*, v.115, n.2, p.1747-1752, 2007.
- DANAIEI, G.; FINUCANE, M.; LU, Y.; SINGH, G.; COWAN, M.; PACIOREK, C.; LIN, J.; FARZADFAR, F.; KHANG, Y.-H.; STEVENS, G.; RAO, M.; ALI, M.; RILEY, L.; ROBINSON, C.; EZZATI, M.; GLOBAL BURDEN OF METABOLIC RISK FACTORS OF CHRONIC DISEASES COLLABORATING, G. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet*, v.378, p.31-40, 2011.
- EVERETT, C.J.; FRITHSEN, I.; PLAYER, M. Relationship of polychlorinated biphenyls with type 2 diabetes and hypertension. *J. Environ. Monit.*, v.13, n.2, p.241-251, 2011.
- FAROMBI, E.O.; SURH, Y.J. Heme oxygenase-1 as a potential therapeutic target for hepatoprotection. *J. Biochem. Mol. Biol.*, v.39, n.5, p.479-491, 2006.
- FRIGO, D.E.; VIGH, K.A.; STRUCKHOFF, A.P.; ELLIOTT, S.; BECKMAN, B.S. Xenobiotic-induced TNF- $\alpha$  expression and apoptosis through the p38 MAPK signaling pathway. *Toxicol. Lett.*, v.155, n.2, p.227-238, 2005.
- GAIKWAD, A.; LONG, D.J.; STRINGER, J.L.; JAISWAL, A.K. In vivo role of NAD (P) H: quinone oxidoreductase 1 (NQO1) in the regulation of intracellular redox state and accumulation of abdominal adipose tissue. *J. Biol. Chem.*, v.276, n.25, p.22559-22564, 2001.
- GUILHERME, A.; VIRBASIS, J.V.; PURI, V.; CZECH, M.P. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell. Biol.* v.9, n.5, p.367-377, 2008.
- HEINLOTH, A.N.; BOORMAN, G.A.; FOLEY, J.F.; FLAGLER, N.D.; PAULES, R.S. Gene expression analysis offers unique advantages to histopathology in liver biopsy evaluations. *Toxicol. Pathol.*, v.35, n.2, p.276-283, 2007.
- HENRIKSEN, E.J.; DIAMOND-STANIC, M.K.; MARCHIONNE, E.M. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free. Radic. Biol. Med.*, v.51, n.5, p.993-999, 2011.
- HSU, S.-C.C.; HUANG, C.-J.J. Changes in liver PPAR $\alpha$  mRNA expression in response to two levels of high-safflower-oil diets correlate with changes in adiposity and serum leptin in rats and mice. *J. Nutr. Biochem.*, v.18, n.2, p.86-96, 2007.
- IBRAHIM, M.; FJÆRE, E.; LOCK, E.-J.; NAVILLE, D.; AMLUND, H.; MEUGNIER, E.; LE MAGUERESSE BATTISTONI, B.; FRØYLAND, L.; MADSEN, L.; JESSEN, N.; LUND, S.; VIDAL, H.; RUZZIN, J. Chronic consumption of farmed salmon containing persistent organic pollutants causes insulin resistance and obesity in mice. *PLoS One*, v.6, p.e25170, 2011.
- JIN, X.; CHEN, M.; SONG, L.; LI, H.; LI, Z. The evaluation of *p,p'*-DDT exposure on cell adhesion of hepatocellular carcinoma. *Toxicology*, v.322, p.99-108, 2014.

- KACEW, S.; SINGHAL, R.L. Role of cyclic adenosine 3':5'-monophosphate in the action of 1,1,1-trichloro-2,2-bis-(p-chlorophenyl)ethane (DDT) on hepatic and renal metabolism. *Biochem. J.*, v.142, n.1, p.145-152, 1974.
- KIYOSAWA, N.; KWEKEL, J.; BURGOON, L.; DERE, E.; WILLIAMS, K.; TASHIRO, C.; CHITTIM, B.; ZACHAREWSKI, T. Species-specific regulation of PXR/CAR/ER-target genes in the mouse and rat liver elicited by o, p'-DDT. *BMC Genomics.*, v.9, p.487, 2008a.
- KIYOSAWA, N.; KWEKEL, J.C.; BURGOON, L.D.; WILLIAMS, K.J.; TASHIRO, C.; CHITTIM, B.; ZACHAREWSKI, T.R. o,p'-DDT elicits PXR/CAR-, not ER-, mediated responses in the immature ovariectomized rat liver. *Toxicol. Sci.*, v.101, n.2, p.350-363, 2008b.
- KNIP, M.; VEIJOLA, R.; VIRTANEN, S.M.; HYOTY, H.; VAARALA, O.; AKERBLUM, H.K. Environmental triggers and determinants of type 1 diabetes. *Diabetes*, v.54, p.S125-136, 2005.
- LA MERRILL, M.; KAREY, E.; MOSHIER, E.; LINDTNER, C.; LA FRANO, M.R.; NEWMAN, J.W.; BUETTNER, C. Perinatal exposure of mice to the pesticide DDT impairs energy expenditure and metabolism in adult female offspring. *PLoS One*, v.9, p.e103337, 2014.
- LEE, D.-H.; STEFFES, M.; SJÖDIN, A.; JONES, R.; NEEDHAM, L.; JACOBS, D. Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. *Environ. Health Perspect.*, v.118, n.9, p.1235-1242, 2010a.
- LEE, D.-H.H.; STEFFES, M.W.; SJÖDIN, A.; JONES, R.S.; NEEDHAM, L.L.; JACOBS, D.R. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. *PLoS One*, v.6, n.1, p.e15977, 2010b.
- LIN, M.C.; ARBEENY, C.; BERGQUIST, K.; KIENZLE, B.; GORDON, D.A.; WETTERAU, J.R. Cloning and regulation of hamster microsomal triglyceride transfer protein. The regulation is independent from that of other hepatic and intestinal proteins which participate in the transport of fatty acids and triglycerides. *J. Biol. Chem.*, v.269, n.46, p.29138-29145, 1994.
- LUCENA, R.A.; ALLAM, M.F.; JIMÉNEZ, S.S.; VILLAREJO, M.L. A review of environmental exposure to persistent organochlorine residuals during the last fifty years. *Curr. Drug Saf.*, v.2, n.2, p.163-172, 2007.
- MIYAWAKI, I.; MATSUMOTO, I.; HORIE, H.; INADA, H.; KIMURA, J.; FUNABASHI, H.; SEKI, T. Toxicological approach for elucidation of clobazam-induced hepatomegaly in male rats. *Regul. Toxicol. Pharmacol.*, v.60, n.3, p.323-331, 2011.
- MORENO-ALIAGA, M.; MATSUMURA, F. Effects of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (p,p'-DDT) on 3T3-L1 and 3T3-F442A adipocyte differentiation. *Biochem. Pharmacol.*, v.63, n.5, p.997-1007, 2002.
- PALMING, J.; SJÖHOLM, K.; JERNÅS, M.; LYSTIG, T.C.; GUMMESSON, A.; ROMEO, S.; LÖNN, L.; LÖNN, M.; CARLSSON, B.; CARLSSON, L.M. The expression of NAD(P)H:quinone oxidoreductase 1 is high in human adipose tissue, reduced by weight loss, and correlates with adiposity, insulin sensitivity, and markers of liver dysfunction. *J. Clin. Endocrinol. Metab.*, v.92, n. 6, p.2346-2352, 2007.
- PATEL, C.J.; BHATTACHARYA, J.; BUTTE, A.J. An Environment-Wide Association Study (EWAS) on type 2 diabetes mellitus. *PLoS One*, v.5, p.2009.
- PORTA, M.; MALATS, N.; JARIOD, M.; GRIMALT, J.O.; RIFÀ, J.; CARRATO, A.; GUARNER, L.; SALAS, A.; SANTIAGO-SILVA, M.; COROMINAS, J. M.; ANDREU, M.; REAL, F.X. Serum concentrations of organochlorine compounds and K-ras mutations in exocrine pancreatic cancer. PANKRAS II Study Group. *Lancet*, v.354, p.2125-2129, 1999.
- RAHIMI, R.; ABDOLLAHI, M. A review on the mechanisms involved in hyperglycemia induced by organophosphorus pesticides. *Pestic. Biochem. Phys.*, v.88, n.2, p.115-121, 2007.
- REDDY, J.K.; HASHIMOTO, T. Peroxisomal  $\beta$ -oxidation and peroxisome proliferator-activated receptor  $\alpha$ : an adaptive metabolic system. *Annu. Rev. Nutr.*, v.21, p.193-230, 2001.
- ROLO, A.P.; PALMEIRA, C.M.; WALLACE, K.B. Interactions of combined bile acids on hepatocyte viability: cytoprotection or synergism. *Toxicol. Lett.*, v.126, n.3, p.197-203, 2002.

- RUBIN, D.; SCHNEIDER-MUNTAU, A.; KLAPPER, M.; NITZ, I.; HELWIG, U.; FÖLSCH, U.R.; SCHREZENMEIR, J.; DÖRING, F. Functional analysis of promoter variants in the microsomal triglyceride transfer protein (MTTP) gene. *Hum. Mutat.*, v.29, n.1, p.123-129, 2008.
- RUEPP, S.U.; TONGE, R.P.; SHAW, J.; WALLIS, N. Genomics and proteomics analysis of acetaminophen toxicity in mouse liver. *Toxicol. Sci.*, v. 65, n.1, p.135-150, 2002.
- RUZZIN, J.; PETERSEN, R.; MEUGNIER, E.; MADSEN, L.; LOCK, E.-J.; LILLEFOSSE, H.; MA, T.; PESENTI, S.; SONNE, S.; MARSTRAND, T.; MALDE, M.; DU, Z.-Y.; CHAVEY, C.; FAJAS, L.; LUNDEBYE, A.-K.; BRAND, C.; VIDAL, H.; KRISTIANSEN, K.; FRØYLAND, L. Persistent organic pollutant exposure leads to insulin resistance syndrome. *Environ. Health Perspect.*, v.118, n.4, p.465-471, 2010.
- RYLANDER, L.; RIGNELL-HYDBOM, A.; HAGMAR, L. A cross-sectional study of the association between persistent organochlorine pollutants and diabetes. *Environ. Health Glob.*, v.4, p.28, 2005.
- SANYAL, S.; AGARWAL, N.; DUDEJA, P.K.; MAHMOOD, A.; SUBRAHMANYAM, D. Effect of a single oral dose of DDT on lipid metabolism in protein-calorie malnourished monkeys. *Indian J. Biochem. Biophys.*, v.19, n.2, p.111-114, 1982.
- SARGIS, R.M. The hijacking of cellular signaling and the diabetes epidemic: mechanisms of environmental disruption of insulin action and glucose homeostasis. *Diabetes Metab. J.*, v.38, n.1, p.13-24, 2014.
- SHAO, S.; LIU, Z.; YANG, Y.; ZHANG, M.; YU, X. SREBP-1c, Pdx-1, and GLP-1R involved in palmitate-EPA regulated glucose-stimulated insulin secretion in INS-1 cells. *J. Cell Biochem.*, v.111, n.3, p.634-642, 2010.
- SHEN, K.; NOVAK, R.F. DDT stimulates c-erbB2, c-met, and STATS tyrosine phosphorylation, Grb2-Sos association, MAPK phosphorylation, and proliferation of human breast epithelial cells. *Biochem. Biophys. Res. Commun.*, v.231, n.1, p.17-21, 1997.
- SOHONI, P.; SUMPTER, J.P. Several environmental oestrogens are also anti-androgens. *J. Endocrinol.*, v.158, n.3, p.327-339, 1998.
- SWAMINATHAN, K. Pesticides and human diabetes: a link worth exploring? *Diabet. Med.*, v.30, n.11, p.1268-1271, 2013.
- TEBOURBI, O.; SAKLY, M.; RHOUMA, K.B. *Pesticides in the modern world - pests control and pesticides exposure and toxicity assessment*. Rijeka, Croatia: Intech Open Access Publisher, 2011. cap 15, p.297-332.
- THAYER, K.; HEINDEL, J.; BUCHER, J.; GALLO, M. Role of environmental chemicals in diabetes and obesity: a national toxicology program workshop review. *Environ. Health Perspect.*, v.120, n.6, p.779-789, 2012.
- WALLACE, D.C. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.*, v.39, p.359-407, 2005.
- WANG, T.; PAPOUTSI, M.; WIESMANN, M. Investigation of correlation among safety biomarkers in serum, histopathological examination, and toxicogenomics. *Int. J. Toxicol.*, v.30, n.3, p.300-312, 2011.
- YAU, D.T.; MENNEAR, J.H. The inhibitory effect of DDT on insulin secretion in mice. *Toxicol. Appl. Pharmacol.*, v.39, n.1, p.81-88, 1976.
- YOUNG, I.S.; WOODSIDE, J.V. Antioxidants in health and disease. *J. Clin. Pathol.*, v.54, n.3, p.176-186, 2001.
- ZHU, H.; LI, Y. NAD(P)H: quinone oxidoreductase 1 and its potential protective role in cardiovascular diseases and related conditions. *Cardiovasc. Toxicol.*, v.12, n.1, p.39-45, 2012.

Received for publication on 26<sup>th</sup> March 2015

Accepted for publication on 14<sup>th</sup> March 2016

