Khuouloud Keshim

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T.C.
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DEPARTMENT OF PHYSIOLOGY

EVALUATION OF HEPATOTOXIC AND NEPHROTOXIC EFFECTS OF HEXACHLOROBENZENE, DDT AND P,P-DDE IN RATS

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KHUOLOUD KESHIM

SUPERVISOR
Prof. Dr. Bayram YILMAZ

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated ..... and numbered .....

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

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To my soulmate Tarek, I dedicate this research to the one who took my hand towards what I wanted and gave me back my confidence in my ability to advance, and for being the first to support me and encouragement me, to my dear husband I offer this effort.

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LIST OF SYMBOLS AND ABBREVIATIONS

AHR	Aryl hydrocarbon receptor
ALKP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
CHOL	Cholesterol
CREA	Creatinine
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyl trichloroethane
GLU	Glucose
HCB	Hexachlorobenzene
LDH	Lactate dehydrogenase
OCP	Organochlorine pesticide
PCB	Polychlorinated pesticide
PFOS	Perfluorooctanesulfonic Acid
POP	Persistent Organic Pollutants
TBIL	Bilirubin
TRIG	Triglycerides
WHO	World health Organization

ABSTRACT

Keshim, Khuoloud, 2022. Evaluation of Hepatotoxic and Nephrotoxic Effects of Hexachlorobenzene, DDT, and p,p-DDE in Rats. Yeditepe University Institute of Health Sciences, Department of Physiology, Master Thesis, İstanbul.

Persistent Organic Pollutants (POPs) pose a threat to the human health because of their long-lasting presence in the environment and bio-accumulative properties in organisms. There have been numerous reports on neurotoxic, carcinogenic, immunotoxin, hepatotoxic, nephrotoxic, cytotoxic, and endocrine disruptor effects of several persistent compounds. In the present study, three OCPs have been selected to be examined for their hepatotoxic and nephrotoxic effects in male rats. While the current literature reports on some indicators, no empirical findings were found for the effects of DDT and DDE on liver enzymes: ALT, AST and ALP. In the experiment, four groups of 8 adult male Sprague-Dawley rats were researched: Control, HCB (5 mg/ kg), p,p-DDE (5mg/ kg), and DDT (5 mg/ kg). Each of the experimental group rats received daily dosages for 18 days. Concentrations of liver and kidney indicators in serum were analyzed. The findings of the experiment shows that the majority of liver indicators: bilirubin (TBIL), cholesterol (CHOL), lactate dehydrogenase (LDH), triglycerides (TRIG), glucose (GLU), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were not significantly affected by the administration of OCP dosages among the DDE, DDT, and HCB groups based on the one-way ANOVA analysis at the 0.05 level. However, results from alkaline phosphatase (ALKP) indicated that its levels were significantly lower for the DDE and DDT group in comparison with the control group at the same significance level. The kidney indicators in the experiment: creatinine (CREA) and urea, were not significantly affected by the administration of OCP dosages among the DDE, DDT, and HCB groups based on the one-way ANOVA analysis at the 0.05 level. It is concluded that OCP dosage and length of exposure play major roles in toxicity levels.

Keywords: Persistent Organic Pollutants (POPs); Liver, Kidney

ABSTRACT (Turkish)

Keshim, Khuoloud, 2022. Sıçanlarda Hekzaklorobenzen, DDT ve p,p-DDE'nin Hepatotoksik ve Nefrotoksik Etkilerinin Değerlendirilmesi. Yeditepe Universitesi Sağlık Bilimleri Enstitüsü, Fizyoloji Anabilim Dalı, Yüksek Lisans Tezi, İSTANBUL.

Kalıcı Organik Kirleticiler (KOK'lar), çevrede uzun süre kalmaları ve organizmalarda biyo-birikim özellikleri nedeniyle insan sağlığı için bir tehdit oluşturmaktadır. Birkaç kalıcı bileşiğin nörotoksik, kanserojen, immünotoksin, hepatotoksik, nefrotoksik, sitotoksik ve endokrin bozucu etkileri hakkında çok sayıda rapor bulunmaktadır. Bu çalışmada, erkek sıçanlarda hepatotoksik ve nefrotoksik etkileri incelenmek üzere üç OCP seçilmiştir. Mevcut literatür bazı göstergeler hakkında rapor verirken, DDT ve DDE'nin karaciğer enzimleri: ALT, AST ve ALP üzerindeki etkilerine dair hiçbir ampirik bulguya rastlanmamıştır. Deneyde, 8 yetişkin erkek Sprague-Dawley sıçanından oluşan dört grup araştırıldı: Kontrol, HCB (5 mg/kg), p,p-DDE (5mg/kg) ve DDT (5 mg/kg). Deney grubu sıçanlarının her biri, 18 gün boyunca günlük dozlar aldı. Serumdaki karaciğer ve böbrek göstergelerinin konsantrasyonları analiz edildi. Deneyin bulguları, karaciğer göstergelerinin çoğunun bilirubin (TBIL), kolesterol (CHOL), laktat dehidrojenaz (LDH), trigliseritler (TRIG), glikoz (GLU), alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) olduğunu göstermektedir.), 0,05 düzeyinde tek yönlü ANOVA analizine dayalı olarak DDE, DDT ve HCB grupları arasında OCP dozajlarının uygulanmasından önemli ölçüde etkilenmemiştir. Bununla birlikte, alkalin fosfatazdan (ALKP) elde edilen sonuçlar, DDE ve DDT grubu için, aynı anlamlılık düzeyinde, kontrol grubu ile karşılaştırıldığında, seviyelerinin önemli ölçüde düşük olduğunu göstermiştir. Deneydeki böbrek göstergeleri: kreatinin (CREA) ve üre, 0,05 düzeyinde tek yönlü ANOVA analizine dayalı olarak DDE, DDT ve HCB grupları arasında OCP dozajlarının uygulanmasından önemli ölçüde etkilenmedi. OCP dozu ve maruz kalma süresinin toksisite seviyelerinde önemli rol oynadığı sonucuna varılmıştır.

Anahtar kelimler: Kalıcı Organik Kirleticiler (KOK); Karaciğer, Böbrek

1. INTRODUCTION AND PURPOSE

1.1 Subject overview

There are a variety of chemicals that are widely used in daily life, industry and agriculture that pollute the environment. Particularly, Persistent Organic Pollutants (POPs) pose a threat to the human health because of their long-lasting presence in the environment and bio-accumulative properties in organisms (1) (2). Dioxins, polychlorinated pesticides (PCBs) and organochlorine pesticides (OCPs) are in the POPs category. In addition to the POPs, metals, plasticizers, butylated hydroxyanisole, butylated hydroxytoluene, parabens and pharmaceutical agents are also among the environmental pollutants (1). There have been numerous reports on neurotoxic, carcinogenic, immunotoxin, hepatotoxic, nephrotoxic, cytotoxic, and endocrine disruptor effects of several persistent compounds such as PCBs and dioxins (1)(2). Endocrine disruptors are defined as exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior (1)(2). A wide range of substances, both natural and synthetic have been documented to cause endocrine disruption, including pharmaceuticals, dioxin and dioxin-like compounds, PCBs, OCPs and plasticizers (2) (3).

1.2 Study purpose and significance

In the present study, three OCPs have been selected to be examined for their hepatotoxic and nephrotoxic effects in male rats. Hexachlorobenzene (HCB) is an OCP that was widely used and polluted the environment (4). Although the use of HCB was discontinued in most countries, however, it is still released into the environment as a byproduct of several industrial applications and processes (5). HCB has still been detected in human biological samples along with PCBs (6). HCB is a toxic and persistent chemical that has been reported to cause neurological, developmental, endocrine and immunological toxicity in animals and humans (5). Animal studies have shown that HCB causes reproductive toxicity and increases the risk for cancer formation. The most notable

targets of its toxicity are the liver, ovary, and central nervous system. HCB shows dioxin-like property and is a weak ligand of AHR (7) (8). Thus, it has endocrine disrupting properties. Hepatotoxic effects of HCB have been reported (5). It has been reported that HCB induces liver foci growth in rats (9). High doses (100 mg/kg for 4 weeks) of HCB administration causes an imbalance between cell proliferation and cell death in the liver (10). HCB has been reported to have no effects on serum ALT and creatinine levels in rats following administration of this OCP through diet (200 ppm) for 8 weeks (11). In a recent study, it has been reported that HCB (16 mg/kg for 28 days, oral gavage) causes significant increases in serum alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels and oxidative stress in the liver tissue (12). HCB had no significant effects on these parameters at a lower dose of 4 mg/kg (28 days, oral gavage). In an epidemiological study conducted in Canada, plasma levels of POPs including HCB have been adjusted with creatinine levels in urine (13). Previously, no adverse effects of chronic HCB exposure on health have been observed in a general population from Spain (14).

DDT was introduced in the 1940s as an efficient insecticide to control transmission of diseases such as malaria. It was widely used as pesticide throughout the world (15). DDT was banned in the USA in the 1970s due to its adverse effects on the environment and human health (16). In the Stockholm Convention in 2001, DDT was banned in many countries. However, it is still recommended by the World Health Organization (17) against malaria vectors to be used at low concentrations. Thus, it is produced and used today (16) (18). According to the Federal Drug Administration (FDA) guidelines published in 2018, DDT and DDE levels in food and feed have been limited to 0.05–5 ppm depending on the commodity (19). Dichlorodiphenyldichloroethylene (DDE) is a common breakdown product of DDT formed by the loss of hydrogen chloride. Both DDT and DDE are POPs that bioaccumulate in the lipid-rich adipose tissue of humans (20) (21) (22). It has been reported that DDT and DDE are endocrine disruptors by acting as androgen receptor antagonist (23) (24) (25). Since these OCPs were widely used in the 20th century, they are still commonly detected in human and animal tissue samples (26) (27) (28). DDE is fat-soluble like other OCPs; therefore, it is rarely excreted from the body, and

concentrations tend to bio-accumulate throughout life. The major exception is the excretion of DDE in breast milk, which transfers a substantial portion of the mother's DDE burden to the young animal or child (29).

Accidental acute poisoning with DDT have been reported in two people with severe metabolic acidosis, acute oliguric renal failure and recurrent convulsions (30). In one of the patients, blood urea nitrogen and creatinine levels were 47 mg/dl and 6.4 mg/dl, respectively. High doses (50 and 100 mg/kg, intraperitoneal for 10 days) of DDT has been tested for nephrotoxicity in male rats (31). Their findings revealed an increase in serum creatinine and urea levels along with histological changes in renal tissue and tubular cells. Increasing global incidence of chronic kidney disease has been attributed to environmental pollution with POPs, especially with DDT. In a prospective human study in Sweden, 10-year follow-up showed that there was a significant decline in glomerular filtration rate (32). In the same study, serum levels of DDT and its metabolite were measured and correlated with renal functions.

It has been suggested that DDT produces toxic effects on liver in rats (33) (34). Hepatotoxic effects of this OCP have recently been reported using an in vitro model (35). Hepatic effects have been reported in 83 farm workers in Pakistan exposed to DDT (36). In another study from Ethiopia, hepatomegaly and ascites were observed in 32 cases, 78% of those were correlated with high levels (>125 ppb) of DDT (37). However, in a previous study conducted in northern Italy, no correlation was found between tissue levels of DDT and liver findings (38). There have been limited studies on the effects of DDT and DDE on liver enzymes, ALT, AST and ALP.

1.3 Aim and Originality

In the present study, hepatotoxic and nephrotoxic effects of HCB, DDT and p,p-DDE will be comparatively investigated in male rat model. In addition to histopathological analysis, effects of these OCPs on serum biochemical parameters of liver (ALT, AST and ALP) and kidney (creatinine and urea) will also be examined.

There have been relatively contradictory reports on hepatotoxic and nephrotoxic effects of HCB, DDT and p,p-DDE. In the present study, hepatotoxic and nephrotoxic effects of these OCPs will be examined in male adult rats following chronic exposure. Effects of these OCPs on serum biochemical parameters of liver (ALT, AST and ALP) and kidney (creatinine and urea) will also be examined.

With the result of this thesis, hepatotoxic and nephrotoxic effects of HCB, DDT and p,p-DDE will be clarified. Since environmental pollutants is a growing health problem worldwide, exposure to these persistent chemicals will need to be avoided. Food will need to be monitored in terms of chemical contamination with such POPs.

1.4 Thesis structure

The research is divided into five main chapters for the delivery of the study content:

- Introduction: a definition of the problem, research aim, and objectives.
- Literature review: general knowledge about POPs and their health implications. Moreover, the most relevant studies on the subject are reviewed for their methods and results.
- Materials and methods: the details of the experiment are specified in terms of animals, chemicals, and procedure.
- Results: the statistical analysis of the collected data are presented and differences between study groups are highlighted.
- Discussion and comparison: a comparison between the research results and the relevant literature, the final findings of the study, and insights for future research.

2. LITERATURE REVIEW

2.1. Persistent Organic Pollutants (POPs)

Persistent organic pollutants (POPs) are organic compounds that can cause pollution to different components of the ecosystem. They are persistent in different environments, stable, and have high toxicity (39). One of the main challenges with POPs is their accumulation into living bodies throughout the food cycle. The issue of POPs was addressed on the international level in 2001, when a treaty banning a group of compounds was signed. These are polychlorinated dibenzofurans (furans), dibenzodioxin (Dioxins), polychlorinatedbiphenyls (PCBs), mirextoxaphane, hexachlorobenzene, heptachlor, endrine, dieldrin, dichlorodiphenyl trichloroethane (DDT), chlordane, and aldrin. Later on, another group of POPs were also banned, including hexabromocyclododecane, endosulfans, perchlorooctasulphuricacid, tetrabromodiphenylether, pentachlorobenzene, n lindane, hexabromodiphenylether, hexacyclochlorohexane, and Chlordecone (40).

POPs were produced through artificial processes as pesticides to be used in agriculture, as well as using them in different industrial processes (41). A small group of POPs are accidentally produced in nature, such as dioxin, during combustion processes. However, the main use as pesticides is main source of POPs spread through the ecosystem, while their use as solvents in industrial processes is considered a secondary source. A minor group of POPs occur naturally, such as biosynthetic pathway and volcanoes (42).

The high fat solubility and halogenation of POPs are the main characteristics that contribute to their transportation and bioaccumulation in the environment. They accumulate in lipid tissues and continue to the process as they move upward in the food chain through long ranges of transport (43). As they move further into the chain, their concentrations become more dangerous and toxic, as well as making the determination of their original source more challenging to determine (44). As shown in Figure 2.1, POPs enter the environmental system in a gaseous form as they move to the atmosphere from water sources, vegetations, and soil. Prior to their deposit, they could travel long distances

due to their resistance to degradation (45), which makes their travel distances long and unpredictable (46). Once they enter the food chain, their accumulations can multiply through to metabolization in certain tissue types (47). Health risk assessments are carried out to understand exposures to POPs and their effects on humans. They are classified into dioxins, polychlorinated pesticides (PCBs), and organochlorine pesticides (OCPs) (48). Worldwide assessments still show that POPs of different types are still produced and accumulated imposing high health risks (49).

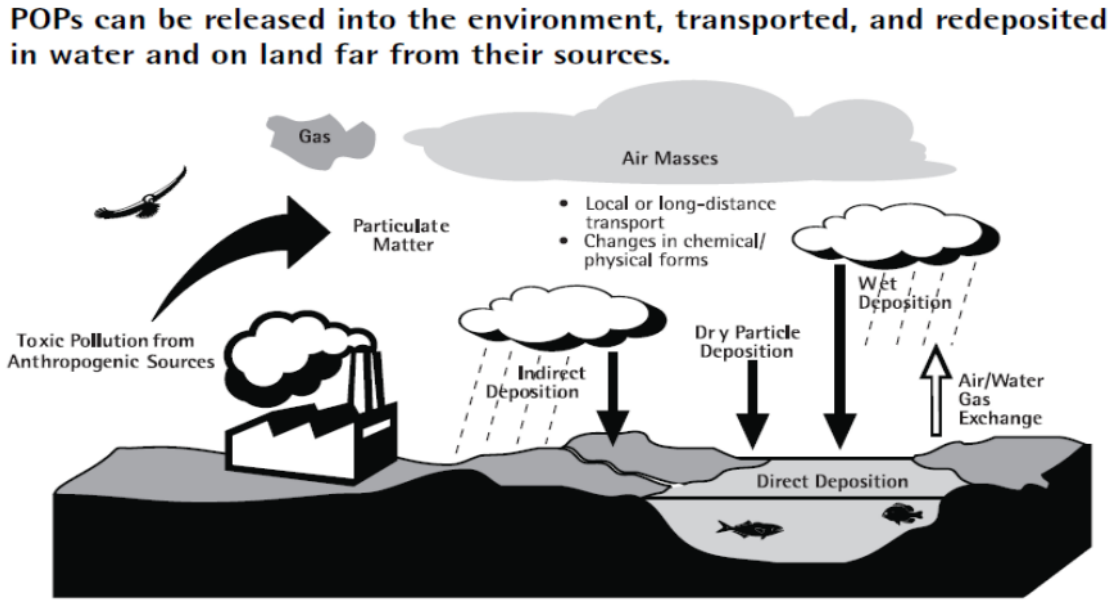


Figure 2. 1. Deposition and transportation of POPs in the environment

Organochlorine pesticides (OCPs) form a major type of POPs, which they were originally produced for agricultural purposes. When their toxic characteristics were discovered, great efforts went into phasing them out (50). Nonetheless, their high persistence in the environment formed a great challenge to these plans. The use of OCPs was popular due to their insecticidal potency. Their adverse effects were realized to include weakening the fertility of the soil through the disturbance of soil decomposers and organisms (51). The agricultural ecosystem was greatly affected as they decreased microbial biomasses, toxifying crops, and causing food chain contamination, as shown in Figure 2.2 (52).

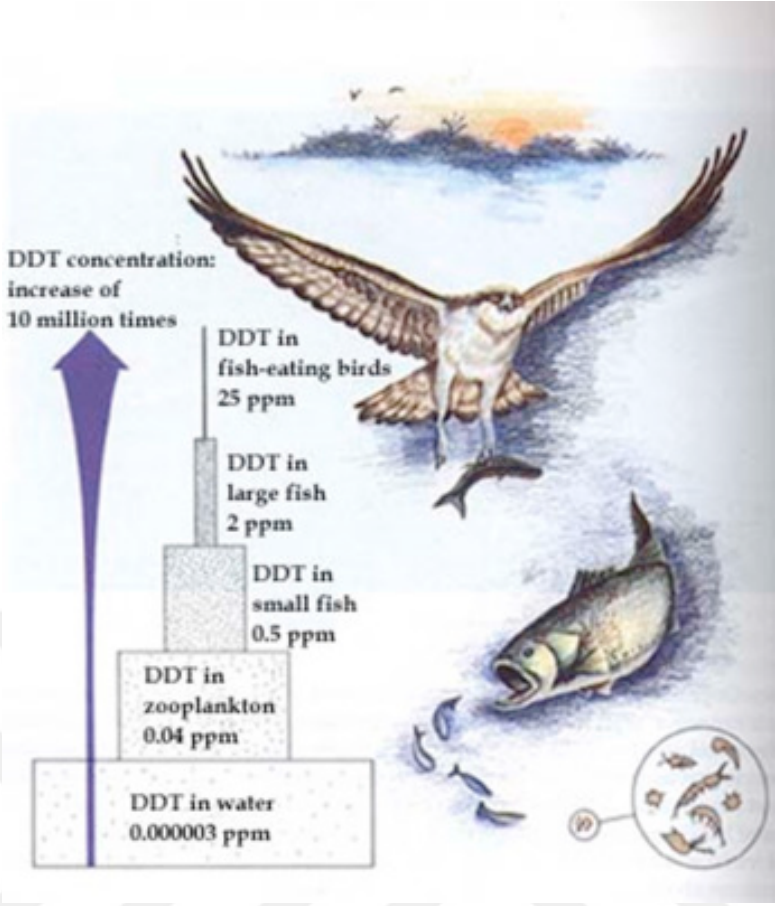


Figure 2. 2. Bioaccumulation of OCPs throughout the food chain

Water bodies form a major medium for the transportation and bioaccumulation of OCPs due to disposition from soil and the atmosphere, as shown in Figure 2.3. Industrial vessels dumping them into the water bodies, as well as agricultural activities, washing of equipment, industrial wastes and effluent releases make them as a collection basin for these compounds (53). Two processes are responsible for the OCPs' impact on the ecosystem: bioaccumulation and biomagnification. Bioaccumulation occurs through the metabolization and exertion of OCPs in organic tissue. Moreover, biomagnification is the movement of these compounds up the food chain through feeding and the increase in their concentrations upwards (54). The routes of production and human intake of OCPs is shown in Figure 2.4.

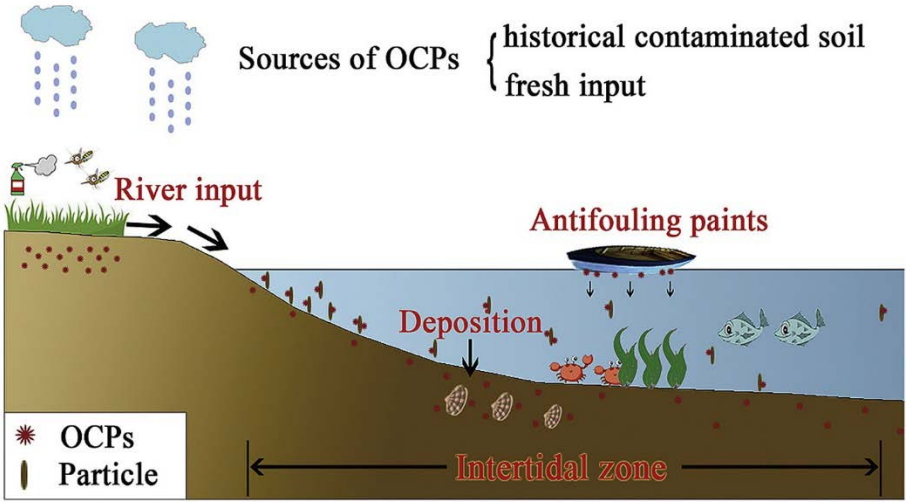


Figure 2. 3. Disposition of OCPs into water bodies

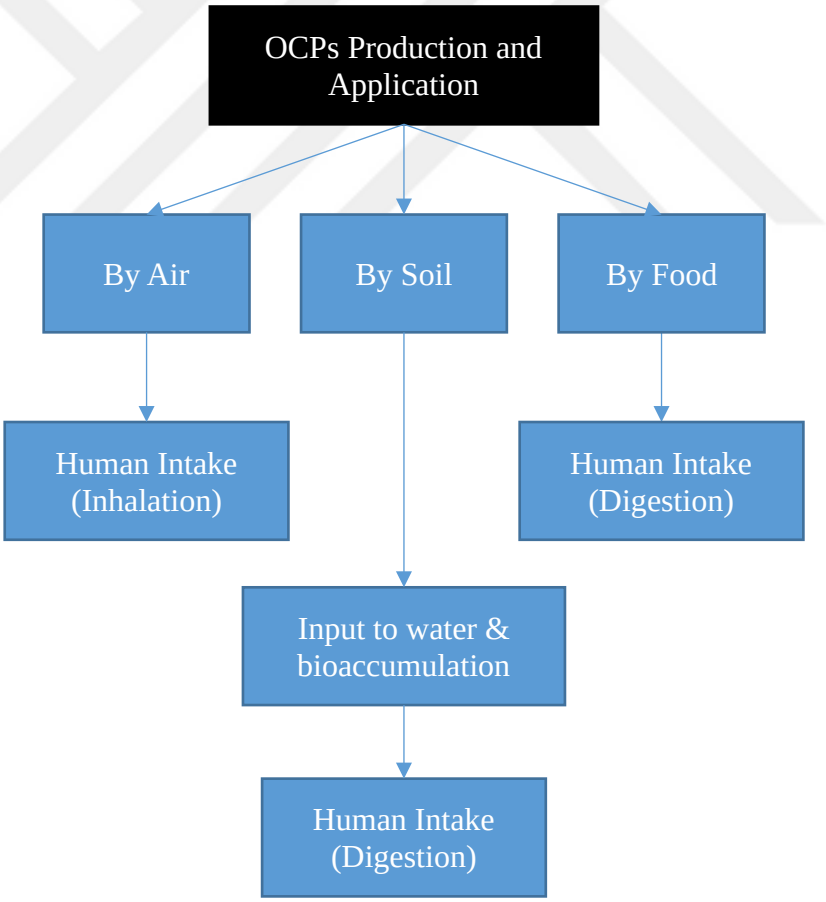


Figure 2. 4. Routes of human intake of OCPs

OCPs are classified into three main types of compounds: DDT analogs, Benzene Hexachloride (HCB), and cyclodiene compounds, including chlordane, heptachlor, aldrin, isodrin, dieldrin, endrin, thiodan, telodrin, and camphene (55). Figure 2.5 shows the classification of OCPs and their examples.

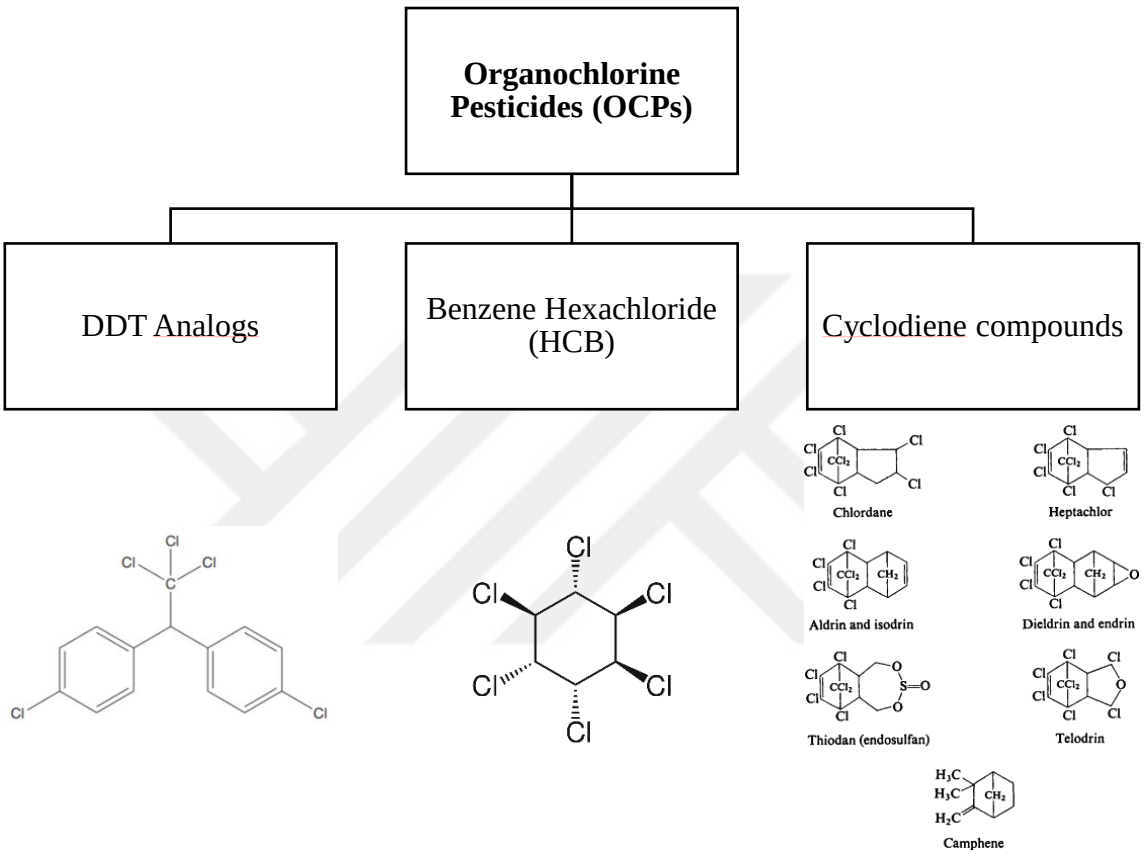
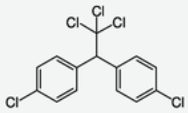
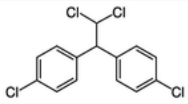
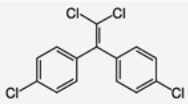
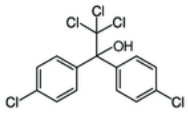
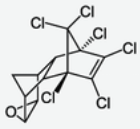
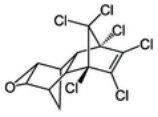
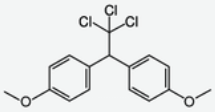
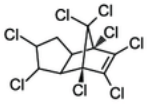
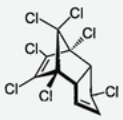


Figure 2. 5. Classification of OCPs

OCPs were developed for different agricultural applications. A compound like DDT was created as an acaricide, while it has high persistance and was classified as moderately hazersous by the World Health Organization (WHO). More hazerdous OCPs like Endrin with moderate persistance were also developed (56). More details on different OCP compounds are shown in Table 2.1.

Table 2. 1. Usage, toxicity, persistance, and WHO classification of most common OCPs

No.	Chemical name	Structure	Toxicity LD_{50}	Use	Persistence in environment	WHO classification based on rat oral LD_{50}
1	Dichlorodiphenyltrichloroethane (DDT) $C_{14}H_9Cl_5$		**Rat** Oral: 113–130 mg/kg Dermal: 2510 mg/kg **Mice** Oral: 150–300 mg/kg **Gunia Pigs** Oral: 300 mg/kg **Rabbit** Oral: 400 mg/kg	Acaricide Insecticide	High Persistence Half life: 2–15 years	Moderately hazardous
2	1,1-dichloro-2,2bis (p-chlorophenyl)ethane (DDD)		**Rat** Oral: 4000 mg/kg	Insecticide	High Persistence Half life: 5–10 years	Acute hazard is unlikely
3	Dichloro diphenyl dichloroethane (DDE)		**Rat** Oral: 800–1240 mg/kg	Insecticide	High Persistence Half life: 10 years	Slightly hazardous
4	Dicofol $C_{14}H_9Cl_5O$		**Rat** Oral: 684–1495 mg/kg **Rabbit** Oral: 1810 mg/kg Dermal: 2.1 g/kg	Acaricide	Moderate persistence Half life: 60 days	Moderately hazardous
5	Endrin $C_{12}H_8Cl_6O$		**Rat** Oral: 3 mg/kg Dermal: 15 mg/kg **Mouse** Oral: 1.37g/kg Intravenous: 2300 g/kg **Goat** Oral: 50 mg/kg **Rabbit** Oral: 60–94 mg/kg	Avicide insecticide	Moderate Persistence Half life: 1Day to 12 Years	Highly hazardous
6	Dieldrin $C_{12}H_8Cl_6O$		**Rat** Oral: 46 mg/kg Dermal: 50–120 mg/kg **Mouse** Oral: 38–77 mg/kg **Dog** Oral: 56–120 mg/kg **Rabbit** Oral: 45–50 mg/kg **Cow** Oral: 25 mg/kg **Duck** Oral: 381 mg/kg	Insecticide	High Persistence Half life: 9 months	Highly hazardous
7	Methoxychlor $C_{16}H_{15}Cl_3O_2$		**Rat** Oral: 5000–6000 mg/kg **Mice** Oral: 2000 mg/kg **Monkey** Oral: 2500 mg/kg	Insecticide	High Persistence Half life:< 120 Days	Acute hazard is unlikely
8	Chlordane $C_{10}H_6Cl_8$		**Rat** Oral: 200 to 700 mg/kg Dermal: 530–690 mg/kg **Mice** Oral: 145– 430 mg/kg Dermal: 153 mg/kg **Rabbit** Dermal: 780 mg/kg	Insecticide	High Persistence Half life: 10 years	Moderately hazardous
9	Heptachlor $C_{10}H_5Cl_7$		**Rat** Oral: 40– 220 mg/kg Dermal: 119–320 mg/kg **Mouse** Oral: 30–68 mg/kg **Guinea pigs** Oral: 116 mg/kg Dermal: 1000 mg/kg **Rabbit** Dermal: 2000 mg/kg	Insecticide	High Persistence Half life: 2 years	Highly – Moderately hazardous

2.2. Effects of POPs on Health

POPs differ in terms of human routes of exposure and effects on human health, which they range from cancer to skin damage, as shown in Table 2.2. More long-term effects, such as comptonization of the immune system and reproductive systems were also found (57).

Table 2. 2. POPs routes of exposure and effects on human health

POP	Classification	Route of Exposure	Heath Effect **Invalid source specified. Invalid source specified.**
Aldrin	OCP	Ingestion inhalation, Skin	Cancer
Chlordane	OCP	Ingestion Inhalation Skin	testicular cancer, changes in their liver function, respiratory infections ,immune system activation, anxiety, depression ,blurry vision
Dieldrin	OCP	Ingestion Inhalation Skin	endocrine disruption. testicular descent , Parkinson breast cancer, immune and reproductive system damage
Endrin	OCP	Ingestion Inhalation Skin	Convulsion, the central nervous system disorder;
Heptachlor	OCP	Ingestion (drinking water and food and breast milk) decrease	Type 1 diabetes
Hexachlorobenzene	OCP	Ingestion Inhalation Skin	liver diseases, skin lesion with discoloration, ulceration, photosensitivity, bone and thyroid effects
Mirex	OCP	Ingestion Inhalation Skin	liver problems, endocrine disruption and cancer
Toxaphane	OCP	Ingestion Inhalation Skin	Thyroid, liver and kidney

POP	Classification	Route of Exposure	Heath Effect **Invalid source specified. Invalid source specified.**
Polychlorinated Biphenyl	Industrial chemical	Ingestion	Skin damage (chloracne), liver damage, immune compromise and motor control problems
Dichlorodiphenyl Trichloro Ethane (DDT)	OCP	Ingestion Inhalation Skin	endocrine disrupting, carcinogenic disruption in human semen, quality menstruating gestational length and duration of lactation thyroid function in pregnancy and childhood
Polychlorinated Dibenzon Dioxins	Unintentional	Ingestion Inhalation Skin	Reproductive, developmental and immune system interference and cancer risks, skin disease (chloracne) liver damage, metabolism alterations, serum lipid level alterations, alteration of thyroid function, diabetes and immunological effects sperm count and motility damage
Polychlorinated Dibenzon Furans	Unintentional	Ingestion Inhalation Skin	carcinogenic, mutagenic and tetragenic

There are nine POP compounds that were classified internationally of being the most hazardous on human health, as well as the most persistent in the environment. These compounds were also called “nasty nine” (58). Table 2.3 provides more information about their routes of exposure and human health effects.

Table 2. 3. Routes of exposure and effects on human health of the “Nasty Nine”

POP	Classification	Route of Exposure	Heath Effect **Invalid source specified.**
Chlodecone	OCP	Ingestion Inhalation Skin	Severe convulsions resulting from degradation of synaptic junctions
Alpha Hexachloro Cyclohexane	OCP	Ingestion Inhalation Skin	Altered thyroid hormone, poor brain development
Hexabromodiphenyl ether	Flame redundant	Ingestion Inhalation Skin	Reproductive (infertility)and neurological problems mental and physical development disorder
Lindane	OCP	Ingestion Inhalation Skin	Skin irritation, seizures burning sensations itching ,dryness and rashes nervous carcinogenic system, liver and kidneys
Beta Hexachlorocyclohexan	OCP	Ingestion Inhalation Skin	Poor brain development human immune system damage
Pentachlorobenzane	Chlorinated aromatic hydrocarbons	Ingestion Inhalation Skin	
Perfluorooctanesulfonic Acid (PFOS)	Anthropogenic fluoro surfactant	Ingestion Inhalation Skin	Immune system cancer, stunted growth, Preeclampsia in pregnant women. Thyroid hormone disorder
Endosulfan	OCP	Ingestion Inhalation Skin	Development defects as sexual maturity delay in boys and sex hormones interference. endocrine disruptor death in humans
Hexabromododecane	Flame redundant	Ingestion Inhalation Skin	Liver and brain damage

2.3. Empirical Studies

In studies no HCB toxicity, ATSDR (5) and Ou (9) reported hepatotoxic effects of through inducing liver foci growth in rats. Giribaldi et al. (10) administrated high HCB doses (100 mg/kg for 4 weeks). The results show that the experimental group reported an imbalance between cell proliferation and cell death in the liver. Furthermore, findings from the epidemiological study of Drysdale et al. (13) showed that plasma levels of POPs including HCB have been adjusted with creatinine levels in urine.

In the same context, other studies showed no significant hepatotoxic effects of HCB. Chalouati et al. (12) administrated HCB to rats (16 mg/kg for 28 days, oral gavage), which caused an increase in serum alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels and oxidative stress in the liver tissue. Moreover, HCB had no significant effects on these parameters at a lower dose of 4 mg/kg (28 days, oral gavage). Sala et al. (14) concluded that chronic HCB exposure did not have any adverse effects in human subjects. Findings from Grassi et al. (11) showed that the administration of HCB with the diets of rat (200 ppm for 8 weeks) showed no effect on serum ALT and creatinine levels.

Hepatotoxicity was also tested for DDT and DDE exposure in the literature. The conclusions of Tomiyama et al. (33) and Harada et al. (34) show that DDT produces toxic effects on liver in rats. An in-vitro model tested by Sun et al. (35) showed Hepatotoxic effects of DDT. Azmi et al. (36) reported Hepatic effect due to DDT exposure in 83 farm workers. Similar findings were reported by Robinson et al. (37), who found that high levels of DDT exposure (>125 ppb) at 78% correlated to hepatomegaly and ascites. Nonetheless, Gallelli et al. (38) found no correlation between DDT exposure and liver indicators.

For DDT and DDE, Kelce et al. (23), Hoffmann & Kloas (24), and Migliaccio et al. (25) were empirical studies that confirmed that those compounds are endocrine disruptors by acting as androgen receptor antagonist. Ozucelik et al. (30) reported two people with severe metabolic acidosis, acute oliguric renal failure, and recurrent convulsion as a result of acute poisoning with DDT. In one of the patients, blood urea nitrogen and creatinine levels were 47 mg/dl and 6.4 mg/dl, respectively. Marouani et al. (31) reported nephrotoxicity in male rats following the administration of high DDT does

(50 and 100 mg/kg, intraperitoneal for 10 days). The results of the study showed an increase in serum creatinine and urea levels along with histological changes in renal tissue and tubular cells. Furthermore, Jayasinghe et al. (32) presented results that indicated a significant decline in glomerular filtration rate through a long-term follow up in human subjects, while serum levels of DDT and its metabolite were measured and correlated with renal functions.

In the literature, no empirical findings were found for the effects of DDT and DDE on liver enzymes: ALT, AST and ALP.

3. MATERIALS AND METHODS

3.1 Animals

Adult male Sprague-Dawley rat model is used. They are allocated in four groups (n=8 rats / group). HCB, DDT and p,p-DDE are dissolved in corn oil and administered by oral gavage to the animals for a period of five weeks at 5 mg/kg dose. Oral gavage administrations are applied on every other day, and each animal have received 18 doses of corresponding organochlorine pesticide by the end of the experiments. Rats in the control group are given the vehicle, corn oil alone. Experiments is terminated 24h after the last OCP administration. The study groups are:

1) Control (Vehicle, corn oil)
2) HCB (5 mg/kg)
3) p,p-DDE (5 mg/kg)
4) DDT (5 mg/kg)

3.2 Chemicals

HCB, DDT and p,p-DDE are obtained from international chemical suppliers. Kits for blood liver and kidney biochemical parameters are purchased. Basic laboratory consumables and chemicals are also commercially obtained. Staining of liver and kidney tissues are processed at the Histology & Embryology laboratory and all necessary consumables, chemicals and re-agents are commercially obtained.

3.3 Preparation and Setup of System

Biochemical analyzer, Electronic scale, Surgical set, Mixer, Heater, Centrifuge, Precision Scale, Micropipettes, Homogenizer, pH meter, Microtome, Microscopy, Tissue Processing Machine, Staining Machine, Refrigerator, -80 Freezer.

3.4 The Experiment

Samples & parameters:

Body weight of all animals will be determined before and during the study. At the end of the study, blood samples will be collected, and serum will be separated.

Liver parameters:

Liver enzymes: Alanine Aminotransferase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (AKLP) .

Renal parameters:

Creatinine and Urea

Histology:

Liver and kidney samples will be obtained and embedded in parafin solution. They will be processed and examined for histological evaluation.

3.5 Statistics

The data from the four study groups are entered into GraphPad Prism (version 9) for data analysis. One-way ANOVA is used to compare the results based on the significant differences between their means at a minimum of $p < 0.05$ significance level.

3.6. Tissue Processing and Morphological Analysis

At the end of the experimental procedure, decapitation was performed for liver and kidney tissue isolation from the experimental and control group. Liver and kidney were trimmed for paraffin tissue processing. The histological sections from liver was processed by Hematoxylin & Eosin stain (H&E) and kidney was processed by Periodic acid–Schiff (PAS) stain.

The fixative was thoroughly removed from the tissues pieces. After removing the fixative, the tissues were processed through graded alcohols, cleared in xylene, and

embedded in paraffin. 5 μm sections are taken from the paraffin blocks on the microtome (Leica, #RM2245). Sections were incubated at 60°C for 1 hour to remove paraffin and 2x20 min placed in xylene for complete removal of paraffin. It was incubated in the 100%, 90%, 80% and 70% alcohol series for 10 minutes, respectively. The sections were washed with distilled water and nucleus was stained with Hematoxylin (Bio Optica, Gill3 #05-06015). Afterwards, sections washed with tap water and stained with eosin histological staining (Sigma, #HT110132). All the sections were washed with distilled water and then sections were incubated 70%, 80%, 90% and 100% ethanol for a few seconds in each. Then, sections were placed 30 min in xylene. The slides were covered with coverslip using xylene-based mounting medium (Bio Optica, #05-BMHM508). Imaging was performed with a Leica DM 6000.

Morphological Results

Liver and kidney morphology between control, DDE, DDT and HCB groups were analyzed under a light microscope by staining with Hematoxylin & Eosin stain (H&E) and Periodic acid–Schiff (PAS) stain, respectively.

The control group of mice showed a normal liver architecture with sinusoidal cords of hepatocytes and central vein in the centre of the hepatic lobule. The cells of the liver are arranged like the spokes of a wheel to form sheets. Portal triad arrangement was normal (Figure 1A and B). DDE (Figure 1 C and D), DDT (Figure 1 E and F) and HCB (Figure 1 G and H) groups showed very low damage with sinusoidal cords of hepatocytes.

The control group of mice showed a normal kidney architecture with normal renal tubules, glomerulus and capsular space (Figure 2A and B). DDE group showing normal kidney architechure (Figure 2C and D), mice of DDT (Figure 2E and F) and HCB (Figure 2G and H) group showing deformed kidney glomerulus and capsular space and tubular arrangement.

4. RESULTS

The study groups were compared based on their mean creatinine concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.1 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

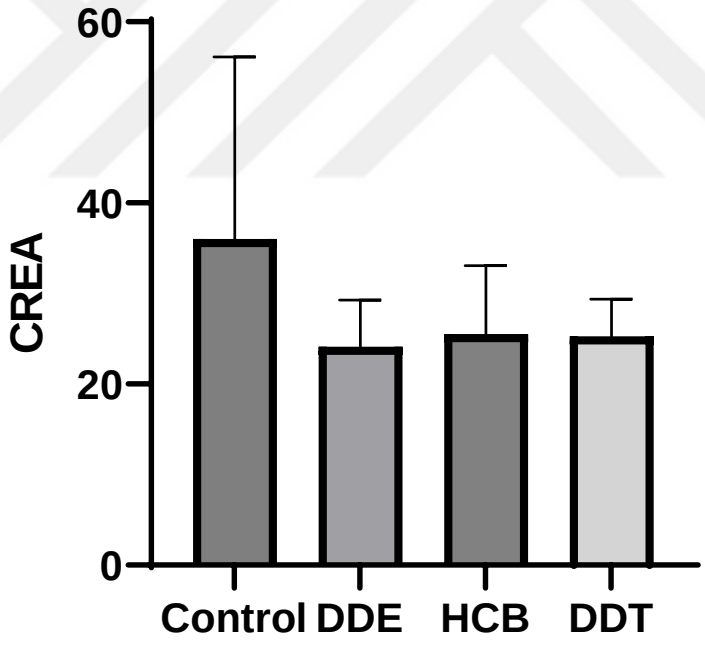


Figure 4. 1. One-way ANOVA comparison between study groups for creatinine concentration in serum

The study groups were compared based on their mean bilirubin concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.2 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

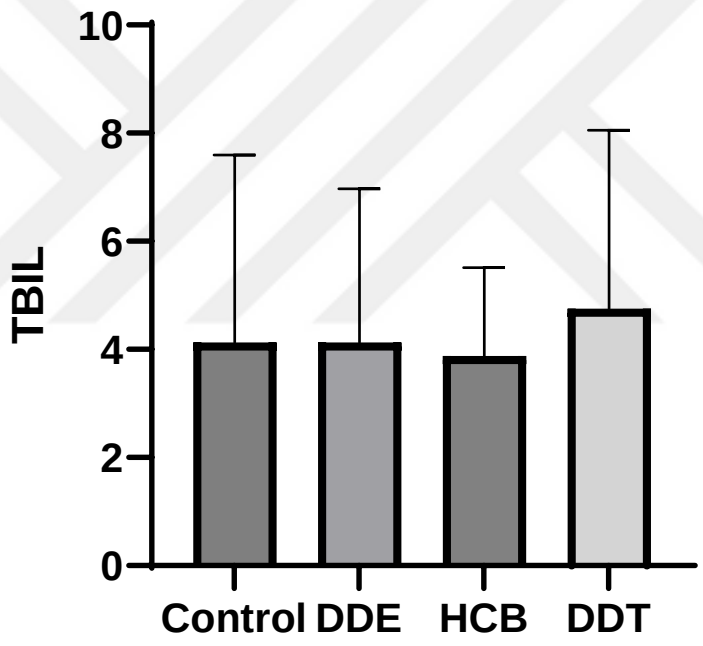


Figure 4. 2. One-way ANOVA comparison between study groups for bilirubin concentration in serum

The study groups were compared based on their mean cholesterol concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.3 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

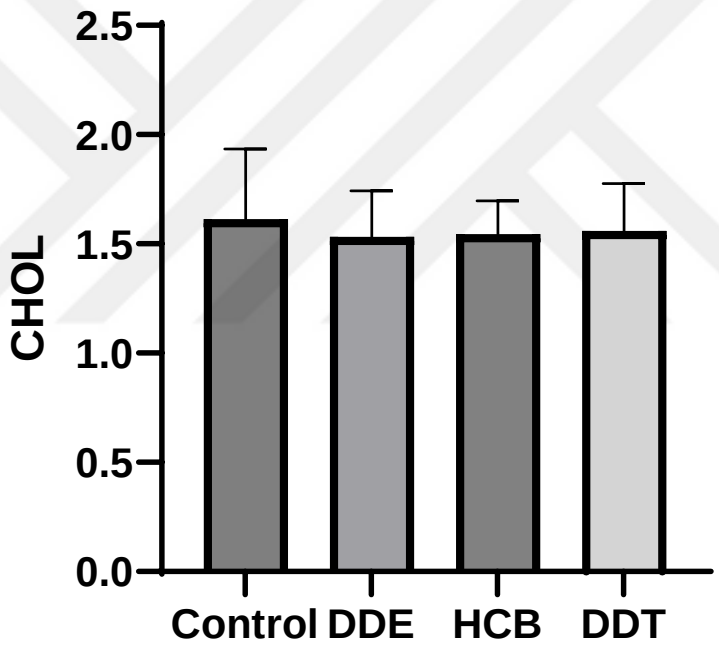


Figure 4. 3. One-way ANOVA comparison between study groups for cholesterol concentration in serum

The study groups were compared based on their mean lactate dehydrogenase (LDH) concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.4 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

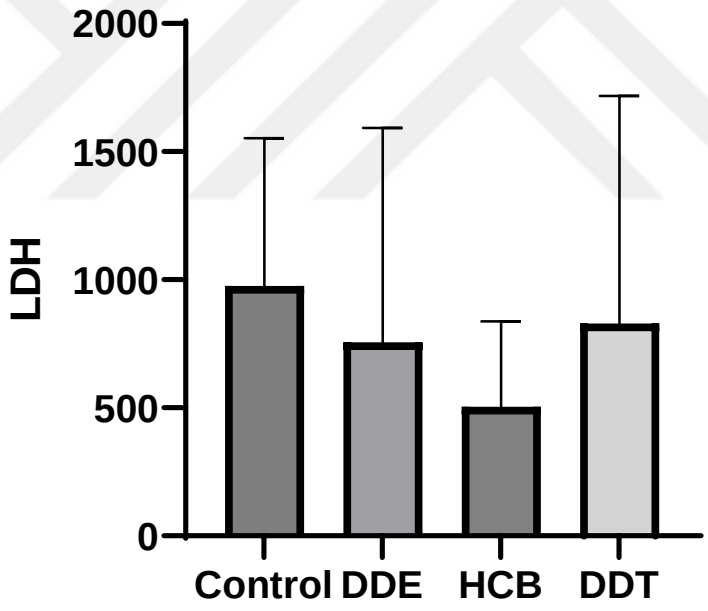


Figure 4. 4. One-way ANOVA comparison between study groups for lactate dehydrogenase (LDH) concentration in serum

The study groups were compared based on their mean triglycerides (TRIG) concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.5 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

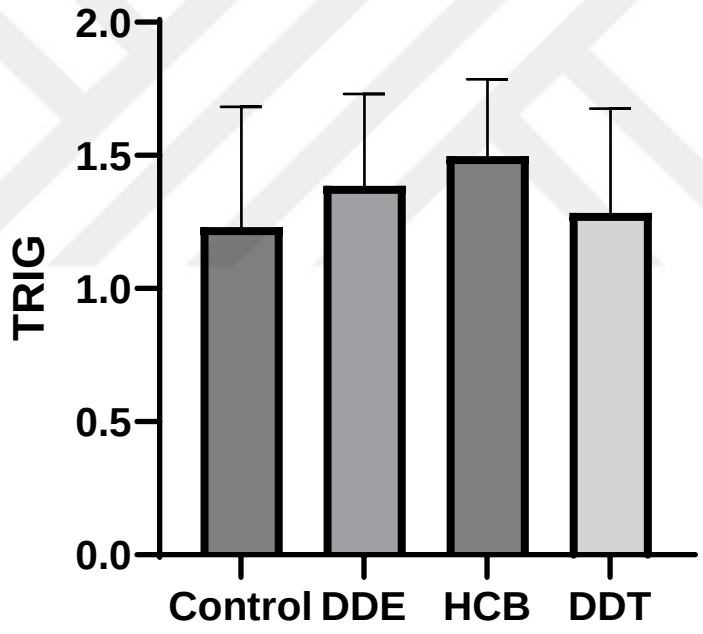


Figure 4. 5. One-way ANOVA comparison between study groups for triglycerides (TRIG) concentration in serum

The study groups were compared based on their mean glucose concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.6 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

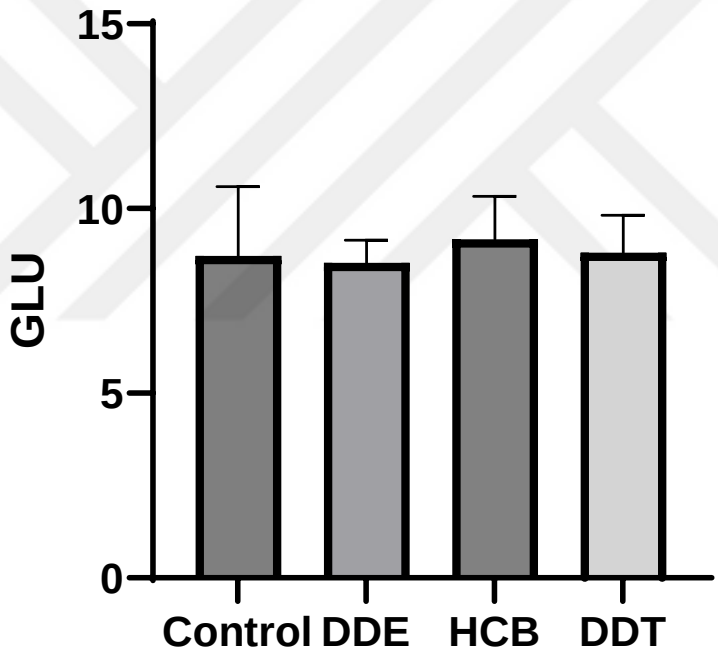


Figure 4. 6. One-way ANOVA comparison between study groups for glucose concentration in serum

The study groups were compared based on their mean alanine aminotransferase (ALT) concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.7 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

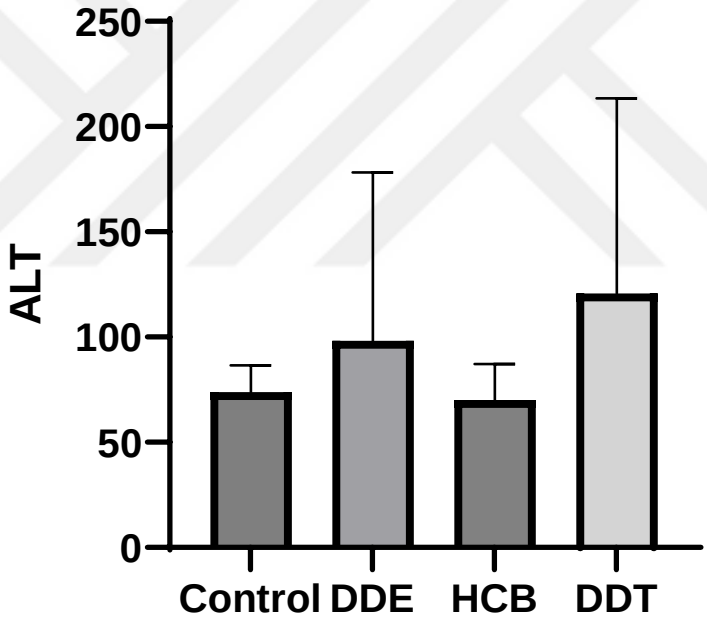


Figure 4. 7. One-way ANOVA comparison between study groups for alanine aminotransferase (ALT) concentration in serum

The study groups were compared based on their mean aspartate aminotransferase (AST) concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.8 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

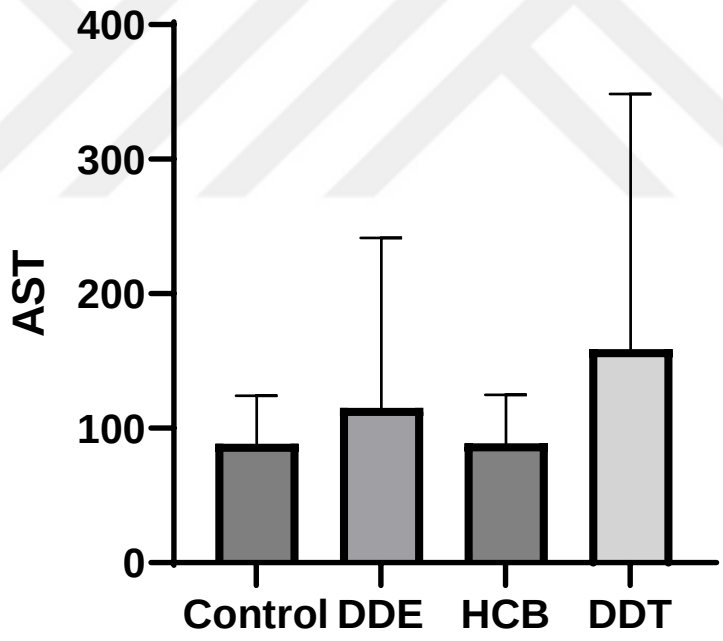


Figure 4. 8. One-way ANOVA comparison between study groups for aspartate aminotransferase (AST) concentration in serum

The study groups were compared based on their mean alkaline phosphatase (ALKP) concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.9 indicates that there are significant differences at the 0.05 level between the control group and each of the DDE and DDT groups.

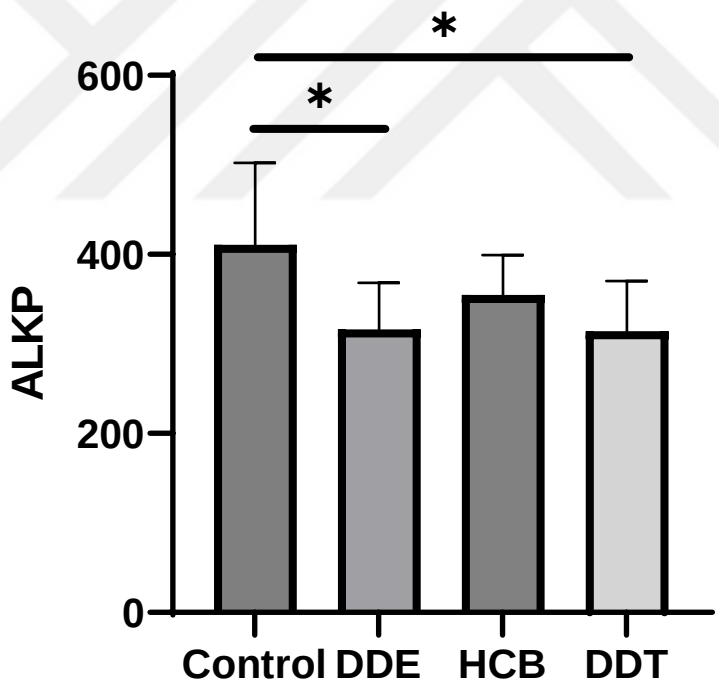


Figure 4. 9. One-way ANOVA comparison between study groups for alkaline phosphatase (ALKP) concentration in serum

The study groups were compared based on their mean urea concentrations in the collected serum samples. The one-way ANOVA analysis shown in Figure 4.10 indicates that there are no significant differences between the four study groups, where $p < 0.05$.

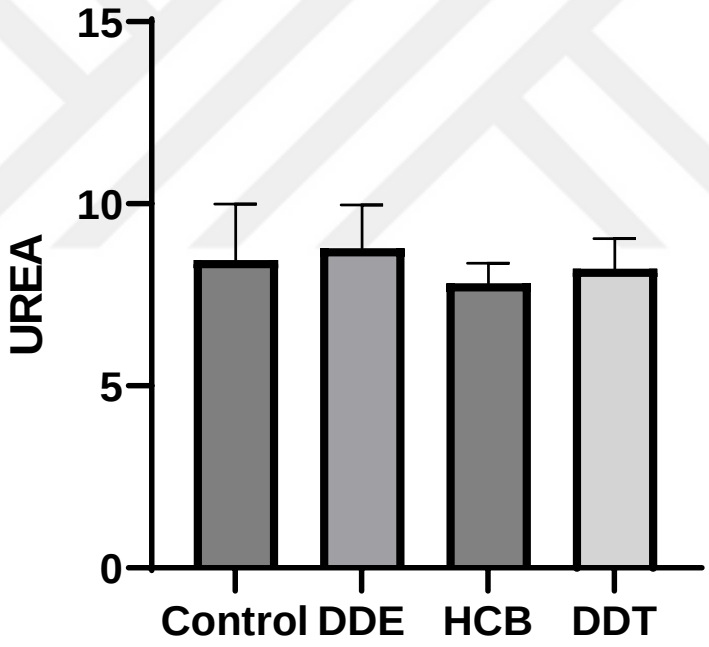


Figure 4. 10. One-way ANOVA comparison between study groups for serum urea concentration

Liver and kidney morphology between control, DDE, DDT and HCB groups were analyzed under a light microscope by staining with Hematoxylin & Eosin stain (H&E) and Periodic acid–Schiff (PAS) stain, respectively. The control group of rats showed a normal liver architecture with sinusoidal cords of hepatocytes and central vein in the center of the hepatic lobule. The cells of the liver are arranged like the spokes of a wheel to form sheets. Portal triad arrangement was normal (Figure 4.11A and B). DDE (Figure 4.11 C and D), DDT (Figure 4.11 E and F) and HCB (Figure 4.11 G and H) groups showed very low damage with sinusoidal cords of hepatocytes.

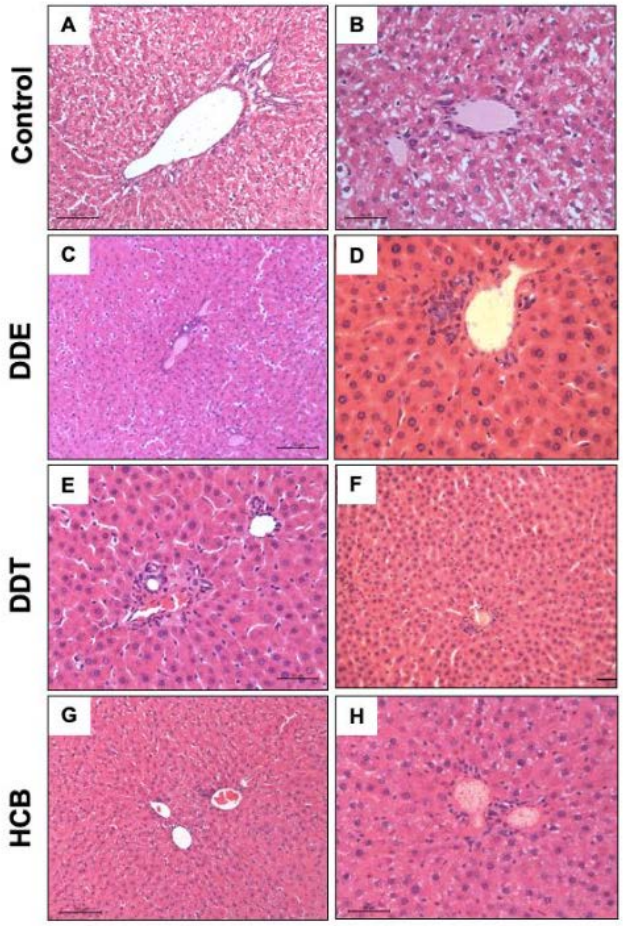


Figure 4. 11. Hematoxylin & Eosin (H&E) staining of liver sections

Histological changes in liver tissues of rats (A-B) control group showing normal liver architecture, (C-D) rats of DDE group, (E-F) DDT group and (G-H) HCB group liver tissues showing very low damage. (A-C-E-G, 10x magnification; B-D-F-H, 40x magnification).

The control group of mice showed a normal kidney architecture with normal renal tubules, glomerulus, and capsular space (Figure 4.12A and B). DDE group showing normal kidney architecture (Figure 4.12C and D), mice of DDT (Figure 4.12E and F) and HCB (Figure 4.12G and H) group showing deformed kidney glomerulus and capsular space and tubular arrangement.

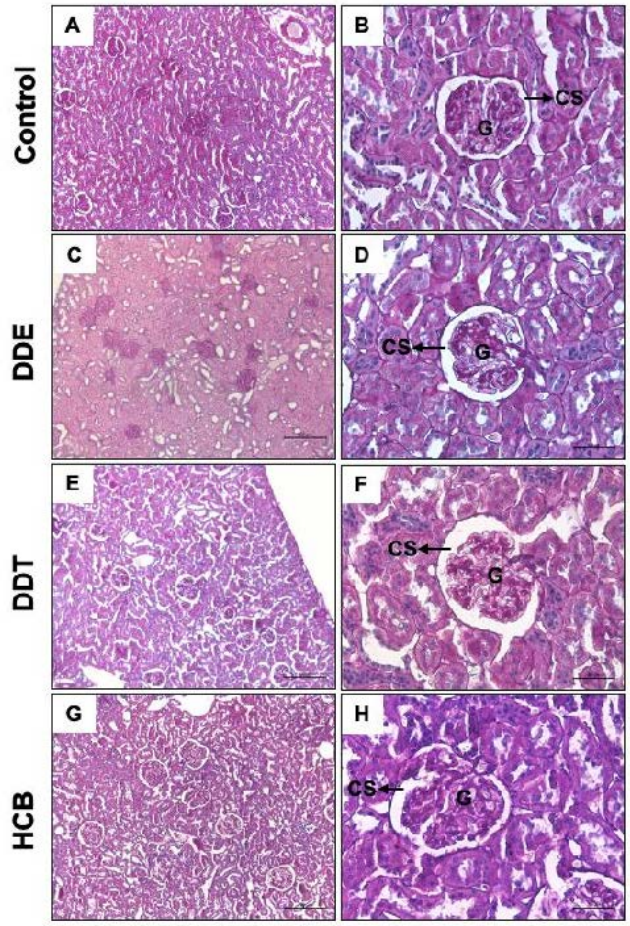


Figure 4. 12. Periodic acid-Schiff (PAS) staining of kidney sections

Histological changes in kidney tissues of rats (A-B) control, (C-D) DDE, (E-F) DDT and (G-H) HCB. Control and DDE group showing normal kidney architecture, rats of DDT and HCB group showing deformed kidney glomerulus and capsular space and tubular arrangement. G-Glomerulus, CS-Capsular space. (A-C-E-G, 10x magnification; B-D-F-H, 40x magnification).

5. DISCUSSION AND CONCLUSION

The findings of the experiment shows that the majority of liver indicators: bilirubin (TBIL), cholesterol (CHOL), lactate dehydrogenase (LDH), triglycerides (TRIG), glucose (GLU), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were not significantly affected by the administration of OCP dosages among the DDE, DDT, and HCB groups based on the one-way ANOVA analysis at the 0.05 level. However, results from alkaline phosphatase (ALKP) indicated that its levels were significantly lower for the DDE and DDT group in comparison with the control group at the same significance level.

There results mainly confirm the hepatic results of Chalouati et al. (12), Sala et al. (14), and Grassi et al. (11) who showed no significant hepatotoxic effects of HCB. Nevertheless, the results of the HCB group contradict the hepatotoxic presented in Ou (9), Giribaldi et al. (10), and Drysdale et al. (13). Additionally, results in the DDE and DDT groups contradicts the findings of Tomiyama et al. (33), Harada et al. (34), Azmi et al. (36), Robinson et al. (37), and Gallelli et al. (38) on most of the liver indicators. The results on alkaline phosphatase (ALKP) confirm that there is a certain extent of hepatotoxicity with DDT and DDE administration.

The kidney indicators in the experiment: creatinine (CREA) and urea, were not significantly affected by the administration of OCP dosages among the DDE, DDT, and HCB groups based on the one-way ANOVA analysis at the 0.05 level. While there were no clear results from the literature on HCB exposure, the results from the DDE and DDT groups contradict the findings of Kelce et al. (23), Hoffmann & Kloas (24), Migliaccio et al. (25) Marouani et al. (31), and Jayasinghe et al. (32), who found different indications of kidney failures.

Two main factors can explain the results of the current research. The first factor is the dosage of OCPs administrated to the rats (5 mg/kg), which is considered low in comparison with other studies that administrated high dosage and found toxic effects (50 to 100 mg/ kg). Moreover, the length of exposure is the second factor as most of the

studies that found toxic effects reported subjects that had long-term exposure to those compounds. In future research, a study can be conducted to understand the effects of these two factors on liver and kidney indicators through increasing the range of experimental groups.

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APPENDIX 1. Curriculum Vitae

Personal Informations

Name		**Surname**	
Place of Birth		**Date of Birth**	
Nationality		**TR ID Number**	
E-mail		**Phone number**	

Education

Degree	**Department**	**The name of the Institution Graduated From**	**Graduation year**
Master	Department of Physiology	**Yeditepe University**	2022
University			
High school			

Languages	**Grades**

Work Experience

Position	**Institute**	**Duration (Year - Year)**

Computer Skills

Program	**Level**

APPENDIX 2. Ethical Committee Approval