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Study of the sub-chronic toxicity of bisphenol A in Wistar females.

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List of abbreviations

AHR	Aryl hydrocarbon receptor
AR	Androgenic receptor
ALP	Alkalin phosphatase
AKT	Protein kinase (PKB)
AOPP	Advence oxidative proteins products
BPA	Bisphenol A
CK-MB	Creatine Kinase
DDT	Dichlorodiphenyltrichloroethane
DES	Diethylstilbestrol
EDCs	Endocrine disruptors chemical
ER	Estrogenic receptor
ERK	Extracellular-regulated kinase
ERE	Estrogen response element
FSH	Follicle-stimulating hormone
GSH	Glutathione
GSH-PX	Glutathione peroxidase
Her	Hormone reponse elements
hERR	Hormone estrogen -related receptor
hPPAR	Hormone peroxisome proliferator activated receptor
HCB	Hexachlorobenzene
IGF-1	Insulin growth factor 1

IGF-2	Insulin growth factor 2
IUPAC	International Union of Pure and Applied Chemistry
GBE	Ginkgo biloba extract
GnRH	Gonadotropin-releasing hormone
GST	Glutathione S transferases
KCL	Chlorure de potassium
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
MDA	Malondialdehyde
mER	Membrane-associated estrogen receptor
MEHP	Mono-2-ethylhexyl phthalate
MT1	Melatonin receptor 1
OC	Osteocalcin
OCPs	Organochlorine pesticides
PBDE-99	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyl
PC	Plasma cell
PE	Phytoestrogens
PPAR	Peroxisome proliferator activated receptor
PR	Progesterone receptor
PSA	Prostate-specific antigen
PVC	Polyvinyl chloride
RBC	Red blood cell

ROS	Reactive oxygen species
RXRs	Retinoid X receptor
SHBG	Stimulating hormone-binding globulin
SGOT	Serum glutamic-oxaloacetic transaminase
TH	Thyroid hormone
T3	Triiodothyronine
T4	Thyroxine
TPO	Thyroperoxidase
WWTP	Waste water treatment plant

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Abstract

Recent studies have indicated that a number of ubiquitous chemicals found in the environment are linked to a myriad of health concerns, including reproductive abnormalities and increases in infertility via their actions as exogenous hormones. Bisphenol A is probably one of the most prevalent synthetics xenoestrogens in the environment. This study aimed to assess the subchronic toxicity of bisphenol A (BPA) in female rats. Female rats were subjected over a 25 days, intraperitoneally to BPA at 50 μg/kg/day and 100 mg/kg/day and orally to filtered water collected from Guelma WWTP. Various parameters were evaluated to understand the potential adverse consequences. Results of oxidative stress cellular biomarkers revealed significant physiological alterations in female rats exposed to the highest dose of BPA. The results showed a significant decrease in GSH and GSH-Px levels and an increase in AOPP and MDA levels in the three organs (liver, kidneys, and ovaries) with a noticeable hepatic and ovarian damage confirmed by the histopathological analysis. The observed subchronic toxicity suggests that BPA exposure can have detrimental effects on female rats' reproductive and general health va oxidative stress mechanism. These findings contribute to our understanding of the potential risks associated with BPA exposure and highlight the need for further research and preventive measures to minimize human exposure.

Keywords: endocrine disruptor, bisphenol A, subchronic toxicity, WWTP, oxidative stress, liver, kidney, ovary.

Résumé

Des études récentes ont indiqué qu'un certain nombre de produits chimiques omniprésents trouvés dans l'environnement sont liés à une myriade de problèmes de santé, y compris des anomalies de la reproduction et une augmentation de l'infertilité via leurs actions en tant qu'hormones exogènes. Le bisphénol A est probablement l'un des xénoestrogènes synthétiques les plus répandus dans l'environnement. Cette étude visait à évaluer la toxicité subchronique du bisphénol A (BPA) chez les rats femelles. Des rats femelles ont été soumis pendant 25 jours, par voie intrapéritonéale à du BPA à 50 µg/kg/jour et 100 mg/kg/jour et par voie orale à de l'eau filtrée collectée à la station d'épuration de Guelma. Divers paramètres ont été évalués pour comprendre les conséquences néfastes potentielles. Les résultats des biomarqueurs cellulaires du stress oxydatif ont révélé des altérations physiologiques significatives chez les rats femelles exposés à la dose la plus élevée de BPA. Les résultats ont montré une diminution significative des taux de GSH et de GSH-Px et une augmentation des taux d'AOPP et de MDA dans les trois organes (foie, reins et ovaires) avec une atteinte hépatique et ovarienne notable confirmée par l'analyse histopathologique. La toxicité subchronique observée suggère que l'exposition au BPA peut avoir des effets néfastes sur la santé reproductive et générale des rats femelles via le mécanisme de stress oxydatif. Ces résultats contribuent à notre compréhension des risques potentiels associés à l'exposition au BPA et soulignent la nécessité de poursuivre les recherches et les mesures préventives pour minimiser l'exposition humaine.

Mots clés : perturbateur endocrinien, bisphénol A, toxicité subchronique, station d'épuration, stress oxydatif, foie, rein, ovaire.

ملخص

أشارت الدراسات الحديثة إلى أن عددًا من المواد الكيميائية الموجودة في كل مكان الموجودة في البيئة مرتبطة بعدد لا يحصى من المشكلات الصحية، بما في ذلك التشوهات الإنجابية وزيادة العقم من خلال أفعالها كهرمونات خارجية. من المحتمل أن يكون Bisphenol A أحد أكثر أنواع الزينوإستروجينات الاصطناعية شيوعًا في البيئة. هدفت هذه الدراسة إلى تقييم السمية شبه المزمنة للبيسفينول أ (BPA) في إناث الجرذان. تعرضت إناث الفئران لمدة 25 يومًا، داخل الصفاق لـ BPA عند 50 ميكروغرام / كجم / يوم و100 مجم / كجم / يوم وشفويًا للمياه المفلترة التي تم جمعها في محطة معالجة مياه الصرف الصحي في قالمة. تم تقييم العديد من المعلمات لفهم العواقب السلبية المحتملة. كشفت النتائج من المؤشرات الحيوية الخلوية للإجهاد التأكسدي عن تغيرات فسيولوجية كبيرة في إناث الفئران المعرضة لأعلى جرعة من .BPA أظهرت النتائج انخفاضًا معنويًا في مستويات GSH و GSH-Px وزيادة في مستويات AOPP و MDA في جميع الأعضاء الثلاثة (الكبد والكلى والمبايض) مع إصابة الكبد والمبيض التي تم تأكيدها بالتحليل. تشير السمية شبه المزمنة الملاحظة إلى أن التعرض لـ BPA قد يؤثر سلبًا على الصحة الإنجابية والعامة لإناث الجرذان من خلال آلية الإجهاد التأكسدي. تساهم هذه النتائج في فهمنا للمخاطر المحتملة المرتبطة بالتعرض لـ BPA وتسليط الضوء على الحاجة إلى مزيد من البحث والتدابير الوقائية لتقليل التعرض البشري.

الكلمات المفتاحية: اضطراب الغدد الصماء، بيسفينول أ، السمية شبه المزمنة، محطة معالجة مياه الصرف الصحي، الإجهاد التأكسدي، الكبد، الكلى، المبيض.

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Introduction

Introduction

Recent studies have indicated that a number of ubiquitous chemicals found in the environment are linked to multiple health problems, including reproductive abnormalities and increases in infertility, cancer, and obesity via their actions as exogenous hormones.

By impacting the endocrine (hormonal) systems, those environmental pollutants, called endocrine disruptors (EDCs) can obstruct the development of both human beings and non-human animal species**.** EDCs are described by the World Health Organization (WHO) as "an exogenous substance or a mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny, or (sub) populations"**(Varticovski et al., 2022).**

A common industrial chemical used as a precursor in the manufacture of consumer goods, such as thermal paper, epoxy resins, and polycarbonate plastics, is bisphenol A (BPA). Over the past few decades, BPA manufacturing has increased significantly around the world, reaching more than 10 billion pounds generated a year **(Wang et al., 2022)**. This increase in manufacturing has facilitated the widespread presence of BPA in consumer goods as well as in the air, land, and water.

The European Chemicals Agency has recently designated BPA as a substance of very high concern and a recognized EDC that can alter hormonal balance even at low levels **(Mustieles et al., 2020)**.

Since BPA is structurally related to diethylstilbestrol (DES) and has estrogenic characteristics, though much less than DES or estradiol, its presence in biological samples is problematic **(Gassman, 2017)**.

Numerous studies have demonstrated its endocrine-disrupting properties and attributed exposure to cytotoxic, genotoxic, and carcinogenic effects; however, the results of these studies are still highly debated and a consensus about BPA’s safety and its role in human disease has not been reached **(Gassman, 2017)**.

Because of the importance of evaluating the effects of these chemicals in living model organisms this research work is proposed to investigate the mechanisms implicated in the toxic effects of BPA after a sub chronic exposure of 25 days on the liver, kidney and the reproductive health of female rats.

In this master dissertation, the research is structured into several key sections. The first section provides an introduction to the topic, including a literature review of endocrine disruptors especially BPA, it's uses, The endocrine system and its disruptors and the toxicity of bisphenol-A. The second section presents the methodology employed to collect and analyze data, outlining the experimental design and data collection procedures. The third section presents the results obtained from the analysis, along with their interpretation and discussion. Finally, the conclusion summarizes the key findings, discusses their significance, and suggests avenues for future research.

Bibliographic Synthesis

Chapter I

The Endocrine System and Its Disruptors

I. Endocrine System

The endocrine system functions as a communication system. In many-celled organisms, communication between cells is essential for their coordinated function. Such communication is based on chemical signals. Neighboring cells communicate via surface molecules and special junctions, whereas communication between distant cells is carried out through the release of chemical signals, and hormones that activate target cells and interact with specific receptors **(Figure 01)**. Chemical signals travel through the bloodstream (circulatory system) to reach target cells **(Demeneix, 2019)**.

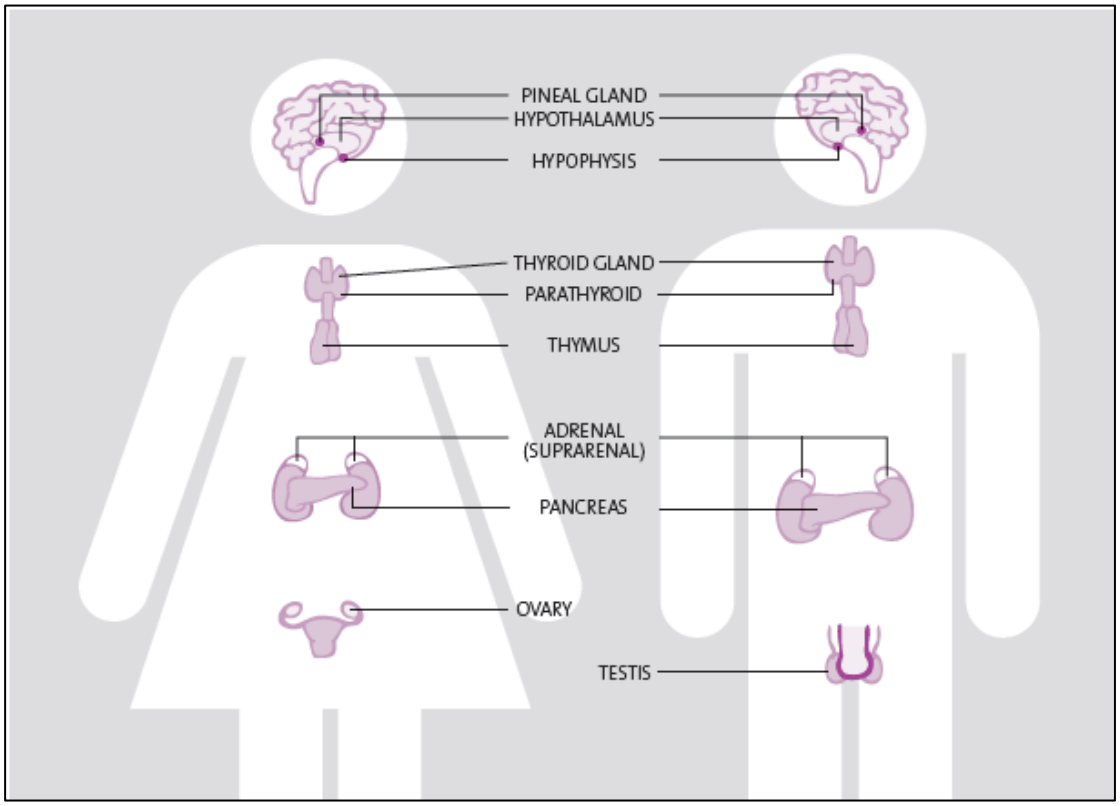

Figure 1: Endocrine system **(Romano Mozo, 2012)**.

1. Hormones

Hormones are molecules that are produced by **(Demeneix, 2019)** endocrine glands, including the hypothalamus, pituitary gland, adrenal glands, gonads, (i.e., testes and ovaries), thyroid gland, parathyroid glands, and pancreas. The term “endocrine” implies that in response to specific stimuli, the products of those glands are released into the bloodstream. The hormones then are carried via the blood to their target cells. Some hormones have only a few specific target cells, whereas other hormones affect numerous cell types throughout the body. The target cells for each hormone are characterized by the presence of certain docking molecules

(receptors) for the hormone that is located either on the cell surface or inside the cell. The interaction between the hormone and its receptor triggers a cascade of biochemical reactions in the target cell that eventually modify the cell's function or activity **(Hiller-Sturmhöfel and Bartke, 1998)**.

1.1. Hormonal Effect

The process by which a substance is released in one organ and transferred via the blood to its target organ is called an endocrine process: at one place synthesis and release occur, then undirected transport in the blood occurs, and finally, reactivity occurs at a distant cell or organ. The science of hormones (of endocrine-acting drugs, of internal secretion) was thus named endocrinology.

Exocrine release, as opposed to endocrine secretion into the blood, characterizes gland production for example, release into the oral cavity, into the gut lumen, or across the skin. Furthermore, odorant release, territory marking, and pheromone secretion are exocrine processes as well. A mediator which affects neighboring cells for example, within a gland or in the intestinal wall, in a lymph node, or within the placenta acts in a paracrine manner. Most regulators with very short biological half-lives show this paracrine effect. Hormones, however, are not very stable in blood. Hypothalamic releasing (RH) hormones such as corticotropin RH, thyrotropin RH, and gonadotropin RH have half-lives in blood below 10min; owing to the short distance to the target organ the pituitary gland (only 2–3cm away) the life span of these hormones is sufficiently long for them to reach the target organ in pulses. The simultaneous arrival of hormones in concentrations above the level needed for activation results in endocrine functions. When an effector substance expresses its effects on the releasing cell for example, in tumor cells, in activated lymphocytes, or again, in the placenta at the interface of maternal and fetal blood these actions are called autocrine actions **(Kleine and Rossmanith, 2016)**.

1.2. Role of Hormones

Hormones have many roles that work together to achieve the exquisite regulation required of many body processes. This regulation by the endocrine system is often the result of different mechanisms at many targets, thereby allowing the body to respond to a diverse variety of concurrent physiologic changes and pathologic insults. The major body processes regulated by hormones include energy production, utilization and storage (intermediary metabolism), growth, development, reproduction, and maintenance of the internal environment **(Belfiore and LeRoith, 2018)**.

1.3. Hormones Classes

1.3.1. Proteins/Peptides Hormones

This first category comprises hormones regulating reproduction (gonadotropins), energy balance (insulin), or blood pressure (angiotensins). These hormones are produced like any other protein: RNA is translated from the hormone gene. This RNA is then processed in the cell nucleus and exported from there as messenger RNA. In the cytosol, this messenger RNA associates with ribosomes. After the initiation of peptide synthesis, the RNA–ribosome complex binds to the endoplasmic reticulum (ER), the signal peptide reaches the inner ER via a pore, and the remainder of the protein is translated into the ER. Almost all protein/peptide hormones undergo posttranslational modifications in the ER and other intracellular compartments such as cleavage of the signal peptide, endopeptidase action by prohormone convertases, single amino acid removal from the C-terminal end via exopeptidases, oxidation of the C-terminal glycine resulting.

in a characteristic feature of many different hormones, amidation of the C-terminus, and finally cyclization of the N-terminal glutamine into a pyroglutamate residue **(Kleine and Rossmanith, 2016).**

1.3.2. Terpenes: Juvenile Hormones and Steroids Hormones

In these two hormone classes, we find estradiol and testosterone (female and male sex hormones), corticosteroids and mineral corticosteroids such as cortisol and aldosterone, and gestagens—for example, progesterone. Insects have their steroids—ecdysone (the hormone that controls molting; ecdysis) and its precursor. All these steroids are derived from cholesterol. Vitamin D3 is produced similarly. In complex biochemical reactions, the precursor squalene is converted into cholesterol. This cholesterol is present in the plasma membrane. A characteristic feature of steroid-forming cells is the expression of a steroidogenic acute regulatory protein, which transfers cholesterol from the plasma membrane into mitochondria, where the first step of vertebrate steroid synthesis occurs: the conversion of cholesterol into pregnenolone. Starting from pregnenolone, all the other human steroids are synthesized in a series of conversions. These steps require several enzymes. The presence or absence of these enzymes is the critical determinant that decides whether in a given cell cortisol or estradiol is made **(Kleine and Rossmanith, 2016)**.

1.3.3 Amino Acid Derivatives

The fourth class of hormones is derived from amino acids. Triiodothyronine and thyroxine are derivatives of tyrosine, as are the catecholamines dopamine, noradrenaline, and adrenaline. Indolamines such as melatonin and related molecules such as serotonin are made from tryptophan in successive steps **(Kleine and Rossmanith, 2016)**.

II. Endocrine Disruptors

1. Definition

An endocrine disruptor is defined as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, which implies that these kinds of chemicals may interfere with normal hormone action and that doing so can hurt the human. This term includes a wide range of substances with very varied qualities that can imitate or antagonize the effects of endogenous hormones, such as estrogens and androgens, or disturb the synthesis and metabolisms of endogenous hormonal receptors. And this definition includes a wide range of chemicals with very different properties, sources, and environmental fates, including natural and synthetic hormones, surfactants, pesticides, antibacterials, personal care products, cosmetics, and plastisol. These chemicals can mimic or antagonize the effects of endogenous hormones, such as estrogens and androgens, or disrupt the synthesis and metabolisms of endogenous hormonal receptors **(Schmitt et al., 2016)**.

2. Mechanisms and Action Principles of Endocrine Disruptors

Recent years have seen great advances in which researchers have managed to describe different ways in which endocrine disruptors can alter hormonal balance.

Those processes include:

• Mimicry of hormones: for example, EDCs that act like estrogen are known as environmental estrogens; examples are Dichlorodiphenyltrichloroethane (DDT), some Polychlorinated biphenyl (PCBs), and many phytoestrogens, nonsteroidal chemical compounds found in vegetables that are similar to human estrogens.

• Antagonizing hormone effects: for example, the anti-estrogenic action.

• Altering hormonal binding and metabolism patterns: for example, the effects of the flame retardant Polybrominated diphenyl ethers (PBDE-99), which alters the synthesis of thyroid hormone (TH).

• Modulating the levels of hormone receptors:

for example, BPA, found in some plastics and epoxy resin, which interacts with estrogen receptors.

The most researched action mechanisms include:

• Estrogenicity/ anti-estrogenicity

• Androgenicity/ anti-androgenic

• Thyroid alteration

• Alteration of hormone receptors: estrogenic receptor (ER), membrane-associated estrogen receptor (mER), androgenic receptor (AR), estrogen-related receptor (ERR), peroxisome proliferator-activated receptor (PPAR), progesterone receptor (PR), retinoid X receptor (RXRs); aryl hydrocarbon receptor (AHR)

• Alteration of retinoic acid, PPAR, and vitamin D routes

• Alteration of target tissues in the brain and the reproductive and cardiovascular systems

• Pancreatic ß-cell (beta-cell) dysfunction

• Endogenous inhibition of hormone metabolism

• Multiple hormone-modifying effects

EDCs are considered “chemical chameleons,” that is, a single EDC has different action mechanisms depending on its concentration.

Therefore, the same EDCs may also have different action mechanisms depending on the specific developmental stage of the affected tissue: To obtain a uterotrophic response (affecting the uterus). Adverse effects may vary depending on the time of exposure and the hormonal balance of the exposed individual, which is determined by among other factors **(Demeneix, 2019)**.

3. Endocrine Disrupting Chemicals

The main consequence of industrialization is the release of substances capable of interfering with the physiological endocrine function [i.e., (EDCs)]. These substances include pesticides, dioxins, pharmaceuticals, metals, phytoestrogens, phthalates, plasticizers, and polychlorinated biphenyls, among others; worldwide, EDCs represent a threat to health and the environment. Among EDCs, BPA interferes in steroid signaling and thus affects several biological functions causing reproductive, developmental, and metabolic dysfunction in humans, animals, and plants. BPA is widely used as a monomer in the production of epoxy resins and polycarbonate plastics and thus is introduced to humans and the environments via storage containers for food and beverages, medical devices, tableware, lenses, DVDs, electronics, sports equipment, thermal paper, dental sealants, etc **(Santoro et al., 2019)**.

3.1. Phytoestrogens

Foods including grains, beans, fruits, vegetables, and, in particular, many soy products are known to contain phytoestrogens, which are substances that have weak estrogenic effects in plants. In the human body, lignan, isoflavones (daidzein, genistein, and glycitein), and coumestans are the three main groups of phytoestrogens (coumestrol). The biological action of phytoestrogens, a plant component that has effects on a variety of hormone-related disorders, is comparable to that of animal estrogen **(Figure 02)**. They bind to the ER with a low affinity, which results in a weak estrogen-like impact. The fact that ERs are found in many tissues, such as the mammary gland, placenta, and reproductive system, suggests that phytoestrogens may have a hormonal effect on particular tissues. ER is thought to encourage cell development, whereas ER does the opposite **(Domínguez-López et al., 2020)**.

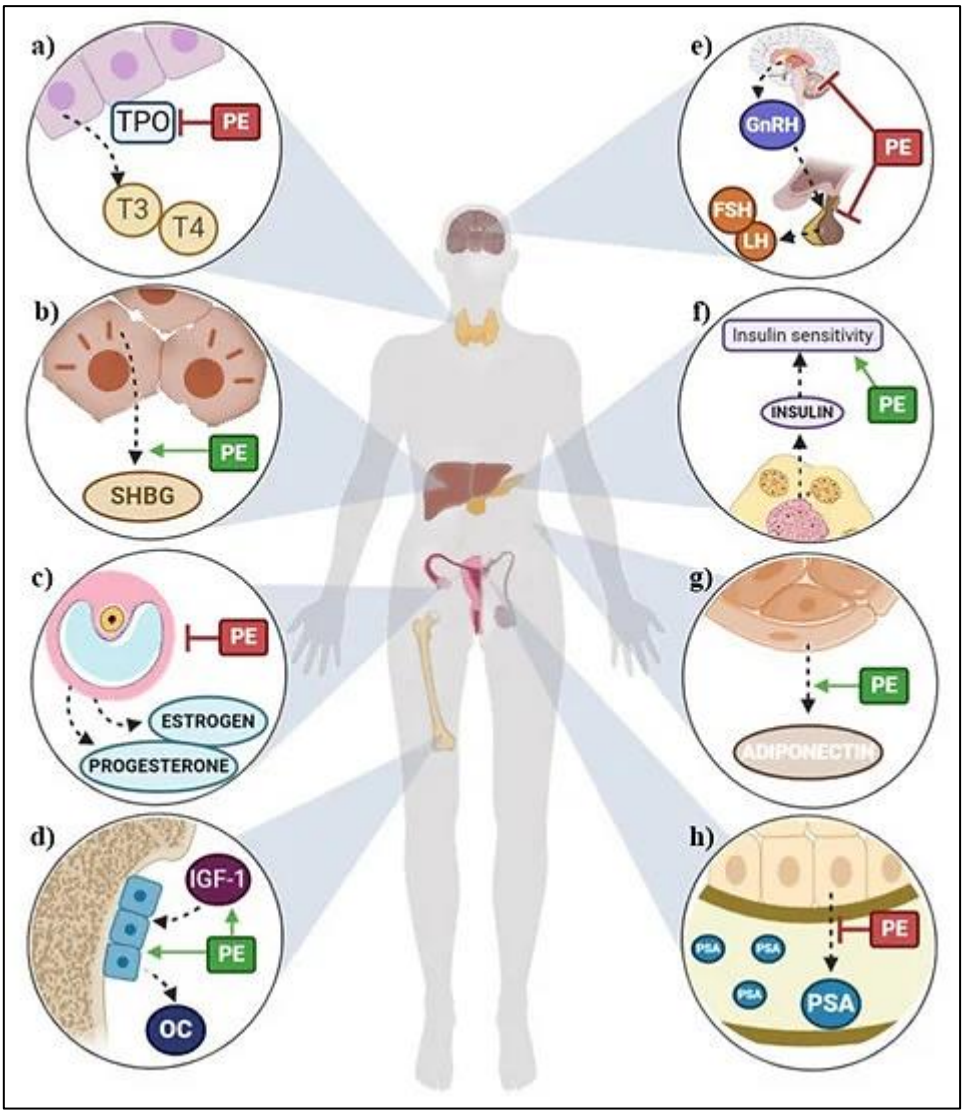

Figure 2: Shows a summary of the possible health effects of phytoestrogens.

(a) thyroids, (b) liver, (c) ovaries, (d) bones, (e) the hypothalamic-pituitary-gonadal axis, (f) pancreas, (g) adipose tissue, and (h) prostate through the manipulation of the endocrine system. Prostate-specific antigen (PSA), FSH (follicle-stimulating hormone), GnRH (gonadotropin-releasing hormone), IGF-1 (insulin growth factor 1), LH (luteinizing hormone), OC (osteocalcin), PE (phytoestrogens), SHBG (stimulating hormone-binding globulin), T3 (triiodothyronine), T4 (thyroxine), and TPO: thyroperoxidase **(Domínguez-López et al., 2020)**.

3.2. Diethylstilbestrol

The pharmacological and therapeutic properties of natural estradiol are produced by diethylstilbestrol, a synthetic non-steroidal estrogen compound that is five times stronger. The normal growth of the female reproductive system, endometrial hyperplasia, and vaginal epithelial keratinization can all be encouraged by DES, which can also increase the uterus'

oxytocin sensitivity **(Figure03)**. DES promotes the release of prolactin and anterior pituitary gonadotropins when administered in low doses. On the other hand, using large dosages of DES will counteract this result. Moreover, DES binds to estrogen or progesterone receptors (ERs) and has an anti-androgenic effect, which has negative consequences on reproduction. The chemical structure of diethylstilbestrol **(Demeneix, 2019)**.

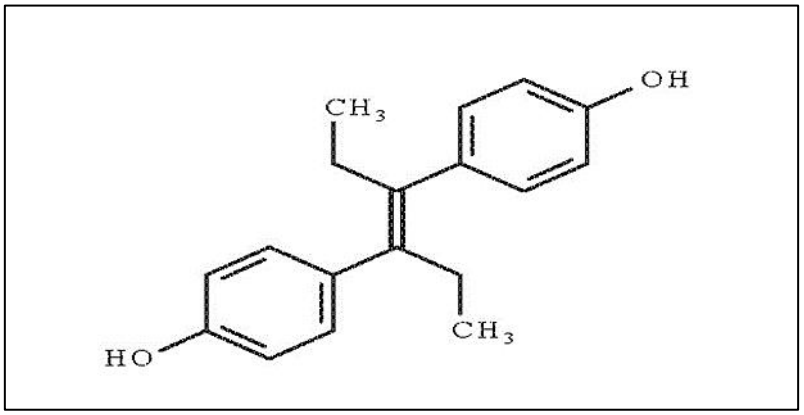

Figure 3: Chemical structure of diethylstilbestrol **(Humans, 2012).**

3.3. Organochlorine Pesticides

Insect, weed, and fungal growth are all controlled by a class of organic molecules known as organochlorine pesticides (OCPs), which include carbon, hydrogen, and chlorine. According to statistics, 40% of all pesticides applied fall under the chemical class of organochlorines. DDT and its metabolites, hexachlorobenzene (HCB), lindane, and dieldrin are among the many chlorinated hydrocarbons that make up this huge category. Although DDT is still commonly used in agriculture, it is now prohibited in many nations. The use of many pesticides in agricultural production has increased soil, water, and air pollution because OCPs have the characteristics of poor water solubility, stable chemical properties, and the ability to adsorb organic molecules in the soil. OCPs gradually spread from fish to humans **(Demeneix, 2019).**

3.4. Phthalates

Phthalates increase reactive oxygen species and alter the expression and activity of antioxidant enzymes that lead to DNA damage. Mono-2-ethylhexyl phthalate (MEHP) exposure, which inhibits trophoblast invasion through activation of PPARγ, is the most important mechanism of early abortion. Moreover, placental ErbB signaling is one of the majors signaling pathways responding to gene expression induced by DNA methylation and phthalate exposure.

The EGF signaling pathway activates numerous intercellular pathways that stimulate placental growth and function, including regulation of trophoblast cell proliferation, differentiation, and invasion.

Exposure to phthalates in early pregnancy alters the placental transcriptome and also alters key placental DNA methylation changes, such as insulin growth factor 2 (IGF2).

A previous study examined human chorionic gonadotropin urine samples to explore a possible association between phthalate exposure and early miscarriage.

Phthalates can cross the placental barrier as a result of continued exposure during pregnancy and increase the risk of developing defects such as low birth weight, cardiovascular disease, cryptorchidism, and subsequent cancer **(Demeneix, 2019).**

3.5. Bisphenol A

BPA has become a target of intense public scrutiny since concerns about its association with human diseases such as obesity, diabetes, reproductive disorders, and cancer have emerged. BPA is a highly prevalent chemical in consumer products, and human exposure is thought to be ubiquitous. One of the contributing factors is a lack of molecular mechanisms or modes of action that explain the diverse and pleiotropic effects observed after BPA exposure. The increase in BPA research seen over the last ten years has resulted in more studies that examine molecular mechanisms and revealed links between BPA-induced oxidative stress and human disease. Here, a review of the current literature examining BPA exposure and the induction of reactive oxygen species (ROS) or oxidative stress will be provided to examine the landscape of the current BPA literature and provide a framework for understanding how induction of oxidative stress by BPA may contribute to the pleiotropic effects observed after exposure **(Gassman, 2017)**.

Chapter II
Bisphenol A Toxicity

I. Definition

Bisphenol A (BPA, 4, 4'-dihydroxy-2, 2-diphenylpropane in IUPAC nomenclature) (CAS No. 80-05-7), composed of two aromatic rings (phenyls) linked by a carbon bridge **(Figure 04)**. This environemental polluant belongs to the family of hydroxylated diphenyl alkanes or bisphenols. Discovered by the Russian chemist Alexandre Dianin in 1891, it comes from the condensation of acetone with two phenols. The reaction is catalyzed by hydrochloric acid or by a resin of Polystyrene **(Tyl et al., 2002)**.

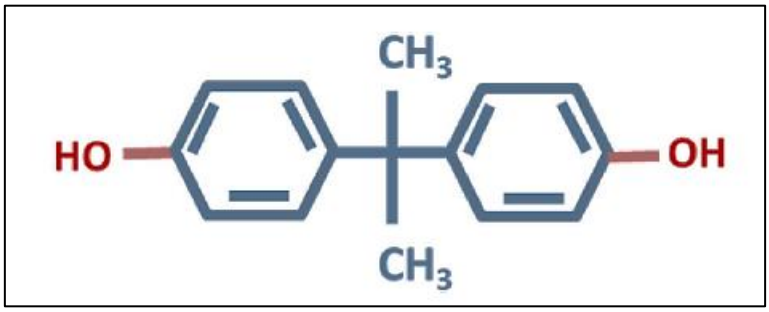

Figure 4: Chemical structure of BPA **(Ma et al., 2019)**.

II. Application of BPA

BPA is a well-known synthetic chemical used worldwide in the manufacture of a wide variety of polymers, including epoxies, polycarbonates, and other polymeric materials. Polycarbonates and epoxies are excellent polymers for critical applications of bisphenols.

Other uses of bisphenol A include the production of various resins (unsaturated polyesters, polysulfones, polyetherimides, and polyacrylates). Global demand for polycarbonate and epoxy resins in 2015 was 64% and 34% respectively. Moreover, demand for these two polymers is expected to grow at an average annual rate of 3% to 4% over the next five years. Furthermore, in recent years, its application has expanded to the processing of optical and electronic materials. Plastic cups, bottles, bowls, food containers, and utensils used in microwave ovens are also synthesized with polymers.

The epoxy resin protects the can from the inside. Therefore, they can be a significant source of food adulteration through direct contact. Storage bottles are also coated with epoxy resin for a similar purpose. Nevertheless, epoxy resins are also successfully used in the paint and ink industry. Epoxy resins are also highly valued in the production of thermal paper, compact discs (CDs), and digital video discs. BPA-derived compounds are used in tickets and

newspapers as antioxidants and stabilizers, and in the textile industry to make infant socks **(Li et al., 2022)**.

III. Exposure to Bisphenol A

BPA is a `ubiquitous' contaminant as it is present in all resources to which humans may be exposed via air, water, and soil. The main reason for the environmental impact is air, soil, and water pollution, as BPA enters the environment through thermal paper recycling and use in related industries. According to the Global BPA Market Report and Forecast 2021-2026, the global BPA market was worth US$ 10. Point sources include sewage treatment plant effluents and landfill leachates, and non-point sources include epoxy and polycarbonate plastic debris entering water bodies (13–30 mg/day) also contaminate the environment. This is caused by the migration of her BPA from packaging into food and beverages, this depends on many factors, including the composition of the various foods, the length of contact time, the temperature of the food during contact, and the type of packaging material.

Additionally, compounds such as epoxies and polyvinyl chloride (PVC) are used in the manufacturing industry to protect the inside of cans from corrosion and rusting from direct contact with various foodstuffs, Residual monomers from BPA migrate to food during high-temperature processing and storage in these bottles due to incomplete polymerization.

The main route of exposure is dietary exposure, including consumption of BPA-contaminated seafood and freshwater fish, fresh food from contaminated areas, food packaged in plastic or canned containers, and consumption of contaminated water and the main reason for this exposure is the daily feeding of canned formula in plastic bottles containing plasma cell (PC).

However, BPA exposure via inhalation and dermal routes accounts for less than 5% of all exposure sources. Breast milk or non-PC bottle-fed had a minimum BPA level compared to PC-free packaged babies. BPA This value was lower than that of infants (6–36 months) who used non-PC foods and ate solid foods. To validate BPA levels, non-canned foods had lower BPA levels (7%). As a preserved food (73%), under nutritional evaluation in adults disclosed can coating materials as a major source of BPA. A total of 12.6 ng/kg per day was calculated for humans. 12.4 ng/kg was ingested from canned food. In addition, dietary trends ranged from 3 to 12.95 ng/kg per day **(Heindel et al., 2020).**

IV. Bisphenol A in the Environment

BPA is a compound used in the manufacture of a wide variety of everyday materials that, when released into the environment, cause multiple detrimental effects on humans and other organisms **(Torres-García et al., 2022).**

1. Food

Food is the most important source of the general population's exposure to BPA. The presence of BPA in edibles is connected with the exposure of animals and raw plant material to BPA, accumulation of BPA in the environment, and contact of food with polymers containing this substance. It is considered that BPA is consumed each day with food, and the exposure of human organisms to BPA by the alimentary canal was estimated from 0.48 to 1.6g/kg/body weight/day **(Michałowicz, 2014).**

2. Dust

Besides the digestive system, humans are exposed to BPA by respiration and dermal contact. BPA is present in the dust because it migrates from articles made from BPA synthetic polymers **(Michałowicz, 2014).** Dust may penetrate human organisms by air passages; this is a potential source of BPA exposure. Nevertheless, it is considered that exposure of the general population to BPA inhaled with dust is considerably lower than that related to food consumption **(He et al., 2009).**

V. Metabolism of BPA:

A study of the metabolism of BPA revealed that BPA enters the liver after ingestion, During the binding process, the insoluble BPA-free material is converted into a water-soluble substance, the conjugated form excreted by the kidneys suggests a mechanism of tissue damage. The present study found that direct exposure to BPA increases tissue oxidative stress over time, as evidenced by the results of oxidative stress enzyme activity.

This also affects the liver cells in the form of alkalin phosphatase (ALP) injury, changes in the levels of lactate dehydrogenase (LDH), urea, serum glutamic-oxaloacetic transaminase (SGOT), and the cellular structure of the liver leading to damage to the liver cells. Cellular structures in organs such as the kidneys and lungs are damaged by oxidative stress, Liver and kidney damage may increase cardiac function. Metabolic and excretory disturbances associated with oxidative stress caused by BPA. Both treatment groups lead to myocardial damage supported by elevated creatine kinas (CK-MB) levels **(Figure 05)**.

In addition, permeability changes in the lungs may have caused this inefficient gas exchange and increased tissue hypoxia. Tissue hypoxia can lead to increased oxidative stress, leading to an increased cardiac workload **(Pant et al., 2020).**

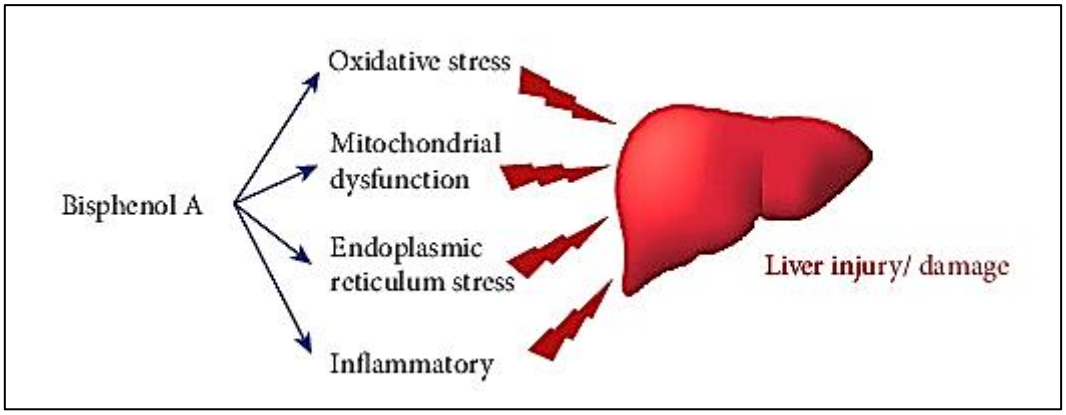

Figure 5: Schematic diagram proposing the possible mechanisms that cause liver injury/damage by BPA **(Abdulhameed et al., 2022).**

VI. Toxicity of BPA

BPA, an organic synthetic compound found in some plastics and epoxy resins, is classified as an Endocrine disrupting chemical. Exposure to BPA is especially dangerous if it occurs during specific "critical Periods" of life when organisms are more sensitive to hormonal changes (i.e., intrauterine, perinatal, juvenile, or puberty periods), it exerts both toxic and estrogenic effects on mammalian cells, on the liver.

The reproductive system is a target of toxicity for BPA, the effects of BPA on the ovary and female reproductive outcomes indicate that BPA is an ovarian toxicant that significantly reduces fertility. Further, some recent studies indicate that prenatal exposure to BPA causes adverse transgenerational effects on ovarian function and fertility in female offspring **(Ni et al., 2022)**.

VII. Current Monoexposure Mechanisms of Action

Studies of BPA exposure have revealed several different molecular mechanisms. BPA can stimulate receptor-mediated effects, induce DNA damage and epigenetic changes, alter oxidation-reduction balance, and induce mitochondrial dysfunction. In addition to receptor-mediated effects, BPA is known to damage DNA contributing to carcinogenesis and teratogenesis **(Sonavane and Gassman, 2019)**.

1. Co-Exposure Effects with BPA

BPA is so common in our environment that interactions with other exogenous and endogenous exposures are quite likely. Daily chemical exposure to humans and even laboratory animals come from their physical surroundings, hygiene practices, and meals. Mammals are particularly exposed to EDCs through soy-based diets in addition to BPA exposure. According to studies, the effects of low-dosage BPA exposure may be the result of its synergistic impact with other estrogen-like chemicals already present **(Sonavane and Gassman, 2019)**. Moreover, the discovery of free circulating BPA in biological samples is alarming because it shares structural similarities with DES and exhibits estrogenic properties, albeit to a much lesser extent than DES or estradiol **(Gassman, 2017).**

2. Co-Exposure with Therapeutics

One of the most contentious endocrine disruptors is BPA, which can interact with nuclear receptors and impair the normal operation of nuclear receptors at extremely low concentrations. The precise chemical mechanism by which BPA disrupts nuclear receptors' ability to function normally is still unknown. Here, molecular dynamics simulations were used to examine the specific method by which BPA interacts with the three common nuclear receptors, hER, hormone estrogen -related receptor (hERR), and hormone peroxisome proliferator activated receptor (hPPAR). According to the findings of the simulations and the computed binding free energy, BPA can bind to these three nuclear receptors **(Figure 06)**. To these three receptors, BPA had somewhat lower binding affinities than E2. To identify BPA as an endocrine disruptor and to find safer alternatives to BPA, the current work supplied the structural evidence necessary **(Li et al., 2015)**. Beyond estrogens, ER and ER may bind a variety of substances with various structures, such as BPA, which has distinct binding preferences and a varied relative binding affinity for different ER subtypes and species **(Cimmino et al., 2020).**

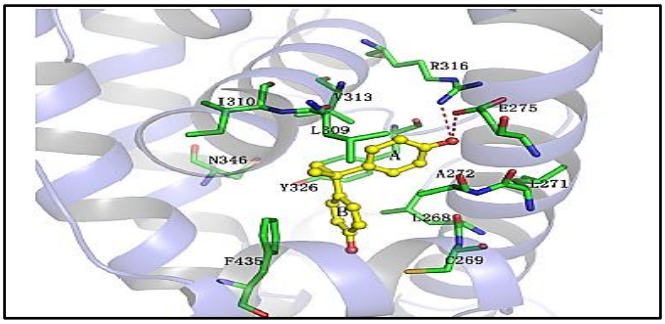

Figure 6: The relationship between hERR and BPA **(Li et al., 2015).**

3. Co-Exposure with Environmental Chemicals

Due to their growing usage in contemporary agriculture and industry, BPA, cadmium (Cd), and dibutyl phthalate (DBP) are recognized environmental degradation products (EDCs) that co-occur in aquatic ecosystems (**Sonavane and Gassman, 2019)**. Environmental disruptors (EDCs) such as BPA and di-(2-ethyl hexyl) phthalate (DEHP) is widely present in the environment and hurt humans. Inhibiting the tumor suppressor gene PTEN raised the expression of the oncogene c-MYC, increasing the expression of HDAC6, and ultimately increasing the susceptibility to thyroid cancers are the major effects of BPA alone and in conjunction with DEHP. By modifying H3K9ac to inhibit PTEN, activate the AKT signaling pathway, and simultaneously upregulate the expression of c-MYC, BPA alone and in combination with MEHP can significantly induce the proliferation of BCPAP cells. Unexpectedly, we discovered that BPA, either alone or in conjunction with MEHP, may greatly increase Nthy-ori3-1 cell proliferation without relying on HDAC6 by activating the ERK signaling pathway **(Figure 07)**. Taken together, these findings not only provide new evidence of the promoting effect of BPA and DEHP on thyroid cancer but also discusses some possible mechanisms underlying these effects **(Zhang et al., 2022)**.

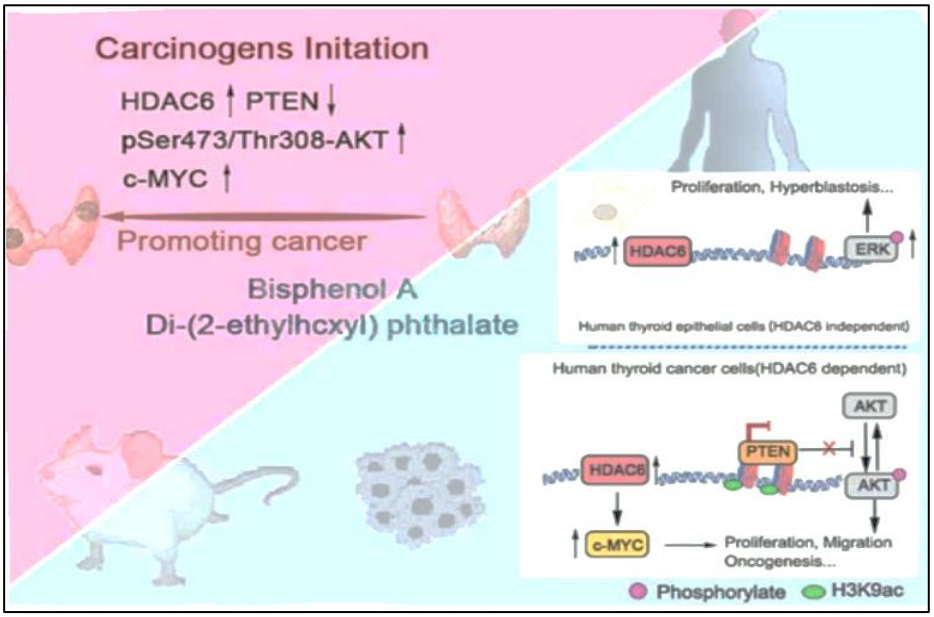

Figure 7: BPA alone and in combination with DEHP **(Zhang et al., 2022).**

4. Co-Exposure with Electromagnetic Radiation or Radioactive Substances

Ionizing radiation (IR) like X-rays and BPA is widely present in the environment and may act together, particularly at low doses. For X-rays, about 60% of the damage occurs due to

indirect effects. In somatic cells of female mice treated with BPA and X-rays, BPA enhanced the genotoxic effect of X-rays at 1 week, as indicated by the increased number of micronucleus (MN). Similar results were observed in their previous study, suggesting low doses of BPA diminished the sensitivity of the genetic material to damage induction. It is important to note that a similar dose of BPA (5 mg/kg b.w.) and X-rays (0.05 Gy) was able to show both harmful and ameliorative effects in somatic cells of male mice, which may be due to the differences in the exposure time. Using COMET assay, the authors reported higher strand breaks in bone marrow lymphocytes of females exposed to X-rays and BPA. On the contrary, male mice exposed to combine treatment for 2 weeks showed reduced DNA damage **(Sonavane and Gassman, 2019)**.

5. Co-Exposure with Other Stressors

Multiple stressors, including hypoxia, are a common occurrence in the natural world, especially when managing wildlife. Zebrafish larvae exposed to BPA in hypoxic conditions showed severe bradycardia and lower cardiac output (heart rate) than under normoxic conditions. At the lowest BPA dose (0.01 mg/L), which resulted in a 25% reduction in arterial RBC velocity both during and after the exposure, vascular parameters were most adversely impacted. Additionally, the later developmental stages of the larvae were more negatively impacted, resulting in 51 and 55% slower arterial and venous RBC velocities, with no impact on developmental parameters. However, no effects on cardiovascular parameters were seen from BPA exposure alone, indicating that BPA-related toxicity plays a minor role. Together, these findings indicate that environmental stressors including hypoxia and BPA, clearly have a more potential effect on cardiovascular function as compared to individual exposure **(Sonavane and Gassman, 2019)**.

Experimental part

Chapter III

Material and Methods

This research aims to study the sub-chronic toxicity induced by BPA in female Wistar rats. The work was carried out in its entirety between the animal house, the biochemistry laboratory of the university 8 Mai 1945, and the anatomopathological service of Ibn Zohr Hospital, Guelma.

1. Material

1.1. Animals

The experiment is carried out on female albino Wistar rats from the animal's house of the university on 8 May 1945, Guelma. At the beginning of the acclimatization period, these rats weighed between 100 and 200 grams, and at the time of the experiment on average 150 to 225 grams. Animal testing was conducted by ethical standards approved by the European Community Council Directive of 24 November 1986 (86-609/EEC) and the Decree of 20 October 1987 (87-848/EEC).

1.1.1. Conditions for Accommodation

The animals were raised in metal cages, lined with bedding made of wood chips. The cages were cleaned and the litter was changed once a day. These animals were acclimatized to the conditions of the pet store, at a temperature of 25 ° C, a natural hygrometry, and a photoperiod (spring). The food brought to the animals is made in the form of sticks made of corn, and barley, as for the drinking water, it is presented in bottles adapted to the cages.

1.1.2. Treatment of Animals

After the adaptation period, the female rats were divided into 4 batches:

- ✓ **Group 01:**(vehicle control n=4): 5% ethanol and corn oil (0.5 cc/day) were administered intraperitoneally for 25 consecutive days.
- ✓ **Group 02:**(low-dose BPA n=6): BPA at 50 µg/kg/day was administered intraperitoneally for 25 consecutive days, BPA in the form of dry powder was dissolved in 5% alcohol and suspended in corn oil based on the procedure found in the literature **(Li et al., 2016)**.
- ✓ **Group 03**:(high-dose BPA n=7): BPA at 100 mg/kg/day was administered intraperitoneally for 25 consecutive days **(Li et al., 2016)**.
- ✓ **Group 04:**(WWTP n=5) was supplemented with treated water (ad libitum) collected from WWTP for 25 days.

The idea behind this treatment is to compare the toxic effects observant in this group to those groups 2 and 3 in order to complete the work of previous study where the results have reported that BPA is not completely eliminated (by 23% reaching 31% at the end of the processing chain) from the water in Guelma WWTP.

1.1.3. Sacrifice and Collecting of Blood and Organs

After a sacrifice, and collecting blood from the heart with the cardiac puncture method for biochemical analysis. An abdominal and then a thoracic incision is made. The liver, kidneys, and ovaries are isolated and stored in the freezer at -20°C until the day of homogenization. A few grams of tissues (liver, kidney, and ovaries) from each rat of the different groups studied were recovered. After grinding and homogenizing the tissues in saline phosphate buffer (pH 7.4). The organ homogenates are then used for the assay of oxidative stress markers: The malondialdehyde (MDA), reduced glutathione, advanced oxidative protein products, glutathione peroxidase assay, and tissue protein assay (liver, kidney, and ovaries).

2. Method of Analysis

2.1. Biochemical Serum Assays

2.1.1. Blood Glucose Level

Two hours before sacrifice, blood samples were collected from the tail of the rat and used to determine serum glucose activity.

2.2 Preparation of the Cytosolic Fraction

At the end of the reperfusion period, a portion of the three organs (liver, kidney, ovaries) were removed for histopathological study. The rest of the three organs' tissue was weighed, cut into small pieces, immersed in three volumes of phosphate buffer (0.1 M, pH 7.4 at 4°C) containing KCl (1.17%), then homogenized with an ULTRA-TURRAX homogenizer (IKA T10, position 6).

A volume of 0.5 mL of trichloroacetic acid (5% TCA) is added to the same volume of crude homogenate and centrifuged at 4°C for 10 min at 4000 rpm. The resulting supernatant is used for the reduced glutathione assay.

Part of the supernatant was used for the MDA assay. The rest of the supernatant was centrifuged at 4°C for 45 min at 10,000 rpm. The resulting supernatant was used for the estimation of antioxidant enzyme activity.

2.2.1. Evaluation of Lipid Peroxidation (MDA)

The malondialdehyde (MDA) rate, considered a final product of lipid peroxidation, was measured according to the colorimetric method described by **Ohkawa et al., 1979**.

The MDA contained in 0.5 ml of the supernatant reacts with 1 ml of thiobarbituric acid (TBA) (0.67%) in the presence of 0.5 ml of trichloroacetic acid (TCA 20%). The reaction mixture was incubated at 100°C for 15 minutes and then cooled. The pink compound formed was extracted by adding 4 ml of n-butanol. The organic phase containing the complex formed between MDA and TBA was then separated by centrifugation at 3000 rpm for 15 min. Its absorbance was measured at 532 nm (Jenway 6305 UV/visible spectrophotometer).The level of MDA in the three organ homogenates is expressed in nmol of MDA/g of tissue using a calibration curve produced from 1,1, 3,3-tetraethoxypropane which during its acid hydrolysis releases MDA.

2.2.2. Evaluation of Protein Oxidation

The level of protein oxidation in the three tissue was assessed by measuring advanced protein oxidation products (AOPP Advanced Oxidation Protein Products) quantified by a spectrophotometric method described by **(Witko-Sarsat et al., 1996)**.

0.2 mL of the supernatant was diluted in phosphate buffer (0.1 M, pH 7.4) and mixed with 0.1 mL of potassium iodide (1.16 M). Two minutes later, 0.2 mL of glacial acetic acid was added and the mixture was centrifuged at 3500 × g for 15 minutes at 4°C. The absorbance of the sample below the lipid phase was measured at 340 nm.

The three-tissue AOPP content was calculated using the extinction coefficient and expressed as nmol/mg protein. It is determined using the following formula:

$$\boldsymbol{AOPP\ Rate = DO \times \frac{Fd}{\varepsilon \times L \times C\ prot\acute{e}ines}}$$

DO: Optical density of the sample at 340 nm.

Δt: time interval in minutes.

Fd: Dilution factor (*Vt/Ve*) or *Vt*: Total volume of the reaction medium, *Vs*: Supernatant volume.

$\mathscr{E}$: Molar extinction coefficient of AOPP (261 mM^{-1} cm^{-1}).

L: Length of tank used (L = 1 cm).

CP: Protein concentration (mg/g of tissu).

2.2.3. Reduced Glutathione Assay (GSH)

Ellman's method was adopted for the evaluation of hepatic cytosolic glutathione levels **(Ellman, 1959)**. It is based on the oxidation of GSH by 5,5-dithio2-nitrobenzoic acid (DTNB) thus releasing 2-nitro 5-mercaptobenzoic acid, which at alkaline pH has an absorbance at 412 nm.

1700 µl of the phosphate buffer (pH 7.4° 0.1 M) is added to 200 µl of the supernatant. Then, 100 µl of the DTNB solution (0.01 M prepared in the phosphate buffer). After shaking and incubation for 5 min at room temperature. The optical density of the supernatant is measured by spectrophotometer at 412 nm against a blank prepared under the same conditions with distilled water. The level of GSH is calculated from a standard curve prepared by GSH at increasing concentrations (from 0.2 to 1 mM). The results are expressed per µmole/g of tissue.

2.2.4. Glutathione Peroxidase Enzymatic Activity Assay (GSH-Px)

The enzymatic activity of glutathione peroxidase (GSH-Px) was measured by the method **(Flohé and Günzler, 1984)**. This method is based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH), the latter is transformed into (GSSG) under the influence of GSH-Px according to the following reaction:

$$\boldsymbol{GSH + H_2O_2 \xrightarrow{GSH-Px} GSSH + H_2O_2}$$

400 µl of the GSH (0.1 mM) is added to 200 ml of the supernatant. Then, 200 µl of the TBS buffer solution (Tris 50 mM NaCl 150 mM pH 7.4). After incubation for 5 min in a water bath at 25°C, 200 µl of H_2O_2: (1.3 mM) is added to initiate the reaction and left to react for 10 minutes. Then 1 ml of TCA (1%) is added to stop the reaction, the mixture is placed on ice for 30 minutes. And is centrifugated for 10 minutes at 3000 rpm.

2.2 ml of TBS buffer solution is added to 480 µl of the supernatant, then 0.32 ml of DTNB (1.0 mM), it mixed and the optical densities at 412 nm after 5 min.

$$GSH - Px = \frac{DO\ ec \times DO\ et\ \times 5}{DOet\ \times cp}\ 0.04$$

2.2.5. Tissue Protein Assay

The protein content of the samples (in the 3 organs) was determined by the method of **(Lowry et al., 1951)**. This very sensitive and very fast method is based on the adsorption of a dye.

A volume of 1 ml of Gornall's reagent is added to 20 µl of each supernatant sample. After stirring followed by incubation for 10 minutes at room temperature, the optical density of the reaction mixture is read at 540 nm against a blank prepared by the reagent, and a standard that contains 50 mg of BSA is dissolved in 1 ml of 0.9% NaCl.

The protein concentration is calculated from a standard curve prepared with bovine serum albumin (BSA) at increasing concentrations (from 0.1 to 1 mg/ml). Results are expressed as mg/ml.

2.3. Relative Organ /Body Weight

By calculating the relative organ body weight, the proportion of an organ's weight is assessed in relation to the overall body weight, providing insights into organ development and potential effects of sub chronic exposure of BPA on organ size.

It depends on the relation between organs and body weight and where we divide each animal’s organ weight by its body weight.

The formula can be expressed as:

Relative Organ Body Weight = (Absolute Organ Weight / Body Weight)

2.4. Histopathological Analysis

For this part of this study, samples of the three organs belonging to rats from the different experimental groups were fixed in 10% formaldehyde, impregnated, and then included in paraffin blocks. After the inclusion step, 5µm sections were made and then stained with hematoxylin-eosin (H&E). The visualization of any lesions affecting the three organs

tissue was carried out using the optical microscope (Olympus C 41) and the histological sections were enlarged forty times (10x and 40 x).

2.5. Statistical Analysis

All data were expressed as mean ± S.E.M., statistical analysis was performed with Graph Pad Prism 6 software. Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test. To statistically interpret the differences between the results of the biochemical parameters a $p<0.05$ was considered statistically significant.

Chapter IV

Discussion

Discussion

BPA is a chemical compound commonly found in various consumer products, such as plastic bottles, food containers, and thermal paper receipts. This pollutant can interfere with processes that are regulated by endogenous hormones **(Alonso-Magdalena et al., 2006)**. It has been reported that the ability of BPA to bind oestrogen receptors may cause various disorders including hypertension, atherosclerosis, liver dysfunction, diabetes, and obesity **(Marmugi et al., 2014)**.

In the present study, we aimed to assess the potential toxic effects of the daily exposure of different BPA doses in female Wistar rats by exploring biochemical and histological changes in the liver, the kidney and the ovary.

Based on our finding, the relative organ body weight of treated animals has not been altered. Actually, all the studies in the field have studied the effect of BPA exposure on the organ weight which could not be compared with the result of our parameter. Indeed, the finding of **Srivastava and Dhagga, 2019** revealed a decrease in the weight of reproductive organs following the oral administration of 600 mg of BPA/kg Bw for 3 months. Another *in vivo* study of induced BPA toxicity has reported an increase in the body weight of rodents **(Angle et al., 2013).**

Regarding the results of serum glucose perturbation, the analysis showed a decrease in glycemia level in both BPA treated groups compared to control animals. These effects on glycemia may be attributed to BPA's ability to disrupt endocrine signalling, particularly through interactions with oestrogen receptors and other hormonal pathways involved in glucose regulation. Research in the field that contradicts our findings is the one conducted by **Carchia and colleagues, 2015**. The teamwork evaluated low doses BPA-induced perturbation of glycemia by toxicogenomics and reported fasting hyperglycaemia due to a reduction of insulin secretion.

Concerning the evaluation of the oxidative status of the experimental animals, the group treated intraperitoneally with 100 mg of BPA /kg for 25 days has experienced the most prominent effects. It was also noticed that livers and kidneys of that group were the most injured organs.

According to our results, exposure to BPA contributed to increased oxidative stress which was first demonstrated by increased lipid peroxidation and subsequently elevated MDA

levels in the three studied organs with a more pronounced effect in the liver and the ovary of high dose treated rodents. The studied xenoestrogen induced loss of cell membrane integrity and therefore causes it to break which is a sign of a possible hepatotoxicity and reprotoxicity. This is supported by a researcher team that demonstrated that the mechanism implicated in the toxicity of BPA is oxidative stress and cellular depletion of antioxidant reserves leading to tissue damage **(Behmanesh et al., 2018)**.

It is well known that oxidative stress occurs when reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide, exceed the body's ability to neutralize them. These ROS can cause damage to various biomolecules, including proteins.

The damaged proteins are one of the crucial parameters that may result from the direct effect of xenobiotics as BPA or its metabolites (e.g. quinones, semiquinones) or the indirect influence of ROS on these macromolecules **(Koszowski et al., 2008)**. Advanced oxidative protein products (AOPPs) are key markers of oxidative stress and can provide information about the oxidative status of the body. They are generated during oxidative stress conditions, where there is an imbalance between the production of reactive oxygen species ROS and the body's antioxidant defences.

The results of the present study, revealed an elevated AOPP levels in female rats exposed to BPA which is in concordance with the increased MDA levels indicating an oxidative statue and potential protein damage. Moreover, higher levels of this biomarker were noticed in animals having wastewater collected from Guelma treatment station as a daily beverage. These results could be due to the presence of a mixture of pollutants in this water leading a sever of oxidative stress.

Previous studies reported that BPA and its analogues increased ROS formation, while only BPA generated hydroxyl radical **(Maćczak et al., 2017)**. It has been proven that hydroxyl radical is mostly involved in protein oxidative damage **(Koszowski et al., 2008)**, therefore the results achieved in the study of **(Maćczak et al., 2017)** may partly explain why BPA caused the strongest changes in protein degradation. In another study, **(Bukowska et al., 2008)** observed that phenoxy acetic acids, which are the precursors of phenols causing protein damage, while **(Maćczak et al., 2017)** reported that BPA and its analogues induced oxidative damage to proteins.

To counteract reactive oxygen species formation, the cell has defence mechanisms such as glutathione. This tripeptide is a crucial antioxidant and detoxifying molecule involved in maintaining cellular redox balance and protecting against toxic chemicals harms **(Masella et al., 2005)**.

According to the results of our study, bisphenol A exposure has been implicated in altering the levels of glutathione in the liver, kidney and ovary of the low and high dose treated rats. The reduced GSH depletion in rats poisoned by BPA are explained by its consumption as much by its direct trapping of free radicals than by its repair of oxidized proteins or by its involvement in the conjugation and detoxification processes of peroxides ensured by GPx and GS-T. These results suggest that reduced GSH could constitute a first-line defence against oxidative stress caused by this estrogenic mimetic **(Valko et al., 2007)**.

Furthermore, recent studies reported that BPA exposure may affect the expression or activity of enzymes involved in GSH metabolism, such as glutathione peroxidase and glutathione reductase, which are responsible for maintaining GSH levels and its recycling. Disruption of these enzymatic processes can contribute to decreased GSH levels.

Reduced glutathione (GSH), which allows the body to get rid of unwanted toxins and pollutants, forming with them a water-soluble compound likely to be excreted in the urine or bile. When glutathione is oxidized to form (GS•) it can react with another glutathione anion to form the oxidized molecule (GS-SG). Subsequently, it can be reduced to GSH again by the NADPH-dependent glutathione reductase **(Valko et al., 2007)**. It is therefore interesting to study the level of GSH in the liver, kidneys, and ovaries to assess the extent of cell recovery from the BPA induced damage.

Glutathione exerts its role in synergy with antioxidant enzymes, including glutathione peroxidase (GSH-Px). In this research, GSH-Px activity have been assayed in the tissue homogenates of the experimental animals. It is noteworthy that GSH-Px, an antioxidant enzyme located in the cytosol, requires two essential components to function effectively in addition to its main role of eliminating lipid peroxides: reduced glutathione and selenium which needs glutathione and selenium **(Iskusnykh et al., 2013)**.

Based on our findings, a significant decrease in the levels of GPx following the administration of BPA has been noticed as a response of the subchronic exposure of BPA at 50µg and 100 mg/kg doses. This result is supported by **Hassan and co, 2012** findings, where they administered a high dose of BPA (50 mg/kg) and found a significant decrease in

glutathione, GSH Px, GSH reductase, and glutathione-S-transferase. As per our study's outcomes, **Moon et al., 2012** observed that hepatic lipid peroxidation increased, whereas GPx levels decreased after administering BPA.

While a majority of BPA is converted into less toxic BPAG and BPAS, the remaining free BPA induces ROS through the enzymatic (H_2O_2/peroxidase and NADPH/CYP_{450}) and non-enzymatic (peroxynitrite/CO_2 and −OCl/HOCl) formation of phenoxyl radicals. Subsequent reactions of these radicals with NADPH or intracellular glutathione (GSH) along with further enzymatic processing produce a variety of radical species, including superoxides, peroxides, and hydroxyl radicals **(Gassman, 2017)**

Figure 9 summarizes all findings related to potential mechanisms by which BPA causes toxicity in female Wistar rats. This could mean disturbances in hormone receptors or other cellular pathways. BPA induces similar biological effects by activating an intracellular signaling pathway and regulating gene expression mediated by cell surface receptors and nuclear receptors. Membrane receptors can mediate BPA-induced activation of the SRC and ERK pathways and the PI3K/AKT pathway to regulate cell proliferation and migration. In addition, BPA can regulate the activity of enzymes such as CYP450 family and antioxidant enzyme. Aromatase can catalyze the conversion of androgen to estrogen to affect the endocrine system, BPA can also affect the antioxidant system, cause the accumulation of ROS and LPO, and damage cell function due to mitochondrial dysfunction. In addition, BPA causes genetic toxicity, including DNA strand breaks, oxidative DNA damage, and chromosomal mutations, etc. In addition to epigenetic changes, BPA-induced gene expression without DNA sequence variation plays an important role. **(Ma et al., 2019)**.

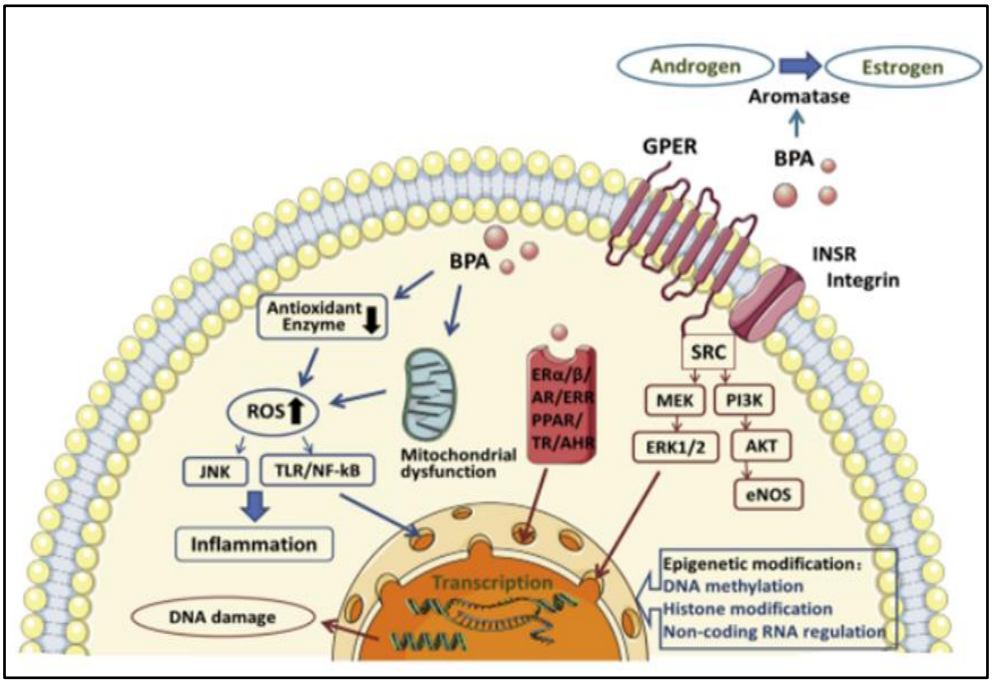

Figure 9: Schematic diagram illustrating the mechanisms of BPA toxicity **(Ma et al., 2019).**

BPA: Bisphenol A, INSR: insulin receptor, ER Estrogen receptor, AR: Androgen receptor; ERR: estrogen related receptor; PPAR: peroxisome proliferator activated receptors; TR: Thyroid hormone receptor. AHR: Aryl hydrocarbon receptor, ROS: reactive oxygen species; JNK: c-Jun N terminal kinase TRL: toll-like receptors; SRC: proto-oncogene tyrosine-protein kinase; MEK: MAPK/ ERK kinase; ERK Extracellular regulated protein kinases; PI3K: phosphatidylinositol-3-kinases; AKT: Protein kinase B. eNOS: Endothelial nitric oxidase synthase.

We aimed to confirm the results obtained below by conducting histopathological analysis of tissue samples to assess the structural changes or abnormalities. When examining the tissue for toxicity induced by BPA, histopathological findings were in line with our research findings concerning the extent of the oxidative statue in the explored tissues, with a more marked damage in the liver and the reproductive organs (ovaries and uteri) of the females treated by the highest dose of bisphenol A. The obtained results were consistent with the findings of previous studies **(Korkmaz et al., 2010)** and **(Shi et al., 2021)**. These investigations have proposed that BPA exposure may lead to liver toxicity in animal models. The recorded histopathological changes associated with the high dose BPA-induced liver toxicity include: hepatic inflammation, hepatic steatosis, liver fibrosis and/ or disruption of liver architecture.

It is well-established that BPA is one of the EDCs that mimic hormones by binding to oestrogen receptors or androgen receptors causing adverse effects on the human and animal body **(Vandenberg et al., 2012)**. Contamination with this xenoestrogen results in increased atresia of the ovarian follicles **(Boone and Tsang, 1998)**. Exposure of neonatal females to 50

mg/kg BPA resulted in abnormal folliculogenesis and follicle degeneration, with a few follicles containing multinucleated cells **(Patisaul et al., 2009)**. Other scholarly work has indicated that BPA ingestion may lead to various adverse effects on the rat ovary, including changes in ovarian morphology, alterations in follicular development, disruptions in hormone levels, and potential negative impacts on reproductive outcomes. These effects could potentially affect fertility and reproductive health in female rats **(Fernández et al., 2010)**.

In addition, studies have suggested that BPA exposure may lead to changes in uterine morphology and histology, such as alterations in the thickness of the uterine wall, disruptions in the epithelial lining, and modifications in glandular structures. These effects could potentially affect the reproductive health and function of female rats **(Hamdy et al., 2018).**

It is worth noting that the specific histopathological changes induced by BPA in the examined organs can vary depending on factors such as the dose, duration of exposure, and individual susceptibility. Additionally, while animal studies provide insights into potential effects, the relevance to human health requires further investigation.

Conclusion

Conclusion

In conclusion, the current study established that oxidative stress is implicated in BPA-induced liver, kidneys and ovaries injury and fibrosis. These results strongly suggest that the daily intraperitoneal administration of 100 mg of BPA/kg of female rat is, in one way or another, harmful to the three explored organs with a more severe effects to the liver and the ovary.

In the light of these results, BPA contact should be avoided. It is also crucial to provide comprehensive training and education to workers about the potential hazards of BPA exposure, including proper handling, storage, and personal protective measures. Workers should undergo periodical monitoring of liver, kidneys and specially ovaries functions.

In the other hand, it seems that the daily exposure of rats to the treated water collected from Guelma wastewater epuration plant did not record harmful effects to experimental animal, as compared with the two other BPA treated groups, allowing us to suggest that bisphenol A could be present in these filtered wastewaters in barely detectable quantities. These results encourage collaboration between industries, researchers, and regulatory agencies to collectively address the challenges posed by BPA exposure.

Therefore, clinical and experimental studies should be conducted in the future to determine prophylactic strategies for people chronically exposed to BPA. In addition, further long-term studies as dose-response relationships, including larger groups, are required to confirm and expand upon the present findings.

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Bibliographic Reference

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Appendices

1. Bisphenol A: [>99.0%]

- IUPAC name: 2,2-Bis (4-hydroxyphenyl) propane
- Formula: C15H16O2
- Molar mass: 228.29 g/mol
- Melting Point: 158°C
- Solubility: Ethanol, corn oil

2. Reagents and products used

- Hydrogen chloride (HCl),
- Methanol, Ethanol
- Sodium chloride (NaCl), potassium chloride (KCl),
- Bovine serum albumin (BSA)
- Trichloroacetic acid (TCA)
- 5,5'dithiodis-2-nitrobenzoic (DTNB)
- GSH
- Formalin
- Distilled water
- 1-Chloro-2,4-dinitrobenzene (CDNB)
- Gornall Reagent
- Hydrogen peroxide H_2O_2
- Thiobarbituric acid (TBA)
- n-Butanol
- Phosphate buffered saline (PBS)
- Tompon phosphate (0.1 M PH=7.4)
- Potassium iodide (KI)
- Glacial Acetic acid
- Tris
- Ethanol
- Xylene
- Eosin
- Mayer's reagent [mercuric chloride (1.36 g) and potassium iodide (5.00 g)].
- Acetone

3. Laboratory equipment

- Desiccator
- Dissection kit
- SIGMA horizontal centrifuge.
- SP-UV 2005 molecular transmission spectrophotometry.
- IKA T18 basic type homogenizer.
- Laboratory water bath
- Electric balance type KERN EMB 2200-O
- Magnetic stirrer with a hot plate.
- Micropipette, spatula
- Conservation boxes
- Petri dishes
- Glassware (lumber, Erlenmeyer, crystallizer, test tube, test tube, dry tube, graduated pipette, quartz tank).
- Vortex