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The role of pesticides in non-alcoholic fatty liver disease (NAFLD).

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THE ROLE OF PESTICIDES IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

By

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ABSTRACT

THE ROLE OF PESTICIDES IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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NAFLD, the most common form of liver disease worldwide, is caused by various factors including industrial chemicals and pesticides exposure. Annually, 5.2 billion pounds of pesticides are used worldwide and can contribute to liver disease, but their role is modestly studied. We hypothesize that pesticides contaminating food supply can worsen diet-induced steatosis *via* xenobiotic receptor activation. Two human and two rodent databases were utilized and 85% of the 330 chemicals identified associated with NAFLD were pesticides. Eight were selected for evaluating hepatic receptor activation *in vitro*. The majority including DDT activated hPXR/CAR and mPXR. DDT (100 mg/kg) was studied *in vivo* in a diet-induced obesity (DIO) model. DDT upregulated Cyp2b10 (CAR target) in control diet-fed mice. DDT decreased adiposity, but it did not affect weight gain, food consumption or insulin resistance. In conclusion, DDT improved steatosis, but it did not affect NAFLD, obesity, liver damage or diabetes caused by DIO.

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CHAPTER 1

INTRODUCTION

Pesticides:

In our daily lives, we are exposed to a variety of chemicals, many of which are pesticides, through food consumption or contact with skin and air. According to the United States Environmental Protection Agency (US EPA), a pesticide is defined as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest". Although pesticides are often restricted to insecticides, the term 'pesticide' also applies to herbicides, fungicides, and various other substances used for controlling pests (1). Pesticide usage is a double-edged sword; they are valuable for controlling pests, but they are also poisonous compounds to humans and animals. Many of these compounds are lipophilic and tend to accumulate in the adipose tissue. However, the body defense mechanism, particularly the liver, is responsible for the detoxification of these xenobiotic compounds through metabolism by cytochrome P450 enzymes, including CYP3A and CYP2B families (2, 3). In contrast, if the pesticides' bioaccumulated concentration reaches lethal levels due to high dose or chronic exposure, they can overwhelm the liver detoxification capacity and cause toxicity. In fact, the WHO reported that at least 3 million cases of pesticide poisoning occur worldwide annually. Furthermore, many pesticides have been

associated with liver disease and elevated levels of liver enzymes (aminotransferases) (4).

Chemical pesticides are classified into four main classes: organophosphate, carbamate, organochlorine and pyrethroid pesticides (5). Our laboratory works with persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) and their association with liver disease. Epidemiological studies have demonstrated associations between pesticides such as OCPs and toxicant associated steatohepatitis (TASH)/nonalcoholic fatty liver disease (NAFLD) as well as serum alanine aminotransferase (ALT) elevation (6). However, the mechanisms by which these compounds potentiate liver disease and damage are poorly studied.

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent form of liver disease worldwide (7-9). It is often associated with obesity, insulin resistance, diabetes and other metabolic disorders primarily through adipo-cytokine dysregulation and hence, NAFLD is considered a hepatic indicator of the metabolic syndrome (10, 11). Histopathologically, NAFLD involves a wide spectrum of liver damage, initiating from steatosis, which is characterized by fatty liver, and transforming to steatohepatitis (12, 13). Our laboratory focuses specifically on nonalcoholic steatohepatitis (NASH), which progresses in a “two hit” model (8). The first hit results in steatosis, an accumulation of macrovesicular or microvesicular triglyceride in at least 5% of hepatic cells, and this can be brought about by excessive consumption of hyper-caloric diets such as high-fructose diet (9, 14, 15). The “second hit” results in hepatic and systemic

inflammation which is accompanied by increased levels of inflammatory cytokines/adipokines, mitochondrial dysfunction and oxidative stress and causes steatohepatitis and fibrosis (13, 16). Steatohepatitis may then progress to fibrosis and cirrhosis (4). The “second hit” can include factors such as industrial chemicals and toxic pollutants leading to the term “toxicant associated steatohepatitis” (TASH) (17, 18). We focus on the role of environmental pollutants as a ‘second hit’ in NAFLD and the mechanisms of their action in the potentiation and progression of steatohepatitis.

TASH was first described in vinyl chloride (VC) workers who reported increased levels of proinflammatory cytokines, insulin resistance, and antioxidant depletion (17, 18). The necrotic hepatocyte death biomarker, cytokeratin 18, was also elevated in these workers (11, 19). Mild to moderate ALT and aspartate aminotransferase (AST) elevation are the only laboratory manifestations reported in most cases of steatohepatitis (14, 18, 20, 21). However, the VC workers with TASH had normal serum ALT and AST levels. On the other hand, abnormalities in ALT and AST levels have been reported in humans and animals exposed to other chemicals such as carbon tetrachloride, dimethylformamide, some minerals and pesticides (22).

Xenobiotic compounds are metabolized by P450 enzymes, which are under the control of xenobiotic receptors, and this metabolic link can add a layer of complexity to both the disease and treatment state. Hepatic xenobiotic receptors, particularly the pregnane xenobiotic receptor (PXR) and the constitutive androstane receptor (CAR), play an important role in xenobiotic

metabolism. Apart from regulating detoxification, PXR has been reported to promote lipogenesis and repress fatty acid-β-oxidation leading to hepatic lipid accumulation (23, 24). On the other hand, CAR reportedly suppresses gluconeogenesis and lipid metabolism and decreases serum triglyceride levels (24). Hence both PXR and CAR appear to play a pivotal role in regulating energy metabolism and their activation/inhibition can lead to NAFLD, obesity and the metabolic syndrome (24). In addition to CAR and PXR, other nuclear receptors such as the peroxisome proliferator-activated receptors (PPARs) regulate genes associated with glucose and lipid metabolism, adipogenesis, insulin sensitivity, cell growth, and differentiation (25, 26). Their activation also affects immune responses and energy homeostasis. In fact, PPARs are important therapeutic targets in treating metabolic disorders such as diabetes (25). The thiazolidinedione drugs, which are PPARγ agonists are used for treating type 2 diabetes, whereas another type 2 drug, metformin, works *via* PPARα-dependent or independent mechanisms (26, 27). Similar to the PPARs, the farnesoid X receptor (FXR), plays an important role in regulating metabolism of bile acids, fat, cholesterol, glucose and xenobiotics (28). In fact, clinical trials on FXR-targeting drugs for treating cholestasis, type 2 diabetes mellitus, NASH or NAFLD and primary bile acid diarrhea are now in Phase I and II trials (28).

Significance of The Study:

Non-alcoholic fatty liver disease (NAFLD) is prevalent in 30% of the US adult population and it is the leading cause of chronic liver disease worldwide (7, 8, 29). According to a community-based study, NAFLD increases the mortality risk among the US population (7). NAFLD was associated with obesity, insulin resistance, diabetes and other metabolic disorders (10, 11). In one study, 100% of obese diabetic patients had steatosis, 50% of those had steatohepatitis and 19% had cirrhosis (30). Furthermore, many industrial chemicals including pesticides have been associated with fatty liver disease.

Elevated serum ALT and AST are traditional biomarkers of NAFLD (4). Abnormalities in ALT and AST were found in humans and animals exposed to OCPs and other chemicals such as carbon tetrachloride, dimethylformamide, minerals and pesticides (4, 22). Because traditional NAFLD biomarkers are not effective in diagnosing TASH, liver biopsies serve as a good diagnostic tool. However, obtaining human liver biopsies can be challenging, therefore, animal models can be a valuable tool in identifying TASH-causing chemicals. Furthermore, the concentration(s) needed and mechanism(s) exhibited by pesticides in their contribution to NAFLD are modestly studied due to the lack of disease manifestations and chemical indicators. Additionally, a comprehensive list of these chemicals is not currently available in literature.

Pesticide exposure and their biological effects are relevant because annual pesticide consumption worldwide was 5.2 billion pounds, out of which 1.1 billion pounds was consumed in the US alone (31). While a large number of

pesticides have been banned in many countries including the US, some still exist in the environment due to their thermodynamic stability and lipophilicity.

The hepatic xenobiotic receptors such as CAR and PXR play an important role in both xenobiotic detoxification and endobiotic metabolism either through direct activation or interaction with other receptors (24). Therefore, elucidating the mode(s) of action of pesticides through CAR and PXR regulation is crucial in terms of NAFLD due to their role in maintaining energy homeostasis (23, 24).

Based on the evidence provided, we hypothesize that pesticides, which contaminate the food supply, may worsen diet-induced steatohepatitis via xenobiotic receptor activation, PXR and CAR. Therefore, to test our hypothesis, 1) we identified pesticides associated with steatohepatitis and NAFLD in a) rodent studies (ToxRefDB and CEBS) and b) human studies (NHANES). 2) We identified pesticides activating PXR and CAR in humans a) *via* data-screening of ToxCastDB database and b) screening assays using HepG2 cells. 3) After screening a list of pesticides, which were associated with NAFLD and activated PXR and CAR, we selected DDT, a relevant POPs, to be studied *in vivo* in a diet-induced obesity (DIO) model.

CHAPTER 2

IDENTIFICATION OF CHEMICALS ASSOCIATED WITH NAFLD

INTRODUCTION

The liver is the first-line of defense against potentially harmful xenobiotics, and it is therefore not surprising that it is also the target organ that is most commonly affected by industrial chemicals (4). Indeed, 33% of the 677 most common workplace chemicals reported in the National Institute of Occupational Safety and Health Pocket Guide are associated with hepatotoxicity (32). The pathologic liver lesions associated with chemical exposures are myriad and range from hepatitis to fibrosis and cirrhosis with liver cancer (22). However, following the description of TASH, it now appears that fatty liver may be the most common pathologic response to chemicals (4, 20, 22, 33, 34).

Recent terms describing fatty liver disease such as “steatosis” and “steatohepatitis” were not well-known before recognizing fatty liver disease as a clinical disease. Consequently, one of the tools used to identify compounds relevant to liver disease, is to search former chemical studies by looking at fatty liver clinical and histopathological biomarkers. With NAFLD, the only clinical biomarker for fatty liver disease is serum ALT elevation. However, diagnosing TASH in humans is challenging for several reasons, namely, the entity is

clinically under-recognized and routine clinical biomarkers are insensitive. Also, out of the 83 million substances and 65 million sequences registered by the chemical abstracts service by 2014, there is no comprehensive list of chemicals that cause TASH (35). As such, TASH is a clinicopathologic diagnosis that relies solely on histologic examination.

The transition from steatosis to steatohepatitis is characterized by centrilobular (zone 3) centered injury and lobular inflammation (lymphocytes with neutrophils and activated Kupffer cells), hepatocyte ballooning and Mallory-Denk bodies and fibrosis (36). While these findings are typically present in hemotoxylin and eosin (H&E) stained slides, other stains such as Oil-Red-O that stains micro-vesicular lipid droplets are used to quantify steatosis. Similar pathologic lesions have been observed in human NAFLD/TASH and in rodent models of steatohepatitis (4).

The purpose of this part of the study is to identify chemicals associated with the development of hepatic steatosis in previously published human and rodent studies. Searchable archive of Human studies from the 2003-2004 NHANES (National Center for Health Statistics) and rodent studies provided in the websites of US Environmental Protection agency (EPA) and the National Institute of Environmental Health Sciences (NIEHS) presented a unique opportunity to accomplish this objective. The identification of environmental chemicals associated with the development of hepatic steatosis/TASH will enable subsequent mechanistic animal studies and clinical translation in exposed humans.

MATERIALS AND METHODS

Data-screening:

The first database screened was the 2003–2004 National Health and Nutrition Examination Survey of the United States population (**NHANES 2003–2004**). The NHANES was evaluated in a cross-sectional cohort study done previously by our group. The National Center for Health Statistics (NCHS) conducts the NHANES as a complex multistage probability sample, and interprets the health and nutrition findings as representative of the non-institutionalized U.S. civilian population (37). Approval for the analysis of the NHANES data was granted by the University of Louisville Institutional Review Board. The following exclusion criteria were used in the study: age < 18 years, positive serum hepatitis B surface antigen, positive serum hepatitis C antibody, elevated transferrin saturation (> 60% for men and > 50% for women), and alcohol consumption ≥ 20 g/day for men and ≥ 10 g/day for women. As classified by the NCHS, the downloaded pollutants data posted prior to December 2008 showed 196 pollutants from 17 subclasses (38). All alanine aminotransferase (ALT) and pollutant levels were measured for each participant and 111 of 196 pollutants were evaluated (38). Elevated ALT was defined as proposed by Prati et al. (>30 IU/L for men and >19 IU/L for women) (38, 39). Subjects were classified into different quartiles for each class of pollutants, with the first quartile composed of subjects with the lowest serum levels of each pollutant. Pollutants within the same subclass were then summed by their ranks depending on the

magnitude of their detectable levels, because of the likelihood of an individual's exposure to more than one pollutant in the same subclass (38). Multivariate-adjusted odds ratios for ALT elevation were measured though the increasing quartiles of chemical exposure and the 1st quartile was used as the reference group (38).

Two more sets of rodent databases compiled by the federal government for environmental chemicals were used in this study. The former was the Environmental Protection Agency (EPA) database known as **ToxRefDB** or the Toxicological Reference Database, which was designed by the National Center for Computational Toxicology (NCCT) and Environmental Protection Agency's (EPA) office of Pesticide programs (OPP). This database includes pesticide registration toxicity data for the past 30 years and $2 billion of animal studies results (40, 41). Using standardized vocabulary, ToxRefDB warehouses detailed study design, dosing, and observed treatment-related effects (41). The ToxRefDB stores also chemical toxicity data in detail in free accessible and searchable databases (41). ToxRefDB also connects with the ACToR (Aggregated Computational Toxicology Resource) in order to link it with public hazard, exposure and risk resources (41). Furthermore, ToxRefDB is connected to the ToxCast, another EPA chemical screening tool used to understand biological processes affected by chemicals (41). The available ToxRefDB database allows aggregation and grouping of chemicals depending on the toxicological outcomes that are specific to the type of the study and target organ/effect categories (e.g., tumorigenicity) (42). ToxRefDB classifies chemicals

by their relative potency depending on specific endpoints and also assigns groups based on the mechanism of action (42). Future improvements of the ToxRefDB are planned, which will connect the relational environment of ToxRefDB with associated chemical structure information (43). Furthermore, searching will develop predictive high-through-put screening bioactivity profiles and genomic signatures (16). Currently, ToxRefDB warehouses searchable pathologic information on 474 studies of pesticides and intermediates. In our study, the 474 rat/mouse studies were queried for histological NAFLD and TASH descriptors including “fatty change”, “Oil red O positive”, “steatosis”, and “lipid deposition”. The data were accessed in Fall 2013 at http://actor.epa.gov/toxrefdb/faces/Home.jsp. The following study types were queried: sub-chronic (SUB), chronic (CHR) and multigeneration reproductive (MGR) in both rat and mouse species. The effect type selected was “pathology (non-neoplastic)”. The effect target was always the “liver” in the search and the effect descriptions were: “fatty change”, “lipid deposition”, “steatosis” and “Oil red O” positivity in increased effect direction. Compound selection was based on the altered NAFLD and TASH descriptors at the Lowest Effect Level (LEL). Compounds and their LELs were arranged and listed in tables (Appx. 1).

The latter rodent database was the **Chemical Effects in Biological Systems (CEBS)** data repository developed by the National Toxicology Program (NTP) and it warehouses about 9000 rodent toxicology studies (44). CEBS combines public toxicogenomics databases such as the study design and timeline, clinical chemistry and histopathology, microarray and proteomics data

(45). CEBS warehouses data from academic, industrial and governmental laboratories and it was mainly developed to allow public and free search though the results of these studies (45, 46). CEBS stores rats, mice and human subjects studies and it contains more than 4000 microarray hybridizations, and 75 2D gel images with details protein identification (45). Furthermore, CEBS contains the clinical chemistry and histopathology data from more than 1500 animals (45). CEBS was accessed at: http://cebs.niehs.nih.gov. The queried assay domain was "histopathology" and the diagnoses selected were "fatty change" and "toxic hepatopathy" as the latter two terms appear to have been used to describe fatty liver in several National Toxicology Program (NTP) reports on polychlorinated biphenyls (47). "Liver and all its parts" was always the target organ selected and all degrees of severity were included. The search initially returned 329 studies, but medications and natural products were subsequently manually excluded. Remaining compounds and their LELs were then arranged and listed in tables in the CEBS databases (Appx. 1-7).

RESULTS

NHANES 2003-2004:

Eight organochlorine pesticides were detected in the serum of the participants of the survey and three of them, trans-nonachlor, heptachlor epoxide and dieldrin, showed increased odds ratios for ALT elevation across quartiles (p trend-adj ≤0.05). The other five pesticides, namely, oxychlordane, hexachlorobenzene, hexachlorocyclohexane, dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyltrichloroethane (DDT) showed a trend towards significance when we compared the 4th quartile vs. unexposed (95% CI). In the 4th quartile, DDE had the highest median lipid-adjusted serum concentration (1535 ng/g) of the eight analyzed pesticides (Appx. 1).

ToxRefDB

At the Lowest Effect Level (LEL), 42 unique pesticides from 474 pesticides were associated with steatosis/TASH pathologic descriptors including “fatty change”, “Oil red O positive”, “steatosis”, and “lipid deposition”. The 42 compounds included 22 fungicides, 13 herbicides, 6 insecticides, and 1 miticide which are given in Appendix 2, along with study design, species, and LEL values. These positive results came from both species (rat = 40 and, mouse = 20) from all queried study designs including sub-chronic (n = 16), chronic (n = 34) and multigeneration reproductive (n = 10). Thus, nearly 10% of ToxRefDB pesticide studies were associated with the development of steatosis based on the use of TASH descriptors. It is possible that, due to the high LEL values associated with some reported liver disease descriptors, only a subset of these pesticides are consequential to human disease at relevant environmental exposures. However, 6 pesticides had LELs less than 10 mg/kg/day, and that increases the likelihood that they could be significant mediators of TASH depending on their crop application patterns. These pesticides were: cyproconazole, dazomet, fluazinam, hexaconazole, pyrasulfotole metabolite (SXX 0665) and acequinocyl. Cyproconazole, dazomet, fluazinam, flusilazole, hexaconazole, paclobutrazol, triadimefon, vinclozolin and fluthiacet-methyl pesticides were associated with the development of steatosis in more than one study in the ToxRefDB database. This reproducibility increases the likelihood that exposures to these chemicals do indeed result in TASH. Two fungicides, dazomet and hexaconazole, were linked to steatosis in 3 studies and had LELs less than 10 in at least 2 studies.

CEBS:

Three hundred twenty nine studies of 81 chemicals reported positive steatosis/TASH descriptors ("toxic hepatopathy" and "fatty change"). These chemicals included 31 solvents, plasticizers, monomers, and chemical intermediates (Appx. 3), 14 miscellaneous chemicals (Appx. 4), 12 pesticides and pesticide intermediates (Appx. 5), 9 fragrances, cosmetics and essential oils (Appx. 6), 9 paints, polishes, dyes and food additives (Appx. 7), and 6 PCBs and dioxin-like compounds (Appx.8). Chemical name, study design, species and LEL values, when known, are provided. Several chemicals from each class produced steatosis with LELs ≤ 10 mg/kg (7/14 pesticides; 6/6 PCBs and dioxin-like compounds; 4/31 solvents, plasticizers, monomers, and chemical intermediates; 3/9 paints, polishes, and dyes; 3/14 miscellaneous chemicals; and 1/9 fragrances; cosmetics, and essential oils). In CEBS, steatosis was reported in 29, mice studies and 57 rat studies, short term (n =9) and chronic (n =72).

DISCUSSION

In the 2003-2004 NHANES population, an unexpected increase in the adjusted odds ratios for ALT elevation was observed in many of the participants. ALT levels were the highest in participants with very high serum levels of some metals and eight OCPs. Interestingly, among the quartile with the highest ALT elevation, the DDE median lipid-adjusted serum concentration was also the highest (1535 ng/g) supporting our decision to use DDT and DDE in further studies. Along with other compounds in the survey, DDT and OCPs are still persistent in the environment. For example, the Blackleaf Chemical site in Louisville, Kentucky was reported by the US EPA and the Kentucky Department of Environmental Protection to be contaminated with dieldrin, other OCPs and metals such as lead and arsenic (48, 49).

Between CEBS and ToxRefDB, 371 studies linked 123 unique environmental chemicals to fatty liver disease in rodents. Pesticides comprised almost 44% (54/123) of these chemicals and 14/55 pesticides led to steatosis with LELs less than 10. While it is not surprising that insecticides were on the list; fungicides and herbicides may be under-recognized mediators of TASH. Fungicides and herbicides are widely used at farms, houses and industry (50). According to the EPA, annual fungicide consumption worldwide is almost 500 million pounds (50). Some fungicides such as the conazoles have been associated with hepatotoxicity and hepatomegaly in rats (51). Furthermore, triadimefon and propiconazole are conazole fungicides that emerged from the

ToxRefDB analysis and they have been reported to induce hepatotoxicity and hepatomegaly (51). Cyproconazole is another fungicide in the ToxRefDB list which is found to cause hepatomegaly, single-cell necrosis and fat vacuolation leading to liver damage (52). Moreover, herbicides are widely used in the United States. Herbicides are mainly classified into: chlorophenoxy, bipyridil, triazine and chloroacetanilide which are also associated in causing or worsening liver disease (22). Many of the herbicides from our study do not belong to the previously mentioned classes providing researchers new targets for studying liver disease. Interestingly, of all pesticides dazomet and hexaconazole were linked with fatty liver disease by relatively low LELs in multiple studies. Dazomet is a fungicide, herbicide and nematicide that causes hepatomegaly combined with large fat droplets due to intermediary- and centro-acinary fatty degeneration in mice and it is also associated with liver damage in long term exposure (53, 54). Hexaconazole is a systemic triazole fungicide mainly used for control of black and yellow sigatoka diseases in bananas (55). Hexaconazole was found to be associated with hepatic enzyme elevation, hepatocellular hypertrophy, hepatic fatty infiltration and fatty changes in rodent and dog studies (55). While it is not surprising that subacute/chronic pesticide exposures resulted in steatosis, it was surprising that 10 multigenerational reproductive studies reported a positive effect. Additionally, this may be the first evidence linking developmental pesticide exposures to fatty liver disease.

CHAPTER 3

ACTIVATION OF HEPATIC XENOBIOTIC RECEPTORS BY PESTICIDES

INTRODUCTION

PXR and CAR were traditionally thought to be xenobiotic detoxification receptors but recent studies have demonstrated their roles in glucose and lipid metabolism (24, 56). PXR and CAR also share common ligands and have overlapping target gene battery (24). Identifying pesticides that can activate CAR and/or PXR is crucial because this can be one of the mechanism(s) by which pesticide can exposure cause hepatic fatty infiltration and damage as seen in studies in CEBS/ToxRefDB databases. Previous work by our laboratory group demonstrated that PCB 153, a known CAR activator, worsened DIO and therefore supported the hypothesis that these compounds can also worsen NAFLD and DIO through nuclear receptor interaction.

The ToxCastDB database contains results of high throughput assays that test the effect of pesticides on human and rodent receptor activation and target gene induction at different doses. Therefore, this database was used to identify pesticides that activate CAR and PXR. Furthermore, after identifying pesticides associated with NAFLD from the database-screening mentioned in the first part of the study, we selected eight pesticides to test for CAR and PXR activation *in*

vitro. The results from this study, supported by information obtained from the ToxCastDB, will allow us to predict the effect of these chemicals in humans.

MATERIALS AND METHODS:

ToxCastDB screening:

The ToxCastDB was established to predict potential toxicity and is a cost-effective approach for prioritizing thousands of chemicals that need toxicity testing (40). The data were collected by the US EPA Office of Pesticide Programs (OPP) and the National Center for Computational Toxicology (NCCT) and they were released in three different phases (57). As mentioned earlier, ToxRefDB was developed from the animal toxicity studies carried out prior to pesticide licensure, compiled as freely accessible and searchable databases (41, 57). ToxRefDB was critical in the development of ToxCastDB because it contains chemicals of known toxicity profiles from the ToxRefDB database (57). ToxCastDB Phase I database contains almost 300 chemicals and phase II includes 300 additional compounds, most of which are pesticides (57). The pesticides selected to be tested in high throughput assays were compiled from previously reported multiple animal toxicity studies. The objective of this process was to generate abundant data to form the basis computational predictive models of toxicity (57). Importantly, all the ToxCastDB pesticides previously met the safety standard for registration (57). Furthermore, the ToxCastDB was used to identify compounds associated with PXR activation, utilizing the NGCG, Attagene, CellzDirect and Novascreen assays which evaluated chemicals that can activate PXR or PXR target gene, CYP3A4. NGCG, Attagene, CellzDirect and Novascreen are the sources of the data in the website. The data were accessed in 2013 at http://actor.epa.gov/actor/faces/ToxCastDB/Home.jsp. The

query was constructed by selecting first ToxCastDB and then “Gene Associated with Assays”, leading to a list of genes. For our search purposes, we selected the human PXR ATG, NCGC and NVS under the “nuclear receptor subfamily 1, group I, member 2” and also the CLZD and NVS under the “cytochrome P450, family 3, subfamily A, polypeptide 4”. The hyperlinks in all cases led to tables of chemicals that activated the receptor and the gene along with the doses used in the studies.

Compound selection:

In the NHANES database, dieldrin, trans-nonachlor, DDE and DDT were associated with ALT elevation. These pesticides were therefore selected for further studies to investigate their interaction with CAR and PXR using transient transfection assays. Another candidate, chlordane, was selected because 3 compounds from the NHANES study namely heptachlor epoxide, trans-nonachlor and oxychlordane, are either components or metabolites of chlordane (58). In addition to these, we selected 3 more pesticides that are still in the market or persistent in the environment. They were selected from the list of compounds identified by ToxCastDB, and include lindane, atrazine and alachlor.

Trans-nonachlor and dichlorodiphenyldichloroethylene (DDE) were purchased from SUPELCO (Bellefonte, PA). Lindane and DDT were purchased from CHEM Service (PA) and chlordane, atrazine and alachlor were purchased from Sigma Aldrich laborchemikal (Tiedel-De Haïn, Seeize). Dieldrin was purchased from Sigma Aldrich (St. Louis, MO).

Plasmid construction:

The reporter plasmids for human (h) PXR, were constructed by using two copies of a direct repeat 4 (DR4), an inverted repeat 1 (IR1) and a direct repeat 1(DR1) response element (RE) respectively. The top strand oligonucleotide was 5' AGAGTTCATGAGAGTTCATGAGAGTTCATGAGAGTTCATG 3' for pGL3-DR4-Luc, 5' AGAGGTCATTGACCTTTAGAGGTCATTGACCTTT 3' for pGL3-IR1-Luc and 5' AACTAGGTCAAAGGTCAAACTAGGTCAAAGGTCAAA 3' for pGL3-DR1-Luc. The bottom complementary strands had Kpn1 and Xho1 overhangs at the 5' and 3' positions respectively. The oligonucleotides were annealed and inserted into Xho1 and Kpn1 restriction sites in the polycloning region of a modified version of pGL3 promoter vector (Promega, Madison, WI). Reporter plasmid for AhR (pXRE-SV40-Luc) was synthesized using the oligonucleotide 5' TCAGGCATGTTGCGTGCATCCCTGAGGCCAGCC 3' inserted into the EcoR1 site of a modified version of pGL3 promoter vector. Expression vector pSG5-hPXR was a generous gift from John Y. Chiang (Department of Integrative Medical Sciences, Northeast Ohio Medical University). Expression vector pCMV6-hCAR (CAR2) was purchased from Origene (Rockville, MD). mPXR was a generous gift from Dr. Steven Kliewer (The University of Texas Southwestern Medical Center) and mCAR was a generous gift from Tom Rushmore (Merck Research Laboratories, West Point, PA). Restriction endonucleases and T4 DNA ligase were purchased from New England BioLabs (Ipswich, MA). Dimethyl sulfoxide (DMSO) was purchased from Fisher BioReagents (Thermo Fisher Scientific, Pittsburg, PA) and rifampicin

(RIF), androstenol and 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime (CITCO) were purchased from Sigma Aldrich (St. Louis, MO). Lipofectamine and Opti-MEM were acquired from Life Technologies Inc (Carlsbad, CA). Oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA).

Cell culture:

HepG2 Cells: The human hepatoma-derived cell line (HepG2) was obtained from American Type Culture Collection (ATCC, Manassas, MD). Cells were grown in Dulbecco's modified Eagle's medium (DMEM, HyClone Laboratories Inc, Thermofisher, Waltham, MA) supplemented with 10% fetal bovine serum (FBS) and 1% antimycotic/antibiotic solution (Mediatech, Manassas, VA). The cells were incubated in a 5% carbon dioxide atmosphere and 95% humidity at 37°C and sub-cultured every 2 days.

Transfection:

Cells were plated in Thermo Scientific Nunc 24-well plates and transfected at 40-60% confluence. The transfection mix per well contained 150 ng β -galactosidase expression plasmid (pCMV-β, Stratagene, CA) as a transfection control, 50 ng receptor expression plasmid and 150 ng reporter plasmid if not otherwise specified. All cells were co-transfected by lipofection using Lipofectamine reagent according to the manufacturer's instructions and Opti-MEM (reduced serum medium) as the transfecting medium. After 4 hour of incubation, the medium was changed to DMEM supplemented with 10% FBS and 1% antimycotic/antibiotic solution then cells were left overnight to recover.

Compounds of interest were then added to the cells and cells were incubated for 24 hours. DMSO was used as a vehicle for all compounds (final concentration <0.5%). mCAR is constitutively active and therefore its activation was measured by the ability of the compound to reverse the inhibition caused by androstenol.

Reporter assay:

Cells were washed twice with phosphate buffered saline (1X), harvested using 50 µL cell lysis buffer (Promega, Madison, WI) and subjected to a single freeze-thaw event. For β -galactosidase assays, cell extracts (5 µL), were incubated with chlorophenol red β -galactopyranoside (CPRG, Roche Diagnostics, Indianapolis, IN) at 37 °C for 30-60 minutes. The enzyme activity was measured spectrophotometrically at 595 nm using the Bio-Tek Synergy HT multi-mode micro plate reader. Luciferase activity assays were performed on cell extracts (5 µL) using the Luciferase Assay System (Promega, Madison, WI). Luminescence was measured using the Orion L micro plate luminometer (Berthold Detection Systems, Pforzheim, Germany) over a 10 second period. Receptor activation was measured by luciferase activity and results were normalized to the amount of β-galactosidase expressed.

Statistical analysis:

All statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software Inc., La Jolla, CA, USA). Data are expressed as mean ± SEM. Multiple group data were compared using One Way ANOVA followed by Bonferroni's post-hoc test for parametric data for all pairwise comparisons (59). $P < 0.05$ was considered statistically significant.

RESULTS

Identification of pesticides that activate hPXR and induce its target gene CYP3A4 (EPA-ToxCast):

Two hundred eighteen different compounds were found to activate hPXR and hPXR target gene CYP3A4 at different doses (Appx. 14). For hPXR activation, 67 compounds including alachlor, were identified by the NGCG assay (Appx. 9), 102 compounds including lindane by the Attagene assay (Appx. 10) and 91 compounds by Novascreen assay (Appx. 11). For CYP3A4 induction, 202 compounds, including alachlor and atrazine were identified by the CellzDirect assay (Appx. 12) and 17 compounds by the Novascreen assay (Appx. 13).

Identified chemicals that were mutual among the NHANES, ToxCast, ToxRef and CEBS.

After data-screening of NHANES, ToxCast, ToxRef and CEBS, we clustered the results and 30 different compounds were found to be associated with fatty liver disease in all the four databases (Table 1).

Table 1: Mutual chemicals between NHANES, ToxCastDB, ToxRefDB and CEBS databases:

#	Chemical Name	#	Chemical Name
1.	Bensulide	2.	Metalaxyl
3.	Buprofezin	4.	Oxadiazon
5.	Butafenacil	6.	Paclobutrazol
7.	Chlorpyrifos-methyl	8.	Propiconazole
9.	Chlorsulfuron	10.	Rimsulfuron
11.	Cyproconazole	12.	Sethoxydim
13.	Dimethomorph	14.	Sulfentrazone
15.	Ethofumesate	16.	Tetramethrin
17.	Fenarimol	18.	Thiacloprid
19.	Fipronil	20.	Thiazopyr
21.	Fluazinam	22.	Triadimefon
23.	Flusilazole	24.	Triadimenol
25.	Fluthiacet-methyl	26.	Trifloxystrobin
27.	Hexaconazole	28.	Triflumizole
29.	Iprodione	30.	Vinclozolin

DDT activates hepatic xenobiotic receptors hPXR, mPXR and hCAR.

Transient transfection assays of HepG2 cells co-transfected with hPXR or mPXR or hCAR were performed for the following pesticides: dieldrin, trans-nonachlor, lindane, alachlor, chlordane, DDT and DDE, at 10 μM and other concentrations to evaluate their interaction with these xenobiotic receptors.

All compounds except atrazine activated hPXR (dieldrin DDT~2 fold; trans-nonachlor, chlordane, DDE and alachlor ~1.5 fold; lindane ~2.5 fold). mPXR was activated by all the compounds except atrazine and dieldrin (Trans-nonachlor and DDT ~2 fold; chlordane and alachlor ~1.7 fold and DDE and lindane ~1.5 fold) (Figs. 1 & 2).

hCAR variant 2 was activated by all the compounds except atrazine, chlordane and DDE (Alachlor ~1.5 fold; DDT ~1.4 fold and Dieldrin, trans-nonachlor and lindane ~1.3 fold) (Fig. 3). On the other hand, mCAR (human variant 1 anthology) was not activated by any of the compounds tested (Fig. 4)

Concentration response relationship was also determined to identify DDT and DDE half maximal effective concentration (EC50) for further studies. The dose response curve was plotted and the EC_{50}s were calculated using the GraphPad Prism version 5.01. DDT was a more potent hPXR activator than DDE with an EC_{50} that was 2.813 μM while the EC_{50} of DDE was 13.00 μM (Fig. 5&6). The results from the *in vitro* study are summarized in Table 2.

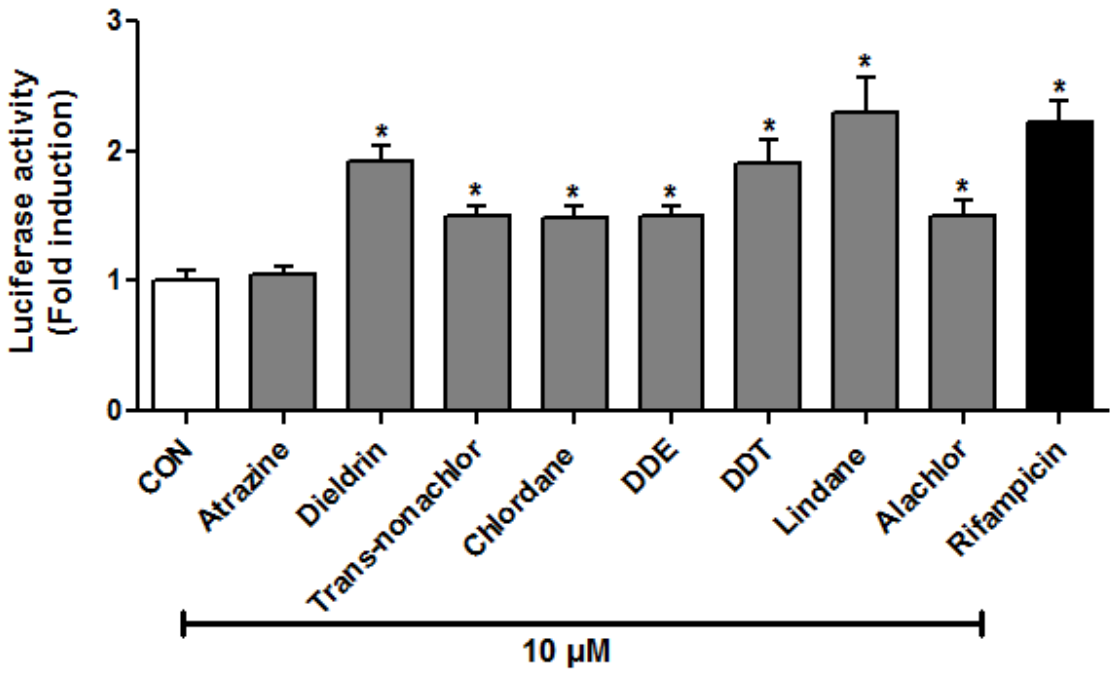

Figure 1. Activation of hPXR by pesticides:

HepG2 cells were co-transfected with pCMVβ, hPXR and pGL_3-DR4-Luc and exposed to each compound at 10 µM. All compounds except atrazine increased the luciferase expression compared to cells exposed to DMSO (solvent control) and the highest fold induction was with dieldrin and DDT (~2-fold). Rifampicin (10 µM) was used as a positive control. *P<0.05.

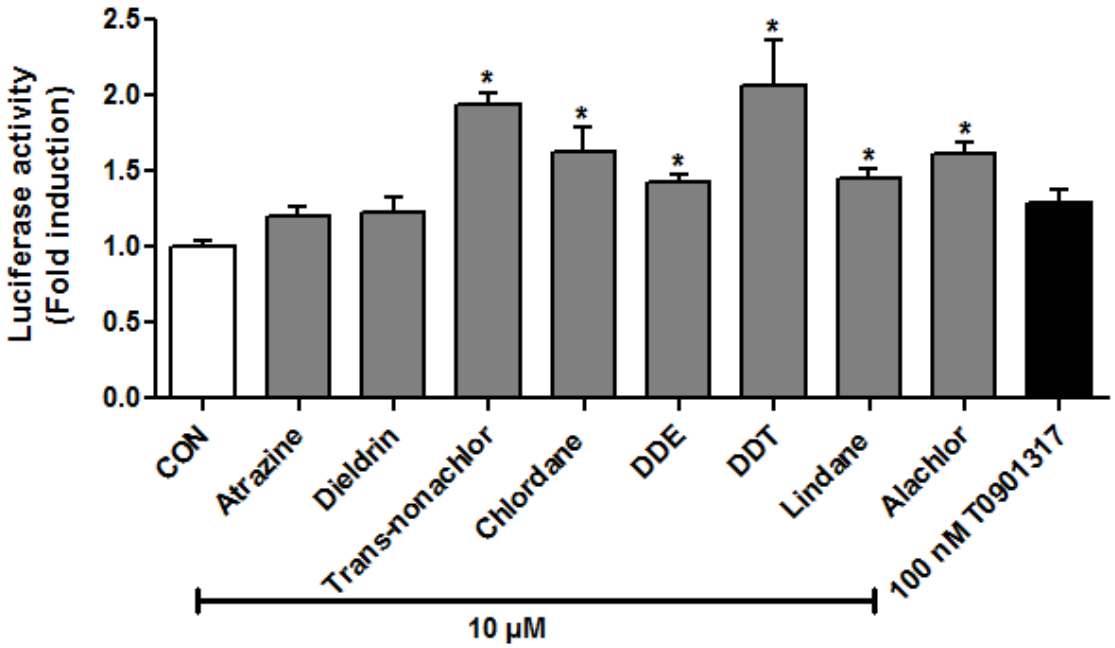

Figure 2. Activation of mPXR by pesticides:

HepG2 cells were co-transfected with pCMVβ, mPXR and pGL_3-DR4-Luc and exposed to each compound at 10 µM . All compounds except atrazine and dieldrin increased the luciferase expression compared to cells exposed to DMSO (solvent control) and the highest fold induction was with Trans-nonachlor and DDT (~2-fold). T0901317 (100 nM) was used as positive control. *P<0.05.

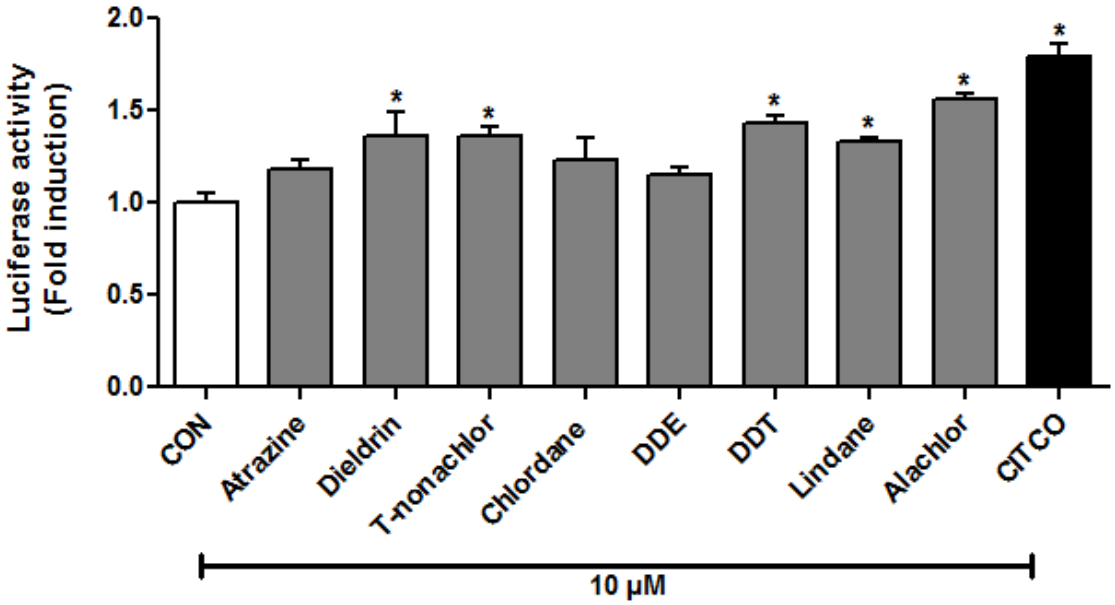

Figure 3. Activation of hCAR2 by pesticides:

HepG2 cells were co-transfected with pCMVβ, hCAR2 and pGL_3-DR4-Luc and exposed to each compound at 10 µM. All compounds except atrazine, chlordane and DDE increased the luciferase induction and the highest fold expression was with Alachlor ~1.5-fold and DDT ~1.4-fold. CITCO (10 µM) was used as positive control. *P<0.05.

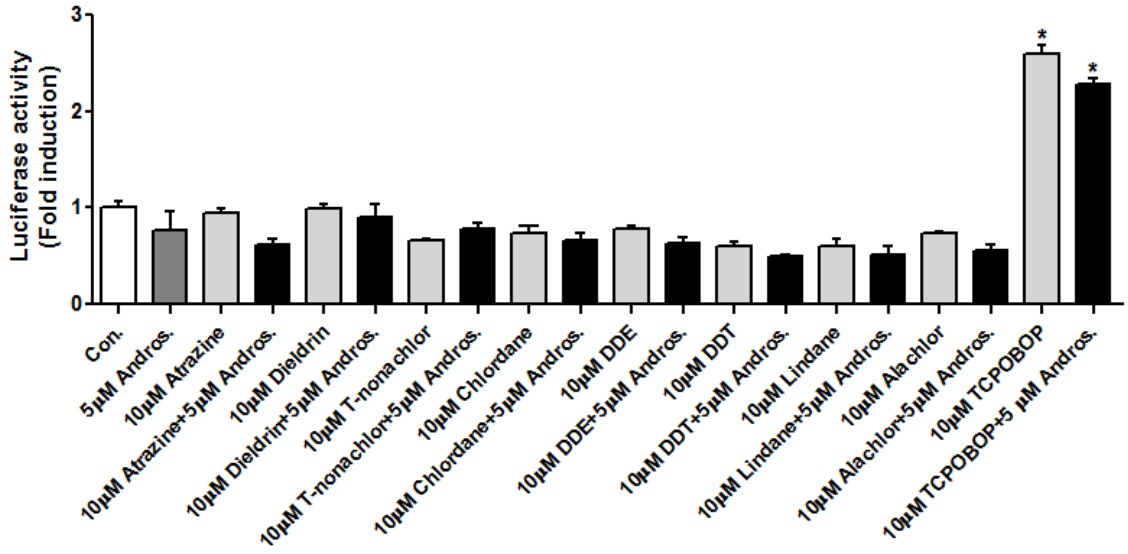

Figure 4. Activation of mCAR by pesticides:

HepG2 cells were co-transfected with pCMVβ, mCAR and pGL_3-DR4-Luc and exposed to each compound at 10 μM with and without 10 μM androstenol. None of the compounds increased luciferase expression significantly. Androstenol (10 μM) was used as a mCAR suppressor and as a negative control and TCPOBOP as a positive control. *P<0.05.

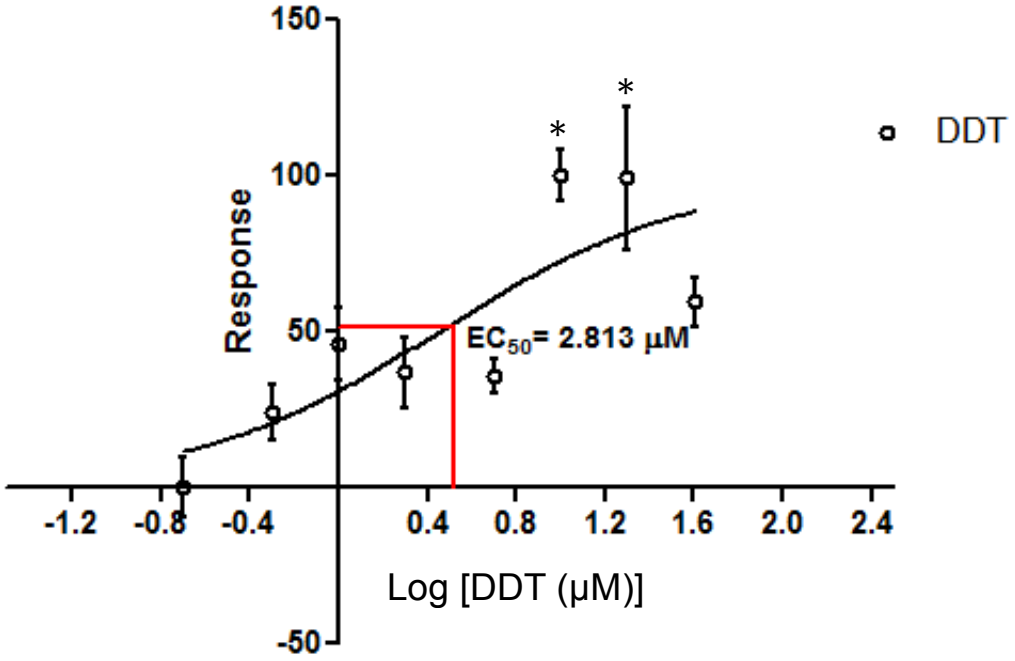

Figure 5. Concentration response curve of hPXR activation by DDT:

HepG2 cells were co-transfected with pCMVβ, hPXR and pGL_3-DR4-Luc and exposed to DDT at concentrations 0.2, 0.5, 1, 2, 5, 10, 20 and 40 µM. hPXR was activated at 10 and 20 µM concentrations compared to cells exposed to DMSO. Rifampicin (10 µM) was a positive control. The EC_{50} of DDT was calculated by GraphPad Prism 5.01 (EC_{50} = 2.813 µM). *$p<0.05$.

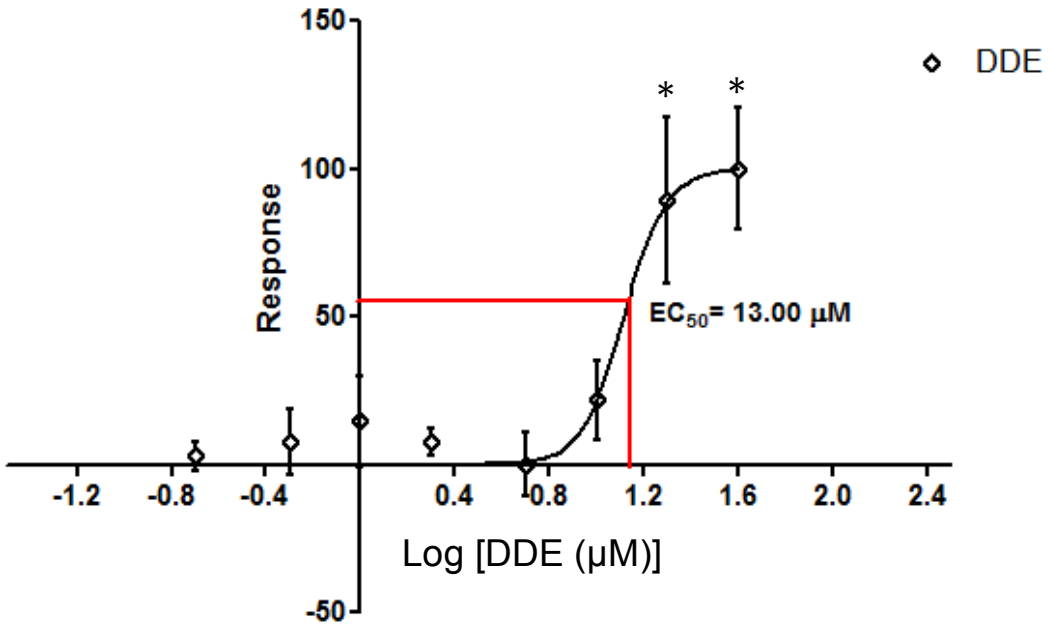

Figure 6. Concentration response curve of hPXR activation by DDE:

HepG2 cells were co-transfected with pCMVβ, hPXR and pGL_3-DR4-Luc and exposed to DDE at concentrations 0.2, 0.5, 1, 2, 5, 10, 20 and 40 µM. hPXR was activated at 20 and 40 µM concentrations compared to cells exposed to DMSO. Rifampicin (10 µM) was a positive control. The EC_{50} of DDT was calculated by GraphPad Prism 5.01 (EC_{50} = 13.00 µM). *P<0.05.

Table 2. Summary of results of the transient transfection assays:

#	Pesticide Name	hPXR	mPXR	hCAR	mCAR
1.	Atrazine	-	-	-	-
2.	Dieldrin	+	-	+	-
3.	Trans-nonachlor	+	+	+	-
4.	Chlordane	+	+	-	-
5.	DDE	+	+	-	-
6.	DDT	+	+	+	-
7.	Lindane	+	+	+	-
8.	Alachlor	+	+	+	-

DISCUSSION

Data screening from the three databases; ToxRefDB, CEBS and NHANES, highlighted the role of pesticides in NAFLD pathogenesis, therefore, inspiring a more detailed investigation into the suggested mechanisms correlated with liver disease. We mined the ToxCastDB database that warehouses high-throughput assays (NGCG, Attagene, CellzDirect and Novascreen), which evaluated chemicals that can activate human PXR or induce its target gene, CYP3A4. Over 200 compounds activated hPXR or induced CYP3A4. Interestingly, some of these compounds are pesticides which are still used in the U.S. or banned but persistent in the environment such as alachlor, an herbicide used in corn fields, lindane, a pesticide in shampoos for lice, and atrazine, a herbicide. Moreover, after screening the databases, 30 chemicals were mutually presented (Table 2).

In the next step of this study, we selected eight pesticides that were relevant to human exposure (NHANES) and human PXR activation (ToxCastDB). Transient transfection assays to study receptor activation demonstrated that almost all the pesticides activated human and/or mouse PXR and human CAR. The compounds selected from the ToxCastDB that were hPXR activators were further validated in our studies and their activation of hPXR was verified. Our results suggest that interaction with PXR might be a crucial molecular mechanism for NAFLD development in humans with compounds such as dieldrin, trans-nonachlor, lindane, alachlor, chlordane, DDT and DDE, but it might

not be with atrazine at the tested dose. Among the eight compounds studied, DDT appeared to be the strongest activator of hPXR, mPXR and hCAR. Likewise, DDE, the major metabolite of DDT, also activated hPXR and mPXR. Therefore, DDT and DDE were further tested at graded concentrations to measure their half maximal effective concentration (EC50); 2.813 μM for DDT and 13.00 μM for DDE.

CHAPTER 4

DDT DID NOT WORSEN NAFLD IN DIET INDUCED OBESITY MODEL

INTRODUCTION

The transient transfection experiments indicated that DDT and its major metabolites DDE are highly associated with the suggested mechanisms of liver disease development, which is consistent with the NHANES report wherein DDE reportedly showed the highest Lipid-adjusted serum levels concentration; 1535 ng/g (Fig. 7). These findings made DDT a good candidate for further evaluation with respect to NAFLD in an animal model of DIO.

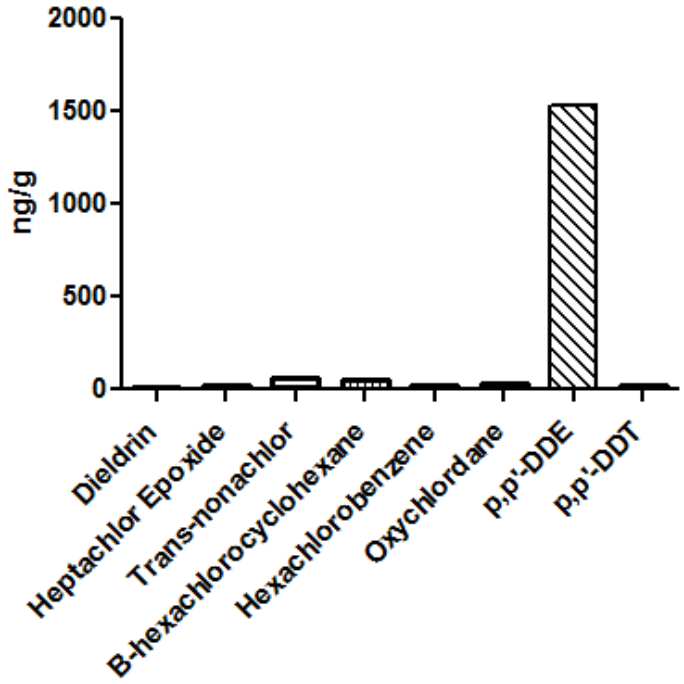

Figure 7. Lipid-adjusted serum levels of organochlorine compounds:

The median lipid adjusted serum levels of organochlorine compounds detected in the 4th quartile of NHANES population showed that DDE had the highest median lipid-adjusted serum concentration (1535 ng/g) compared to the other 7 organochlorine compounds.

Both human and animal studies have shown induction of CYP450s by DDT, in particular CYP2B and CYP3A (60). DDT induction of P450 is thought to be primarily through CAR activation, suggesting that DDT can also exhibit the phenobarbital-like hepatic tumor promoting activities as seen in rats and mice (60-63).

DDT is an organochlorine pesticide first discovered by the Swiss scientist Paul Hermann Muller in 1939 (64). DDT was used mainly as an insecticide to control malaria and typhus and its usage has saved millions of lives (64). DDT was banned in the U.S. and many other countries in the early 1970s because it was reported in high concentrations in aquamarine animals and it was also found to cause sever developmental toxicity to birds (64, 65). However, DDT is still used in many African countries to control malaria. DDT is a highly lipophilic persistent organic pollutant (65). DDT bio-accumulates in living organisms' fat tissue and hence, it still persists in the environment, and one way of exposure in mammals is through breast milk (60). In fact, it has been stated that there is no living organism on the planet that is free from DDT (22, 66).

DDT toxicity is directed mainly to freshwater and marine microorganisms, fishes, amphibians and birds (66). DDT residues were found in almost all the U.S. great lakes. In fact, DDT was banned in the U.S. in 1972 after many investigations, research and reports of the bioaccumulation of DDT and its metabolites in toxic levels in birds and aquamarine organisms, such as fish, invertebrates, and plants, as a food source for humans and animals. The DDT tissue accumulation in the aquamarine organisms was reported in concentrations

much higher than those in the physical environment. Studies have also shown that DDT use killed aquatic invertebrates in field situations. Therefore, by the 1960s and 1970s, DDT was known to be significantly prevalent in the aquatic ecosystems and in some aquatic animals and plants in the U.S. (65). In addition, in 1958, wild birds death took place after DDT applications and when the DDT residues were measured in these animals, they were similar to those measured in poultry fed DDT containing diet in a study in 1947. DDT levels bioaccumulation in birds can be dangerous when DDT and its residue DDD (dichlorodiphenyldichloroethane) levels reach 30 ppm or more in the brain. Under stress, such as migration, birds mobilize the stored DDT in the fat increasing the risk of reaching toxic serum and brain DDT levels. Furthermore, around the period when DDT was heavily used, it was reported that the number of Osprey birds decreased noticeably in the East Coast nesting colonies. The reported decrease in the number of these birds was reasoned to the death of these animals as well as the failure of their eggs to hatch due to thin eggshells and embryonic death. The phenomenon was also repeatable in laboratory animals. Additionally, the increase in the numbers of birds after the reduction and ban of DDT application was also reported in may studies. DDT and its metabolites were also reported to be accumulated in tissues, such as the brain, liver, adipose, muscles and others in mammals including rodents, rabbits, deer, bears and others. Moreover, it was also reported that DDT decreases the oxygen evolved from the phytoplankton, which are the main source of world's oxygen (65).

Both DDT and DDE are classified by the International Agency for Research on Cancer (IARC) as probable Group 2B carcinogens (67). In fact, in a clinical trial in Linxian, China, 168 of the trial subjects developed liver cancer, and compared to control subjects, they had higher rate of Hepatitis B surface antigen (HBsAg) positivity (68). Additionally, DDT is associated with hepatic tumorigenesis in mice at higher doses (60). DDT has also been linked to neurotoxicity in animals and neonatal exposure in humans affects neurocognition (60). DDT was also reported to cause abnormalities in sperm characteristics. Furthermore, DDE is antiandrogenic while DDT is estrogenic; however, studies found that DDT displays no teratogenic effect (60).

Technically, DDT is a mixture of 3 main compounds, DDT, DDE (dichlorodiphenyldichloroethylene) and DDD; with DDT representing the highest proportion of the mixture while both DDE and DDD are DDT metabolites. DDT and its major metabolite, DDE, have long half-lives, approximately 7 and 10 years in humans, respectively (69, 70). Seventy to eighty percent of DDT is absorbed in the gastrointestinal tract but this is dependent on the vehicle used. DDT metabolism in rodents occurs *via* two different routes, urinary and hepatic. DDA is the major urinary metabolite of DDT while DDE and DDD are the major hepatic metabolites (60). The LD_{50} of DDT in mice reported by ATSDR ranges between 152.3 - 1466 mg/kg/day. Different LD_{50}s of DDT in mice were reported in different studies and the wide range is reasoned to the use of mice of different strains, ages and genders in these studied (71).

The purpose of this study is to determine if DDT exposure results in NAFLD either by itself or in conjunction with high fat diet (HFD).

MATERIALS AND METHODS

Animals and diets:

The protocol of the animal study was approved by the University of Louisville Institutional Animal Care and Use Committee (IACUC). In this 12-week study, 11 week old male C57Bl/6J mice from the Jackson Laboratory (Bar Harbor, ME, USA) were divided into 4 study groups (n=10) based on diet and DDT exposure. Mice were fed either a control diet (CD): 10.2% kCal from fat (TD.06416 Harlan Teklad) or a high fat diet (HFD): 42% kCal from fat (TD.88137 Harlan Teklad). DDT (CHEM Service, PA) was administered on Weeks 3, 5, 7 and 9 by i.p. (intraperitoneal) injections in corn oil (*vs.* corn oil alone) at four individual doses of 25 mg/kg (100 mg/kg cumulative). Mice were housed in a temperature and light controlled-room (12 hour light/dark) with food and water. Animal weight and food consumption were measured every week in this 12-week study. The animals were euthanized with sodium pentobarbital (40 mg/kg body weight, i.p. injections) at the end of Week 12. Prior to euthanasia, the animals were fasted for 6 h (5:00 AM - 11:00 AM) and % fat composition and lean tissue weight were measured by a dual energy X-ray absorptiometry (DEXA) scanner (Lunar PIXImus densitometer, WI, USA).

Glucose tolerance test (GTT):

One week before euthanizing the animals, GTT was performed. On the day of the test, mice were fasted for 6 h (5:00 AM - 11:00 AM). A hand-held glucometer (ACCU-CHECK Aviva, Roche, Basel, Switzerland) was used to measure the fasting blood glucose levels using 1-2 µL blood via tail snip (59, 72). Animals

were then injected with 1 mg glucose/g body weight in sterile saline as i.p. injections and blood glucose levels were measured at 5, 15, 30, 60, 90 and 120 min after the injection.

Liver and white adipose tissue histological studies:

Liver sections were fixed in 10% buffered formalin for 48 h and then embedded in paraffin for histological examinations. The tissues then were sectioned and after drying they were then stained with hematoxylin-eosin (H&E). After drying, the stained tissues were examined under light microscopy at 10X and 40X magnification. Photomicrographs were captured using a Nikon Eclipse E600 Microscope (59).

Cytokine and adipokine measurement:

Plasma cytokine and adipokine levels were measured with the Milliplex Plasma Cytokine and Adipokine Kits (Millipore Corp, Billerica, MA, USA) on the Luminex IS 100 system (Luminex Corp, Austin, TX, USA), as per manufacturer's instructions. The Piccolo Xpress Chemistry Analyzer using the Lipid Panel Plus reagent discs (Abaxis, Union City, CA, USA) was used to measure plasma aspartate transaminase (AST), alanine transaminase (ALT), low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (TG) and total cholesterol levels (59).

Real-time PCR:

Total RNA was extracted from animal liver tissue samples by homogenizing the tissues using the RNA-STAT 60 protocol (Tel-Test, Austin, TX, USA) (59). The QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA, USA) was used to

synthesize cDNA from the total RNA (59). Hepatic gene expression was measured with StepOnePlus (Applied Biosystems) using Taqman Universal PCR Master Mix (Life Technologies, Carlsbad, CA, USA) (59). Primer sequences from Taqman Gene Expression Assays (Applied Biosystems, Foster City, CA) were as follows: tumor necrosis factor alpha (TNFα) (Mm00443258-m1), fatty acid synthase (FAS) (Mm00662319-m1), carnitine palmitoyl transferase 1A (CPT1A) (Mm01231183-m1), cytochrome P450s [Cyp4a10 (Mm02601690-gH), Cyp2b10 (Mm01972453-s1), Cyp3a11 (Mm007731567-m1), CD36 (Mm01135198-m1), interleukin 6 (IL-6) (Mm00446190-m1), monocyte chemo attractant protein-2 (MCP-2) (Mm01297183-m1) and tissue plasminogen activator inhibitor (tPAI-1) (Mm00435860-m1) (59). mRNA levels were normalized relative to the amount of GAPDH mRNA, and expression levels in mice fed control diet and administered vehicle were set at 100% (59). Gene expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ method (59, 73).

Statistical Analysis:

Statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software Inc., La Jolla, CA, USA). Data are expressed as mean ± SEM. Multiple group data were compared using two Way ANOVA followed by Tukey test for all pairwise comparisons (59). $P < 0.05$ was considered statistically significant.

RESULTS

DDT did not induce obesity in mice fed either CD or HFD.

Bodyweights (BW) were measured and the increase in BW with time was calculated. The BW gain with time for CD-fed mice was considered 100%. CD-fed mice did not show an increase in BW with time and this was not affected by DDT co-exposure. HFD feeding resulted in an increase in BW with time (163.94±4.69%, $p < 0.001$) but this was not affected by DDT co-exposure (145.29±5.61%) (Fig. 8). Likewise, HFD feeding resulted in an increase in food consumption (kCal/mouse) (43.95±0.99, $p<0.001$) and this was not affected by DDT co-exposure (47.61±0.99) (Fig. 9).

Fat tissue and lean tissue weight (g) were measured by scanning the animals using the DEXA scanning analyses. DDT exposure in CD-fed mice decreased the fat tissue weight (6.31±1.085, $p=0.027$) *vs.* CD only. HFD feeding increased the fat tissue weight in mice (15.68±1.085, $p<0.001$) but DDT co-exposure had no effect (11.56±12.297) (Fig. 10). Neither HFD feeding nor DDT exposure affected lean tissue weight in any group (Fig. 11). Epididymal weight/body ratio weight (EW/BW) was calculated and HFD feeding increased the EW/BW (0.068±0.005, $p<0.001$) but DDT had no effect on it (0.056±0.006) (Fig. 12). The liver weight to body weight ratio (LW/BW) ratio was also calculated and HFD feeding increased the LW/BW (0.048±0.002, $p=0.022$). However, DDT co-exposure resulted in a decrease in the LW/BW caused by HFD (0.040±0.003, $p=0.038$). There was a significant interaction between HFD and DDT (Fig. 13).

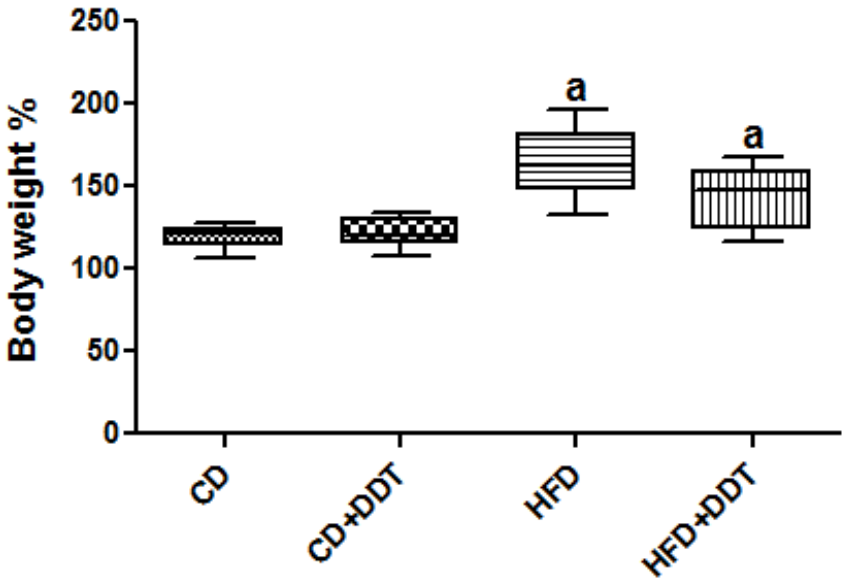

Figure 8. DDT did not affect body weight gain in either CD- or HFD- fed mice:

CD-fed mice did not show an increase in BW with time and this was not affected by DDT co-exposure. HFD feeding resulted in an increase in BW with time ($p < 0.001$) and DDT co-exposure did not affect that. The mice weights in the 12th week of the study were compared to the initial body weights to calculate the percentage of the body weight gain. Data are expressed as mean±SEM and analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD effect).

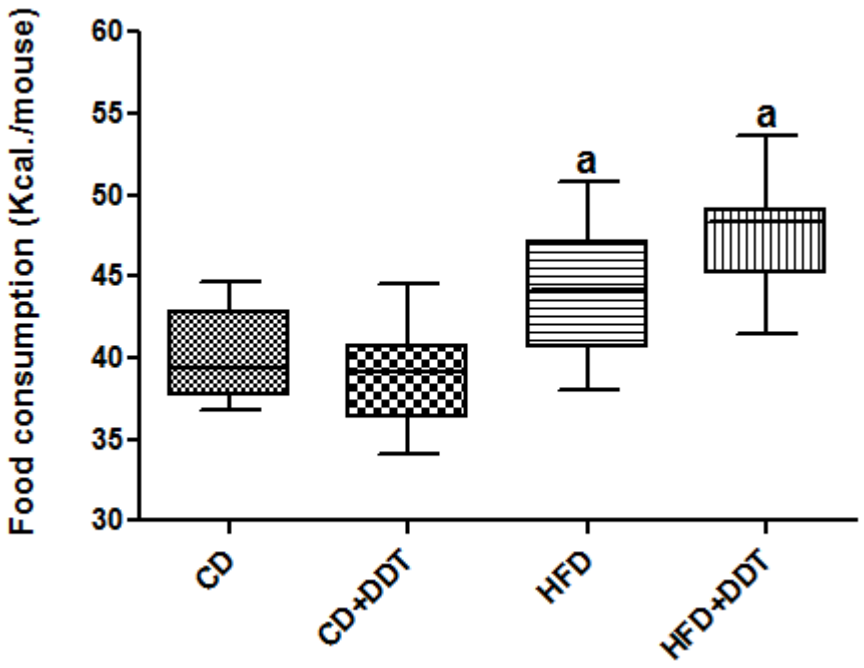

Figure 9. DDT did not affect food consumption in either CD- or HFD- fed mice:

The food consumption (kCal/mouse/week) increased significantly with HFD feeding ($p<0.001$) and it was not affected by DDT co-exposure. The food consumption of animals was measured every week during the 12 week study then the average of food consumption was calculated and converted into kCal for each mouse. Data are expressed as mean±SEM and analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD effect).

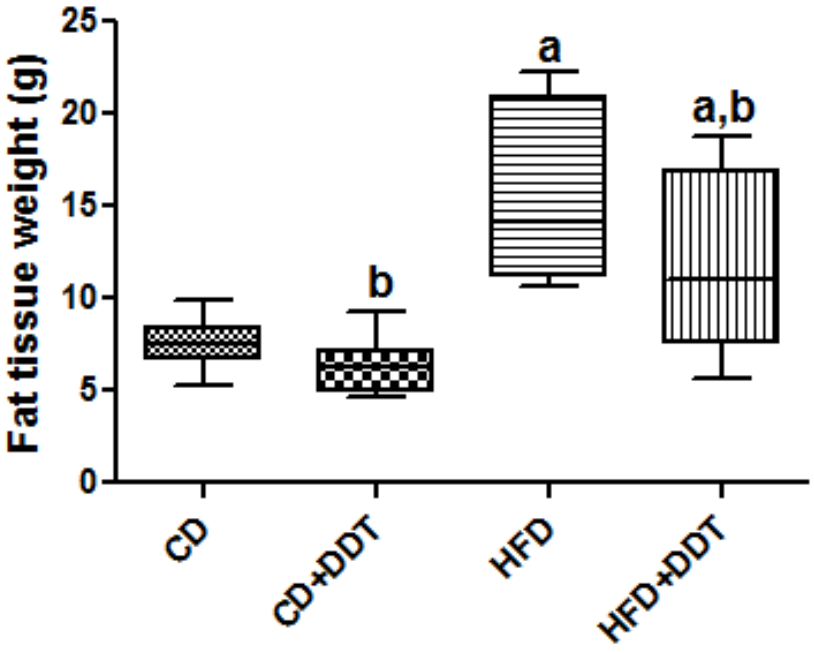

Figure 10. DDT decreased fat tissue weight in both CD- and HFD-fed mice: After 12 weeks, DDT exposure in CD-fed mice decreased the fat tissue weight ($p=0.027$) *vs.* CD only. HFD feeding increased the fat tissue weight in mice ($p<0.001$) but DDT co-exposure had no effect. The Dual energy X-ray absorptiometry (DEXA) scanning analyses was used for measurements and the data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD effect, and b: due to DDT effect).

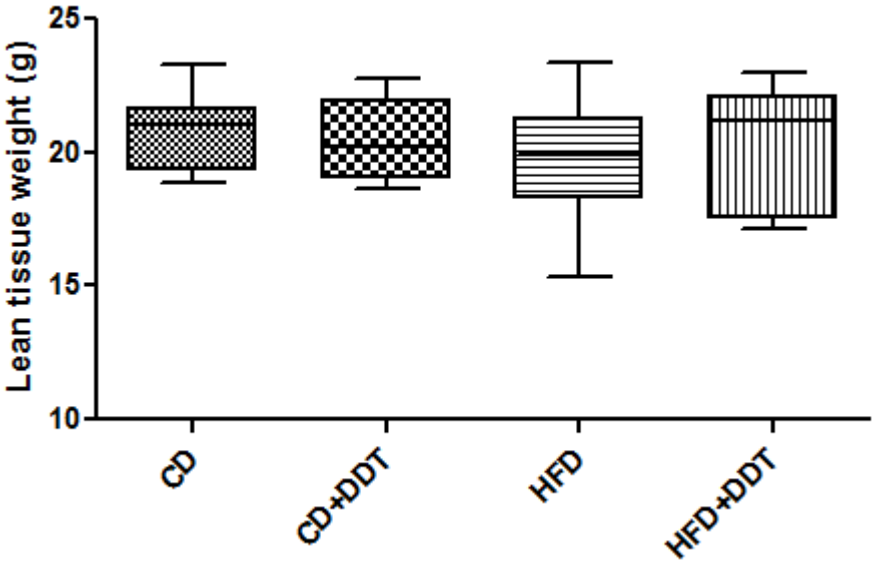

Figure 11. Neither DDT nor HFD affected the lean tissue weight:

After 12 weeks, neither HFD feeding nor DDT exposure affected lean tissue weight in CD or HFD mice. The Dual energy X-ray absorptiometry (DEXA) scanning analyses was used for measurements and the data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA and the data showed no significance.

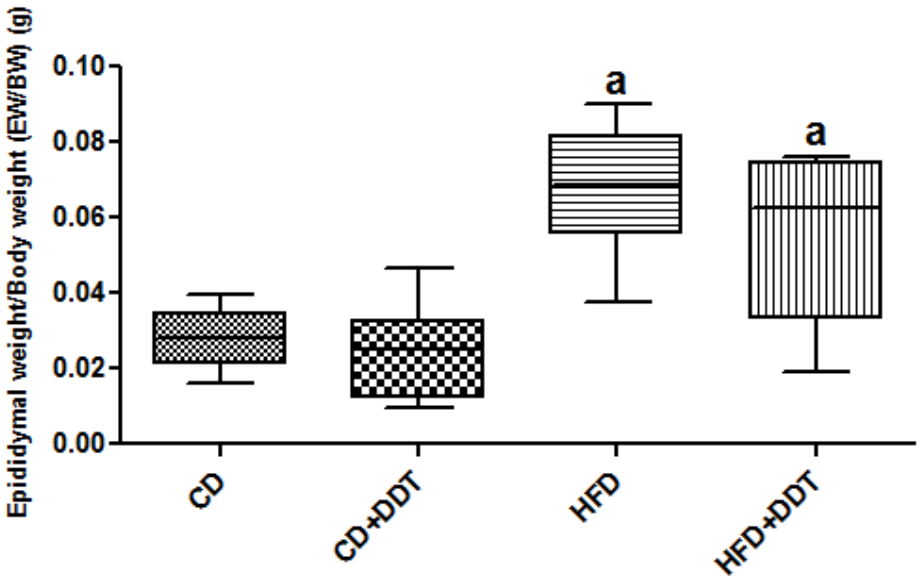

Figure 12. DDT did not affect the epididymal weight per body weight ratio in CD- and HFD-fed mice:

HFD feeding increased the EW/BW ($p<0.001$) but DDT had no effect on it. Weights of white adipose tissue were measured after euthanization and the data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD).

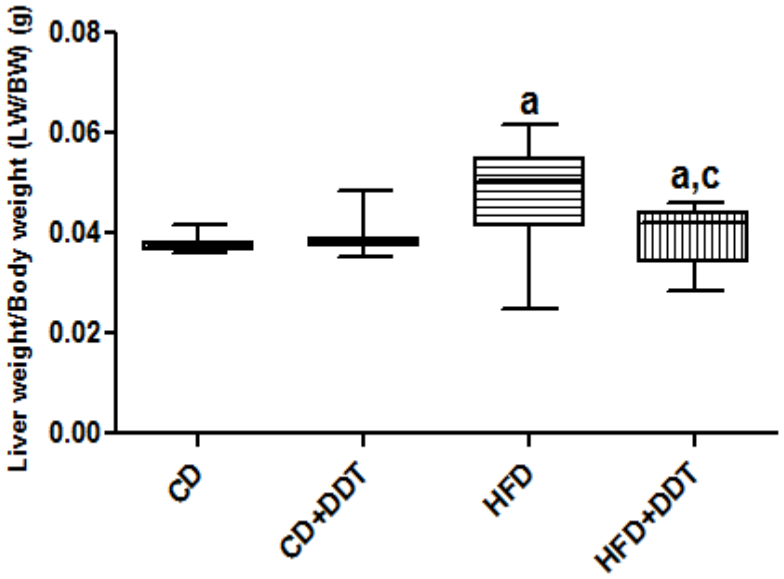

Figure 13. DDT decreased the liver weight per body weight ratio in HFD-fed mice:

HFD feeding increased the LW/BW ($p=0.022$). DDT co-exposure resulted in a decrease in the LW/BW caused by HFD ($p=0.038$). Weights of livers were measured after euthanization and data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD, and c: interaction between HFD and DDT).

Effect of DDT exposure on liver injury.

Plasma ALT and AST levels were measured using the Piccolo Xpress Chemistry Analyzer. The data demonstrated that DDT exposure resulted in decreased plasma ALT levels in both the CD- and HFD- fed mice (CD+DDT: 48.80±25.59 U/L and HFD+DDT: 52.57±30.59 U/L, p=0.048) (Fig. 14). On the other hand, neither DDT nor HFD affected plasma AST levels (Fig. 15).

Steatosis and liver injury were also assessed by H&E staining of liver sections. CD-fed group with or without DDT exposure showed no evidence of steatosis. However, HFD-fed groups with or without DDT exposure developed steatosis with some liver sections. Interestingly, some of HFD-fed mice co-exposed to DDT showed less or no steatosis. Additionally, there was no sign of inflammation in any of the groups. (Fig. 16).

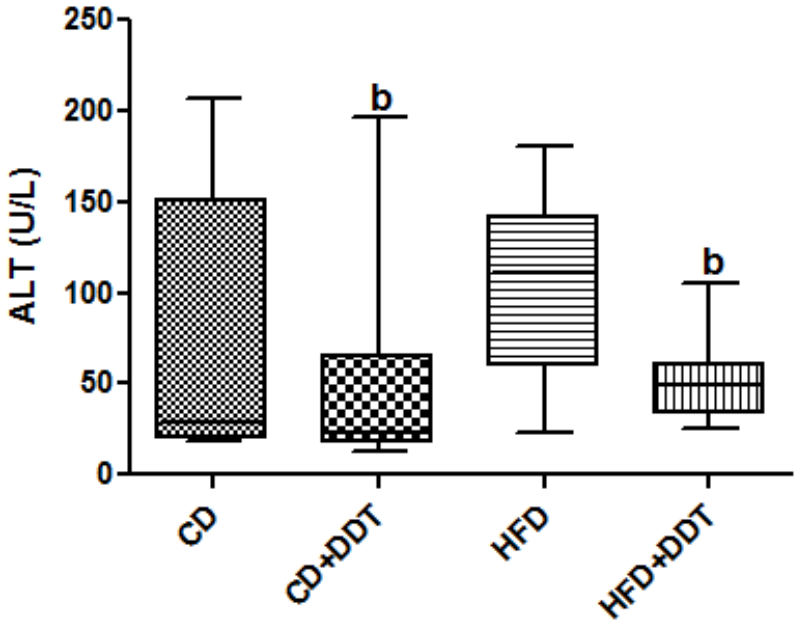

Figure 14. DDT decreased ALT levels in CD and HFD animals:

DDT exposure resulted in decreased plasma ALT levels in both the CD- and HFD- fed mice (p=0.048). The Piccolo Xpress Chemistry Analyzer was used to measure plasma levels of ALT and data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA. (P<0.05, b: due to DDT effect).

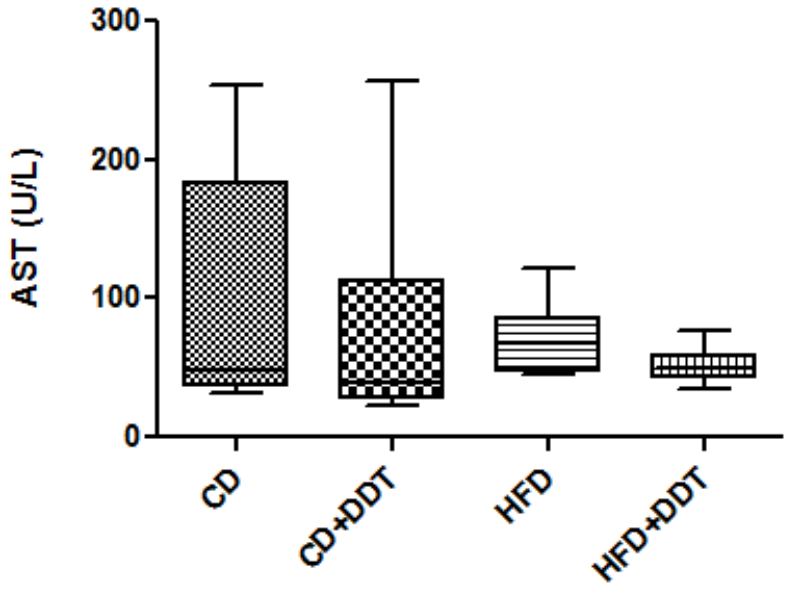

Figure 15. Neither DDT nor HFD affected AST plasma levels:

Neither DDT nor HFD affected plasma AST levels. The Piccolo Xpress Chemistry Analyzer was used to measure plasma levels of ALT and data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA and the data showed no significance.

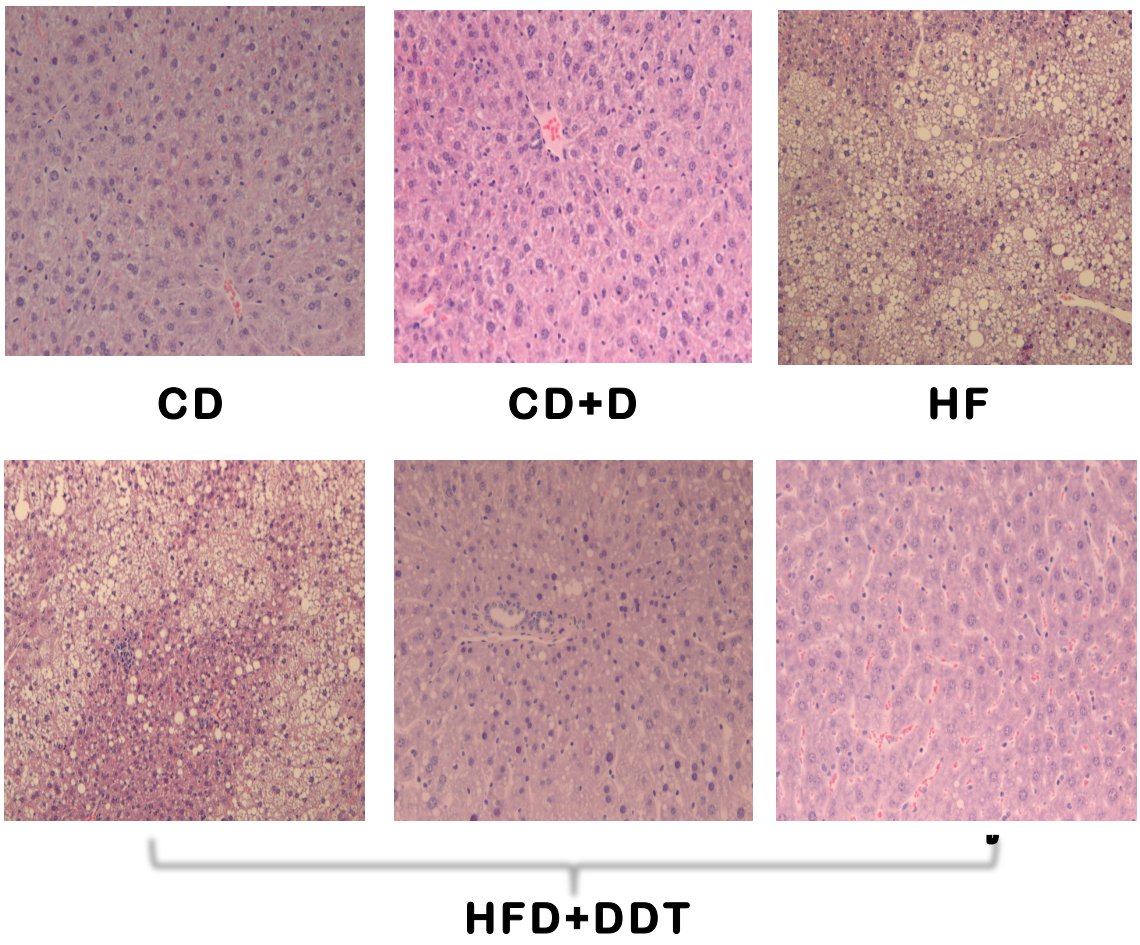

Figure 16. H&E staining of liver tissues:

The H&E staining of the liver tissues of CD-fed group with or without DDT exposure showed no evidence of steatosis. HFD-fed groups with or without DDT exposure developed steatosis, but some of HFD-fed mice co-exposed to DDT showed less or no steatosis. There was no sign of inflammation in any of the groups.

Effects of DDT on glucose tolerance test (GTT).

Glucose tolerance test (GTT) was performed one week prior to euthanizing the animals. HFD feeding increased blood glucose levels (mg/dL) but this was not affected by DDT co-exposure (Fig. 17). Likewise, HFD feeding increased fasting blood glucose levels (211.70±9.109 mg/dL, $p=0.009$) but this was not affected by DDT co-exposure (207.57±10.887 mg/dL) (Fig. 18).

Among the adipokines, plasma resistin levels were not altered in either the CD or HFD groups with or without DDT exposure (Fig. 19) whereas HFD groups showed increased plasma leptin levels (HFD: 13444.78±1270.850 pg/mL and HFD+DDT: 9405.10±1640.660 pg/mL, $p<0.001$) (Fig. 20). DDT exposure in both the CD and HFD mice increased plasma adiponectin levels (CD+DDT: 25.14±1.862 and HFD+DDT: 24.36±2.404, $p=0.049$) (Fig. 21).

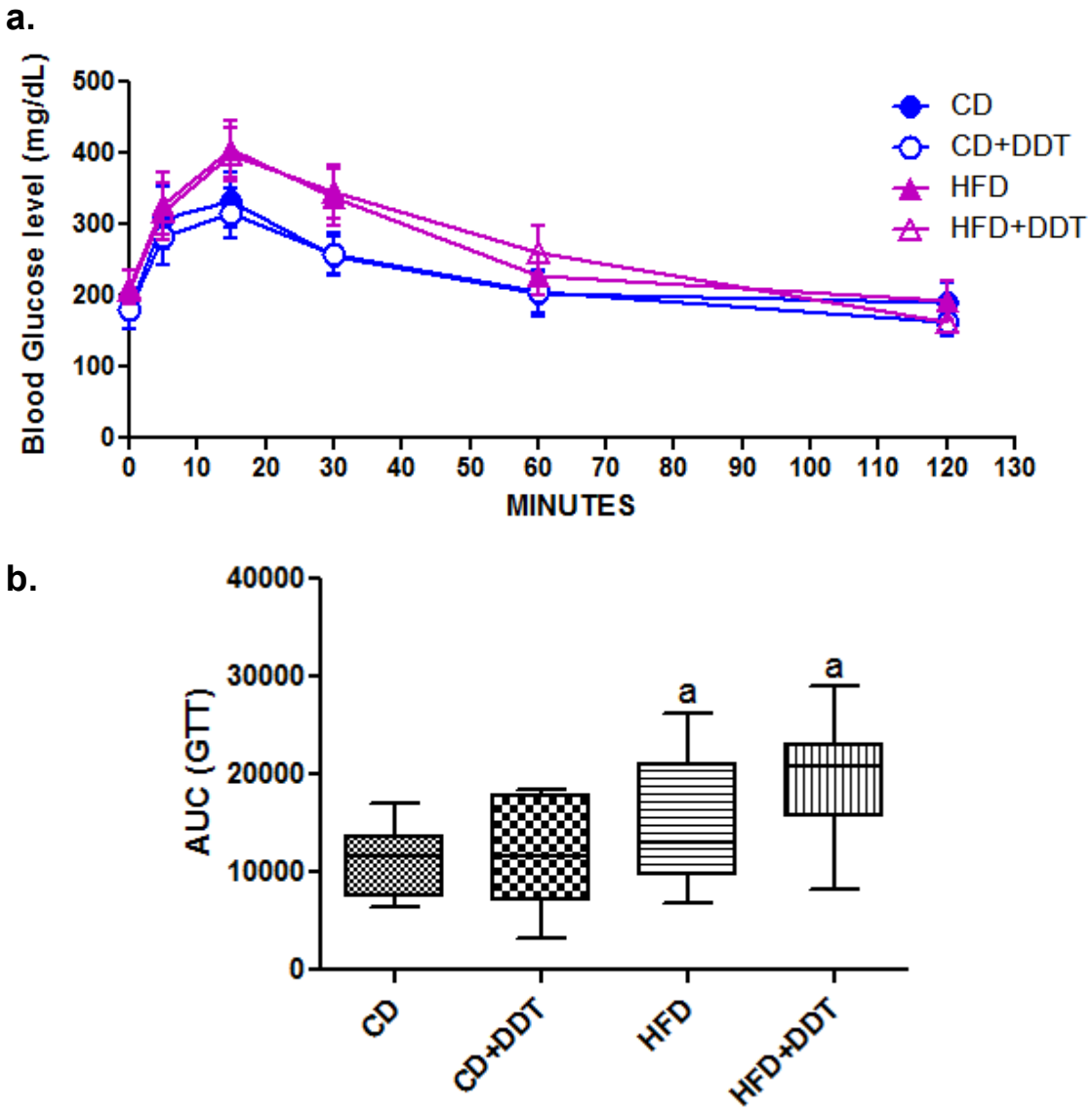

Figure 17. DDT did not affect GTT in either CD- or HFD-fed mice:

a. HFD feeding increased blood glucose levels (mg/dL) but this was not affected by DDT co-exposure at all the time points starting from time 0 and at 5, 15, 30, 60, and 120 minutes after injecting 1 mg glucose/g body weight. b. The area under the curve (AUC) of the GTT. A hand-held glucometer (ACCU-CHECK Aviva, Roche, Basel, Switzerland) was used to measure the blood glucose levels and data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD).

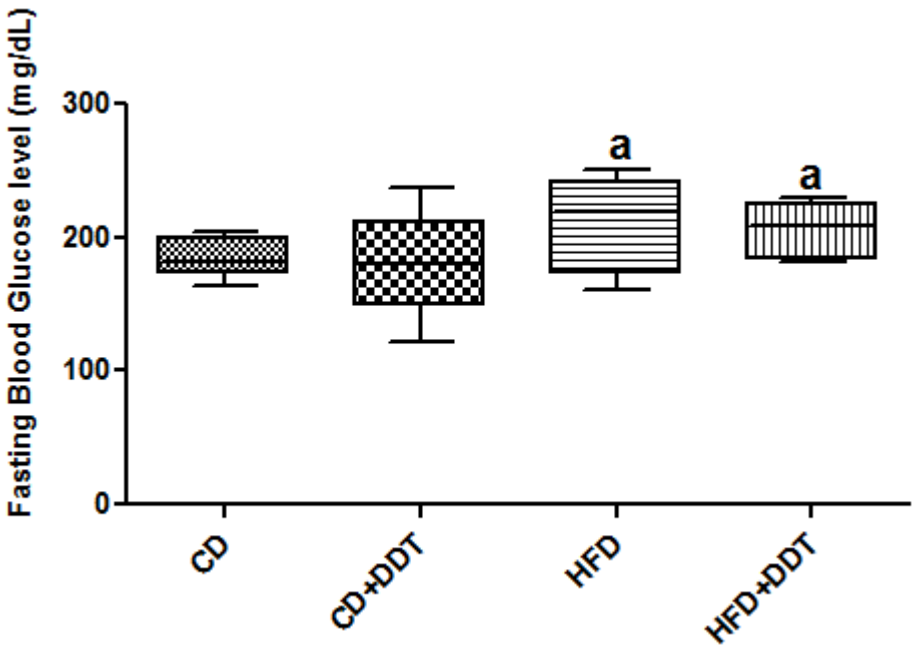

Figure 18. DDT did not affect fasting blood glucose levels:

HFD groups had high fasting blood glucose levels (p=0.009), but DDT had no effect in either CD- or HFD-fed animals. A hand-held glucometer (ACCU-CHECK Aviva, Roche, Basel, Switzerland) was used to measure the fasting blood glucose levels. Data are expressed as mean±SEM and Two Way ANOVA was used for statistical analysis. (P<0.05, a: due to HFD).

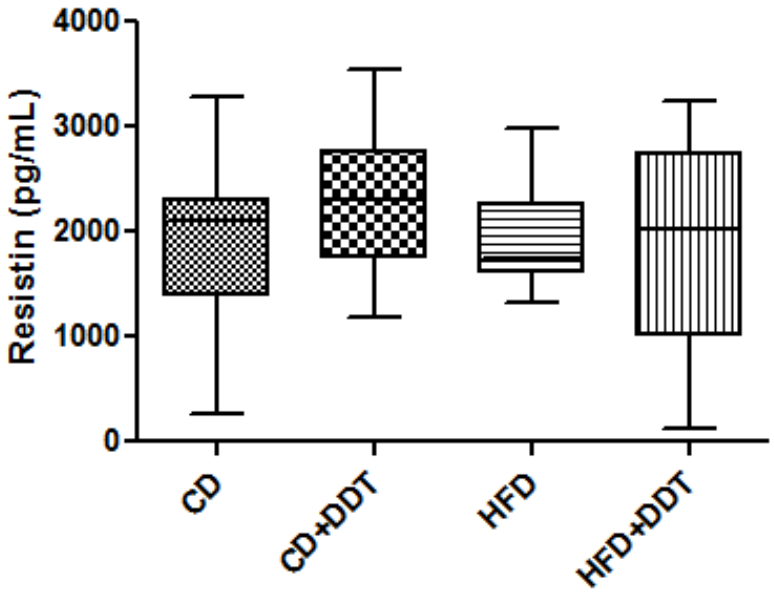

Figure 19. Neither DDT nor HFD affected plasma resistin levels:

Plasma resistin levels were not altered in either the CD or HFD groups with or without DDT exposure. Luminex IS 100 system was used to measure the plasma levels of resistin. Data are expressed as mean±SEM and Two Way ANOVA was used for statistical analysis and the data showed no significance.

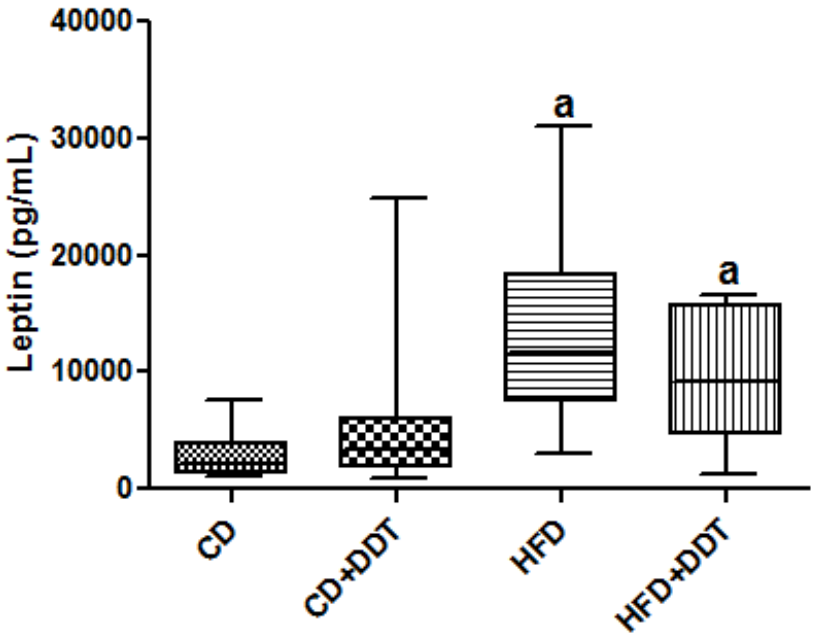

Figure 20. DDT did not affect plasma levels of leptin:

HFD groups showed increased plasma leptin levels ($p<0.001$) and DDT had no effect. Luminex IS 100 system was used to measure the plasma levels of leptin. Data are expressed as mean±SEM and Two Way ANOVA was used for statistical analysis. ($P<0.05$, a: due to HFD).

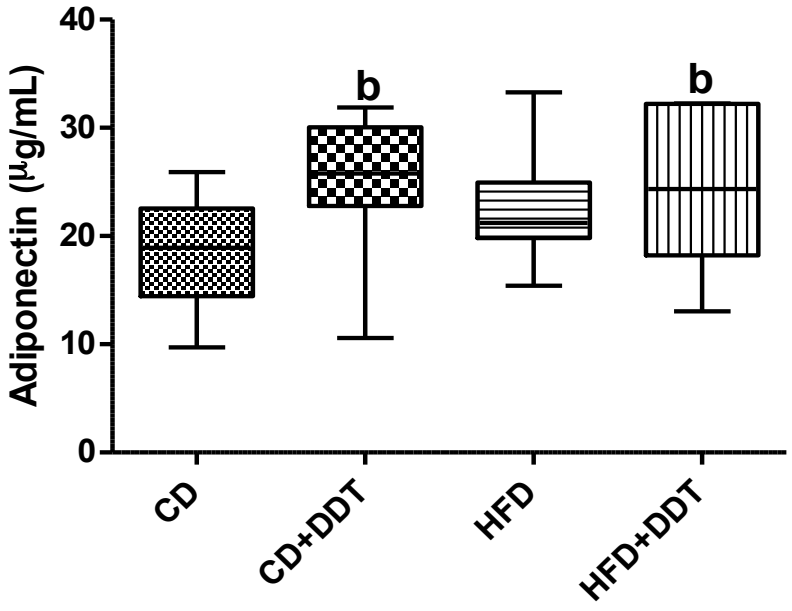

Figure 21. DDT exposure increased plasma levels of adiponectin in CD- and HFD- fed mice:

DDT exposed mice fed CD or HFD had high plasma adiponectin levels (p=0.049) compared to unexposed mice. Luminex IS 100 system was used to measure the plasma levels of adiponectin. Data are expressed as mean±SEM and Two Way ANOVA was used for statistical analysis. (P<0.05, b: due to DDT effect).

DDT had no effect on plasma cholesterol/triglyceride levels or on hepatic/systemic inflammation.

Plasma cholesterol, triglycerides and high density lipoprotein (HDL) levels were measured using the Piccolo Chemistry Analyzer. HFD feeding increased plasma total cholesterol (HFD: 133.70±7.89 mg/dL and HFD+DDT: 106.5±9.43 mg/dL, $p<0.001$) and HDL levels (HFD: 85.00±7.16 mg/dL and HFD+DDT: 78.60±7.16 mg/dL, $p=0.012$) (Fig. 22& 23). On the contrary, HFD feeding decreased plasma triglyceride levels (HFD: 43.40±3.945 mg/dL and HFD+DDT: 40.71±4.715 mg/dL, $p=0.008$) (Fig. 24).

Plasma cytokine levels and their hepatic mRNA levels were measured using the Luminex IS 100 system and RT-PCR respectively. HFD feeding decreased both plasma IL-6 levels (HFD: 7.74±14.45 pg/mL and HFD+DDT: 19.61±18.65 pg/mL, $p=0.002$) and hepatic IL-6 expression (HFD: 0.60±0.458) and HFD+DDT: 0.50±5.520, $p=0.040$) in DDT-exposed and unexposed mice (Fig. 25 &26). On the other hand, DDT exposure decreased plasma tPAI-1 levels in both CD and HFD groups (CD+DDT: 1285.22±339.512 and HFD+DDT: 951.76±438.301, $p=0.047$) (Fig. 27). Neither HFD feeding nor DDT exposure affected TNFα plasma and hepatic mRNA levels (Fig. 28 & 29). Similarly, plasma MCP-1 levels and hepatic MCP-2 mRNA levels were unchanged by diet or DDT exposure (Fig. 30 & 31). The inflammatory cytokines measurements of the unexposed CD-fed mice were higher than those of the HFD-fed mice showing that the control group mice must had some sort of infection that increased their serum and hepatic cytokine levels.

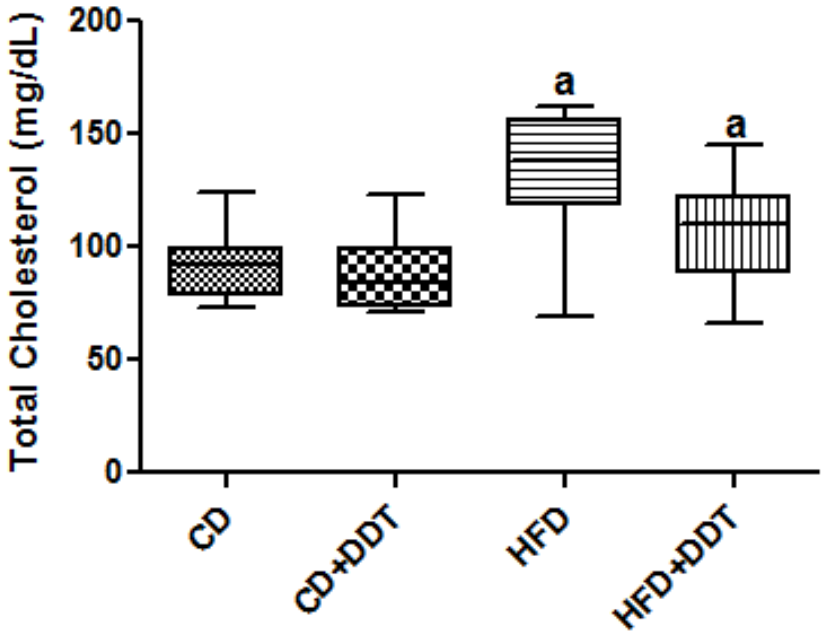

Figure 22. DDT did not affect total cholesterol plasma levels:

HFD fed groups had high cholesterol levels ($p<0.001$) while DDT had no effect. The Piccolo Xpress Chemistry Analyzer was used to measure plasma levels of total cholesterol and data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD).

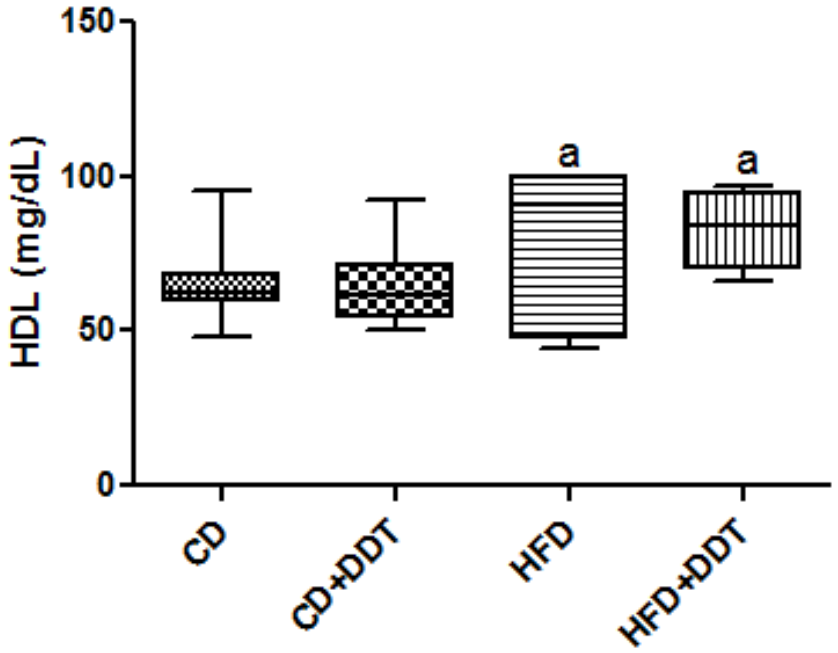

Figure 23. DDT did not affect the plasma levels of HDL:

HFD groups had high HDL plasma levels (p=0.012) while DDT had no effect. The Piccolo Xpress Chemistry Analyzer was used to measure plasma levels of HDL and data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA. (P<0.05, a: due to HFD).

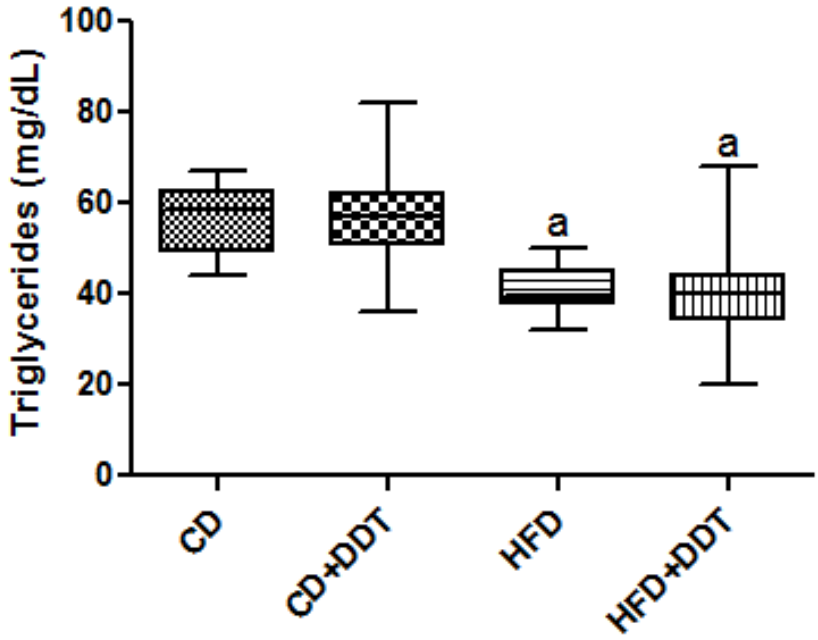

Figure 24. DDT did not affect triglycerides plasma levels:

HFD fed animals had low plasma levels of triglycerides (p=0.008) while DDT had no effect. The Piccolo Xpress Chemistry Analyzer was used to measure plasma levels of triglycerides and data are expressed as mean±SEM. Analysis was performed using Two Way ANOVA. (P<0.05, a: due to HFD).

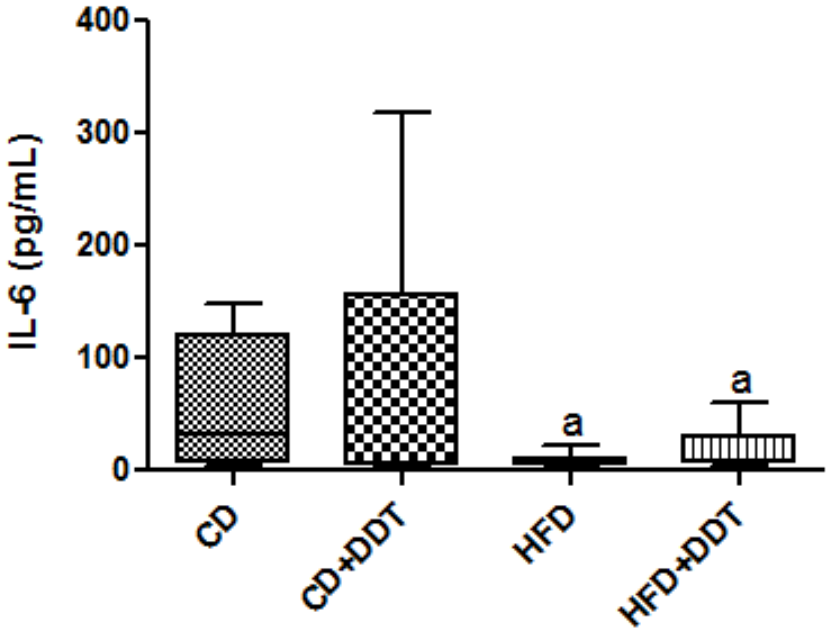

Figure 25. DDT did not affect IL-6 plasma levels:

HFD-fed mice had low plasma IL-6 levels (p=0.002) while DDT had no effect. Luminex IS 100 system was used to measure the plasma levels of IL-6. Data are expressed as mean±SEM and Two Way ANOVA was used for statistical analysis. (P<0.05, a: due to HFD).

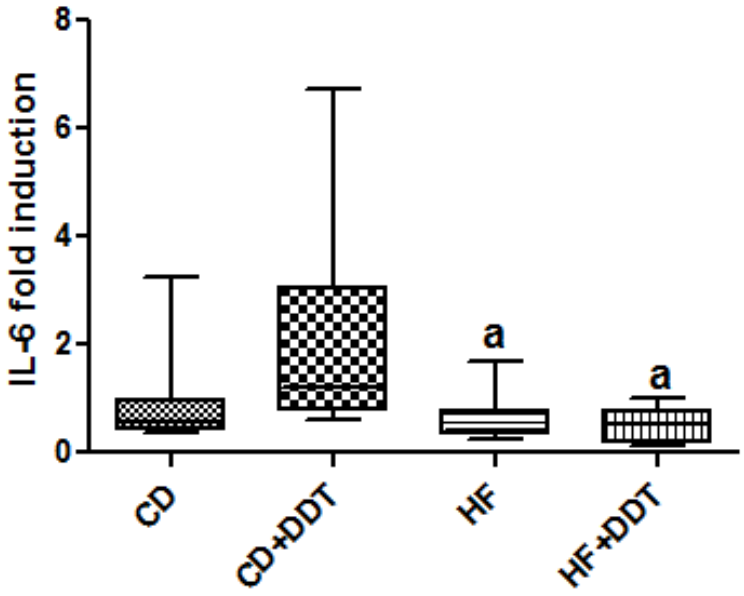

Figure 26. DDT did not affect hepatic IL-6 expression:

Hepatic IL-6 expression was decreased in HFD fed groups (p=0.040) while DDT had no effect. Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA. (P<0.05, a: due to HFD).

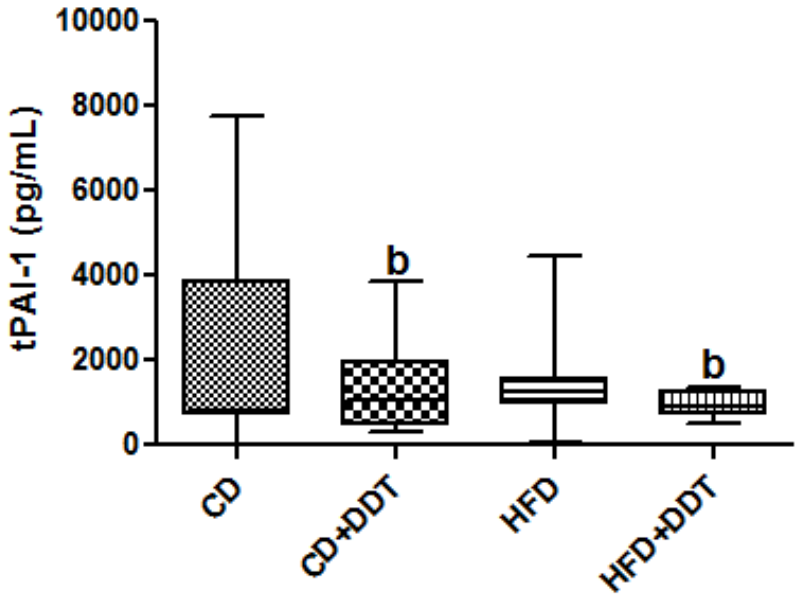

Figure 27. DDT decreased plasma levels of tPAI-1:

DDT exposure decreased plasma tPAI-1 levels in both CD and HFD groups ($p=0.047$). Luminex IS 100 system was used to measure the plasma levels of tPAI-1. Data are expressed as mean±SEM and Two Way ANOVA was used for statistical analysis. ($P<0.05$, a: due to HFD, b: due to DDT effect and c: interaction between HFD and DDT

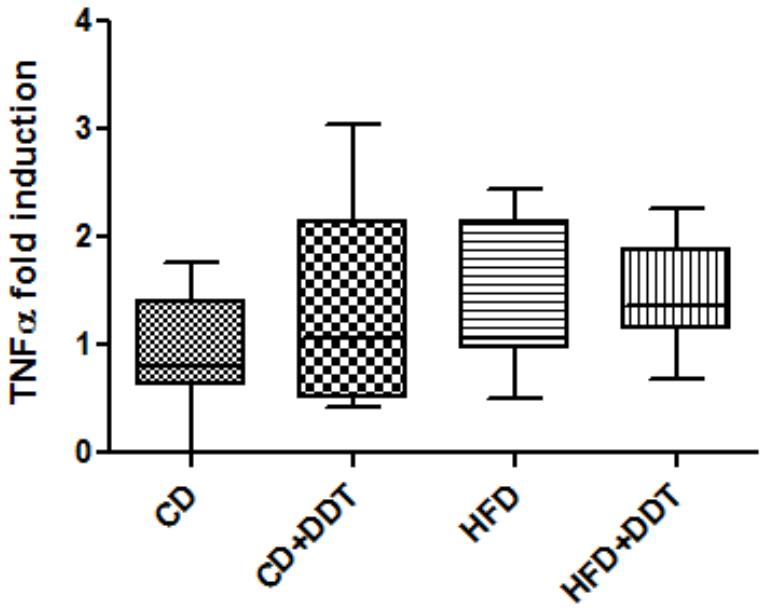

Figure 28. Neither HFD nor DDT affected the TNFα hepatic expression:

Neither DDT nor HFD affected the TNFα hepatic expression. Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA and the data showed no significance.

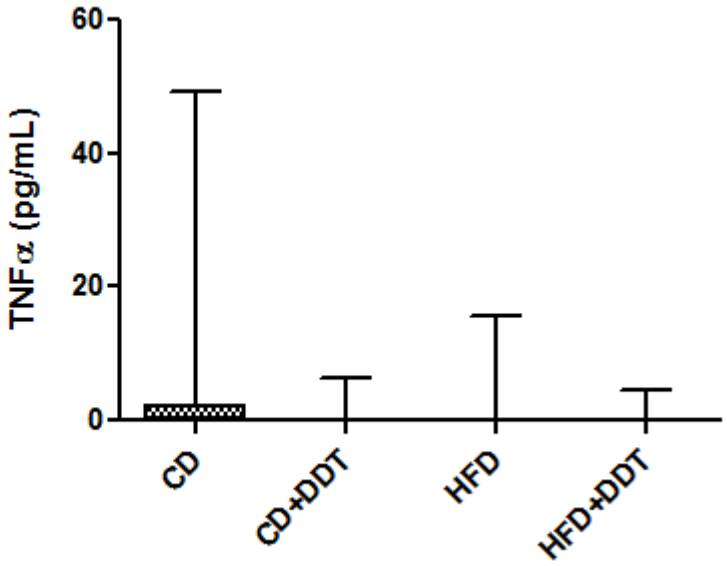

Figure 29. Neither HFD nor DDT affected TNFα plasma levels:

Neither HFD feeding nor DDT exposure affected TNFα plasma levels. Luminex IS 100 system was used to measure the plasma levels of TNFα. Data are expressed as mean±SEM and Two Way ANOVA was used for statistical analysis and the data showed no significance.

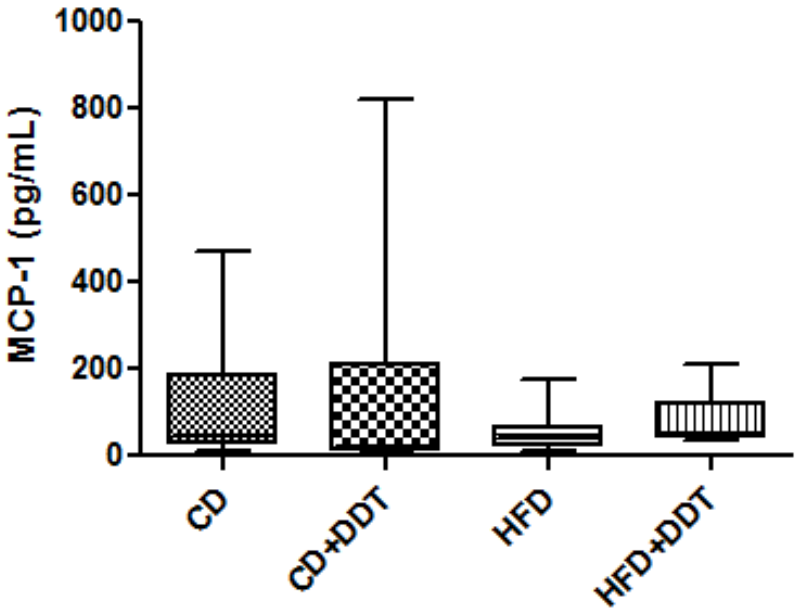

Figure 30. Neither HFD nor DDT affected MCP-1 plasma levels:

MCP-1 plasma levels were not affected by either HFD or DDT. Luminex IS 100 system was used to measure the plasma levels of MCP-1. Data are expressed as mean±SEM and Two Way ANOVA was used for statistical analysis and the data showed no significance.

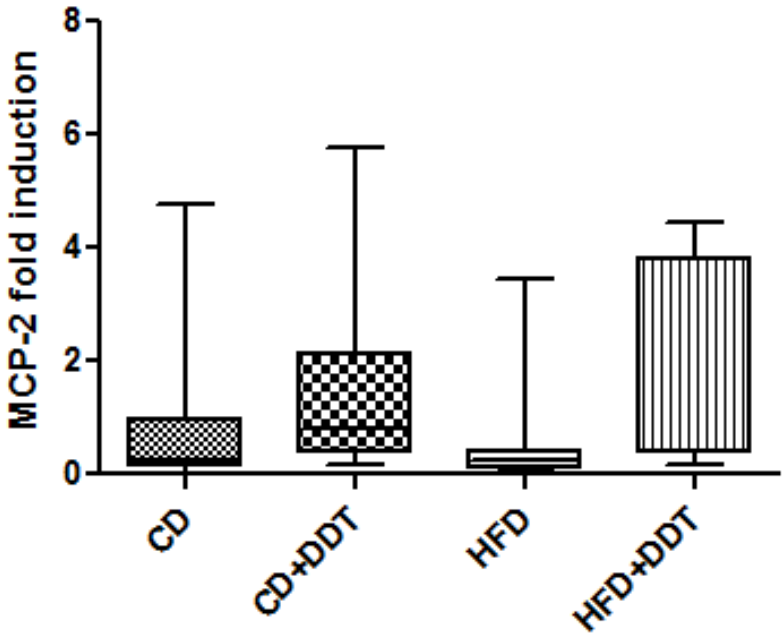

Figure 31. Neither HFD nor DDT affected hepatic MCP-2 expression:

MCP-2 liver expression levels were not affected by either HFD or DDT. Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA and the data showed no significance.

Effects of DDT on PPARα and LXR target genes.

Hepatic mRNA levels of PPARα target genes, namely carnitine palmitoyltransferase I (CPT1a), an enzyme that regulates mitochondrial fatty acid beta-oxidation and Cyp4a10, an enzyme that regulates peroxisomal fatty acid oxidation were measured (74). DDT exposure resulted in decreased hepatic expression of CPT1A mRNA irrespective of the diet type given (CD+DDT: 0.31±0.110 and HFD+DDT: 0.35±0.131, $p<0.001$), suggesting that fatty acid oxidation was compromised in DDT-exposed mice (Fig. 32). Likewise, hepatic Cyp4a10 mRNA expression was lowered in both the DDT-exposed groups (CD+DDT: 0.76±0.436 and HFD+DDT: 0.97±0.52, $p=0.005$). However, HFD alone resulted in increased Cyp4a10 mRNA expression (0.77±0.44, $p=0.006$) and there was a significant interaction between HFD and HFD+DDT ($p=0.017$) (Fig. 33).

Hepatic mRNA levels of liver-X-receptor (LXR) target genes were also evaluated, including fatty acid synthase (FAS), an enzyme that catalyzes fatty acid synthesis, and CD36, a fatty acid binding protein required for cellular fatty acid uptake (75). HFD consumption led to downregulation of hepatic FAS in both DDT-exposed and unexposed mice (HFD: 0.40±0.242 and HFD+DDT 0.25±0.29, $p=0.001$) (Fig. 34). On the other hand, DDT exposure in CD-fed mice increased hepatic CD36 (2.351±0.598, $p=0.031$). Likewise, HFD consumption also resulted in increased of hepatic CD36 (HFD: 2.518±0.567 and HFD+DDT: 3.925±0.678, $p=0.017$) (Fig. 35).

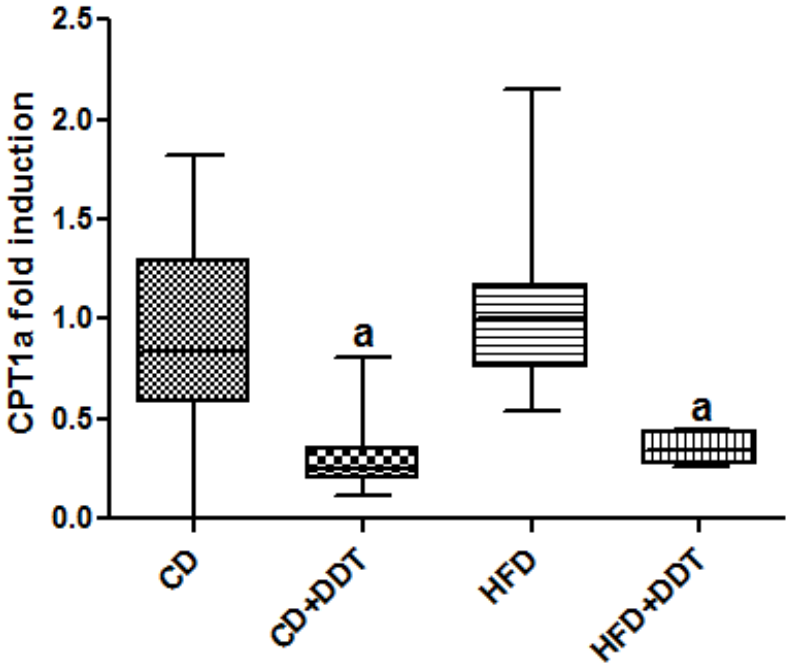

Figure 32. DDT decreased hepatic expression of CPT1a in both CD- and HFD- fed groups:

DDT co-exposed animals fed either CD or HFD had low expression of hepatic CPT1a ($p<0.001$). Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD).

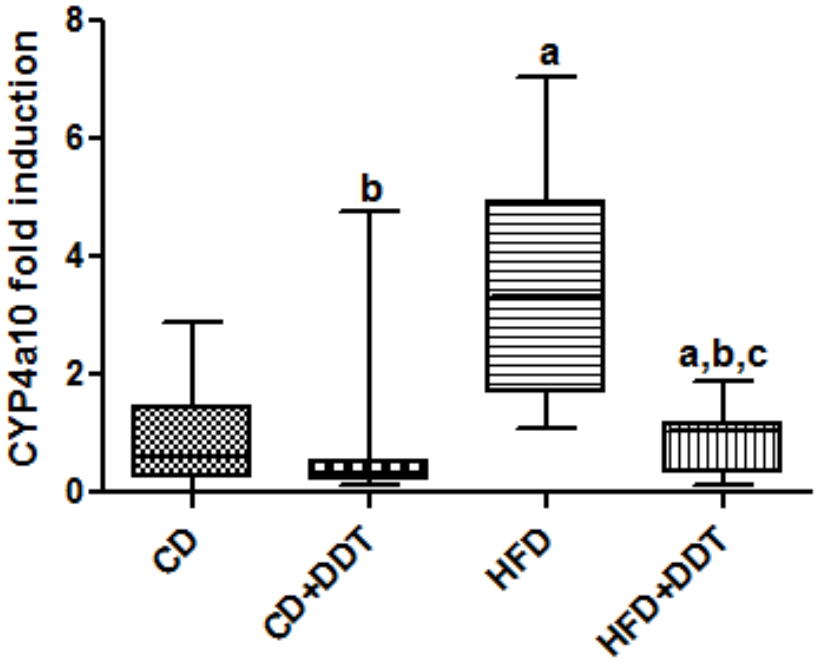

Figure 33. DDT decreased hepatic expression of Cyp4a10 in both CD- and HFD- fed groups:

Hepatic Cyp4a10 expression was lowered in both the DDT-exposed groups (p=0.005). HFD alone group had in high Cyp4a10 expression (p=0.006) and there was a significant interaction between HFD and HFD+DDT (p=0.017). Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA. (P<0.05, a: due to HFD, b: due to DDT effect and c: interaction between HFD and DDT).

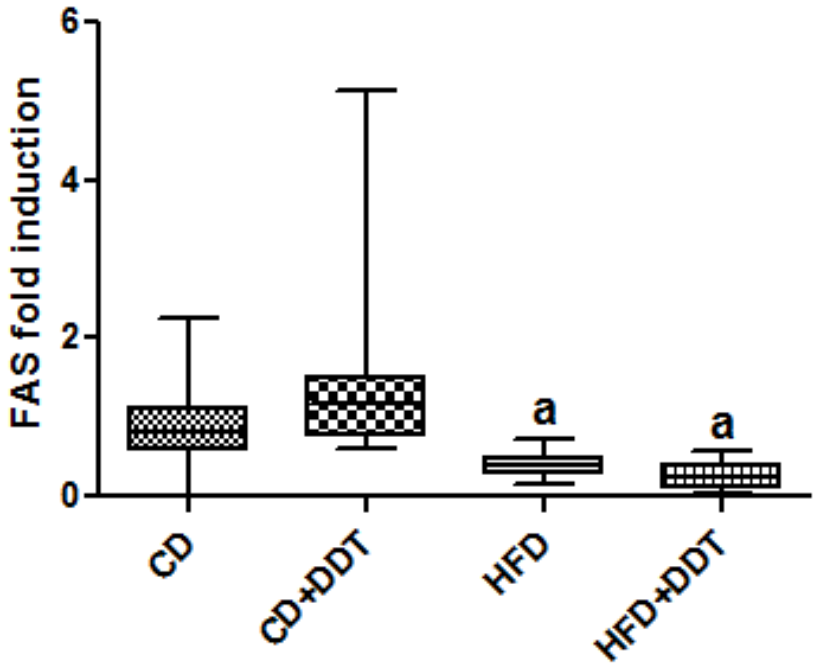

Figure 34. HFD decreased the hepatic expression of FAS:

HFD-fed groups had low levels of hepatic FAS (p=0.001) while DDT had no effect. Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA. (P<0.05, a: due to HFD).

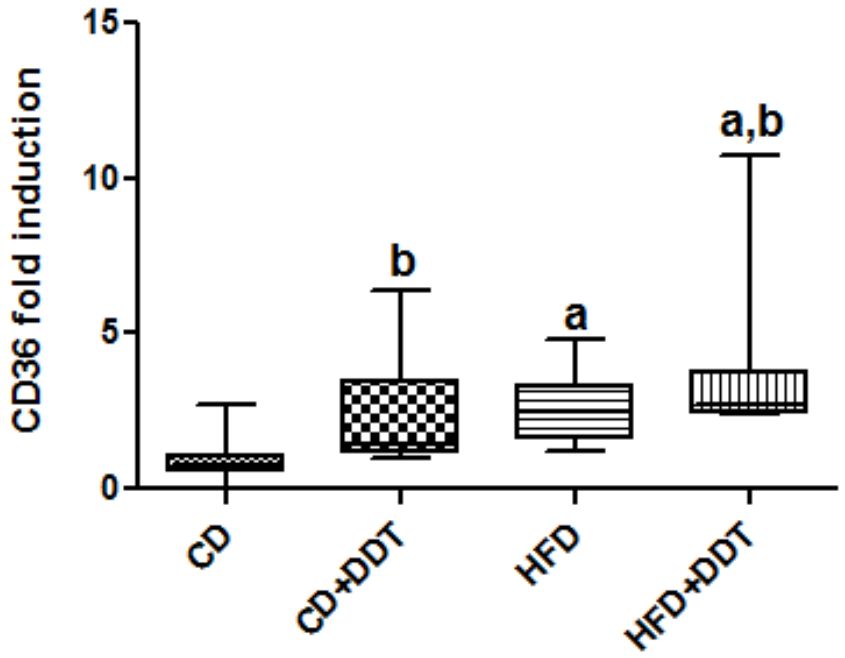

Figure 35. DDT and HFD increased the hepatic expression of CD36:

Hepatic expression of CD36 was increased with DDT (p=0.031) and HFD (p=0.017). Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA. (P<0.05, a: due to HFD, b: due to DDT effect).

DDT exposure induced Cyp2b10, a CAR target gene.

Hepatic expression of Cyp2b10, a CAR target gene, was measured to determine if DDT activated CAR in these animals. DDT exposure led to increased of Cyp2b10 in CD-fed mice (83.82±24.474, p=0.026), indicating CAR activation in these animals (Fig. 36). Likewise, HFD consumption also resulted in increased of Cyp2b10 (73.65±24.474, p=0.049). Interestingly, DDT co-exposure did not potentiate Cyp2b10 increased by HFD. Rather, there was a significant interaction between HFD and DDT (p=0.036), suggesting that DDT co-exposure in HFD-fed mice displayed downregulated Cyp2b10 (HFD+DDT: 42.404±29.252).

Hepatic expression of Cyp3a11, a PXR target gene, was also measured to assess PXR activation in these animals. DDT exposure did not alter Cyp3a11 expression in CD-fed mice (Fig. 37). However, HFD feeding resulted in increased of hepatic Cyp3a11 but DDT had no effect on this (HFD: 5.79±0.803 and HFD+DDT: 4.38±0.959, p<0.001). The results indicated that DDT did not activate hepatic PXR in these animals.

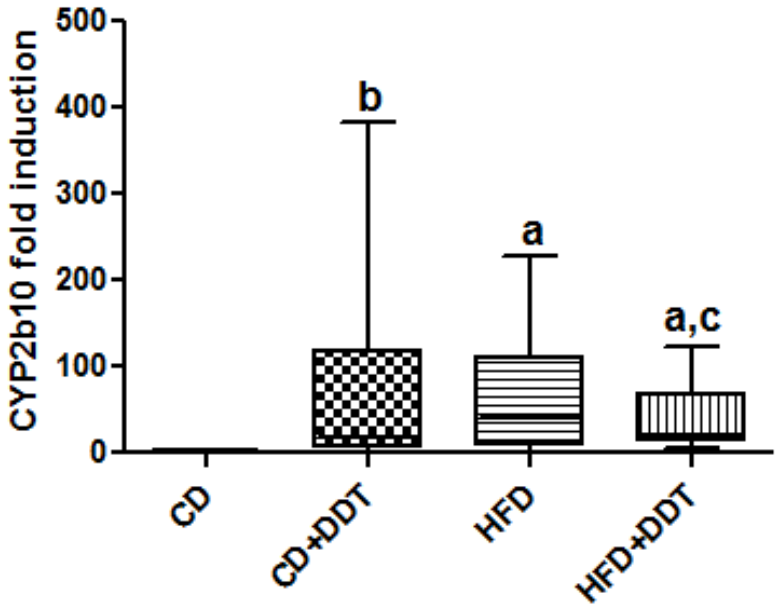

Figure 36. DDT and HFD increased the hepatic expression of Cyp2b10:

DDT exposure increased Cyp2b10 in CD- (p=0.026) and HFD- fed mice (p=0.049). There was a significant interaction between HFD and DDT (p=0.036). Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA. (P<0.05, a: due to HFD, b: due to DDT effect and c: interaction between HFD and DDT).

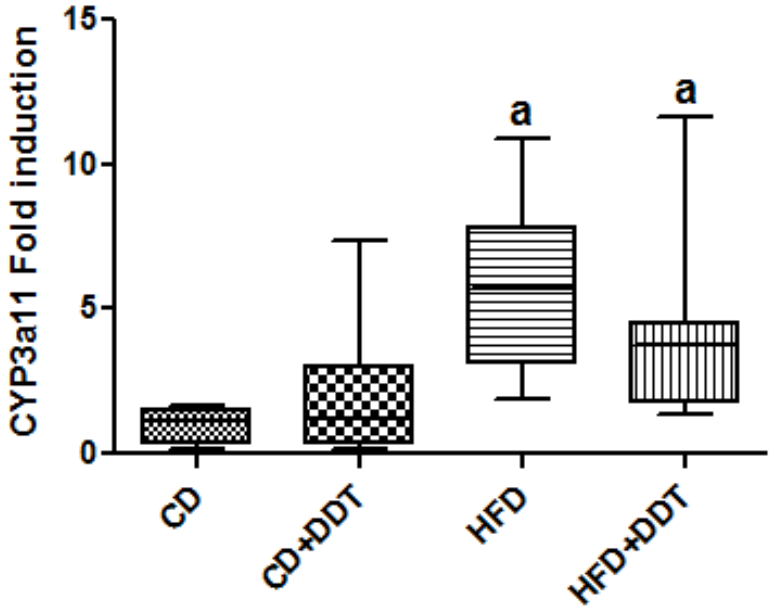

Figure 37. HFD increased the hepatic expression of Cyp3a11:

HFD increased the hepatic Cyp3a11 expression ($p<0.001$) while DDT exposure did not alter Cyp3a11 expression. Real-time PCR was used for levels of expression measurement. Data are expressed as mean±SEM and statistical analysis was performed using Two Way ANOVA. ($P<0.05$, a: due to HFD).

DISCUSSION

The *in vivo* study on DDT demonstrated that DDT did not worsen obesity or liver injury caused by HFD feeding. However, the DDT-exposed mice displayed lower plasma ALT levels, higher plasma adiponectin levels and no insulin resistance. DDT exposure also lowered the fat tissue weight in HFD-fed mice. Additionally, there was no evidence of either hepatic or systemic inflammation observed with DDT exposure. Furthermore, DDT appeared to be a CAR activator in our study. These results indicated that DDT exposure appeared to be protective from DIO, which is counter-intuitive to our initial hypothesis. However, these findings are not surprising, given the fact that CAR activation has been closely related to protect against insulin resistance and obesity-related disorders (76). In terms of dosage, we used a cumulative dose of 100 mg/kg. This was designed to be well below the LD_{50} of DDT which was reported to range from 152.3 - 1466 mg/kg/day to prevent acute toxicity (60). It is possible that using a dose >100 mg/kg may have resulted in different outcomes than those observed in this study.

DDT exposure did not affect body weight or food consumption. The protective effect of DDT in terms of adiposity was seen in the HFD-fed animals that displayed lower fat weight and decreased liver weights. DDT exposure also resulted in lower plasma ALT levels. Plasma adiponectin levels were also higher in the DDT-exposed mice irrespective of the diet type. Adiponectin is an adipokine that regulates glucose and fatty acid catabolism and its levels are

inversely proportional to body fat composition. Increased adiponectin levels observed in DDT-exposed mice may be a plausible reason for a decrease in fat weight in CD-fed mice. Adiponectin also has anti-inflammatory function, which is consistent with the absence of inflammation seen in the liver tissues of the DDT-exposed mice (77). Moreover, DDT exposure also decreased plasma tPAI-1 levels, confirming the absence of liver injury. DDT exposure did not affect leptin levels. Leptin, another adipokine, regulates hunger and satiety and since its levels remained unaltered, food consumption was not significantly affected as well. Insulin resistance has been linked to high resistin levels in obese mice (78). Consistent with our results that showed that DDT did not cause insulin resistance, DDT-exposed animals' plasma resistin levels were not affected.

DDT exposure did not contribute to elevated plasma cholesterol and HDL. Hepatic expression of the lipogenic gene FAS was not affected by DDT, indicating that DDT did not cause lipogenesis in these animals. Paradoxically, another lipogenic gene, CD36, required for fatty acid uptake by cells was increased. CD36 plays an important role in the immune system, coagulation cascade, atherosclerosis and lipid metabolism (79). In lipid metabolism, CD36 binds HDL, LDL and VLDL and it also works as a scavenger for oxidized LDL in macrophages (80, 81). PPARα targets, CPT1a and Cyp4a10, were downregulated in DDT-exposed mice indicating that the fat burning machinery was compromised in these animals. Surprisingly, this did not cause nor worsen steatosis, and one of the reasons could be the protective effects exerted by CAR activation. Malabsorption of dietary fat in the HFD+DDT co-

exposed mice may be another explanation for the absence of steatosis in this group.

DDT induced Cyp2b10 in CD-fed mice, indicating CAR activation as mentioned earlier. In contrast, DDT did not activate PXR, since Cyp3a11 was not induced. CAR activation is consistent with insulin sensitivity and decreased lipogenesis, which is concordant with our findings. The transient transfection studies on HepG2 cells mentioned previously demonstrated that DDT did not activate murine CAR. However, Mutoh *et al* showed the ability of phenobarbital to activate CAR indirectly through inhibition of the epidermal growth factor receptor (EGFR). We speculate that DDT might be acting though the same mechanism as phenobarbital to activate CAR indirectly, and this does not necessitate a direct ligand interaction (82, 83). CAR activation was also shown to decrease PPARα expression and hence, downregulation of its target genes (CPT1a and Cy4a10) and this was also observed in our animals (76, 84, 85). In addition, previous studies demonstrated that phenobarbital, a CAR activator, downregulated CPT1 levels in mice but this was not seen in CAR knockout mice, which further supports our findings (82).

Additionally, there was no evidence of either hepatic or systemic inflammation observed with DDT exposure or unexposed animals. In fact, the measured levels of inflammatory cytokines in the serum and hepatic were higher in CD-fed animals, which indicates that these animals had some sort of infection. Therefore, they study should be repeated with the unexposed CD-fed animals only in order to get valid evaluation of the results from the other groups. Another

limitations in this study is that we were using a DIO model to investigate DDT hepatic toxicity in mice. However, humans are exposed to different chemicals simultaneously. Another alternative approach is to investigate the effects of DDT in a mixture of chemicals or using another hit apart from HFD. Choosing a higher dose and a more chronic exposure is another potential approach.

In conclusion, DDT did not decrease body weight or food consumption despite downregulation of PPARα target genes. The HFD+DDT group of mice also exhibited similar food consumption patterns as HFD group but showed lower adiposity. Moreover, DDT did not cause insulin resistance or worsen NAFLD. Despite reducing the expression of fatty acids β-oxidation genes, DDT improved steatosis in HFD-fed mice. However, DDT did not improve diabetes caused by HFD feeding. Furthermore, DDT appeared to contribute to these effects through CAR activation. However, further investigation is required in terms of CAR direct *vs.* indirect activation and if using higher doses could consequently activate PXR as well.

SUMMARY

NAFLD is the most common cause of liver disease worldwide. Toxicant associated steatohepatitis (TASH) is a recently identified form of non-alcoholic fatty liver disease and it is mainly associated with chemical exposure. However, the mechanisms by which environmental chemicals contribute to liver disease are not well-studied due to the lack of manifestations and chemical indicators in addition to a comprehensive lists of chemicals.

Three hundred seventy one studies archived in federal databases ToxRefDB and CEBS linked 123 unique environmental chemicals to fatty liver disease in rodents. Pesticides composed almost 44% of these chemicals. Moreover, the compounds found associated with ALT elevation in the 2003-2004 NHANES were 3 metals and 8 OCPs in addition to more than 200 pesticides identified from the ToxCast DB database. Pesticides have been associated with NAFLD in many studies and we therefore decided to study the role of some pesticides on NAFLD.

Most of the eight compounds studied activated hPXR, mPXR and hCAR and DDT was the strongest activator. Moreover, the main metabolite of DDT, DDE, was detected in considerable concentrations in the NHANES participants with high ALT levels. Therefore, DDT was selected to be studied in a DIO mice model.

Upon DDT exposure (12 weeks, 100 mg/kg), Cyp2b10 (CAR target) was increased in control diet-fed mice. DDT did not increase Cyp3a11 (PXR target) in any group. DDT did not decrease body weight or food consumption, but HFD+DDT mice showed lower adiposity. DDT did not cause insulin resistance or worsen NAFLD.

In conclusion, more than 300 environmental chemicals, mostly pesticides, were linked to fatty liver disease. The *in vivo* studies of DDT showed that it improved steatosis, but it had no effect on NAFLD, obesity, liver damage or diabetes caused by DIO. DDT appeared to contribute to these effects through CAR activation. However, further investigation is required in terms of CAR direct *vs.* indirect activation and if using higher doses could consequently activate PXR as well.

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APPENDICES

APPENDIX 1

Adjusted* Odds Ratios (95% CI) for ALT Elevation by Exposure Quartile (With Median Concentration Levels and Number of Cases/Total Number) for Pollutant Subclasses Lead, Cadmium, and Mercury and Organochlorine Pesticides in Adult NHANES 2003-2004.

#	Pollutant	Detection Rate (%)	Not Detectable	Detectable				P_{trend}	$P_{trend-adj}$ †
				1st	2nd	3rd	4th		
1.	Dieldrin	88.5	--	3.90[a]	5.90	8.20	14.15	0.007	0.027
	Lipid Adj (ng/g)		13/62	38/129[b]	38/119	53/141	67/155		
			Referent	1.6 (0.7-3.5)[c]	1.8 (0.9-3.6)	2.2 (1.1-4.4)	3.1 (1.3-7.2)		
2.	Heptachlor Epoxide	61.7	--	3.60	5.60	8.90	16.70	0.001	0.009
	Lipid Adj (ng/g)		56/214	29/82	35/112	44/106	48/97		
			Referent	1.4 (0.8-2.4)	1.3 (0.7-2.2)	1.9 (1.1-3.2)	2.6 (1.3-5.0)		
3.	Trans-nonachlor	93.9	--	5.90	12.85	27.50	57.50	0.050	0.093
	Lipid Adj (ng/g)		11/37	35/139	54/138	54/137	54/153		
			Referent	0.7 (0.4-1.3)	1.6 (0.8-3.2)	1.7 (0.6-4.6)	1.6 (0.6-3.8)		
4.	B-hexachlorocyclo-hexane	75.4	--	4.30	8.80	19.10	52.40	0.082	0.093
	Lipid Adj (ng/g)		36/146	33/97	56/133	43/117	43/116		
			Referent	1.6 (1.0-2.8)	2.3 (1.4-3.8)	1.8 (1.0-3.4)	1.7 (0.9-3.5)		
5.	Hexachloro-benzene	99.9		9.90	13.75	17.60	24.60	0.075	0.093
	Lipid Adj (ng/g)			44/148	47/142	60/167	59/150		
				Referent	1.1 (0.8-1.6)	1.2 (0.9-1.6)	1.4 (1.0-2.0)		
6.	Oxychlordane	83.8	--	5.00	10.50	19.30	35.50	0.053	0.093
	Lipid Adj (ng/g)		25/106	40/117	42/123	61/142	44/125		
			Referent	1.8 (0.9-3.7)	2.1 (0.8-5.7)	3.5 (1.4-8.4)	2.7 (0.9-8.2)		
7.	p,p'-DDE	99.7	--	82.40	183.00	464.00	1535.0	0.062	0.093
	Lipid Adj (ng/g)		0/1	43/155	52/153	57/148	57/149		
				Referent	1.4 (0.8-2.4)	1.7 (1.0-2.8)	1.7 (0.9-2.9)		
8.	p,p'-DDT	79.0	--	3.50	5.10	7.70	19.30	0.095	0.095
	Lipid Adj (ng/g)		35/135	35/112	42/111	54/121	42/126		
			Referent	1.3 (0.7-2.4)	1.7 (0.8-3.8)	2.2 (1.5-3.3)	1.2 (0.7-1.9)		

* ORs were adjusted for age, sex, race, poverty income ratio, HOMA-IR, and BMI.
[a] Median concentration levels.
[b] Number of cases / total number.
[c] Adjusted odds ratios with 95% confidence intervals.
† Additionally adjusted for multiple comparisons.

1st quartile: ≤ 25th percentile, 2nd quartile: 25th - ≤ 50th percentile, 3rd quartile: 50th - ≤ 75th percentile, 4th quartile: >75th percentile.

Abbreviations: CI; confidence interval

APPENDIX 2

Pesticides associated with fatty liver disease in ToxRefDB.

#	Chemical Name	Study Design and species	LEL (mg/kg/day)
Fungicides:			
1.	Bromuconazole	Mouse-Subchronic	68.1
2.	Cyproconazole	Rat-Chronic Rat-MGR*	8.29 15.6
3.	Dazomet	Rat-Chronic Mouse-Chronic Rat-MGR*	69.9 2.78 3.71
4.	Diethyl 4,4'-o-phenylenebis (3-thioallophanate)	Mouse-Chronic	300
5.	Difenoconazole	Mouse-Chronic	819
6.	Dimethomorph	Rat-Subchronic	14.2
7.	Famoxadone	Mouse-Chronic	274
8.	Fenarimol	Rat-Chronic Mouse-Chronic	14.6 86
9.	Fluazinam	Rat-Chronic Rat-MGR*	40 9.7
10.	Flusilazole	Rat-Subchronic Rat-Chronic	55 13
11.	Hexaconazole	Rat-Subchronic Rat-Chronic Rat-MGR*	25 4.7 5
12.	Iprodione	Mouse-Chronic	604
13.	Propiconazole	Rat-Chronic	96.4
14.	Metalaxyl	Rat-MGR*	62.5
15.	Oxytetracycline hydrochloride	Rat-Chronic	1250
16.	Paclobutrazol	Mouse-Chronic Rat-MGR*	113 62.5
17.	Propanoic acid, 2-(2,4-dichlorophenoxy)-, (R)-	Rat-Subchronic	144
18.	Triadimefon	Rat-Chronic Mouse-Chronic	114 550
19.	Triadimenol	Rat-Subchronic	39.6
20.	Trifloxystrobin	Mouse-Chronic	274
21.	Triflumizole	Mouse-Chronic	67.4
22.	Vinclozolin	Mouse-Chronic Rat-MGR*	1230 290

#	Chemical Name	Study Design and species	LEL (mg/kg/day)
Herbicides:			
23.	Bensulide	Rat-Subchronic	100
24.	Butafenacil	Rat-Chronic	13
25.	Chlorsulfuron	Rat Chronic	309
26.	Ethofumesate	Rat-Subchronic	1900
27.	Fluthiacet-methyl	Rat-Subchronic Rat-Chronic Mouse-Chronic Rat-MGR*	216 130 37 31.8
28.	Mesosulfuron-methyl	Mouse-Chronic	1360
29.	Oxadiazon	Rat-Chronic	50.9
30.	Pyrasulfotole metabolite (SXX 0665)	Rat-MGR*	9.48
31.	Rimsulfuron	Rat-Chronic	121
32.	Sethoxydim	Mouse-Chronic	41.2
33.	Sulfentrazone	Rat Subchronic	199
34.	Tepraloxydim	Rat-Subchronic	383
35.	Thiazopyr	Rat-Subchronic	201
Insecticides:			
36.	Buprofezin	Rat-Subchronic	316
37.	Chlorpyrifos-methyl	Mouse-Chronic	41.5
38.	d-cis,trans-Allethrin	Mouse-Chronic	350
39.	Fipronil	Rat-Subchronic	19.9
40.	Tetramethrin	Rat-Subchronic	57.9
41.	Thiacloprid	Mouse-Chronic	234
Miticide:			
42.	Acequinocyl	Mouse-Chronic	7

Chemicals are arranged according in alphabetic order in each class and their LELs are provided according to the screened ToxRefDB studies.

***MGR: Multigeneration Reproductive**

APPENDIX 3

Solvents, plasticizers, monomers, and Chemical Intermediates associated with fatty liver disease in CEBS.

#	Chemical Name	Study Design and species	LEL (mg/kg)
1.	2,2-Bis(Bromomethyl)-1,3 propanediol	Rat-Chronic	25,000
2.	4-Vinyl-1-cyclohexene diepoxide	Rat-Chronic	50
3.	4,4'-Thiobis(6-tert-butyl-m-cresol)	Mouse-Chronic	250
4.	Alpha-Methylstyrene	Mouse-Chronic	300
5.	Dibutyl phthalate	Rat-Short term	600
6.	Divinylbenzene	Mouse-Chronic	10
7.	Glycidol	Mouse- Short term	100
8.	Isoprene	Rat-Chronic	220
9.	Resorcinol	Rat-Chronic	50
10.	Sodium selenite	Rat-Short Term	4
11.	Tetrabromobisphenol A	Mouse-Short Term	100
12.	Tetrafluoroethylene	Rat-Chronic	156
13.	Tricresyl phosphate	Rat-Chronic	75
14.	Trimethylolpropane triacrylate	Rat-Chronic	0.3
15.	Vinyl toluene	Rat-Chronic Mouse-Chronic	100 25
16.	1-Amino-2,4-dibromoanthraquinone	Rat-Chronic	20,000
17.	4,4'-Diamino-2,2'-stilbenedisulfonic acid, disodium salt	Mouse-Chronic	6250
18.	p-Nitrobenzoic acid	Mouse-Chronic	1250
19.	p-Nitrotoluene	Mouse	1250
20.	2-Methylimidazole	Rat-Chronic	1000
21.	Methyl isobutyl ketone	Mouse-Chronic	900
22.	Toluene	Rat-Chronic Mouse-Chronic	600
23.	Barium chloride dehydrate	Rat-Chronic	500
24.	Styrene-acrylonitrile trimer	Rat-Short term	250
25.	Decalin	Mouse-Chronic	100
26.	3,3'-Dimethoxybenzidine dihydrochloride	Rat-Chronic	80
27.	bis(2-Chloroethoxy)methane	Rat-Chronic	75
28.	1-Bromopropane	Mouse-Chronic	62.5
29.	Tribromomethane	Mouse-Chronic	50
30.	Sodium dichromate dihydrate (VI)	Rat-Chronic	14.3

		mg/L
31. 1,2-Dihydro-2,2,4-trimethylquinoline (monomer)	Mouse-Short term	3.6

Chemicals are arranged in alphabetic order and their LELs are provided according to the screened CEBS studies.

APPENDIX 4

Miscellaneous chemicals associated with fatty liver disease in CEBS.

#	Chemical Name	Study Design and species	LEL
1.	Polysorbate 80	Rat-Chronic	25,000 mg/kg
2.	T-Butylhydroquinone	Mouse-Chronic	1,250 mg/kg
3.	Benzophenone	Rat-Chronic	312 mg/kg
4.	Cumene hydroperoxide	Rat-Short term	100 mg/kg
5.	Isobutyl nitrite	Mouse-Chronic Rat-Chronic	37.5 mg/kg
6.	N,N-Dimethyl-p-toluidine	Rat-Chronic	6 mg/kg
7.	Sodium azide	Rat-Chronic	5 mg/kg
8.	Tetranitromethane	Rat-Chronic	2 mg/kg
9.	Vanadium oxide	Mouse-Chronic	1 mg/M3
10.	Nickel (II) oxide	Rat-Chronic	0.63 mg/m3
11.	Nickel sulfate hexahydrate	Rat-Chronic	0.25 mg/m3
12.	Indium phosphide	Rat-Chronic	0.03 mg/m3
13.	Gallium arsenide	Rat-Chronic	0.01 mg/M3
14.	3,3'-Dimethylbenzidine dihydrochloride	Rat-Chronic	0.003**

Chemicals are arranged in alphabetic order and their LELs are provided according to the screened CEBS studies.

**** Units were not provided in the CEBS search.**

APPENDIX 5

Pesticides associated with fatty liver disease in CEBS.

#	Chemical Name	Study Design and species	LEL (mg/kg)
1.	1,2-Dibromo-2,4-dicyanobutane	Rat-Chronic	2
2.	1,2,3-Trichloropropane	Mouse-Chronic	6
3.	1,2,3-Trichloropropane	Rat-Chronic	3
4.	3,3',4,4'-Tetrachloroazobenzene	Rat-Chronic	10
5.	Beta-Picoline	Rat-Chronic	312.5 mg/L
6.	Formamide	Rat-Chronic	20
7.	Fumonisin B1	Mouse-Chronic	80
8.	Hexachloroethane	Rat-Chronic	10
9.	Monochloroacetic acid	Rat-Chronic	10
10.	Naphthalene	Rat-Chronic	10
11.	p,p'-Dichlorodiphenyl sulfone	Mouse-Chronic	30
12.	Triethanolamine	Mouse-Chronic	630

Pesticides are arranged in alphabetic order and their LELs are provided according to the screened CEBS studies.

APPENDIX 6

Fragrances, cosmetics and essential oils associated with fatty liver disease in CEBS.

#	Chemical Name	Study Design and species	LEL (mg/kg)
1.	3,4-Dihydrocoumarin	Mouse-Chronic	200
2.	Beta-Myrcene	Mouse-Chronic	250
3.	Dipropylene glycol	Rat-Chronic	25,000
4.	Estragole	Mouse-Short term	37.5
5.	Hydroquinone	Rat-Chronic	25
6.	Isoeugenol	Rat-Chronic Mouse-Chronic	75
7.	Methyl trans-styryl ketone	Mouse-Chronic	10
8.	Methyleugenol	Rat-Short term	150
9.	Tris(2-Chloroethyl) phosphate	Rat-Chronic	44

Chemicals are arranged in alphabetic order and their LELs are provided according to the screened CEBS studies.

APPENDIX 7

Paints, polishes, dyes and food additives associated with fatty liver disease in CEBS.

#	Chemical Name	Study Design and species	LEL (mg/kg)
1	2-Butoxyethanol	Rat-Chronic	31.2
2	2,4-Diaminophenol dihydrochloride	Mouse-Chronic	0.038
3	Benzyl acetate	Rat-Chronic	3,000
4	C.I. Acid red 114	Rat-Chronic	0.007
5	C.I. Direct blue 15	Rat-Chronic	0.125**
6	C.I. Direct blue 218	Rat -Chronic Mouse-Chronic	1,000
7	HC yellow 4	Rat-Chronic	25,000
8	Malachite green	Rat-Chronic	600
9	Pyrogallol	Rat-Chronic	5

Chemicals are arranged in alphabetic order and their LELs are provided according to the screened CEBS studies.

**** Units were not provided in the CEBS search.**

APPENDIX 8

PCBs and dioxin-like compounds associated with fatty liver disease in CEBS.

#	Chemical Name	Study Design and species	LEL (mg/kg)
1.	2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)	Rat-Chronic	0.01
2.	Dioxin mixture	Rat-Chronic	10
3.	PCB 118	Rat-Chronic	0.1
4.	PCB 126	Rat-Chronic	0.00001
5.	PCB 153	Rat-Chronic	0.01
6.	Pentachlorodibenzofuran (PECDF)	Rat-Chronic	0.000006

Chemicals are arranged in alphabetic order and their LELs are provided according to the screened CEBS studies.

APPENDIX 9

Pesticides associated Activate hPXR in NCGC-PXR ToxCastDB.

#	Name of Chemical	Dose (µM)	#	Name of Chemical	Dose (µM)
1.	Alachlor	15.4	2.	Fosthiazate	10.6
3.	Bensulide	1.57	4.	Hexaconazole	31.2
5.	Bensulide	1.57	6.	Hexythiazox	18.6
7.	Bensulide	1.57	8.	Imazalil	36.5
9.	Bifenthrin	28.4	10.	Indoxacarb	7.96
11.	Bisphenol A	20.5	12.	Isazofos	5.53
13.	Buprofezin	5.44	14.	Isoxaben	0.479
15.	Butachlor	1.95	16.	Metam-sodium hydrate	31.4
17.	Butralin	36.1	18.	Methoxyfenozide	5.99
19.	Chlorothalonil	17.7	20.	Metolachlor	0.517
21.	Clofentezine	11.8	22.	Napropamide	0.479
23.	Coumaphos	3.74	24.	Oryzalin	7.37
25.	Cyanazine	6.79	26.	Oxadiazon	5.49
27.	Cyfluthrin	19.8	28.	Oxyfluorfen	20.8
29.	Cypermethrin	18.3	30.	Parathion	17.2
31.	Cyproconazole	23.0	32.	Permethrin	7.57
33.	Cyprodinil	28.8	34.	Phosalone	11.6
35.	Allethrin (d-cis,trans)	11.1	36.	Prallethrin	20.0
37.	DEHP (Diethylhexyl phthalate)	20.8	38.	Prochloraz	6.81
39.	Dimethenamid	1.42	40.	Prometryn	36.0
41.	Diniconazole	9.61	42.	Propetamphos	19.1
43.	Dithiopyr	30.9	44.	Propyzamide	24.1
45.	S-Bioallethrin	19.2	46.	Pyraflufen-ethyl	33.3
47.	Endosulfan	10.8	48.	Rotenone	17.0
49.	Esfenvalerate	27.0	50.	TCMTB	39.0
51.	Ethalfluralin	14.7	52.	Tebufenozide	21.3
53.	Ethofumesate	17.1	54.	Tebupirimfos	3.2
55.	Etoxazole	1.81	56.	Tetraconazole	22.7
57.	Fenamiphos	28.7	58.	Thiazopyr	1.01
59.	Fenarimol	20.3	60.	Triadimenol	7.33
61.	Fenpropathrin	4.59	62.	Tribufos	11.1
63.	Fipronil	12.6	64.	Triflumizole	32.0
65.	Fludioxonil	16.1	66.	Triticonazole	4.67
67.	Flumetralin	1.35			

Chemicals are arranged in alphabetic order.

APPENDIX 10

Pesticides associated Activate hPXR in ATG_PXR_TRANS ToxCastDB.

#	Name of Chemical	Dose (μM)	#	Name of Chemical	Dose (μM)
1.	Alachlor	5.7	2.	Fluthiacet-methyl	53.0
3.	Ametryn	34.0	4.	Flutolanil	34.0
5.	Azoxystrobin	4.3	6.	Hexythiazox	8.5
7.	Benfluralin	32.0	8.	Imazapic	51.0
9.	Bensulide	1.4	10.	Imidacloprid	72.0
11.	Bensulide	0.56	12.	Indoxacarb	7.3
13.	Bensulide	0.91	14.	Iprodione	11.0
15.	Bentazone	47.0	16.	Isazofos	4.5
17.	Bifenazate	11.0	18.	Isoxaben	1.0
19.	Bifenthrin	4.1	20.	Lactofen	26.0
21.	Bisphenol A	14.0	22.	Lindane	22.0
23.	Buprofezin	3.8	24.	Linuron	40.0
25.	Butachlor	1.1	26.	Malathion	31.0
27.	Butralin	7.7	28.	Metalaxyl	14.0
29.	Carfentrazone-ethyl	43.0	30.	Methoxyfenozide	2.4
31.	Chlorothalonil	0.22	32.	Metolachlor	6.6
33.	Chlorpropham	45.0	34.	MGK	18.0
35.	Cinmethylin	6.6	36.	Molinate	62.0
37.	Clomazone	35.0	38.	Napropamide	1.3
39.	Coumaphos	3.9	40.	Oryzalin	2.9
41.	Cyazofamid	9.4	42.	Oxadiazon	4.9
43.	Cyfluthrin	25.0	44.	Oxyfluorfen	20.0
45.	Cypermethrin	10.0	46.	Parathion	24.0
47.	Cyprodinil	30.0	48.	Pendimethalin	37.0
49.	Allethrin (d-cis,trans)	11.0	50.	Phosalone	9.9
51.	Diazinon	33.0	52.	Piperonyl butoxide	15.0
53.	Dichlobenil	58.0	54.	Pirimiphos-methyl	12.0
55.	Diclosulam	34.0	56.	Prallethrin	3.6
57.	MEHP (Phthalic acid, mono-2-ethylhexyl ester)	62.0	58.	Prochloraz	3.6
59.	DEHP (Diethylhexyl phthalate)	38.0	60.	Prodiamine	5.7
61.	Disulfoton	38.0	62.	Profenofos	5.0
63.	Dithiopyr	2.3	64.	Prometryn	25.0
65.	Diuron	58.0	66.	Propazine	34.0
67.	S-Bioallethrin	3.6	68.	Propetamphos	7.1
69.	Esfenvalerate	4.6	70.	Propyzamide	39.0
71.	Ethalfluralin	17.0	72.	Pyraflufen-ethyl	29.0

73.	Ethofumesate	37.0	74.	Resmethrin	23.0
75.	Etoxazole	3.3	76.	Sulfentrazone	34.0
77.	Fenamidone	47.0	78.	Tebupirimfos	13.0
79.	Fenamiphos	14.0	80.	Tetramethrin	43.0
81.	Fenarimol	49.0	82.	Thiazopyr	0.5
83.	Fenhexamid	24.0	84.	Thiobencarb	25.0
85.	Fenitrothion	47.0	86.	Triadimefon	26.0
87.	Fenpropathrin	11.0	88.	Triadimenol	4.4
89.	Fenpyroximate (Z,E)	12.0	90.	Tri-allate	25.0
91.	Fenthion	39.0	92.	Triasulfuron	22.0
93.	Fipronil	28.0	94.	Tribufos	23.0
95.	Fludioxonil	23.0	96.	Trifloxystrobin	60.0
97.	Flufenacet	13.0	98.	Trifluralin	14.0
99.	Flumetralin	4.3	100.	Triticonazole	10.0
101.	Flusilazole	36.0	102.	Zoxamide	2.2

Chemicals are arranged in alphabetic order.

APPENDIX 11

Pesticides associated Activate hPXR in NVS_NR_hPXR ToxCastDB.

#	Name of Chemical	Dose (µM)	#	Name of Chemical	Dose (µM)
1.	HPTE	23.0	2.	Malathion	22.0
3.	3-Iodo-2-propynylbutylcarbamate	6.9	4.	Mancozeb	1.5
5.	3-Iodo-2-propynylbutylcarbamate	15.0	6.	Maneb	0.11
7.	Abamectin	18.0	8.	Methamidophos	21.0
9.	Alachlor	8.6	10.	Methidathion	15.0
11.	Azinphos-methyl	18.0	12.	Methoxyfenozide	5.9
13.	Azoxystrobin	4.7	14.	Methylene bis(thiocyanate)	2.2
15.	Bensulide	0.62	16.	Metiram-zinc	3.3
17.	Bensulide	0.69	18.	Milbemectin (mixture)	6.4
19.	Bensulide	1.0	20.	Napropamide	0.18
21.	Buprofezin	5.5	22.	Oryzalin	4.5
23.	Butachlor	1.3	24.	Oxadiazon	7.5
25.	Butafenacil	2.4	26.	Pendimethalin	15.0
27.	Butylate	47.0	28.	PFOS (Perfluorooctane sulfonic acid)	38.0
29.	Cacodylic acid	15.0	30.	Permethrin	29.0
31.	Captan	3.5	32.	Phosalone	10.0
33.	Chlorpyrifos oxon	19.0	34.	Pirimiphos-methyl	9.4
35.	Clodinafop-propargyl	5.5	36.	Prallethrin	25.0
37.	Clothianidin	19.0	38.	Prochloraz	5.0
39.	Coumaphos	6.5	40.	Profenofos	27.0
41.	Cyclanilide	13.0	42.	Prometryn	13.0
43.	Cyprodinil	48.0	44.	Propargite	20.0
45.	Dimethenamid	39.0	46.	Propetamphos	46.0
47.	Diniconazole	20.0	48.	Propiconazole	14.0
49.	Dithiopyr	0.31	50.	Pymetrozine	17.0
51.	Emamectin benzoate	7.1	52.	Pyraclostrobin	7.0
53.	Ethalfluralin	8.1	54.	Resmethrin	6.6
55.	Etoxazole	12.0	56.	Spirodiclofen	0.43
57.	Fenarimol	18.0	58.	TCMTB	1.6

59.	Fenhexamid	1.9	60.	Tebupirimfos	35.0
61.	Fentin	0.86	62.	Tetraconazole	44.0
63.	Fluazifop-butyl	21.0	64.	Tetramethrin	18.0
65.	Fluazinam	0.26	66.	Thiazopyr	0.17
67.	Flufenacet	14.0	68.	Thiobencarb	28.0
69.	Flumetralin	1.8	70.	Thiodicarb	1.1
71.	Flumiclorac-pentyl	2.1	72.	Thiophanate-methyl	48.0
73.	Fluoxastrobin	12.0	74.	Thiram	12.0
75.	Flutolanil	7.1	76.	Triadimefon	7.6
77.	Folpet	8.2	78.	Tri-allate	9.7
79.	Fosthiazate	49.0	80.	Tribufos	40.0
81.	Hexaconazole	49.0	82.	Triclosan	13.0
83.	Indoxacarb	3.2	84.	Triflumizole	38.0
85.	Isazofos	16.0	86.	Trifluralin	28.0
87.	Isoxaben	0.45	88.	Triflusulfuron-methyl	34.0
89.	Lindane	27.0	90.	Vinclozolin	6.7
91.	Malaoxon	33.0			

Chemicals are arranged in alphabetic order.

APPENDIX 12

Pesticides associated Activate hPXR target gene CYP 3a4 in CLZD_CYP3A4 ToxCastDB.

#	Name of Chemical	Dose (µM)	#	Name of Chemical	Dose (µM)
1.	3-Iodo-2-propynylbutylcarbamate	0.58	2.	Imazalil	0.588
3.	Abamectin	5.65	4.	Imazapyr	1.13
5.	Acetochlor	0.396 5.67 0.598	6.	Imazethapyr	14.1
7.	Acifluorfen	9.15	8.	Imidacloprid	29.2
9.	Alachlor	2.99 4.03	10.	Indoxacarb	0.467 8.39 10.2
11.	Ametryn	5.21 6.23	12.	Iprodione	5.77 13.1
13.	Asulam	9.64	14.	Isazofos	0.46 0.788 2.75
15.	Atrazine	10.9 12.6 28.8	16.	Isoxaben	0.0992 0.225 1.17
17.	Azinphos-methyl	0.493 6.12 7.13	18.	Isoxaflutole	6.99 16.1
19.	Azoxystrobin	10.2 11.8	20.	Lactofen	5.12 7.73 9.5
21.	Benfluralin	7.39 11.2 12.1	22.	Lindane	1.45 4.05
23.	Bensulide	0.472 1.08 2.45	24.	Linuron	5.44 12.9
25.	Bensulide	0.783 0.649	26.	Malaoxon	5.31 11.3
27.	Bensulide	0.469 0.659 12.0	28.	Malathion	0.0635
29.	Bifenazate	0.9	30.	Mancozeb	6.51
31.	Bifenthrin	4.16 4.65 11.9	32.	Metalaxyl	8.73 10.4

33.	Bisphenol A	3.53	34.	Methidathion	5.26 9.62
35.	Boscalid	1.17 5.67 10.5	36.	Methoxychlor	6.58 14.0 20.3
37.	Bromacil	6.29 8.82	38.	Methoxyfenozide	0.729 6.02 7.2
39.	Buprofezin	10.8 11.9	40.	Metolachlor	0.488 9.61 12.3
41.	Butachlor	8.35	42.	Metribuzin	3.95
43.	Butafenacil	0.296 0.512 3.87	44.	MGK	12.2 17.7
45.	Butralin	2.16 4.31 5.15	46.	Milbemectin (mixture)	0.442 0.447 29.2
47.	Butylate	0.951	48.	Molinate	6.28
49.	Carbaryl	8.42	50.	Myclobutanil	5.24 5.93 6.07
51.	Carboxin	5.35 8.35	52.	Napropamide	4.43 28.3
53.	Carfentrazone-ethyl	10.3 12.1	54.	Nitrapyrin	0.424
55.	Chlorethoxyfos	8.24 8.49 10.3	56.	Norflurazon	4.37 5.42 12.2
57.	Chloroneb	0.542	58.	Oryzalin	12.6 12.7
59.	Chlorpropham	9.29 13.7 27.7	60.	Oxadiazon	0.568 0.586
61.	Chlorpyrifos oxon	5.21 7.71	62.	Oxasulfuron	29.2
63.	Chlorpyrifos-methyl	12.1	64.	Oxyfluorfen	5.87 8.47 10.7
65.	Chlorsulfuron	1.69	66.	Oxytetracycline dihydrate	5.35
67.	Cinmethylin	7.65 12.8	68.	Paclobutrazol	7.6
69.	Clodinafop-propargyl	28.7	70.	Parathion	4.88 5.27 11.9

71.	Clofentezine	5.0 5.17 10.1	72.	Parathion-methyl	3.61 12.5
73.	Clomazone	9.04 28.3	74.	Pendimethalin	18.5
75.	Clopyralid-olamine	4.87 9.73	76.	Penoxsulam	6.61 11.0 11.7
77.	Coumaphos	2.87 3.47	78.	PFOS (Perfluorooctane sulfonic acid)	28.8 28.9
79.	Cyanazine	5.87 5.94	80.	PFOA (Perfluorooctanoic acid)	2.37
81.	Cyfluthrin	12.3 28.5	82.	Permethrin	2.15
83.	Cypermethrin	4.44 11.2 12.7	84.	Phosalone	7.94
85.	Cyproconazole	7.68 9.77	86.	Piperonyl butoxide	2.23 2.97 9.62
87.	Cyprodinil	28.0	88.	Pirimicarb	6.5
89.	Cyromazine	5.06	90.	Pirimiphos-methyl	5.24 10.6
91.	Allethrin (d-cis,trans)	6.31	92.	Prallethrin	3.56 6.53 14.6
93.	Diazinon	0.532 4.18 4.63	94.	Prodiamine	0.494 5.65 13.6
95.	Diazoxon	9.92 29.6	96.	Profenofos	6.73 8.97
97.	DBP (Dibutyl phthalate)	4.17	98.	Prometon	3.05 4.85 11.1
99.	Dichlobenil	0.408	100.	Prometryn	7.19 6.98
101.	Dichloran	6.07	102.	Propanil	12.4
103.	Diclofop-methyl	10.3	104.	Propargite	1.59
105.	Diclosulam	5.97 8.6	106.	Propazine	5.66 7.8 7.11
107.	Dicofol	5.87 6.9 13.6	108.	Propetamphos	1.03 3.17 4.91

109.	MEHP (Phthalic acid, mono-2-ethylhexyl ester)	5.77 11.9	110.	Propiconazole	0.533 2.96
111.	Diethyltoluamide	4.62 11.1 12.4	112.	Propoxur	7.04 8.81
113.	Dimethenamid	5.32 6.19 6.85	114.	Propoxycarbazone-sodium	28.0
115.	Dimethomorph	0.503 3.36 3.62	116.	Propyzamide	8.7 23.9
117.	Diniconazole	0.513 0.612 2.63	118.	Prosulfuron	6.34 9.19
119.	Disulfoton	14.8 16.8 26.7	120.	Prosulfuron	4.01 4.59
121.	Dithiopyr	1.29 2.62	122.	Prosulfuron	5.36
123.	Diuron	5.27 9.37	124.	Pyraclostrobin	4.62
125.	S-Bioallethrin	8.75 9.7 13.3	126.	Pyraflufen-ethyl	5.03 7.28 11.6
127.	Endosulfan	5.15 6.45 8.57	128.	Pyrimethanil	6.48 9.18
129.	Esfenvalerate	3.37 8.1 15.0	130.	Pyriproxyfen	8.01 12.2 27.8
131.	Ethalfluralin	5.17 13.7	132.	Pyrithiobac-sodium	10.9 13.4
133.	Ethametsulfuron methyl	0.461	134.	Quinoxyfen	9.68
135.	Ethofumesate	7.0 10.1	136.	Quintozene	3.99
137.	Ethoprop	4.43 7.0	138.	Resmethrin	4.78
139.	Etoxazole	2.23 4.64 7.19	140.	Rimsulfuron	9.4
141.	Fenamiphos	5.55 5.77	142.	Rotenone	1.5
143.	Fenarimol	0.461 0.589	144.	Sethoxydim	5.59 5.92 27.8

145.	Fenbuconazole	1.06 2.02	146.	Spirodiclofen	0.437 25.6
147.	Fenhexamid	0.0165	148.	Spiroxamine	4.3 6.42 6.83
149.	Fenitrothion	7.39 7.87	150.	Sulfentrazone	1.61 3.38 7.26
151.	Fenoxaprop-ethyl	5.52	152.	Tebufenozide	0.972 5.17 6.75
153.	Fenpropathrin	4.46 5.13 13.3	154.	Tebufenpyrad	6.05 24.6
155.	Fenthion	4.25 14.7	156.	Tebupirimfos	0.558 1.46 0.446
157.	Fentin	4.0	158.	Tefluthrin	4.5 10.5
159.	Fipronil	0.568 0.757	160.	Tetraconazole	0.495 0.471
161.	Fluazifop-butyl	12.0	162.	Tetramethrin	3.63 7.09
163.	Fluazinam	0.475	164.	Thiacloprid	9.55
165.	Fludioxonil	6.97 9.63	166.	Thiamethoxam	9.18
167.	Flufenacet	7.59	168.	Thiazopyr	0.547 12.7
169.	Flufenpyr-ethyl	6.69 7.86 9.98	170.	Thidiazuron	5.9
171.	Flumetralin	0.51 3.44 5.6	172.	Thiobencarb	4.47 12.1
173.	Flumetsulam	11.3 15.4 16.0	174.	Thiophanate-methyl	5.09 4.89 8.36
175.	Flumiclorac-pentyl	6.37	176.	Tralkoxydim	5.84 5.97 14.6
177.	Flumioxazin	4.77	178.	Triadimefon	5.67 5.82
179.	Fluometuron	0.42 10.2	180.	Triadimenol	0.408 2.39 3.62
181.	Fluoxastrobin	0.27	182.	Tri-allate	5.61

	0.475		7.08
183. Flusilazole	0.62	184. Triasulfuron	4.41 5.91
185. Fluthiacet-methyl	2.43 4.79 5.32	186. Tribufos	0.523 12.5
187. Flutolanil	12.1 12.8	188. Triclosan	4.39
189. Forchlorfenuron	28.2	190. Trifloxystrobin	11.8 12.6
191. Fosthiazate	10.0 12.2 12.7	192. Trifloxysulfuron-sodium	7.44
193. Halosulfuron-methyl	4.54 7.12	194. Triflumizole	0.534 0.764
195. Hexaconazole	3.37 5.33	196. Trifluralin	3.31 11.0 11.6
197. Hexazinone	4.37 8.64	198. Triflusulfuron-methyl	11.8
199. Hexythiazox	3.2 3.93	200. Triticonazole	1.56 4.09
201. Icaridin	6.82	202. Zoxamide	4.87 7.55

Chemicals are arranged in alphabetic order.

APPENDIX 13

Pesticides associated Activate hPXR target gene CYP3A4 in NVS_ADME_hCYP3A4 ToxCastDB.

#	Name of Chemical	Dose (μM)	#	Name of Chemical	Dose (μM)
1.	HPTE	13.0	2.	Imazalil	0.1
3.	Bensulide	0.37	4.	Malathion	4.4
5.	Bensulide	0.49	6.	MGK	3.1
7.	Bensulide	0.7	8.	Milbemectin (mixture)	9.6
9.	Chlorothalonil	0.39	10.	Paclobutrazol	4.3
11.	Cyproconazole	5.7	12.	Piperonyl butoxide	0.95
13.	Diniconazole	1.3	14.	Tetraconazole	18.0
15.	Fenarimol	16.0	16.	Triflumizole	0.46
17.	Fenbuconazole	5.7			

Chemicals are arranged in alphabetic order.

APPENDIX 14

Pesticides associated Activate PXR and CYP3a4 in humans in ToxCastDB.

#	Name of Chemical	Dose (μM)	#	Name of Chemical	Dose (μM)
1.	3-Iodo-2-propynylbutylcarbamate	6.9 15.0 0.58	2.	Imazethapyr	14.1
3.	Abamectin	0.598 5.65 18.0	4.	Imidacloprid	29.2 72.0
5.	Acetochlor	0.396 5.67 0.598	6.	Indoxacarb	0.467 3.2 7.96 7.3 8.39 10.2
7.	Acifluorfen	2.99 4.03 5.7 8.6 9.15 15.4	8.	Iprodione	5.77 11.0 13.1
9.	Allethrin (d-cis,trans)	6.31 11.0 11.1	10.	Isazofos	0.46 0.788 2.75 4.5 5.53 16.0
11.	Ametryn	6.23 5.21 34.0	12.	Isoxaben	0.225 0.0992 0.45 0.479 1.0 1.17
13.	Asulam	9.64	14.	Isoxaflutole	6.99 16.1
15.	Atrazine	10.9 12.6 28.8	16.	Lactofen	5.12 7.73 9.5 26.0
17.	Azinphos-methyl	0.493 6.12	18.	Lindane	1.45 4.05

		7.13 18.0			22.0 27.0
19.	Azoxystrobin	4.3 4.7 10.2 11.8	20.	Linuron	5.44 12.9 40.0
21.	Benfluralin	7.39 11.2 12.1 32.0	22.	Malaoxon	5.31 11.3 33.0
23.	Bensulide	0.37 0.469 0.472 0.49 0.56 0.62 0.649 0.659 0.69 0.7 0.783 0.91 1.0 1.08 1.4 1.57 2.45 12.0	24.	Malathion	0.0635 4.4 22.0 31.0
25.	Bentazone	47.0	26.	Mancozeb	1.5 6.51
27.	Bifenazate	0.9 11.0	28.	Maneb	0.11
29.	Bifenthrin	4.1 4.16 4.65 11.9 28.4	30.	MEHP (Phthalic acid, mono-2-ethylhexyl ester)	5.77 11.9 62.0
31.	Bisphenol A	3.53 14.0 20.5	32.	Metalaxyl	8.73 10.4 14.0
33.	Boscalid	1.17 5.67 10.5	34.	Metam-sodium hydrate	31.4
35.	Bromacil	6.29	36.	Methamidophos	21.0

		8.82			
37.	Buprofezin	3.8 5.44 5.5 10.8 11.9	38.	Methidathion	5.26 9.62 15.0
39.	Butachlor	1.1 1.3 1.95 8.35	40.	Methoxychlor	6.58 14.0 20.3
41.	Butafenacil	0.296 0.512 2.4 3.87	42.	Methoxyfenozide	0.729 2.4 5.9 5.99 6.02 7.2
43.	Butralin	0.951 2.16 4.31 5.15 7.7 36.1 47.0	44.	Methylene bis(thiocyanate)	2.2
45.	Cacodylic acid	15.0	46.	Metiram-zinc	3.3
47.	Captan	3.5	48.	Metolachlor	0.488 0.517 6.6 9.61 12.3
49.	Carbaryl	8.42	50.	Metribuzin	3.95
51.	Carboxin	5.35 8.35	52.	MGK	3.1 12.2 17.7 18.0
53.	Carfentrazone-ethyl	10.3 12.1 43.0	54.	Milbemectin (mixture)	0.442 0.447 6.4 9.6 29.2
55.	Chlorethoxyfos	8.24 8.49 10.3	56.	Molinate	6.28 62.0
57.	Chloroneb	0.542	58.	Myclobutanil	6.07 5.24

					5.93
59.	Chlorothalonil	0.39 0.22 17.7	60.	Napropamide	0.18 0.479 1.3 4.43 28.3
61.	Chlorpropham	9.29 13.7 27.7 45.0	62.	Nitrapyrin	0.424
63.	Chlorpyrifos oxon	5.21 7.71 19.0	64.	Norflurazon	4.37 5.42 12.2
65.	Chlorpyrifos-methyl	12.1	66.	Oryzalin	2.9 4.5 7.37 12.6 12.7
67.	Chlorsulfuron	1.69	68.	Oxadiazon	0.568 0.568 4.9 5.49 7.5
69.	Cinmethylin	6.6 7.65 12.8	70.	Oxasulfuron	5.87 8.47 10.7 20.0 20.8 29.2
71.	Clodinafop-propargyl	5.5 28.7	72.	Oxytetracycline dihydrate	5.35
73.	Clofentezine	11.8 5.17 5.0 10.1	74.	Paclobutrazol	4.3 7.6
75.	Clomazone	9.04 28.3 35.0	76.	Parathion	4.88 5.27 11.9 17.2 24.0
77.	Clopyralid-olamine	4.87 9.73	78.	Parathion-methyl	3.61 12.5
79.	Clothianidin	19.0	80.	Pendimethalin	15.0 18.5

					37.0
81.	Coumaphos	2.87 3.47 3.74 3.9 6.5	82.	Penoxsulam	6.61 11.0 11.7
83.	Cyanazine	5.87 5.94 6.79	84.	Permethrin	2.15 7.57 29.0
85.	Cyazofamid	9.4	86.	PFOA (Perfluorooctanoic acid)	2.37
87.	Cyclanilide	13.0	88.	PFOS (Perfluorooctane sulfonic acid)	28.8 28.9 38.0
89.	Cyfluthrin	12.3 19.8 25.0	90.	Phosalone	7.94 9.9 10.0 11.6
91.	Cypermethrin	4.44 10.0 11.2 12.7 18.3 4.44	92.	Piperonyl butoxide	0.95 2.23 2.97 9.62 15.0
93.	Cyproconazole	5.7 7.68 9.77 23.0	94.	Pirimicarb	6.5
95.	Cyprodinil	28.8 30.0 48.0	96.	Pirimiphos-methyl	5.24 9.4 10.6 12.0
97.	Cyromazine	5.06	98.	Prallethrin	3.56 3.6 6.53 14.6 20.0 25.0
99.	DBP (Dibutyl phthalate)	4.17	100.	Prochloraz	3.6 5.0 6.81
101	DEHP (Diethylhexyl phthalate)	20.8 38.0	102.	Prodiamine	0.494 5.65

				5.7 13.6
103	Diazinon	0.532 4.18 4.63 33.0	104.	Profenofos	5.0 6.73 8.97 27.0
105	Diazoxon	9.92 29.6	106.	Prometon	3.05 4.85 11.1
107	Dichlobenil	0.408 58.0	108.	Prometryn	13.0 25.0 36.0
109	Dichloran	6.07	110.	Prometryn	6.98 7.19
111	Diclofop-methyl	10.3	112.	Propanil	12.4
113	Diclosulam	5.97 8.6 34.0	114.	Propargite	1.59 20.0
115	Dicofol	5.87 6.9 13.6	116.	Propazine	5.66 7.11 7.8 34.0
117	Diethyltoluamide	4.62 11.1 12.4	118.	Propetamphos	1.03 3.17 4.91 7.1 19.1 46.0
119	Dimethenamid	1.42 5.32 6.85 6.19 39.0	120.	Propiconazole	0.533 2.96 14.0
121	Dimethomorph	3.36 3.62 0.503	122.	Propoxur	7.04 8.81
123	Diniconazole	0.513 0.612 1.3 2.63 9.61 20.0	124.	Propoxycarbazone-sodium	28.0

125	Disulfoton	14.8 16.8 26.7 38.0	126.	Propyzamide	4.01 4.59 5.36 6.34 8.7 9.19 23.9 24.1 39.0
127	Dithiopyr	0.31 1.29 2.3 2.62 30.9	128.	Pymetrozine	17.0
129	Diuron	5.27 9.37 58.0	130.	Pyraclostrobin	4.62 7.0
131	Emamectin benzoate	7.1	132.	Pyraflufen-ethyl	5.03 7.28 11.6 29.0 33.3
133	Endosulfan	5.15 6.45 8.57 10.8	134.	Pyrimethanil	6.48 9.18
135	Esfenvalerate	3.37 4.6 8.1 15.0 27.0	136.	Pyriproxyfen	8.01 12.2 27.8
137	Ethalfluralin	8.1 5.17 13.7 14.7 17.0	138.	Pyrithiobac-sodium	10.9 13.4
139	Ethametsulfuron methyl	0.461	140.	Quinoxyfen	9.68
141	Ethofumesate	7.0 10.1 17.1 37.0	142.	Quintozene	3.99
143	Ethoprop	4.43 7.0	144.	Resmethrin	4.78 6.6 23.0

145	Etoxazole	1.81 2.23 3.3 4.64 7.19 12.0	146.	Rimsulfuron	9.4
147	Fenamidone	47.0	148.	Rotenone	1.5 17.0
149	Fenamiphos	5.55 5.77 28.7 14.0	150.	S-Bioallethrin	3.6 9.7 8.75 13.3 19.2
151	Fenarimol	0.461 0.589 16.0 18.0 20.3 49.0	152.	Sethoxydim	5.59 27.8
153	Fenbuconazole	1.06 2.02 5.7	154.	Spirodiclofen	0.43 0.437 25.6
155	Fenhexamid	0.0165 1.9 24.0	156.	Spiroxamine	4.3 6.42 6.83
157	Fenitrothion	7.39 7.87 47.0	158.	Sulfentrazone	1.61 3.38 7.26 34.0
159	Fenoxaprop-ethyl	5.52	160.	TCMTB	1.6 39.0
161	Fenpropathrin	4.59 4.46 5.13 11.0 13.3	162.	Tebufenozide	0.972 5.17 6.75 21.3
163	Fenpyroximate (Z,E)	12.0	164.	Tebufenpyrad	6.05 24.6
165	Fenthion	4.25 14.7 39.0	166.	Tebupirimfos	0.446 0.558 1.46 3.2 13.0 35.0

167	Fentin	0.86 4.0	168.	Tefluthrin	4.5 10.5
169	Fipronil	0.568 0.757 28.0 12.6	170.	Tetraconazole	0.495 0.471 18.0 22.7 44.0
171	Fluazifop-butyl	12.0 21.0	172.	Tetramethrin	3.63 7.09 18.0 43.0
173	Fluazinam	0.475 0.26	174.	Thiacloprid	9.55
175	Fludioxonil	6.97 9.63 16.1 23.0	176.	Thiamethoxam	9.18
177	Flufenacet	7.59 13.0 14.0	178.	Thiazopyr	0.17 0.5 0.547 1.01 12.7
179	Flufenpyr-ethyl	6.69 7.86 9.98	180.	Thidiazuron	5.9
181	Flumetralin	0.51 1.35 1.8 3.44 4.3 5.6	182.	Thiobencarb	4.47 12.1 25.0 28.0
183	Flumetsulam	11.3 15.4 16.0	184.	Thiodicarb	1.1
185	Flumiclorac-pentyl	2.1 6.37	186.	Thiophanate-methyl	4.89 5.09 8.36 48.0
187	Flumioxazin	4.77	188.	Thiram	12.0
189	Fluometuron	10.2 0.42	190.	Tralkoxydim	5.84 5.97 14.6
191	Fluoxastrobin	0.27	192.	Tri-allate	5.61

		0.475 12.0			7.08 9.7 25.0
193	Flusilazole	0.62 36.0	194.	Triadimefon	5.82 5.67 7.6 26.0
195	Fluthiacet-methyl	2.43 4.79 5.32 53.0	196.	Triadimenol	0.408 2.39 3.62 4.4 7.33
197	Flutolanil	7.1 12.1 12.8 34.0	198.	Triasulfuron	4.41 5.91 22.0
199	Folpet	8.2	200.	Tribufos	0.523 11.1 12.5 23.0 40.0
201	Forchlorfenuron	28.2	202.	Triclosan	4.39 13.0
203	Fosthiazate	10.0 10.6 12.2 12.7 49.0	204.	Trifloxystrobin	11.8 12.6 60.0
205	Halosulfuron-methyl	4.54 7.12	206.	Trifloxysulfuron-sodium	7.44
207	Hexaconazole	3.37 5.33 31.2 49.0	208.	Triflumizole	0.46 0.534 0.764 32.0 38.0
209	Hexazinone	4.37 8.64	210.	Trifluralin	3.31 11.0 11.6 14.0 28.0
211	Hexythiazox	3.2 3.93 8.5 18.6	212.	Triflusulfuron-methyl	11.8 34.0

213 HPTE	13.0 23.0	214. Triticonazole	1.56 4.09 4.67 10.0
215 Icaridin	6.82	216. Vinclozolin	6.7
217 Imazalil	0.588 0.1 36.5	218. Zoxamide	2.2 4.87 7.55
219 Imazapic	1.13 51.0		

Chemicals are arranged in alphabetic order.

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ABSTRACTS

1. Data Mining For Pesticides And Environmental Chemicals Associated With NAFLD. **L. Al-Eryani**, B. Wahlang, H.B. Clair, J. J. Guardiola, K.C. Falkner, R.A. Prough and M. Cave-AASLD (2013).
2. Database Mining For Pregnane Xenobiotic Receptor (PXR) Ligands Using ToxCast Database And PXR Activation By Organochlorine Pesticides. **L. Al-**

Eryani, B. Wahlang, H.B. Clair, J. J. Guardiola, K.C. Falkner, R.A. Prough and M. Cave-AASLD (2013).

3. Identification Of Xenobiotic Receptor Agonists Which Could Contribute To Nonalcoholic Fatty Liver Disease – **L. Al-Eryani**, B. Wahlang, H.B. Clair, J. J. Guardiola, K.C. Falkner, R.A. Prough, J.C. States and M. Cave - Research Of Louisville, University Of Louisville (2013).
4. Hepatic Receptor Activation By Polychlorinated Biphenyls - Implications For Xenobiotic/Energy Metabolism And Nonalcoholic Fatty Liver Disease. B. Wahlang, K.C. Falkner, H.B. Clair, **L. Al-Eryani**, J.J. Guardiola, R.A. Prough, and M. Cave-AASLD (2013).
5. Identification Of Environmental Chemicals Which Could Contribute To Nonalcoholic Fatty Liver Disease By Nuclear Receptor Activation**. L. Al-Eryani**, B. Wahlang, K.C. Falkner, H.B. Clair, R.A. Prough, J.C. States and M. Cave- SOT (2014).
6. Aroclor 1260 Exposure Worsens Hepatic And Systemic Inflammation In An Animal Model Of Diet-Induced Obesity And Nonalcoholic Fatty Liver Disease. B. Wahlang, M. Song, J. Beier, **L. Al-Eryani**, H.B. Clair, J.J. Guardiola, K.C. Falkner, R.A. Prough, and M. Cave-AASLD (2013).

PUBLICATIONS

1. Human Receptor Activation By Aroclor 1260, A Polychlorinated Biphenyl Mixture. Banrida Wahlang, K. Cameron Falkner, Heather B. Clair, **Laila A. Al-Eryani**, Russell A. Prough, J. Christopher States, Denise M. Coslo, Curtis J. Omiecinski and Matt Cave.

2. Organochlorine Pesticide, Lead, and Mercury Exposures Are Associated with Liver Disease In The United States General Adult Population. Matt Cave, Savitri Appana, **Laila Al-Eryani**, Mihir Patel, Keith Cameron Falkner, Craig J. McClain, Guy Brock. (Under review for publication)

3. Identification Of Environmental Chemicals Associated With The Development Of Toxicant Associated Steatohepatitis In Rodents. **Laila Al-Eryani**, Banrida Wahlang, K.C. Falkner, J. J. Guardiola, H.B. Clair, R.A. Prough and M. Cave. (Under review for publication)

PROFESSIONAL MEMBERSHIPS/POSITIONS

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Member, Society of Toxicology (SOT)	2014- Present