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Assessment of The toxic Effect of Environmental Pollution by 2 ,3 ,7 ,8 - Tetrachlorodibenzo-p-dioxin (TCDD) on The Female Reproductive System by Pathological and Biochemical Assay in Albino Female Rats

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Abstract

TETRACHLORODIBENZO-P-DIOXIN (TCDD), dioxin 2, 3, 7, 8, and other 75 tricyclic aromatic chemicals that pose serious risks and are structurally linked, one of these compounds is persistent in the environment, accumulates in the food chain, and acts toxically in a similar way. The research aimed to examine the toxic pathological consequences of TCDD in albino female rats' reproductive system for the purpose of the current study. The experiment utilized an interventional design and divided the sixty female rats into randomly and equally two groups. The first group was the control group, while the second group received the medication orally for 120 days, at 0.1 mg / Kg. b.w of TCDD. All animals were sacrificed under slight ether anesthesia for blood collection for biochemical assay, especially for sex hormones and reproductive system. The ovary and uterus were immediately removed and examined to record any abnormal size, color adhesion and consistency. The results elucidated that the biochemical assay revealed all sex hormones significantly decreased $p \leq 0.05$. The reproductive organs of the second group showed severe damage characterized by ovary hyper-cellularity of the granulose layer and increased follicular development in the cortex layer. Uterus characterized by endometrial gland cystic dilation, necrosis, abscess in endometrial and myometrium, lymphocytic granuloma with extensive hemorrhage in endometrial and myometrium. These findings suggest that the administration of TCDD induced pronounced hazardous effects in the female reproductive system, sex hormones and pathological effects in genitalia.

Keywords: Dioxin, toxic effect, Reproductive System.

Introduction

TCDD are prevalent and long-acting environmental pollutants, and there is still cause for concern over their background levels. Growing research from epidemiological and experimental investigations indicates that prenatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic dioxin congener, might reduce the male/female ratio of kids [1]. Because chemicals and their byproducts can produce reactive oxygen species (ROS) when ingested, inhaled, or absorbed, chemical pollutants and their metabolites can have harmful effects [2]. Reactive oxygen species (ROS) can overwhelm cellular antioxidants and cause oxidative stress, which causes substantial cellular damage in the form of DNA damage from free radicals, lipid oxidation, and extensive protein damage in the cells that are subjected to it [3]. The 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is one of 75 tricyclic aromatic compounds having one to eight chlorine atoms. There are four chlorine atoms in this specific TCDD isomer at positions 2, 3, 7, and 8. Dioxins are not known to be biosynthetically created by living organisms, nor do they possess any beneficial industrial properties. PCDD/PCDFs are unintentionally produced by industrial processes that include bleaching paper and pulp with chlorine and making certain insecticides, herbicides, and fungicides, even though that natural processes like forest fires and volcano explosion may produce them. TCDD has no recognized commercial uses, however, it is used

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as a research chemical. Both as a pesticide against insects and fungi that ruin wood, as well as a flame retardant, it was investigated but never put to use commercially [4].

Dioxins are typically found in food, and the bulk of a person's daily consumption comes from meat and dairy products. The permissible daily dosage of dioxins is 0.001-0.01 pg TCDD/kg/day based on human data from highly exposed industrial cohorts; nevertheless, the WHO published a revised tolerated daily intake of 1-4 pg/kg/day [5]. Based on the low dosages of developmental changes in animal studies [6]. The majority of the harmful and metabolic effects of dioxins and similar aromatic hydrocarbons are mediated through the aryl hydrocarbon receptor (AhR) [7]. Several different tissues have the AhR. The nuclear transporter for aryl hydrocarbons is bound by ligand-binding AhR as it moves to the nucleus (ARNT). Specific DNA dioxin response elements (DREs) are bound by the AhR-ARNT complex [8]. The adult rat's lung, thymus, kidney, and liver all had the highest amounts of AhR mRNA expression, whereas the heart and spleen had the lowest levels. Although it has been demonstrated to be strongly expressed in the lung, heart, pancreas, and liver, with lesser expression levels in the brain, kidney, and skeletal muscle, the human placenta was previously thought to have the greatest expression [9]. The developmental effects of TCDD on developing immunological, neurological, and reproductive systems appear to be the most delicate ones seen in experimental animals [10]. Additionally, one of the most important targets for dioxins is the endocrine system [11]. Luteinizing hormone (LH) is present in vertebrates and it is content in the anterior pituitary gland of rats is (6-7) Mg and the essential role in the succession of the menstrual cycle including ovulation and preparation of the uterus to implant fertilized egg [12]. Follicle-stimulating hormone (FSH) generated by anterior pituitary gland response to gonadotropin hormone (GnRH) and is secreted from hypothalamus [13]. The majority of species are sensitive to TCDD's developmental toxicity, however, their sensitivity levels vary. Fish have been shown to have altered organ systems across the board; among the craniofacial impacts of TCDD are malformed anterior nasal features, slowed mandibular growth, and an absence of tooth development. In fish and birds, cardiovascular toxicity has been found [14].

Material and Methods

TCDDs supplied at a level of 0.1 mg/kg B.W. and imported from Biomer France. Following the acetone's evaporation, freshly dissolved TCDD was mixed with corn-acetone oil at a volume of 1 mg/kg B.W [15]. Two groups of sixty adult female albino rats were dividing randomly with 60 days, the first served as group's control group (c) had a regular pallet; for 120 days, the second group (TCDD) received an oral dose of 0.1 mg daily. From January 2023 to May 2023.

Blood collection: Blood were collected at day 120 of the experiment according to the collection protocol [16], for hormonal assay.

FSH, LH, progesterone and estrogen: Were measured by BioMerieux France minivitas [17].

All of the chemicals (99% purity) used in the Science and Technology Ministry's laboratory in Baghdad, Iraq, were either of the best grade available for analysis or came from Sigma Chemical Co. (St. Louis, Missouri, USA).

Histopathological changes: At the end of experiment (120) days all animal were scarified under slight ether anesthesia and reproductive systems (Ovum, Uterus) were swiftly extracted and dissected to note any abnormalities in size, color, consistency, or adherence. Subsequently embedded in paraffin and stained using a standard stain (hematoxylin and eosin) after being fixed in 10% formalin, thrown in ascending grades of ethanol (70, 80, 90, 100%), and then thrown in xylene.

Statistical analysis

Data analysis by using computer statistical program SPSS and sigma stat program. Tow way analysis variance was used $p \leq 0.05$ [18].

Result

Hormonal assay

Table (1) below showed the effect of TCDD on rat female hormones , all sex hormone (LH, progesterone, estrogen, FSH) revealed significant decreased $p \leq 0.05$ at day 120 of experiment when compared with control group.

Histopathological changes:

No important histopathological lesions were observed in uterus and ovary of 1st group (control group), while 2nd group at (120) days showed.

Ovary: Ovary of administered group showed active function characterized by different stages of follicular development with thickness and hypercellularity of granulose layer and cell, (Fig. 1). Other section showed areas of necrosis with mononuclear cells infiltration mostly macrophages and & lymphocytes (Fig. 2).

Uterus: Large area of dark basophilic cells aggregation (lymphocytic granuloma) with necrosis of granulose cells (figure 3), other section presented

accumulation of neutrophils as abscess formation with necrosis (Figs. 4, 5).

Cystic dilation of endometrial gland with lymphocytic infiltration (Fig. 6). Large abscess presented in endometrium (Fig.7). While severe endometrium and myometrium hemorrhage has been shown in (Fig. 8).

TABLE 1. The effects of Dioxin on rat female sex hormones

Treated group	LH mL u/mL	Progesterone Pg/mL	FSH mL u/mL	Estrogen Pg/mL
1st group	1.10 ± 0.03^{Aa}	0.31 ± 0.02^{Aa}	1.32 ± 0.21^{Aa}	1.25 ± 0.14^{Ab}
2nd group	$0.23.\pm0.02^{Bb}$	0.22 ± 0.01^{Bb}	0.26 ± 0.02^{Ba}	0.20 ± 0.22^{Bc}

Values represent mean ± SE (n = female rat/ group)

The same letters in one row refer no significant difference ($p\leq0.05$). The different letter in one row refer to significant difference $p\leq0.05$.

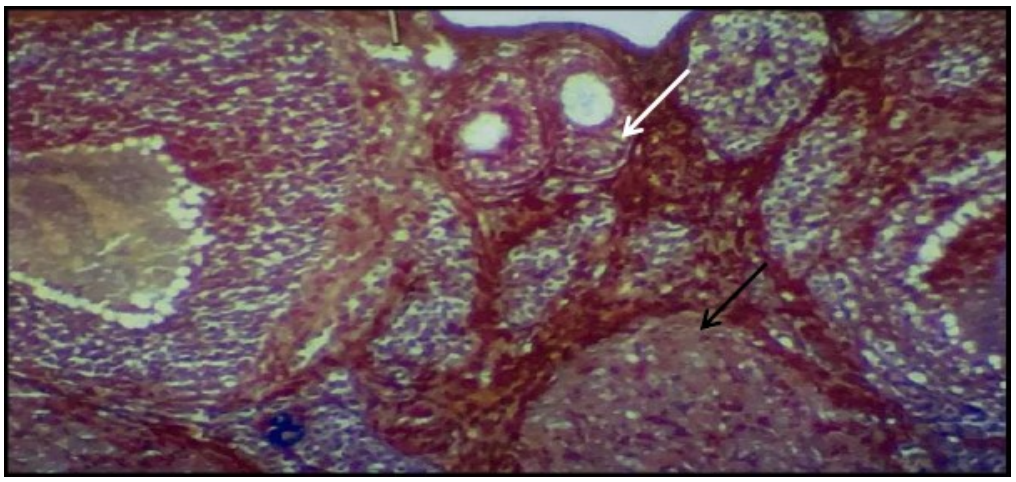

Fig. 1. Ovary Section of 2nd group (TCDD administration) showed: white arrow- multiple stage of follicular development. black arrow Hyper cellularity of granulose layer. (H&E stain) (×400).

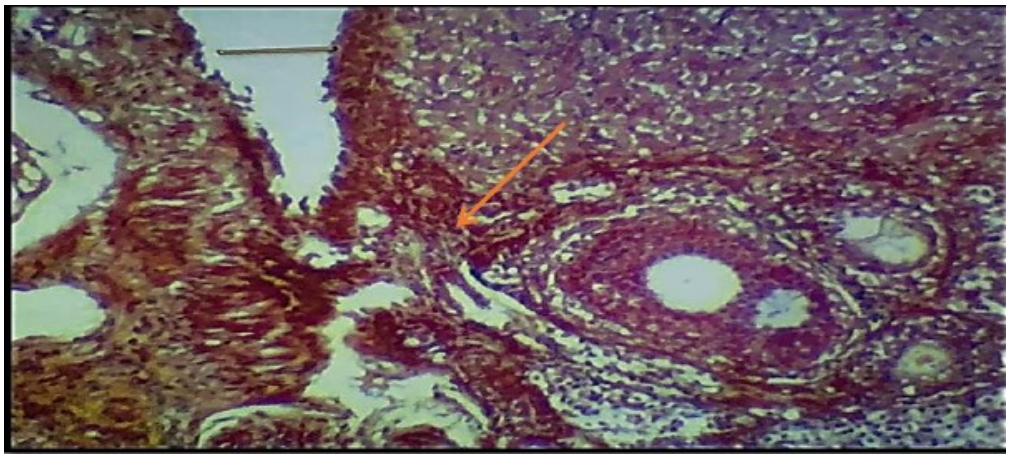

Fig. 2. Ovary Section of 2nd group (TCDD administration) showed mononuclear cells infiltration mostly macrophages and lymphocytes red arrow. (H&E stain) (×400).

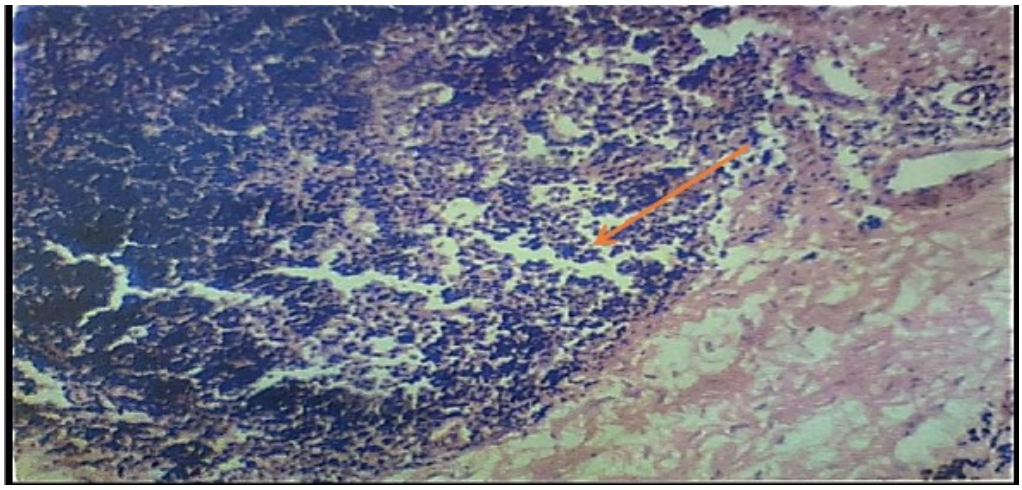

Fig. 3. Uterus Section of 2nd group (TCDD administration) the red arrow showed dark basophilic lymphocytic granuloma. (H&E stain) (×400).

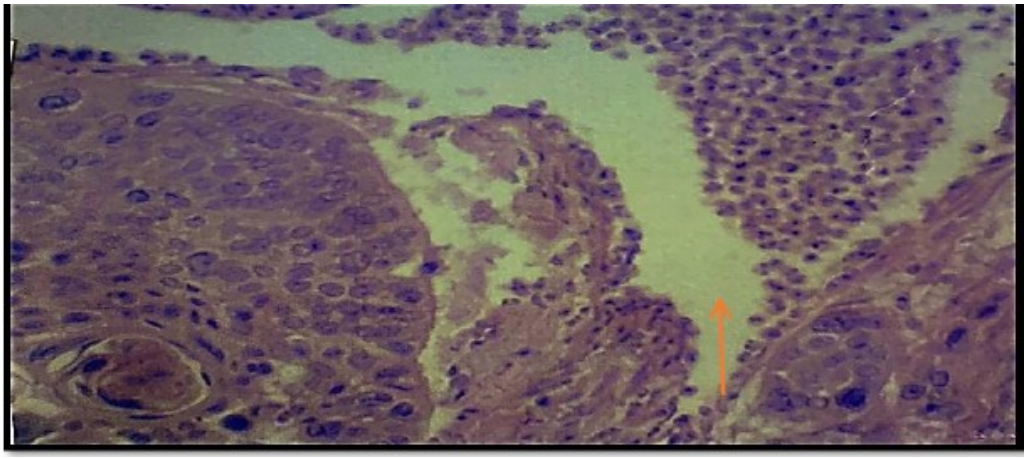

Fig. 4. Uterus section of 2nd group (TCDD administration) the red arrow showed abscess formation. (H&E stain) (×400).

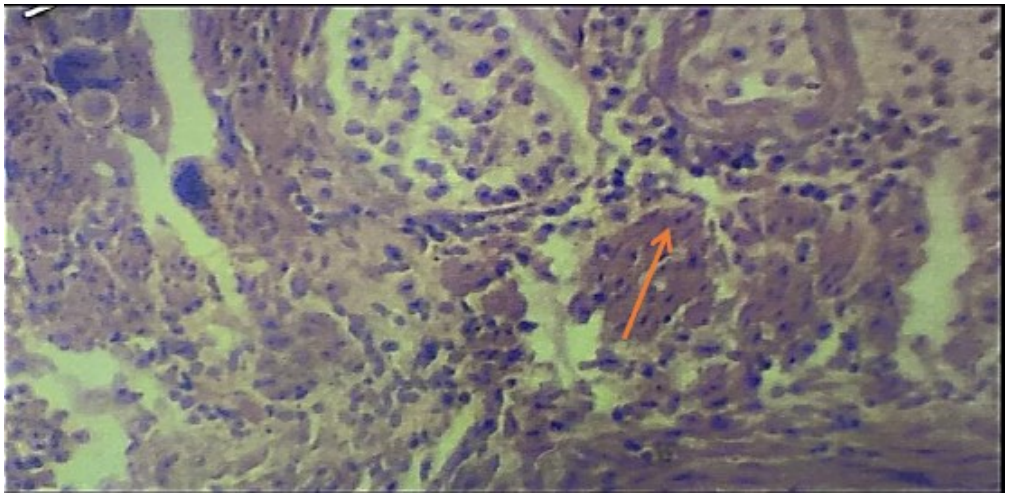

Fig. 5. Uterus section of 2nd group (TCDD administration) the red arrow showed: infiltration of polymorphic cells mostly neutrophils (H&E stain) (×400).

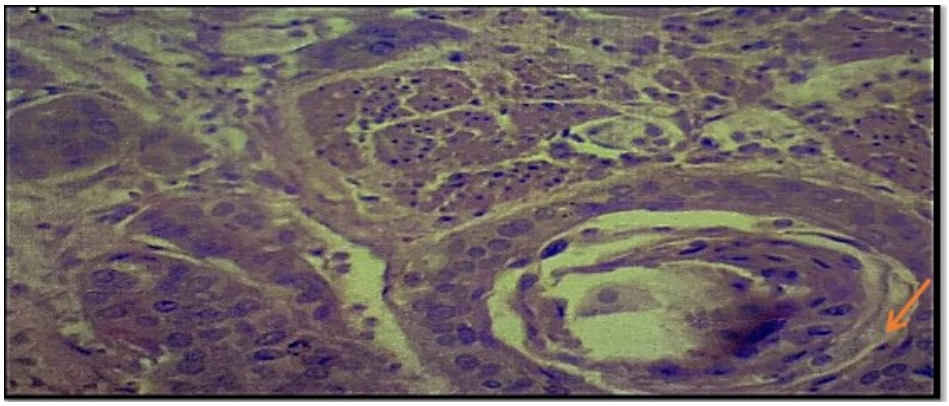

Fig. 6. Uterus section of 2nd group (TCDD administration) the arrow showed endometrial gland, with cystic dilation. (H and E stain) 400 X.

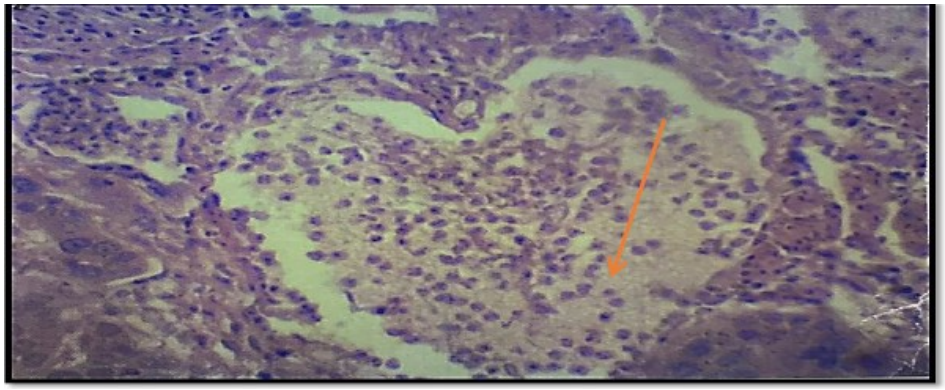

Fig. 7. Uterus section of 2nd group (TCDD administration) the arrow showed large abscess in endometrium consist of dead neutrophil. (H&E stain) (×400).

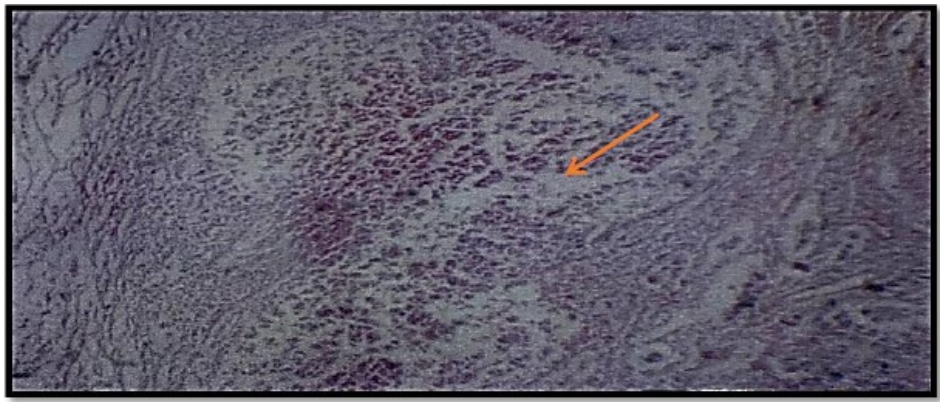

Fig. 8. Uterus section of 2nd group (TCDD administration) the arrow showed endometrium and myometrium hemorrhage and necrosis. (H&E stain) (×400).

Discussion

It has been demonstrated that TCDD and related halogenated aromatic hydrocarbons (HAHS) affect several vertebrate endocrine systems, including sex steroid hormone and its receptors [19, 20]. In the majority of animal species, TCDD and estrogen interact reduced the possibility of a confounding factor in the chicken model and counteracted the impact of exogenous estrogen [21]. In rhesus monkeys, early prenatal exposure to TCDD resulted in abortions. There have been reports of behavioral changes and reduced learning capacities in fish, rats, and monkeys. Rats' hormone and immunological systems were disturbed, and their sexual behavior became more feminine, following TCDD exposure. Moreover, it has been noted that water fleas exposed to TCDD have a reduced ability to reproduce [21]. TCDD has significant negative impacts on the immune system. Similar to mice, even months after

exposure, a single dosage of TCDD (2.5 µg/kg) impaired the immune system as seen by a greater titer of IgM. TCDD has been demonstrated to encourage many cancer forms in a variety of experimental animals, including rats, mice, and hamsters [22]. Dioxin absorbed through the gastrointestinal and excretion from intestine because of their lipophilic nature dioxin accumulate in fat and liver metabolized by oxidation or reductive DE chlorination and conjugation excretion in bile and feces [23]. TCDD has an anti-estrogen impact on the vital site for development and reproductive as the most sensitive end point for dioxin. TCDD expose causes reproductive endocrine disruption [24]. Prolonged exposure at very low doses can damage the immune system. TCDD cause oxidative damage in rat reproductive system by causing necrosis, abscess, hemorrhage and lymphocytic granuloma [25], mostly due to oxidative stress of TCDD causing tissue inflammation and damage. The most delicate negative effects of dioxins are those that arise during the development of various tissues, such as teeth, bones, and sexual organs [26]. When cellular antioxidants are overpowered by reactive oxygen species (ROS), a condition known as "oxidative stress" results. Cells under oxidative stress experience significant cellular damage as a result of lipid oxidation, extensive protein damage, and DNA breakage caused by ROS [3].

Conclusion

The study concludes Dioxin 2 ,3 ,7 ,8 - tetrachlorodibenzo-p-dioxin gave adverse impact on reproductive system through increase the risk of abortion and generated harmful free radical effects directly on the tissues by causing many damage and pathological lesion like necrosis and degenerative changes on cells, moreover the free radical can induced mutation by interact with cell DNA.

Competing Interest

The authors declares that there is no conflict interest.

Author contribution

Hashim M. Obaid: Research article, funding the acquisition and preparing materials, **Qusai Saleh Jumma**: Statistical analysis, review and editing. **Hassan H. Khorshid**: Explain the finding, **Bushra. I. al. Kaisi**: Experiment design.

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تقييم التأثير السام للتلوث البيئي بـ 2،3،7،8- رباعي كلورو ثنائي بنزو ب-ديوكسين (TCDD) على الجهاز التناسلي الأنثوي عن طريق الفحص المرضي والكيميائي الحيوي في إناث الجرذان البيضاء.

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رباعي كلورو ثنائي بنزو ب-ديوكسين (TCDD)، والديوكسين 2، 3، 7، 8، واحد من 75 مادة كيميائية عطرية ثلاثية الحلقات تشكل مخاطر جسيمة ومرتبطة هيكليا، أحد هذه المركبات ثابت في البيئة، ويتراكم في السلسلة الغذائية، و يعمل بشكل سام بطريقة مماثلة. هدف البحث إلى دراسة النتائج السمية المرضية لمرض TCDD في الجهاز التناسلي لأنثى الجرذان البيضاء لغرض الدراسة الحالية. استخدم التصميم التداخلي وقسمت أنثى الجرذ الستين إلى مجموعتين بشكل عشوائي ومتساوي. اعتبرت المجموعة الأولى السيطرة، في حين تلقت المجموعة الثانية الدواء عن طريق الفم لمدة 120 يوما، بجرعة 0.1 ملغم / كغم. بـ-بـ من الديوكسين. تم التضحية بجميع الحيوانات تحت التخدير الطفيف وجمع الدم لإجراء فحوصات كيميائية حيوية خاصة للهرمونات الجنسية والجهاز التناسلي وتم إزالة المبيض والرحم على الفور وفحصهما لتسجيل أي حجم غير طبيعي والتصاق اللون والاتساق. أظهرت النتائج أن الفحص الكيموحيوي أظهر انخفاض معنوي في جميع الهرمونات الجنسية $p<0.05$ وأظهرت الأعضاء التناسلية للمجموعة الثانية تلفاً شديداً تميز بفرط خلوية المبيض في الطبقة الحبيبية وزيادة نمو الجريبات في الطبقة القشرية. يتميز الرحم بتوسع كيسي في غدة بطانة الرحم، ونخر، وخراج في بطانة الرحم وعضل الرحم، ورم حبيبي لمفاوي مع نزيف واسع النطاق في بطانة الرحم وعضل الرحم. تشير هذه النتائج إلى أن تناول TCDD يسبب تأثيرات خطيرة واضحة على الجهاز التناسلي الأنثوي والهرمونات الجنسية والتأثير المرضي على الأعضاء التناسلية.

الكلمات المفتاحية: الديوكسين، التأثير السمي، الجهاز التناسلي.