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**RESEARCH ARTICLE**

# Hematotoxicity induced by sub-chronic dose of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Swiss albino mice (*Mus musculus)*

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## *Abstract*

Present study was designed to determine the acute oral toxicity ($LD_{50}$) and investigate the effect of sub-chronic dose (0.5µg/Kg body wt.) of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) on the haematological parameters in Swiss albino mice. The $LD_{50}$ was found 5.087 µg/Kg body wt. in Swiss albino mice. The effect of sub-chronic dose of TCDD on the haematological parameters showed statistically very significant ($P$<0.01) decrease in the levels of RBC (red blood cell), Hb (Haemoglobin), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration), and HCT (Hematocrit). Whereas, statistically significant ($P$<0.05) increase in the level of WBC (White blood cell) was observed as compared to control group. The research finding suggests that exposure of sub-chronic dose of TCDD significantly altered the haematological parameters, which is relevant to risk evaluation of TCDD that may be used as a predictive values for its toxicity in mammalian system including human being.



## INTRODUCTION

The presence of toxic pollutant in environment is the result of increased anthropogenic activity. Among the pollutant released, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a widespread, persistent, and highly toxic. It is formed as an unintentional by-product of incomplete combustion and various chemical processes (Ziegler, 1997). Major sources of environmental dioxin pollution are herbicide and fungicide manufacturing, the paper and cellulose industry, thermal reactions of chlorinated aromatic compounds, and transformer or condenser break-down (Kulkarni et al., 2008). It is persistent, slowly degradable in environment, and generates toxicity for a longer duration of time (ten-Tusscher et al., 2000). It causes adverse effect like haematological disturbances, malignancy, congenital malformation, and immune suppression etc. (Vos et al., 1997; Neubert et al., 1993) at different doses and at different period of time. It has also been reported that acute and sub-acute exposure of TCDD on mammal causes undesirable effects like hepatotoxicity, teratogenicity, interference with lipid metabolism, chloracne, neurobehavioral disturbance, endocrine disruption, wasting syndrome, and reproductive toxicity (Schecter et al., 2006; Senft et al., 2002; Pluim et al., 1994).

Analysis of blood parameters is relevant to risk evaluation as the predictive value for toxicity. Haematological parameters analysis have been widely used in the diagnosis of a range of diseases induced by industrial compounds,

drugs, dyes, heavy metals, pesticides, and several others (Badraoui et al., 2011; Olson et al., 2000), as blood being a medium for the transport of intercellular and intracellular materials.

Therefore, in the present study, an effort has been made to determine acute oral toxicity ($LD_{50}$), and sub-chronic exposure of TCDD on some of the haematological parameters in Swiss albino mice (*Mus musculus*) such as red blood cell (RBC) and white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haemoglobin (Hb), and hematocrit (HCT) were monitored.

## Material and Methods

**Test Chemicals**

The test chemical, TCDD (2,3,7,8-Tetrachlorodibenzo-*p*-dioxin) was purchased from Accustandard, USA (CAS 1746-01-6; molecular weight, 321.9; purity, 99% as analyzed by GC-MS). It was dissolved in corn oil (Sigma Chemical Co., St. Louis and Neishel chemical Pvt. Ltd., India) which was used as vehicle.

**Experimental animal**

Healthy Swiss albino mice of both sexes, weighing between 30-35g were obtained from a random-bred colony at the Mahavir Cancer Sansthan, Patna, Bihar, India. They were maintained under the optimal condition at the temperature (24 ±1°C), humidity (55 ± 5%), and lighting (12-h light/dark cycle). Food and tap water were given *ad libitum* during the study. Study were carried out as per CPCSEA guidelines (Approval No.-1129/bc/07/CPCSEA).

**Acute toxicity determination ($LD_{50}$)**

Six experimental groups of mice have been formed with six mice in each group. The groups were numbered as Group-1 to Group-6. All the groups were treated at different concentration of TCDD for seven consecutive days. The distribution of doses to each treated groups has been summarized as: Group-I (10µg/Kg body wt.), Group-II (8µg/Kg body wt.), Group-III (6 µg/Kg body wt.), Group-IV (3 µg/Kg body wt.), Group-V (2µg/Kg body wt.), and Group-VI (1µg/Kg body wt.).

**Sub-chronic toxicity study**

Based on the finding of $LD_{50,}$ sub-chronic dose was selected as 0.5µg/Kg body wt. and administered to the mice for 2 and 4 weeks. Treatment volume was determined on the basis of body weight. After acclimatization for a week experimental mice were again divided into three groups and classified as: Group-I (Normal) received food and tap water *ad libitum*, Group-II (Control) received corn oil in addition to food and tap water *ad libitum*, and Group-III (Treated) received sub-chronic (0.5µg/Kg body wt.) dose of TCDD dissolved in corn oil (vehicle) in addition to food and tap water *ad libitum*. Each group consists of six mice and repetation was made three times.

**Haematological Parameters**

Blood samples were collected from normal, control and sub-chronic dose of TCDD administered groups of mice at intervals of 2 and 4 weeks by ocular vein puncture for the determination of haematological parameters. The estimation of the levels of red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and hematocrit (HCT) were done by using EDTA (ethylenediaminetetraacetate) as an anticoagulant. Haematological parameters were assessed using Cell Counter (Medonic M-16 Series) in the Department of Haematology, Mahavir Cancer Sansthan, Patna (Bihar).

**Statistical analysis:** Data was analyzed and experimental values ware expressed as the mean ± SEM and *P* value was calculated using one way analysis of variance (ANOVA) by using SPSS (11.5 version) software. $P<0.05$ was considered statistically significant and $P<0.01$ was considered statistically very significant.

## Result and Discussion

In the present study the acute oral toxicity ($LD_{50}$) of the TCDD was found to be 5.087 µg/Kg body wt. using Probit analysis (EPA PROBIT ANALYSIS PROGRAM, used for calculating LC/EC value, version 1.5) (Table-1). However, acute oral toxicity study in rat was found to be 20 µg/Kg body wt. (NTP, 2000).

The present study indicates the hematotoxic effects of sub-chronic dose of TCDD in Swiss albino mice. The decrease in the levels of RBC, Hb, MCV, MCH, MCHC and HCT were observed and found to be statistically very ($P$<0.01) significant (Table-2). Similar kind of reduction in the haematological parameters due to TCDD has also been reported in a study on human, guinea pigs, female rats, Zebrafish, goats, and mice (Eraslan et al., 2009; Uboh et al., 2012; Li et al., 2010; Belair et al., 2001; Carney et al., 2006; Leijs et al., 2008; Chu et al., 2007). Decrease in the levels of RBC, Hb, and HCT have also been observed in the case of other persistent organic pollutant due to TBDD dioxin in both sexes of rats (Seigo et al., 2006).

Decrease in the levels of RBC and Hb may be due to the altered haematopoiesis (Badraoui et al., 2011), that results in excessive destruction or decrease in the synthesis of RBC. As a result of destruction of RBC, free radicals are generated (Fibach et al., 2008; Stohs, 1990; Bassem et al., 2008) and caused oxidative stress. Free radicals induced the production of reactive oxygen species, reactive nitrogen species, and lipid per-oxidation (Zahran, 1997). This ultimately results into RBC cell membrane damage, and caused anemia (Helliwell and Mediline, 1994). Disorders of erythropoiesis and Hb synthesis may be the consequence of the endocrine disorders (Geusau et al., 2001).

A very significant ($P$<0.01) decrease in levels of MCV, MCH, and MCHC were observed in this study. The decrease in the levels of MCH, MCHC and MCV may be due to the failure in blood osmoregulation and plasma osmolarity (Pohjanvirta et al., 1989). The levels of MCH, MCHC, and MCV found in this study represent the characteristic features of anaemic condition in the treated groups.

However, observation shows that the level of WBC increased significantly ($P$<0.05) in the sub-chronic dose of TCDD administered groups (Group-III) as compare to control Group-II. It may be due to an increase in phagocytic activity towards destructive RBCs, resulting into activation of immune system of the body by innate defence capability of mice against toxins (TCDD). Reduction in erythrocytes and increase in WBC possibly directly associated with marked increase in aryl hydrocarbon receptor (AhR) expression to mediate toxic effect (Crawford et al., 1997). Haematological alterations have also been observed due to Dioxin (TCDD) in human with the decreased level of erythrocytes and increased level of WBC (Geusau et al., 2001).

From the present investigation it can be inferred that at low dose and at less time duration, TCDD is capable of inducing significant alteration in haematological parameters of the experimental animal model Swiss albino mice.

**Table-1:** Environmental Protection Agency PROBIT analysis program used for calculating LC/EC values (Version 1.5) for TCDD

| Concentration of TCDD | No of subject | Observed response | Expected response |
|---|---|---|---|
| 10 µg/Kg body wt. | 6 | 5 | 4.722 |
| 8 µg/Kg body wt. | 6 | 4 | 4.219 |
| 6 µg/Kg body wt. | 6 | 3 | 3.463 |
| 3 µg/Kg body wt. | 6 | 2 | 1.601 |
| 2 µg/Kg body wt. | 6 | 1 | 0.814 |
| 1 µg/Kg body wt. | 6 | 0 | 0.166 |
| Estimated LC/EC 50.0 of TCDD was 5.087 µg/Kg body wt. | | | |

**Table-2:** Effect of oral administration of sub- chronic dose of TCDD on haematological profile of Swiss albino mice

| Sl.No. | Haematological parameters | Group-I | 2 Weeks | | 4 Weeks | |
|---|---|---|---|---|---|---|
| | | | Group-II | Group-III | Group-II | Group-III |
| 1. | RBC($10^6$/$mm^3$) | 9.106±0.078 | 9.32±0.116 | 8.306**±0.041 | 9.20±0.11 | 7.24**±0.21 |
| 2. | MCV(µ$m^3$) | 41.06±0.118 | 41.52±0.216 | 37.90**±0.087 | 41.335±0.1321 | 35.9**±0.104 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3. | WBC($10^3$/$mm^3$) | 5.50±0.097 | 5.329±0.023 | 9.385*±0.090 | 5.267±0.021 | 13.2*±0.042 |
| 4. | Hb(g/dl) | 14.43±0.176 | 14.54±0.23 | 9.383**±0.081 | 14.9±0.012 | 8.89**±0.042 |
| 5. | MCH(Pg) | 15.23±0.012 | 15.02±0.094 | 14.18**±0.056 | 15.12±0.024 | 11.2**±0.031 |
| 6. | MCHC(g/dl) | 36.2±0.023 | 34.0*±0.035 | 30.62**±0.065 | 33.46*±0.036 | 31.43**±0.11 |
| 7. | HCT(%) | 38.42±0.012 | 38.64±0.175 | 31.45**±0.116 | 37.1*±0.85 | 29.6**±0.42 |

Route of administration: oral; values expressed as mean±SEM (n=6); *Significant ($P$<0.05): **very significant ($P$<0.01) as compared to control (Group-II); RBC: Red blood cell; MCV: Mean corpuscular volume; WBC: White blood cell; Hb: Hemoglobin; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; HCT: Hematocrit; Group-I: normal; Group-II: control (corn oil treated); Group-III: treated (sub-chronic dose of TCDD).

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