



## **EFFECTS OF TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) ON BLOOD CONSTITUENTS AFTER SHORT AND LONG TERM ORAL APPLICATION IN ALBINO RATS**

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### **ABSTRACT:**

The deterioration of environmental quality through contamination of air, water, soil and food has existed as a serious problem under the ever-increasing population and industrialization of the society. Dioxins are considered of the most dangerous environmental pollutants that persist and bioaccumulate in different environmental compartments. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was shown to be highly toxic compound to different animal species. The environmental and health effects of this compound which, is a member of a large family of halogenated aromatic hydrocarbons, have been studied. In this study, the effects of TCDD on the hemogram of albino rats have been studied after oral exposure to sublethal doses for short and long term. In the first experiment, rats were once orally intubated with 4.4 µg/kg body weight TCDD in corn oil while in the second one; rats were intubated 0.44 µg/kg body weight TCDD in corn oil day after day for 12 weeks.

Exposure of albino rats to TCDD results in variable degree of anemia as significant decrease in RBCs, Hb and PCV has been recorded in acutely toxicated animals. This decrease indicates microcytic hypochromic anemia in acutely TCDD-orally-exposed rats. Meanwhile, in long term toxicity animals, there was significant increase in RBCs and PCV accompanied with decrease in Hb concentration which indicates macrocytic hypochromic anemia. Total Leucocytic count showed significant decrease in animals acutely or chronically treated with TCDD after 24 hours and till the end of the experiments. These results were accompanied with hypoplasia of bone marrow of the tested animals as significant decrease was recorded in lymphocytes, monocytes and eosinophils count as well as their percentages.

TCDD has myelotoxic effects on bone marrow appeared in the form of hypoplasia as well as apoptosis of its cellularity. Lymphocytes, monocytes, eosinophils and megakaryocytic series were severely affected by feeding TCDD. These effects shown to be time-dependant as it increases with the elongation of the time of exposure. Anemia together with bone marrow affection and other parameters of impairment of hepatic functions are indicative for hematotoxic effects of TCDD.

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### **INTRODUCTION:**

Ecotoxicology is considered as a sequence of interactions and effects. The adverse biological

effects of TCDD in animals include carcinogenesis as well as teratogenesis, reproductive and immune dysfunction. They

occur in very low concentrations in the environment but due to their very high toxicity and physiological activity, particularly of one member of this family, 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), this group of substances has attracted considerable attention. A wide variety of chemicals known to or likely results in the formation of dioxins have been identified. Pesticides have been identified as the most significant group, particularly the phenoxy acetic acid group (2,4-D: 2,4,5-T: silvex and erbon). During the commercial synthesis of these products, a reaction sequences indicating the formation of 2,3,7,8-TCDD has been present (Connell and Miller, 1984).

There is a powerful influence of TCDD exposure on enzyme systems in many organisms. Induction of xoxazolamine hydroxylase enzyme indicates that TCDD has a high probability of being a carcinogen. The effects of TCDD act through receptor-mediated mechanisms as it has its own distinct receptor, the aryl hydrocarbon receptor (AhR) and there is considerable evidence that the biological activity of TCDD is mediated through the Ah receptor (Abbott *et al.*, 1994). TCDD is found throughout the world in practically all media including air, soil, water, sediments and food especially dairy products, meat, fish and shell fish. Human exposure to TCDD may occur through diet food from animal origin as well as from the surrounding environment. Bioaccumulation, biomagnifications and bioconcentration of TCDD and related compounds through food chain play an evident role in its toxicity (Abd El-Nasser *et al.*, 2005). TCDD administration has many adverse effects on hemobiotic system as anemia was the first symptom of its toxicity in various animal species. It induces apoptic cell death in the circulating erythrocytes (Skamoto *et al.*, 1997). Wanda *et al.*, (1998) and Allavain and Gosiewicz

(1999) stated that the adverse effects of TCDD on both RBCs indices including their number, Hb content, PCV and WBCs count. They added that lymphocytes, monocytes, macrophages and neutrophils numbers are also affected. The owed these effects to myelotoxic effects of TCDD on bone marrow which leads to bone marrow hypoplasia as well as apoptosis to its cellularity. Hans *et al.*, (1999) stated that exposure to high doses of TCDD cases drastic decrease in the number and percentage of monocytes and granulocytes. Meanwhile, Chu *et al.*, (2001) recorded many toxic effects of TCDD including significant decrease in Hb contents and PCV in rats exposed to a small dose of it. They also recorded bone marrow toxicity at low doses of TCDD in the form of hypoplasia and depression of colony formation of macrophages granulocytes progenitor cells and pleuripotent stem cells associated with altered lymphopoietic development. Yoon *et al.*, (2001) recorded significant decrease in AhR-mRNA levels in bone marrow and suggested that the biological toxicity of TCDD on bone marrow could be attributed to it.

The present investigation was carried out to through light on the effect of short and long term oral application of dioxins on the blood constituents in albino rats.

## **MATERIALS AND METHODS:**

### **Chemical:**

TCDD (>98%Purity) was obtained from Grey Hound Company Laboratories, England. The compound was dissolved in corn oil as vehicle in a concentration of 10 ml/ kg to keep the dose volume constant.

### **Animals and experimental design:**

160 male albino rats, 150 g weight and 12 weeks age were obtained from the animal house, Faculty of Medicine, Assiut University. Animals were classified into four groups, 40 rats each, the first group was used for the first experiment and the second for the long term experiment, while third and fourth groups were intubated corn oil and kept as parallel control. Drinking water and conventional diet were provided *ad libitum*. In the first experiment, animals were exposed once to 4.4 µg TCDD/kg body weight by gavages. The dosing volume was 10 ml/kg body weight. Blood with anticoagulant (heparin) and bone marrow samples were collected at 12, 24, 48, 72, 96 and 144 hours post exposure (n≥6). In the second experiment, animals received a dose of 0.44 µg TCDD/kg body weight day after day for twelve weeks. Samples were collected 4, 6, 8, 10 and 12 weeks post exposure as well as 2 and 4 weeks after the cessation of TCDD application (n≥5). Animals from the control groups were submitted to the same regimen of sampling for comparison. Erythrocytes and Leucocytic counts, Hb concentration and PCV percentage were determined. Erythrocytes and leucocytic counts, Hb concentration and PCV percentage were determined standard methods of hematology previously described by Coles (1986). Differential Leucocytic count was also taken into consideration as well. Moreover, MCV, MCH, and MCHC were calculated

mathematically. Bone marrow films were prepared, stained and examined according to Winter, (1965).

## RESULTS:

The obtained results are illustrated in the following tables; 1, 2, 3 (A and B), 4, 5 and 6 (A and B). These results indicated that both acute and long term toxicity of TCDD have adverse effects on hemogram and bone marrow cellularity. These effects could be summarized in a significant oligocythaemia represented as decrease in both RBCs and hemoglobin concentration in acute toxicity group of animals in comparison to the control animals. In long term treated group of animals, there was a significant polycythaemia of erythrocytes than the control ones.

There was a significant decrease in WBCs count as well as lymphocytes, eosinophils and monocytes percentages in both acute and long-term toxicities. Bone marrow examination revealed significant increase in metamyeloblasts, promyeloblasts, segmented and band neutrophils series continue till the end of the administration. Restoration of normal values of bone marrow cellularity occurred after 4 weeks from the stoppage of administration in long term toxicated animals and did not occur at all in acutely affected animals as it compared to control groups.

Table (1): Shows the acute effects of TCDD on the hemogram of albino rats

| Time (hours) | Groups  | RBCs (10 <sup>6</sup> /µl) | Hb Conc. (g/dl) | PCV (%)   | MCV (fl)  | MCH (pg)  | MCHC (gm/dl) |
|--------------|---------|----------------------------|-----------------|-----------|-----------|-----------|--------------|
| 12           | Acute   | 7.96±0.32                  | 10.0±0.0        | 36.2±1.1  | 45.4±1.5  | 12.5±0.5  | 27.0±0.9*    |
|              | Control | 8.50±0.86                  | 10.5±0.5        | 37.7±0.8  | 55.8±3.0  | 11.5±1.8  | 20.6±1.0     |
| 24           | Acute   | 5.63±0.21*                 | 06.9±0.8*       | 33.5±0.8  | 59.5±2.9* | 11.5±1.6* | 20.6±1.0     |
|              | Control | 8.89±0.68                  | 10.2±0.3        | 38.3±1.5  | 51.8±1.2  | 09.9±0.8  | 19.2±1.2     |
| 48           | Acute   | 4.85±0.99*                 | 06.6±0.4*       | 32.5±0.8* | 69.4±1.6* | 13.9±1.9* | 20.3±1.5     |
|              | Control | 8.16±0.86                  | 09.7±0.6        | 37.7±0.6  | 54.6±2.0  | 09.8±1.3  | 18.0±1.2     |
| 72           | Acute   | 6.96±0.87                  | 08.6±0.7        | 33.2±1.7* | 56.2±3.0* | 09.2±0.5  | 19.9±1.3     |
|              | Control | 8.20±0.68                  | 10.0±0.5        | 37.7±0.6  | 52.0±1.2  | 09.4±0.3  | 18.0±0.9     |
| 96           | Acute   | 7.96±0.88                  | 08.8±0.5        | 38.8±1.7  | 50.5±4.0* | 10.3±1.4  | 22.1±1.6*    |
|              | Control | 8.20±0.67                  | 10.0±0.5        | 38.3±0.6  | 55.5±5.0  | 10.2±1.2  | 18.4±0.3     |

|     |         |           |           |          |          |          |           |
|-----|---------|-----------|-----------|----------|----------|----------|-----------|
| 144 | Acute   | 7.50±0.54 | 08.2±0.5* | 38.2±1.1 | 50.9±1.4 | 10.9±1.0 | 21.4±1.7* |
|     | Control | 8.10±0.28 | 09.8±0.2  | 38.3±0.6 | 55.0±1.0 | 11.5±1.0 | 18.3±1.9  |

\* Means significance at  $P \leq 0.05$  to  $0.01$  in comparison to control animals.

**Table (2): Shows the acute toxic effects of TCDD on total and differential leucocytic counts of albino rats**

| Time (hours) | Groups  | WBCs ( $10^3/\mu\text{l}$ ) | Lymphocytes (%) | Neutrophils (%) | Eosinophils (%) | Monocytes (%) |
|--------------|---------|-----------------------------|-----------------|-----------------|-----------------|---------------|
| 12           | Acute   | 4.5±0.33                    | 78.3±1.80       | 14.33±1.91      | 3.71±0.75       | 3.66±0.52     |
|              | Control | 4.7±0.76                    | 79.6±0.57       | 12.33±0.59      | 4.45±0.06       | 4.22±0.34     |
| 24           | Acute   | 3.0±0.76*                   | 72.2±1.77*      | 22.32±1.76*     | 2.66±0.81       | 2.82±0.75     |
|              | Control | 5.0±0.50                    | 78.6±1.23       | 14.18±2.35      | 3.56±0.57       | 3.66±0.57     |
| 48           | Acute   | 1.8±0.62*                   | 71.5±1.16*      | 25.12±1.89*     | 1.50±0.16*      | 1.88±0.98     |
|              | Control | 5.0±0.76                    | 78.0±1.22       | 14.33±2.00      | 4.00±0.13       | 3.67±0.57     |
| 72           | Acute   | 1.6±0.24*                   | 71.3±1.25*      | 26.03±1.74*     | 1.12±0.75*      | 1.55±0.53*    |
|              | Control | 5.0±0.62                    | 78.4±1.00       | 14.00±0.16      | 3.46±0.57       | 4.14±0.03     |
| 96           | Acute   | 2.3±0.80*                   | 74.3±0.81*      | 20.34±1.37*     | 2.83±0.65*      | 2.53±0.54*    |
|              | Control | 4.6±0.26                    | 80.0±0.53       | 12.24±0.57      | 4.11±0.22       | 3.65±0.57     |
| 144          | Acute   | 2.8±0.86*                   | 75.2±2.00*      | 18.43±2.11*     | 3.21±0.67       | 3.16±0.75     |
|              | Control | 4.7±0.24                    | 79.0±1.21       | 14.33±2.64      | 3.33±0.56       | 3.34±0.57     |

**Table (3 A): Shows the acute toxic effects of TCDD on Neutrophilic series cells percentages of bone marrow cells of albino rats**

| Time (hours) | Groups  | Myeloblasts | Metamyeloblasts | promyloblast | Segmented cells | Band cells  |
|--------------|---------|-------------|-----------------|--------------|-----------------|-------------|
| 12           | Acute   | 0.80±0.11   | 7.58±0.22       | 5.33±0.38    | 21.23±1.45      | 29.95±0.92  |
|              | Control | 0.81±0.13   | 7.50±0.29       | 5.17±0.15    | 21.40±1.04      | 19.50±1.00  |
| 24           | Acute   | 0.80±0.12   | 7.00±0.24*      | 5.44±0.33    | 22.20±1.55      | 29.50±0.83  |
|              | Control | 0.81±0.15   | 7.61±0.22       | 4.76±0.74    | 21.50±0.98      | 29.46±1.11  |
| 48           | Acute   | 0.81±0.14   | 7.24±0.25       | 5.41±0.33    | 22.58±0.76      | 29.02±1.02  |
|              | Control | 0.81±0.21   | 7.61±0.27       | 5.13±0.32    | 20.63±1.41      | 30.26±1.21  |
| 72           | Acute   | 0.81±0.12   | 7.14±0.29*      | 5.35±0.41    | 21.53±1.33      | 31.13±0.95* |
|              | Control | 0.81±0.11   | 8.58±0.30       | 5.15±0.48    | 20.13±0.72      | 28.66±0.76  |
| 96           | Acute   | 0.81±0.10   | 7.18±0.21*      | 5.23±0.24    | 21.62±1.35      | 29.61±0.71  |
|              | Control | 0.81±0.16   | 7.82±0.20       | 5.32±0.23    | 20.15±0.77      | 27.48±1.11  |
| 144          | Acute   | 0.81±0.22   | 7.32±0.17       | 5.33±0.40    | 21.85±1.12      | 29.21±0.73  |
|              | Control | 0.80±0.20   | 7.21±0.17       | 5.25±0.25    | 21.26±1.21      | 30.53±1.02  |

**Table (3 B): Shows the acute toxic effects of TCDD on eosinophils, lymphocytes, monocytes, RBCs and megakaryocytic series cells percentages of bone marrow of albino rats**

| Time (hours) | Groups  | Eosinophils | Lymphocytes | RBCs        | Monocytes  | Megakaryocytes |
|--------------|---------|-------------|-------------|-------------|------------|----------------|
| 12           | Acute   | 2.29±0.55   | 09.61±0.47  | 20.02±0.71  | 0.67±0.51* | 0.83±0.40*     |
|              | Control | 2.26±0.46   | 09.99±0.55  | 20.67±0.64  | 1.55±0.50  | 1.33±0.57      |
| 24           | Acute   | 1.93±0.45*  | 09.24±0.41  | 16.15±0.83* | 0.54±0.54* | 0.67±0.51*     |
|              | Control | 2.81±0.14   | 09.38±0.57  | 18.83±0.28  | 1.00±0.22  | 1.02±0.29      |
| 48           | Acute   | 1.66±0.22   | 07.55±1.01* | 14.16±0.98* | 0.67±0.55* | 0.33±0.55*     |
|              | Control | 1.99±0.21   | 09.19±0.26  | 19.66±0.57  | 1.52±0.51  | 0.67±0.57      |
| 72           | Acute   | 1.38±0.24*  | 07.96±0.64* | 12.83±0.68* | 0.69±0.53  | 0.50±0.54*     |
|              | Control | 2.86±0.29   | 09.66±0.43  | 20.38±0.07  | 1.00±0.22  | 1.00±0.33      |
| 96           | Acute   | 1.76±0.13*  | 07.98±0.32* | 19.97±1.00  | 0.68±0.52  | 0.83±0.40*     |
|              | Control | 2.35±0.47   | 09.76±0.23  | 20.32±1.51  | 1.00±0.11  | 1.01±0.22      |
| 144          | Acute   | 1.88±0.16*  | 08.43±0.38* | 19.86±0.33  | 0.69±0.56  | 0.83±0.44      |
|              | Control | 2.49±0.44   | 10.11±0.21  | 20.19±0.18  | 1.00±0.08  | 0.67±0.57      |

\* Means significance at  $P \leq 0.05$  to  $0.01$  in comparison to control animals.

**Table (4): Shows the long-term effects of TCDD on the hemogram of albino rats**

| Time (weeks)     | Groups  | RBCs ( $10^6/\mu\text{l}$ ) | Hb Conc. (g/dl) | PCV (%)   | MCV (fl)  | MCH (pg)  | MCHC (gm/dl) |
|------------------|---------|-----------------------------|-----------------|-----------|-----------|-----------|--------------|
| 4 <sup>th</sup>  | Tested  | 11.0±0.49                   | 08.8±0.3*       | 37.6±2.0  | 47.1±3.7* | 10.9±0.8  | 23.3±1.7     |
|                  | Control | 10.0±0.55                   | 10.3±0.5        | 38.0±2.0  | 55.0±3.0  | 10.0±0.6  | 20.3±1.3     |
| 6 <sup>th</sup>  | Tested  | 14.9±1.44*                  | 06.5±0.5*       | 48.8±3.9* | 32.7±1.4* | 04.4±0.5* | 13.4±1.3*    |
|                  | Control | 11.5±2.00                   | 10.0±2.0        | 37.5±2.5  | 52.5±1.5  | 13.0±1.0  | 18.5±0.6     |
| 8 <sup>th</sup>  | Tested  | 16.2±2.75*                  | 07.5±1.0*       | 52.4±2.3* | 32.7±4.3* | 04.7±1.1* | 14.3±2.4*    |
|                  | Control | 10.5±2.22                   | 10.5±0.6        | 38.3±2.0  | 53.1±5.0  | 10.5±1.0  | 19.3±0.5     |
| 10 <sup>th</sup> | Tested  | 15.4±2.06*                  | 06.9±0.9*       | 53.6±1.3* | 35.2±5.0* | 04.4±0.2* | 12.8±1.9*    |
|                  | Control | 10.0±1.21                   | 10.5±0.4        | 39.5±1.0  | 55.5±2.0  | 11.6±0.5  | 20.4±0.4     |
| 12 <sup>th</sup> | Tested  | 12.3±0.82*                  | 07.8±1.0*       | 52.2±1.5* | 42.6±2.2* | 06.4±1.1* | 14.8±2.0*    |
|                  | Control | 10.5±2.09                   | 10.9±1.2        | 38.5±0.3  | 56.0±3.3  | 12.5±1.2  | 19.9±2.0     |
| 14 <sup>th</sup> | Tested  | 12.6±0.83*                  | 06.0±0.5*       | 51.0±1.1* | 40.5±2.2  | 04.6±0.3* | 11.7±0.2*    |
|                  | Control | 10.5±1.07                   | 10.5±0.9        | 38.0±1.2  | 54.5±3.0  | 10.6±1.0  | 18.5±1.3     |
| 16 <sup>th</sup> | Tested  | 10.4±0.65                   | 08.9±0.8        | 41.8±1.2  | 40.0±2.1  | 08.5±0.9* | 21.5±3.2     |
|                  | Control | 10.3±0.63                   | 09.9±0.9        | 38.0±1.0  | 52.5±1.3  | 10.6±0.5  | 20.0±3.0     |

**Table (5): Shows the long-term toxic effects of TCDD on total and differential leucocytic counts of albino rats**

| Time (weeks)     | Groups  | WBCs ( $10^3/\mu\text{l}$ ) | Lymphocytes (%) | Neutrophils (%) | Eosinophils (%) | Monocytes (%) |
|------------------|---------|-----------------------------|-----------------|-----------------|-----------------|---------------|
| 4 <sup>th</sup>  | Tested  | 5.2±0.72                    | 78.8±1.80       | 18.65±0.54*     | 3.88±0.44       | 3.87±0.44     |
|                  | Control | 5.0±0.18                    | 79.0±0.57       | 13.66±0.89      | 3.68±0.54       | 3.45±0.89     |
| 6 <sup>th</sup>  | Tested  | 1.7±0.25*                   | 61.2±1.80*      | 40.83±2.44*     | 1.84±0.83*      | 1.64±0.98*    |
|                  | Control | 5.0±0.83                    | 78.4±0.57       | 14.64±1.11      | 3.48±0.89       | 3.66±0.54     |
| 8 <sup>th</sup>  | Tested  | 2.2±0.65*                   | 59.4±1.77*      | 38.62±3.46*     | 2.45±0.89*      | 1.83±0.44*    |
|                  | Control | 4.8±0.67                    | 79.5±1.23       | 13.63±3.05      | 3.68±0.54       | 3.41±0.54     |
| 10 <sup>th</sup> | Tested  | 1.6±0.41*                   | 55.6±1.16*      | 40.56±4.81*     | 1.84±0.44*      | 2.11±0.89*    |
|                  | Control | 5.1±0.49                    | 79.0±1.22       | 13.57±3.86      | 3.26±0.83       | 3.68±0.83     |
| 12 <sup>th</sup> | Tested  | 1.6±0.73*                   | 54.3±1.25*      | 43.21±6.73*     | 1.24±0.09*      | 1.77±0.97*    |
|                  | Control | 4.8±0.50                    | 78.0±1.00       | 14.27±2.86      | 3.80±0.44       | 3.24±0.54     |
| 14 <sup>th</sup> | Tested  | 1.7±0.65*                   | 56.4±0.81*      | 39.28±4.37*     | 1.65±0.89*      | 2.03±1.00*    |
|                  | Control | 5.1±0.56                    | 78.6±0.53       | 13.43±5.06      | 3.65±0.54       | 3.62±0.54     |
| 16 <sup>th</sup> | Tested  | 2.8±0.43*                   | 62.5±2.00*      | 32.02±3.31*     | 2.89±0.44       | 2.44±0.54*    |
|                  | Control | 4.7±0.59                    | 78.7±1.21       | 14.83±3.06      | 3.40±0.54       | 3.42±0.45     |

**Table (6 A): Shows the long-term toxic effects of TCDD on Neutrophilic series cells of bone marrow of albino rats**

| Time (weeks)     | Groups  | Myeloblast | Metamyeloblast | Promyeloblast | Segmented cells | Band cells  |
|------------------|---------|------------|----------------|---------------|-----------------|-------------|
| 4 <sup>th</sup>  | Tested  | 0.90±0.15  | 8.33±0.41*     | 6.21±0.25*    | 24.94±1.25*     | 36.55±1.13* |
|                  | Control | 0.81±0.13  | 7.35±0.45      | 4.92±0.39     | 22.08±1.03      | 29.23±0.86  |
| 6 <sup>th</sup>  | Tested  | 0.97±0.32  | 9.00±0.35*     | 7.12±0.22*    | 26.54±1.35*     | 40.33±1.45* |
|                  | Control | 0.81±0.14  | 7.35±0.48      | 5.07±0.25     | 21.08±1.60      | 29.72±1.28  |
| 8 <sup>th</sup>  | Tested  | 0.98±0.21  | 9.00±0.24*     | 7.75±0.22*    | 27.15±1.19*     | 41.05±1.42* |
|                  | Control | 0.81±0.13  | 7.32±0.38      | 5.03±1.43     | 22.48±0.78      | 30.19±1.28  |
| 10 <sup>th</sup> | Tested  | 0.95±0.02  | 8.86±0.24*     | 9.05±0.65*    | 28.34±1.96*     | 38.72±1.69* |
|                  | Control | 0.81±0.15  | 7.47±0.29      | 5.27±0.25     | 21.06±0.89      | 30.18±0.93  |
| 12 <sup>th</sup> | Tested  | 0.98±0.11  | 8.76±0.43*     | 7.92±0.36*    | 27.72±1.90*     | 38.72±1.65* |
|                  | Control | 0.82±0.12  | 7.10±0.23      | 5.27±0.21     | 21.80±0.68      | 29.84±0.71  |
| 14 <sup>th</sup> | Tested  | 0.86±0.13  | 7.70±0.31      | 6.28±0.58*    | 22.65±1.50      | 29.63±0.90  |
|                  | Control | 0.81±0.13  | 7.41±0.31      | 5.23±0.24     | 21.35±1.00      | 28.32±1.02  |
| 16 <sup>th</sup> | Tested  | 0.82±0.19  | 6.92±0.41      | 5.72±0.56     | 21.99±1.64      | 29.19±1.12  |

|  |         |           |           |           |            |            |
|--|---------|-----------|-----------|-----------|------------|------------|
|  | Control | 0.83±0.11 | 7.28±0.23 | 5.33±0.43 | 21.80±0.71 | 28.85±0.78 |
|--|---------|-----------|-----------|-----------|------------|------------|

\* Means significance at P ≤ 0.05 to 0.01 in comparison to control animals.

Table (6 B): Shows the long-term toxic effects of TCDD on eosinophils, lymphocytes, monocytes, RBCs and megakaryocytic series cells of bone marrow of albino rats

| Time (weeks)     | Groups  | Eosinophils | Lymphocytes | RBCs        | Monocytes  | Megakaryocytes |
|------------------|---------|-------------|-------------|-------------|------------|----------------|
| 4 <sup>th</sup>  | Tested  | 1.77±0.43*  | 08.22±0.57  | 22.52±1.54  | 0.40±0.54* | 0.41±0.54*     |
|                  | Control | 2.48±0.47   | 09.74±0.61  | 19.87±0.58  | 1.28±0.44  | 1.22±0.44      |
| 6 <sup>th</sup>  | Tested  | 1.16±0.22*  | 06.87±0.27* | 27.45±2.00* | 0.64±0.54* | 0.26±0.44*     |
|                  | Control | 2.34±0.48   | 09.00±0.26  | 19.23±0.66  | 1.00±0.06  | 1.01±0.08      |
| 8 <sup>th</sup>  | Tested  | 0.84±0.47*  | 06.42±0.96* | 30.25±2.02* | 0.64±0.45* | 0.44±0.54*     |
|                  | Control | 2.60±0.45   | 09.88±0.23  | 20.03±0.97  | 1.00±0.22  | 1.00±0.03      |
| 10 <sup>th</sup> | Tested  | 0.60±0.54*  | 05.64±0.65* | 42.25±2.33* | 0.43±0.04* | 0.22±0.44*     |
|                  | Control | 2.31±0.40   | 09.59±0.52  | 20.65±0.85  | 1.21±0.44  | 1.21±0.44      |
| 12 <sup>th</sup> | Tested  | 0.20±0.44*  | 06.06±0.35* | 39.62±2.12* | 0.47±0.54* | 0.20±0.43*     |
|                  | Control | 2.31±0.39   | 09.49±0.61  | 21.07±0.74  | 1.26±0.75  | 1.00±0.70      |
| 14 <sup>th</sup> | Tested  | 2.19±0.44   | 08.00±0.54  | 25.38±1.00  | 0.75±0.67* | 0.88±0.54      |
|                  | Control | 2.62±0.43   | 10.37±0.77  | 21.93±0.48  | 1.24±0.75  | 1.13±0.44      |
| 16 <sup>th</sup> | Tested  | 2.11±0.28   | 09.20±0.25  | 20.82±2.12  | 1.13±0.22  | 0.86±0.54      |
|                  | Control | 2.21±0.43   | 10.00±0.59  | 21.68±1.33  | 1.32±0.41  | 1.00±0.86      |

\* Means significance at P ≤ 0.05 to 0.01 in comparison to control animals.

## DISCUSSION:

Dioxins are a class of substances never intentionally released to the environment which are formed as a result of contamination of commercial chemical products. Dioxins can be formed as combustion product from burning vegetation treated with phenoxy acetic acid herbicides 2,4,5-T and 2,4-D. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a wide spread environmental contaminant that produces adverse biological effects including haematotoxicity. Contamination of food and water by these compounds through bioaccumulation in the food chain may lead to harmful effects in both man and animal (Kreuzer *et al.*, (1997). As detailed below, this study found that microcytic and macrocytic hypochromic anemia have been recorded following acute and long term TCDD oral exposure. Anemia could be attributed to apoptotic cell death in circulating erythrocytes that induced by acute toxic effects of TCDD. Diminished life span of erythrocytes in acute toxicity as the bone marrow can not compensate

the increased rate of destruction. This concept was based upon the myelotoxic effects on the bone marrow which resulted in a significant decrease and hypoplasia of erythrocytes series. Bone marrow examinations at the level of erythrocyte series confirm this concept. Similar results were recorded by Allavain and Gosiewcz (1999) who found that TCDD associated with dose-dependant cases decreases in total number of hematopoietic cells in bone marrow. This concept was explained by Wanda *et al.*, (1998) who stated that TCDD mainly has Ah-receptor mediated myelotoxic effects. This action consists of DNA fragmentation and cell death by Ca<sup>2+</sup> dependant protein synthesis pathway. Anemia could also be attributed to TCDD induced oxidative stress through its effect on AhR mediated pathway, which lead to hematotoxicity resulted in disruption of redox regulation (Yoon *et al.*, 2001) or induction of  $\alpha$ -aminolevulinic acid synthetase which is the initial and rate-limiting enzyme in heme synthesis (Conolly and Anderson 1991).

However, the increased number of RBCs and their cell volume in long term toxicity of TCDD is attributed to the compensatory mechanism of bone marrow to regenerate the destructed cells in peripheral blood and converse the toxic effect on the erythrocytes series. Jubb and Kennedy (1995) attributed the increase in the number of circulating erythrocytes to the hypoxia of the tissues which stimulates the physiological compensation of bone marrow to produce new circulating erythrocytes. Macrocytosis was attributed to the release of immature erythrocytes into the blood stream with low hemoglobin contents, these cells were larger than the mature ones and accompanied with intense and increase in erythrocytes series of bone marrow. These effects seem to be time and dose dependent as restoration to normal values was occurred 96 to 144 hours in acute toxicity and 4 weeks after the stoppage of TCDD administration in long-term toxicity.

Total white blood cells counts showed significant decrease in all exposed animals as a result of TCDD administration. Lymphocytes, monocytes and eosinophils percentages revealed significant decrease as well, while neutrophils percentage showed significant increase in long-term treated group of mice. Hochstein et al., (1998) reported that TCDD was responsible for a significant decrease in WBCs counts. Hans *et al.*, (1999) reported that exposure to high doses of TCDD induced a decrease in both monocytes and granulocytes percentages. The toxic effects of TCDD on total and differential leucocytic count are attributed to its effects on bone marrow as it has a myelotoxic effects. Marrow hypoplasia and apoptosis of bone marrow cells are the major toxic effects as mentioned by Allavain and Gosiewicz (1999). Bone marrow examination revealed significant decrease in lymphocytic, eosinophilic, and megakaryocytic

series while neutrophilic series including myeloblast, metamyeloblast, promyeloblast, segmented and band cell series showed significant increase. These effects on the bone marrow could be attributed to the direct effect of TCDD on AhR as Allavain and Gosiewicz (1999) proved that bone marrow stromal cells express functional AhR which plays an important role in the support and direction of lymphopoiesis. They added that TCDD treatment could alter lymphopoietic development, resulting in decrease of the total number of hematopoietic cells and lymphocytes. Restoration of normal values of total Leucocytic count, lymphocytes, neutrophils, eosinophils and monocytes percentages has not been achieved till the 96 hours after acute toxicity; while it was achieved four weeks after the stoppage of long term toxicity. Bone marrow cells retrain its normal vales also after the stoppage of toxicity.

Severe lymphopenia recorded in this study indicates the immunotoxic effects of TCDD as immunosuppression in exposed animals and increased liability to infection. Neutrophilia, recorded only in long-term exposure was not related to direct effect rather than as a compensatory response to the recoded lymphopenia. Anemia occurs in combination with leucopenia is an indication of the depression of the bone marrow following the application of TCDD. Bone marrow is the tissue composed of rapidly dividing cells and TCDD by its toxic action interferes with nucleic acid metabolism and inhibits nucleic acid synthesis and cell division or maturation. Marrow or hematopoiesis depression seems to be temporary even after the very small doses of TCDD as it returns to its normal values after the stoppage of application. In conclusion, this study demonstrated that albino rats exposed to TCDD in acute and long-term toxicities

developed variable degrees of anemia and hypoplasia of the bone marrow compartments.

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## تأثير الديوكسين على مكونات الدم في الفئران البيضاء بعد التعرض عن طريق الفم لفترات قصيرة وطويلة

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شهد العالم خلال الأربعين سنة الماضية زيادة دراماتيكية في تصنيع الكيماويات العضوية التي تحتوي على الكلور والبولي فنييل (البلاستيك) وتعد استخدامها. من أهم هذه المواد المبيدات الحشرية ومبيدات الحشائش والأعشاب والبلاستيك الذي يصنع من مركب الكلوريد عديد الفينائل. وعندما تحرق هذه المركبات والمنتجات البلاستيكية ينتج الديوكسين كمركب ثانوي، ومنها ينتشر على الهواء وذرات التراب الهائلة ليحط على المحاصيل الزراعية التي يتغذى عليها الحيوان والإنسان. هذه الدراسة تهدف إلى بحث الآثار الصحية الضارة لمركب الديوكسين على مكونات الدم، وذلك في حالات التعرض لهذا المركب لفترات قصيرة، وبشكل حاد أو لفترات طويلة وبشكل مزمن. يعتبر الديوكسين من أكثر المواد ثباتاً في البيئة التي يوجد بها ويعد مركب الـ 2,3,7,8-رباعي الكلور ثنائي البنزين ديوكسين من أخطر هذه المركبات سمية ويسمى اختصاراً TCDD. وقد تمت دراسة التأثيرات السامة للديوكسين على الدم ونخاع العظام ومكوناتهما، وذلك اثر تعرض الفئران البيضاء لجرعة واحدة من الديوكسين مذابة في زيت الذرة مقدارها 4.4 ميكروجرام/كيلوجرام من وزن الحيوان لمدة ستة أيام أو عدة جرعات (0.44 ميكروجرام/كيلوجرام) يوم بعد يوم لمدة 12 أسبوع عن طريق الفم باستخدام أنبوب اللي المعدي الخاص بالفئران. كما تم الاحتفاظ بمجموعة ضابطة من الحيوانات للمقارنة (أعطيت زيتاً فقط).

تم أخذ عينات الدم باستخدام مانع التجلط الهيبارين بعد 12، 24، 48، 72، 96، 144 ساعة من المعاملة في مجموعة التسمم الحاد، وبعد 4، 6، 8، 10، 12، 14، 16 أسبوع في مجموعة التسمم طويل المدى، وكذلك تم عمل شرائح خلوية من نخاع العظام في نفس التوقيت. وذلك لمعرفة عدد كرات الدم الحمراء والبيضاء والنسبة المئوية لكل نوع وقياس تركيز الهيموجلوبين في الدم والمحتوى الخلوي منه لكرات الدم الحمراء. أظهرت الدراسة انخفاضاً معنوياً في عدد كرات الم الحمراء وتركيز الهيموجلوبين بها، وكذلك الحجم الخلوي مما يشير إلى حدوث أنيميا في هذه الحيوانات. كذلك سجلت الدراسة انخفاضاً شديداً في العدد الكلي لكرات الدم البيضاء وكذلك النسبة المئوية لكل نوع منها على حده مما يرجح نقص المناعة في هذه الحيوانات.

أظهرت نتائج فحص خلايا نخاع العظم انخفاضاً معنوياً في الخلايا المكونة له مما يؤكد عجز النخاع عن تعويض العجز الحادث في كرات الدم الناتج عن تعرض الفئران للديوكسين واستمرار حالة الأنيميا مع استمرار التعرض للديوكسين. ومن مجمل ما رصد في هذه الدراسة من آثار سامة لمادة الديوكسين. نخلص إلى ضرورة تفادي وجود هذه المادة ولو بأجزاء صغيرة جداً في طعام الحيوان والإنسان على حد سواء حيث لا توجد حدود دنيا للكمية المجدثة لهذه الآثار. وكذلك التأكد من عدم وجوده في المواد الغذائية المستوردة من الخارج والتوقف فوراً عن حرق النفايات العضوية والبلاستيكية في محارق مكشوفة والتوعية بمخاطر ذلك على صحة المواطنين.

ومما هو جدير بالذكر أنه من مجمل ما رصد في هذا البحث وغيره من الأبحاث السابقة عن هذه المادة الكيماوية المسماة بالديوكسين أنه عندما تصل مستوياته داخل دهون الجسم إلى قيم بسيطة أقل من أجزاء من الترليون يصبح مادة شديدة الفتك بالإنسان أو الوراثية والوظيفية للخلية، وبالتالي يسبب مدى واسعاً من الآثار المفجعة والتي تصيب الحيوان، وذلك عن طريق الارتباط بمستقبلات الهرمونات في الخلايا مما يجعله يعدل أو يغير من الميكانيكية تتراوح بين السرطان وضعف المناعة إلى اعتلال الجهاز العصبي إلى الاجهاض وتشويههم المواليد حيث أنه لا توجد حدود دنيا لهذا المركب الكيماوي.