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Toxicity evaluation of dioxin-contaminated animal-origin foods from an Indian industrial zone in male Wistar rats

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Dietary intake of animal-origin foods (AOF) is the primary pathway for non-occupational human exposure to dioxins and dioxin-like polychlorinated biphenyls (dl-PCBs). Despite known health risks, the specific toxicological impacts of consuming locally contaminated foods remain understudied. This study evaluated the toxicity associated with the consumption of dioxin-contaminated eggs (15 pg TEQ/g), milk (10 pg TEQ/g), and fish (3 pg TEQ/g) sampled from semi-urban areas in South India. Male Wistar rats were utilised as model organisms and divided into sub-acute (n=20) and sub-chronic (n=40) exposure phases. Pathological, haematological, immunological, biochemical, and oxidative stress parameters were systematically analysed across dosing groups. The group dosed with 10 pg TEQ/g exhibited dyslipidemia—characterised by elevated cholesterol and LDL alongside decreased HDL—and concurrent elevations in SGOT, globulin, and urea. The low-dose group (3 pg TEQ/g) demonstrated similar hepatic strain, paired with elevated oxidative stress markers, including CAT and 8-OHdG. In the 15 pg TEQ/g and 10 pg TEQ/g groups, haematological deviations were observed. Biochemical profiles revealed persistent elevations in cholesterol, SGPT, and urea. Furthermore, a marked suppression of glutathione reductase activity and a decline in serum testosterone levels were observed. These findings underscore the critical need for epidemiological surveillance and long-term risk assessment in hot spot areas.

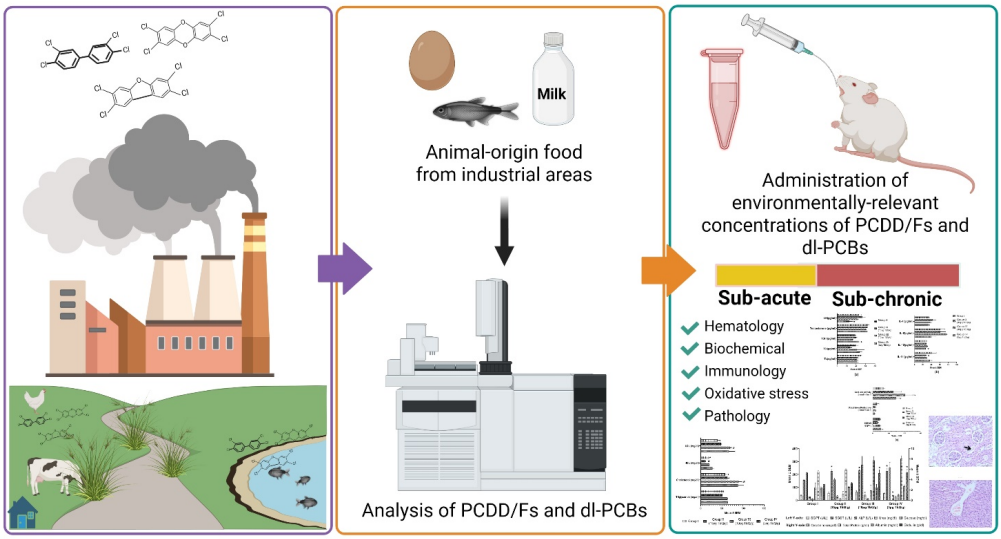
Keywords: Animal-origin foods (AOF), Dioxins, Food contamination, Sub-chronic toxicity, Wistar rats

Animal-origin foods (AOFs), also referred to as animal-source, animal-derived, or foods of animal origin, are foods obtained wholly or partly from animals. They typically include meat and meat products, milk and dairy products, eggs and egg products, and fish and seafood, with dairy sometimes classified as a distinct subgroup within this category[1-4]. These foods are nutrient-rich components of the human diet as they provide good-quality protein, micronutrients, including vitamin B 12, vitamin D, vitamin A, iron, zinc, calcium, iodine, selenium, and long-chain omega-3 fatty acids, which are vital for growth, brain development, a healthy immune system, and overall well-being[2]. Based on ICMR-NIN 'My Plate' recommendations, to meet a 2000-kilo-calorie diet/day, the intake of milk and milk products should be 250-300 ml/day, and 85g of pulses, with eggs/fish/meat substituting a portion[5]. However, due to their high lipid content, AOFs may also serve as reservoirs of lipophilic environmental contaminants, such as dioxins and become a primary route of exposure[6].

Dioxins, mainly polychlorinated dibenzo dioxins/furans (PCDD/Fs) and dioxin-like (dl) polychlorinated biphenyls (PCBs), are the byproducts of industrial activities like waste incineration, metal smelting, and chemical manufacturing [7-9]. Owing to high lipophilicity and resistance to enzymatic degradation in the human system attributed to their molecular planarity, they accumulate in the fat storage areas of the body with longer half-lives, for instance, 7 years for the most potent congener, 2,3,7,8-tetrachlorodibenzo[1,4]-dioxin (TCDD)[6,9]. Exposure to these compounds results in a wide variety of toxic manifestations in humans,

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Abbreviations : HpCDF, Heptachlorodibenzofuran; HxCDD, Hexachlorodibenzo-p-dioxin; HxCDF, Hexachlorodibenzofuran;OCDD, Octachlorodibenzo-p-dioxin; OCDF, Octachlorodibenzofuran; PCB, Polychlorinated Biphenyls; PeCDD, Pentachlorodibenzo-p-dioxin; PeCDF, Pentachlorodibenzofuran; TCDD, Tetrachlorodibenzo-p-dioxin; TCDF, Tetrachlorodibenzofuran; TEF, Toxic Equivalency Factor



Graphical abstract

including endocrine disruption activity, teratogenicity, and reproductive toxicity, as evidenced by *in vivo* studies on laboratory animals[10].

The toxicity of dioxin-like compounds (PCDDs, PCDFs, and PCBs) is expressed as Toxic Equivalent (TEQ), relative to 2,3,7,8-TCDD, the most toxic congener. TEQ is calculated by multiplying each congener's concentration by its Toxicity Equivalency Factor (TEF) and summing the results to estimate overall toxicity[11]. Dioxin contamination in AOFs highlighted the presence of dl-POPs contamination in freshwater fish (0.015 to 54 pg TEQ/g *ww*), marine fish and fish products (0.00015 to 350 pg TEQ/g *ww*), eggs (0.0027 to 361 pg TEQ/g fat), milk and dairy products (0.002 to 3.6 pg TEQ/g fat), and meat and meat products (0.008 to 0.18 pg TEQ/g)[3].

A recent report points out that, on average, an adult from the urban and rural population of India consumes 67 – 157 mL milk or milk products/day and 10 – 44 g eggs and flesh foods/day[5]. In India, specifically, only four studies have investigated dioxin levels in AOFs over the past two decades, and none of which included a toxicological evaluation of the detected levels[12-15]. To the best of our knowledge, the present study represents the first comprehensive *in vivo* risk assessment of dioxin-contaminated eggs, milk, and fish from the Indian subcontinent.

Materials and Methods

Chemicals

The dose formulations for oral administration were made with PCDD/Fs (EDF-5008), NO PCBs (EC-4986) and MO PCBs (EC-4987) native standard mixtures purchased from Cambridge Isotopic Laboratories (CIL), Massachusetts, USA, using corn oil as the vehicle. High-purity nonane (E-Merck, Germany), corn oil (E-Merck, Germany), ELISA kits of Luteinizing Hormone (LH), Thyroid Stimulating Hormone (TSH); Triiodothyronine (T3), Tetraiodothyronine (T4), Interleukin-1 (IL-1), IL-6, IL-10, and IL-11 (G-Biosciences, Germany) and Oxidative stress parameter kits (Cayman Chemicals, USA) were procured for the study.

Experimental animals

Male Wistar albino rats (n=60), aged 4-6 weeks, were used for the experiment. The rats were housed in an accredited animal house at a temperature range of 20-25°C, relative humidity of 55 ± 5% and a photoperiod of 12:12 h light-dark cycle and underwent acclimatisation for one week. The rats received a conventional laboratory diet with unlimited access to drinking water. The animals were fasted overnight before the initial dose administration and for 3-4 h after administration. The study protocol for sub-acute and sub-chronic was approved by the Institutional Animal Ethics Committee (IAEC) of Jubilee Mission Medical College and Research Institute JMMCRI, Thrissur, Kerala, India (RP-02/2022(i)/IAEC/JMMC&RI).

Experimental design

PCDD/Fs and dl-PCBs levels reported in milk, egg and fish samples from industrial areas of Southern India were used to determine the administration dose in sub-acute and sub-chronic toxicological

assessments in the present study. The quantitative analysis of PCDD/Fs and PCBs was performed with a gas chromatograph-triple quadrupole mass spectrometer (GC-MS/MS) (8890/7010B), equipped with a 7693A automatic liquid sampler (Agilent Technologies, USA) as described elsewhere[14,15]. In brief, the administered levels of sum PCDD/Fs and dl-PCBs were 15 pg TEQ/g, 10 pg TEQ/g, and 3 pg TEQ/g, representing the levels reported in eggs (15 pg TEQ/g fat), milk (10 pg TEQ/g fat), and fish (3 pg TEQ/g *ww*) samples[16]. A detailed description of the derived values for the dosage administered to rats, the preparative steps, and the congener concentrations employed in the present study is provided in the supplementary file. Dose calculations were made using WHO_{2005}-TEF values of PCDD/Fs and dl-PCBs congeners. The dosage administered to each group is as detailed in (Table 1).

The aforementioned dosage was administered orally to the respective groups. The control group received only corn oil. The feed and water intake were monitored regularly. The body weight of the rats was recorded on the initial day and the final day (28^{th} day/90^{th} day) of administration.

Animals were observed daily by a veterinary practitioner for physical and behavioural changes during and after dose administration in the sub-acute and sub-chronic study. At the end of subacute and sub-chronic studies, the rats were left overnight fasting with access to *ad libitum* water. Rats were anaesthetised using halothane inhalation, and blood was collected *via* retro-orbital plexus puncture. The blood samples for haematological analysis were collected in tubes containing trisodium EDTA as an anticoagulant. The remaining blood samples were collected in appropriate vials, allowed to clot, and centrifuged at 1,900 × g at 4°C for 10 min. The resulting supernatants were carefully transferred to fresh tubes and stored for subsequent serum biochemistry analyses[16]. Following the blood collection, all animals were subjected to necropsy. The organs for histopathological examinations were immediately fixed in 10% formalin, and the remaining samples were stored in 1X PBS at -80°C for further analysis. The serum and organ samples were transported to CSIR-NIIST, Thiruvananthapuram, Kerala, India, at -20°C.

Table 1 — Grouping of Animals

Duration of study	Groups	Dose
	Group I	Control
Sub-acute	Group II	15 pg TEQ/g
(28 days, n = 5)	Group III	10 pg TEQ/g
	Group IV	3 pg TEQ/g
	Group V	Control
Sub-chronic	Group VI	15 pg TEQ/g
(90 days, n = 10)	Group VII	10 pg TEQ/g
	Group VIII	3 pg TEQ/g

The haematological analysis was conducted on the whole blood samples using a 3-part differential haematology analyser (Mindray BC-20s, China). The biochemical tests were performed using a fully automated biochemical analyser (Erba EM Destiny 180, Germany) and ELISA kits. The tissues of all major internal organs were dehydrated, blocked in paraffin wax and sectioned at a thickness of 4 – 6 μm. The tissue sections were subjected to haematoxylin and eosin (H&E) staining as per the protocol and examined under a light microscope[17]. The images were then digitally recorded using a CX43 optical microscope (Olympus, Japan).

Statistical analysis

All the data were expressed in Mean ± Standard Error Mean (SEM) for each group using statistical software (IBM SPSS Statistics, Version 22). The parameters were analysed for the test of normality and homogeneity of variance, and those that qualified the tests were compared using an independent two-tailed *t*-test, and those that failed the tests were compared using non-parametric Kruskal-Wallis and Mann-Whitney U tests. $P \leq 0.10$ was considered significant. A less stringent significance level was used since this is an exploratory study with a small sample size[18,19].

Results

During the sub-acute observation period, there were no changes in behavioural patterns or in feed or water intake. During the necropsy, no macroscopic changes in the organs were observed. In Group II, there was a significant increase ($P \leq 0.10$) in platelet (PLT) and Serum Glutamic-Oxaloacetic Transaminase (SGOT) levels, along with a decrease ($P \leq 0.10$) in high-density lipoprotein (HDL) and interleukin-11 (IL-11) levels. In Group III, there was an increase in low-density lipoprotein (LDL), cholesterol (CHOL), SGOT, urea, globulin (GLOB), and catalase (CAT) activity ($P \leq 0.10$). Similarly, Group IV rats exhibited increased SGOT, urea, GLOB, thyroid-stimulating hormone (TSH), CAT activity, 8-hydroxy-2'-deoxyguanosine (8'-OHdG), and PLT levels, alongside a decrease in luteinizing

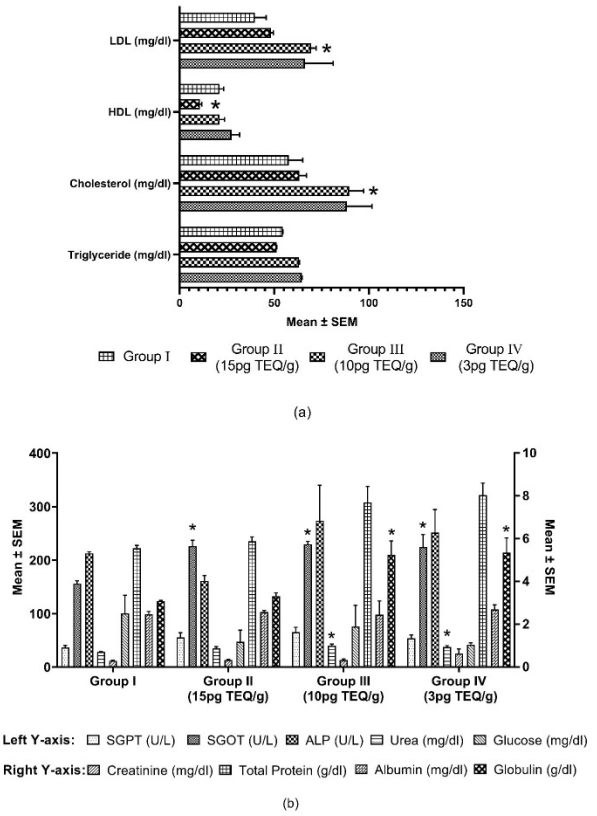


Fig. 1 — (a) Lipid profile, (b) Biochemical Parameters after sub-acute treatment. Adult male rat (n = 3) received daily oral gavage of vehicle or ∑ PCDD/Fs and dl-PCBs at 15,10 or 3 pg TEQ/g for 28 days. Two-tailed T test or Mann-Whitney U tests based on test of normality and homogeneity of variance were used to compare between Group I and other groups. Values represent means ± SEMs, **P*≤ 0.1 compared to Group I. Abbreviations: LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; SGPT: Serum Glutamic Pyruvic Transaminase; SGOT: Serum Glutamic-Oxaloacetic Transaminase; ALP: Alkaline Phosphatase

hormone (LH) and IL-10 (*P*≤ 0.10). The data are represented in (Fig. 1(a) and 1(b), Fig. 2(a-c), and Table 2). In the histopathological analysis of the liver, mild hepatocyte degeneration with cytoplasmic vacuolations was observed in Group IV (Fig. 3d). The kidneys of Groups III and IV exhibited mild degeneration of tubular epithelial cells (Fig. 4c and d).

A sub-chronic exposure to environmentally-relevant concentrations of dioxins resulted in a significant decrease in weight gain in Group VI rats (*P*≤ 0.10). No changes in the rats' behaviour, food intake, or water consumption were noted during this period, and no macroscopic organ alterations were observed at necropsy. Group VI showed increases in haematological parameters, including red blood cell (RBC) and lymphocyte levels, and a decrease in PLT, neutrophils, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) levels (*P*≤ 0.10). Liver function parameters such as Serum glutamic pyruvic transaminase (SGPT), SGOT, urea, CHOL levels, and inflammatory markers like IL-6 and IL-11 were elevated in Group VI (*P*≤ 0.10), while testosterone and GR levels were decreased (*P*≤ 0.10). In Group VII, increases were observed in CHOL, SGPT, urea, albumin (ALB), RBC, and lymphocytes (*P*≤ 0.10). Conversely, there was a significant decline in haematological parameters such as PLT, neutrophils, eosinophils, MCV, MCH, as well as other parameters, including creatine (CRE), testosterone, and Glutathione reductase (GR) activity (*P*≤ 0.10). Group VIII showed increases in CHOL, IL-6, and IL-11, along with decreases in TSH, thyroxine (T4), GR, and packed cell volume (PCV) (*P*≤ 0.10). The data for these observations are presented in (Fig. 5a & b, Fig. 6a-c, and Table 3).

Discussion

Unintentional sources of exposure lead to the entry of dioxins into the ecosystem, which eventually reaches aquatic organisms, foraging animals, and, ultimately, humans. In India, limited studies have focused on dioxin contamination in AOFs from contaminated zones[12-15]. To the best of our knowledge, none of the studies focused on the toxicity evaluation of dioxins at environmentally relevant concentrations. A recent investigation into dioxin monitoring in AOFs in Kerala, India, revealed concerning levels of dioxin contamination[14,15]. In light of the carcinogenic and systemic toxicity of dioxins to animals and humans, a comprehensive toxic evaluation study using Wistar rats was conducted to understand the toxic effects of dioxin concentration observed in AOFs. The results of this study indicated variation in haematological and clinical chemistry parameters, demonstrating that at environmentally-relevant dosage levels of dioxins, there was limited sub-acute and sub-chronic toxicity.

In the subacute exposure groups, an elevation in PLT levels was observed. The sub-chronic exposure groups exhibited increased levels of lymphocytes and RBC, while simultaneously demonstrating reductions in PLT counts, neutrophils, eosinophils, MCV, and MCH. These findings indicate distinct haematological responses associated with varying durations of exposure. Previous research has found that the hematopoietic system is one of the critical sites affected by exposure to TCDD [20]. Rats exposed to various concentrations of TCDD (0.1 µg to 3,000 ng

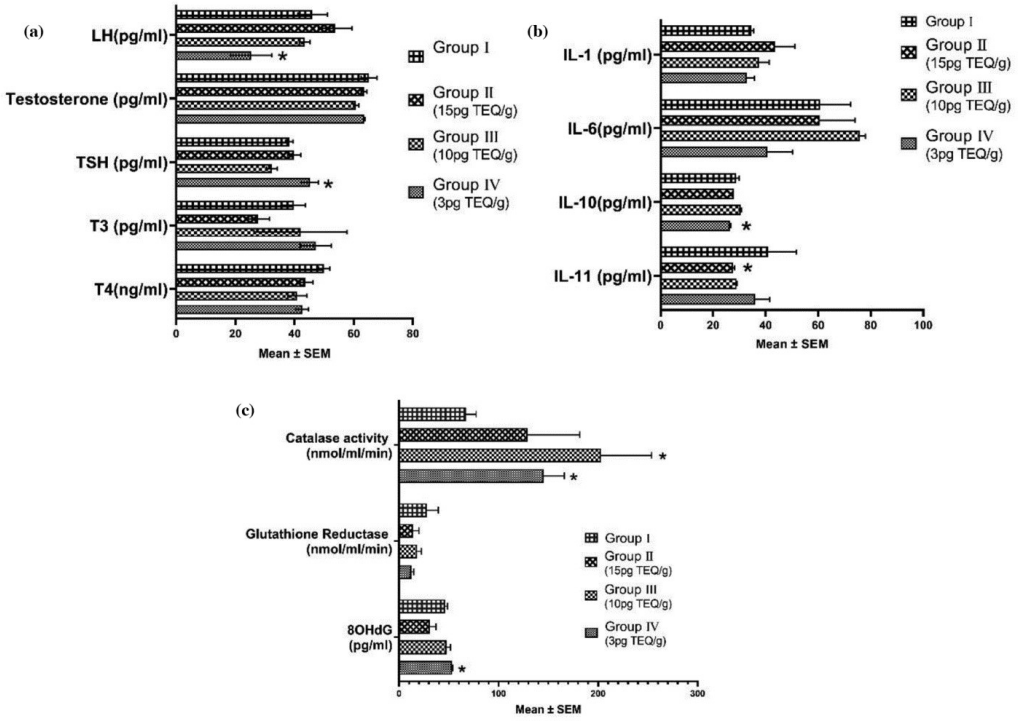


Fig. 2 — (a) Hormone levels in serum, (b) Serum inflammatory markers, and (c) Serum oxidative stress marker levels after sub-acute treatment. Adult male rat (n = 3) received daily oral gavage of vehicle or ∑ PCDD/Fs and dl-PCBs at 15,10 or 3 pg TEQ/g for 28 days. Two-tailed T test or Mann-Whitney U tests based on test of normality and homogeneity of variance were used to compare between Group I and other groups. Values represent means ± SEMs, *$P \leq 0.1$ compared to Group I. Abbreviations: LH: Luteinizing Hormone; TSH: Thyroid Stimulating Hormone; T3: Triiodothyronine; T4: Tetraiodothyronine; IL: Interleukin; 8OHdG: 8-hydroxy-2'-deoxyguanosine

Table 2 — Changes in hematological parameters after sub-acute treatment

Parameter	Group I	Group II (15 pg TEQ/g)	Group III (10 pg TEQ/g)	Group IV (3 pg TEQ/g)
Haemoglobin(g/dl)	13.57 ± 0.56	13.30 ± 0.31	14.23 ± 0.35	12.65 ± 0.41
RBC (million/mm^3)	7.20 ± 0.38	6.93 ± 0.22	7.17 ± 0.25	6.32 ± 0.28
Packed cell volume (%)	42.5 ± 1.93	41.00 ± 0.41	43.33 ± 1.20	39.50 ± 1.66
Platelet(lakh/mm^3)	5.05 ± 1.32	8.40 ± 0.60*	8.31 ±0.85	8.06 ± 0.20*
Neutrophils (%)	10.0 ± 1.22	9.75 ± 1.38	9.33 ± 2.33	8.75 ± 0.25
Lymphocyte (%)	86.75 ±2.13	86.50 ± 1.85	87.67 ± 3.33	87.25 ± 0.85
Eosinophil (%)	3.25 ± 1.03	3.75 ± 0.48	3.00 ± 1.00	4.00 ± 0.82
Mean corpuscular volume (fL)	59.0 ± 1.08	59.50 ± 0.65	59.33 ± 0.88	62.00 ± 0.91
Mean Corpuscular Haemoglobin (pg)	18.75 ±0.25	19.00 ± 0.00	19.33 ± 0.33	20.00 ± 0.41
Mean corpuscular haemoglobin concentration(g/dl)	32.0 ± 0.40	32.25 ± 0.48	32.67 ± 0.33	32.25 ± 0.63

Adult male rat (n = 3), Values represent means ± SEMs, *$P \leq 0.1$ compared to Group I

TCDD/kg) showed a significant decrease in PLT count[20,21]. Exposure to 1000 ng TCDD resulted in reduced haemoglobin, PCV, MCH, MCV, and PLT, with no synergistic effects from PCBs[22]. The variations in the haematological parameters were more pronounced in the sub-chronic exposure group than in the sub-acute exposure group.

Research indicates that exposure to dioxins may be associated with hyperlipidemia[23,24]. TCDD reduced LDL receptor activity in the hepatic plasma membrane of guinea pigs and rats, resulting in decreased LDL binding and internalisation, contributing to elevated cholesterol levels[25-27]. Group III showed a significant reduction in HDL levels, while LDL and CHOL levels increased relative to the control group. Elevated liver enzymes are often observed in individuals with heightened levels of LDL and triglycerides, along with decreased HDL levels[28,29], which aligns with the findings of this study. The co-occurrence of elevated liver enzymes and abnormal lipid profiles indicates an

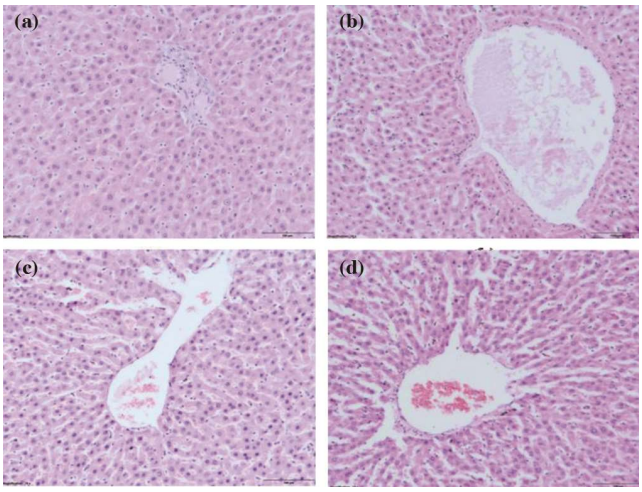


Fig. 3 — Histopathology of Liver. Normal hepatocytes, sinusoids, central vein and portal vessels of (a) Group I, (b) Group II, (c) Group III. (d) mild degeneration of hepatocytes having cytoplasmic vacuolations in Group IV. Adult male rat (n = 3) received daily oral gavage of vehicle or ∑ PCDD/Fs and dl-PCBs at 15,10 or 3 pg TEQ/g for 28 days

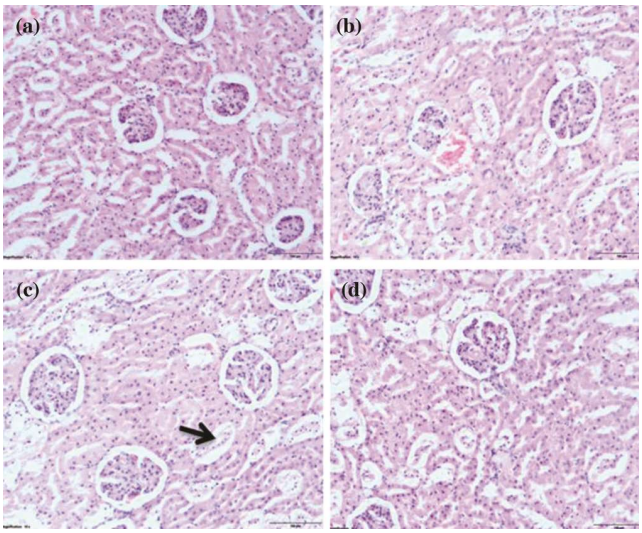


Fig. 4 — Histopathology of the Kidney. Normal histological structures in the cortex and medulla of (a) Group I and (b) Group II; mild degeneration of tubular epithelial cells of (c) Group III; and (d) Group IV. Adult male rat (n = 3) received daily oral gavage of vehicle or ∑PCDD/Fs and dl-PCBs at 15,10 or 3 pg TEQ/g for 28 days

increased risk of liver disease and cardiovascular complications[30,31]. Furthermore, these symptoms may be indicative of non-alcoholic fatty liver disease (NAFLD), metabolic dysfunction-associated fatty liver disease (MAFLD), or pre-diabetic conditions[31,32]. Earlier studies have shown the association between dioxin exposure and the incidence of NAFLD and type 2 diabetes mellitus[33,34].

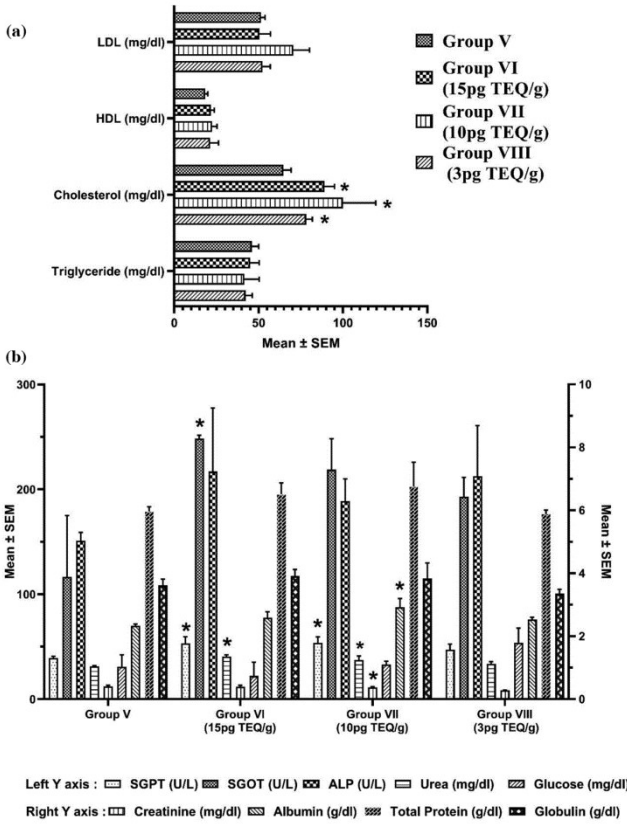


Fig. 5 — (a) Lipid profile (b) Biochemical Parameters after sub-chronic treatment. Adult male rat (n = 3) received daily oral gavage of vehicle or ∑ PCDD/Fs and dl-PCBs at 15,10 or 3 pg TEQ/g for 90 days. Two-tailed T test or Mann-Whitney U tests based on test of normality and homogeneity of variance were used to compare between Group V and other groups. Values represent means ± SEMs, *$P \leq 0.1$ compared to Group V. Abbreviations: LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; SGPT: Serum Glutamic Pyruvic Transaminase; SGOT: Serum Glutamic-Oxaloacetic Transaminase; ALP: Alkaline Phosphatase

The elevated levels of liver markers are attributed to a loss of functional integrity or leakage of cytosolic contents of hepatic cells through lipid peroxidation resulting in increased levels of H_2O_2 and oxidative damage to the cell membrane[35]. The study also shows that groups III and IV exhibit significantly elevated serum globulin levels, reflecting the increased secretory activity of the liver[36]. This rise in globulin levels consequently lowers the albumin-to-globulin ratio (AGR), a marker of inflammation[37]. On histopathological examination, groups III and IV exhibited mild degeneration of the liver and kidneys. Parenchymal degeneration, vacuolisation, and steatosis in hepatocytes are evident upon dioxin exposure[38]. Sub-acute oral dosing of 22–300 ng TCDD/kg/day had caused mild hepatocellular

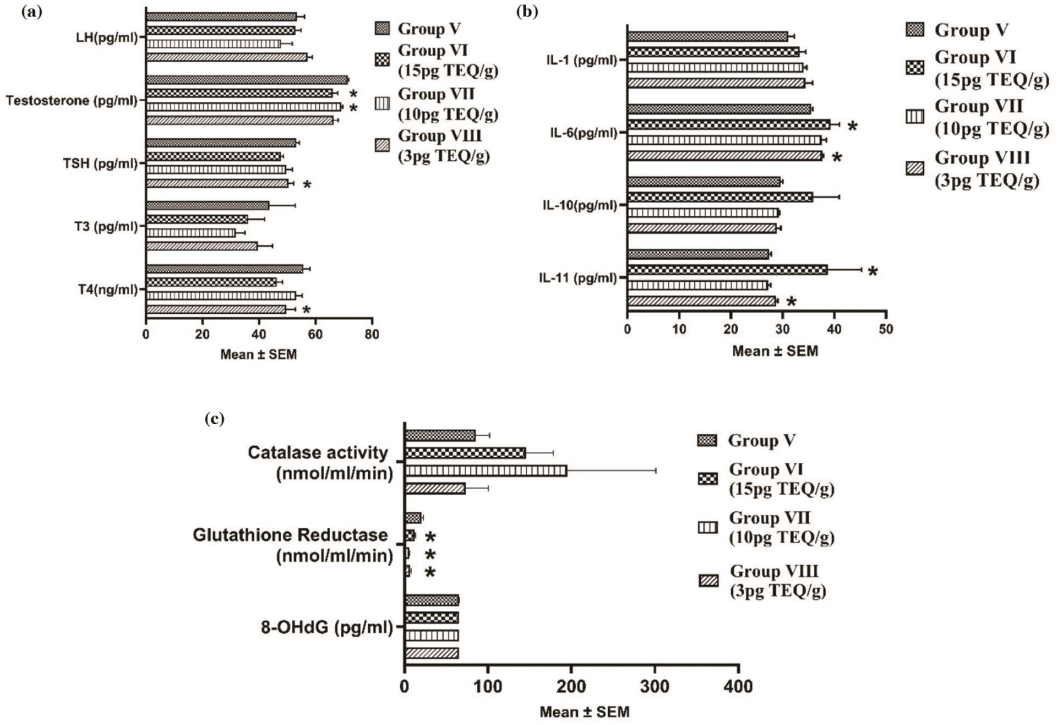


Fig. 6 — (a) Hormone levels in serum. (b) Serum inflammatory markers. (c) Serum oxidative stress marker levels after sub-chronic treatment. Adult male rat (n = 3) received daily oral gavage of vehicle or ∑ PCDD/Fs and dl-PCBs at 15,10 or 3 pg TEQ/g for 90 days. Two-tailed T test or Mann-Whitney U tests based on test of normality and homogeneity of variance were used to compare between Group V and other groups. Values represent means ± SEMs, *$P \leq 0.1$ compared to Group V. Abbreviations: LH: Luteinizing Hormone; TSH: Thyroid Stimulating Hormone; T3: Triiodothyronine; T4: Tetraiodothyronine; IL: Interleukin; 8OHdG: 8-hydroxy-2'-deoxyguanosine

Table 3 — Changes in hematological parameters after sub-chronic treatment

Parameters	Group V	Group VI (15pg TEQ/g)	Group VII (10pg TEQ/g)	Group VIII (3pg TEQ/g)
Haemoglobin (g/dl)	14.60 ± 0.33	13.72 ± 0.25	13.82 ± 0.31	13.00 ± 0.40
RBC (million/mm^3)	5.48 ± 0.18	7.20 ± 0.23*	7.17 ± 0.31*	5.37 ± 0.23
Packed cell volume (%)	47.25 ± 1.44	44.00 ± 0.41	45.00 ± 1.23	40.00 ± 0.91*
Platelet (lakh/mm^3)	11.18 ± 0.59	7.92 ± 0.36*	7.92 ± 0.77*	10.15 ± 0.65
Neutrophils (%)	12.75 ± 1.80	8.50 ± 0.64*	7.25 ± 0.25*	16.00 ± 1.08
Lymphocyte (%)	83.25 ± 2.63	89.00 ± 0.41*	91.25 ± 0.48*	78.50 ± 1.32
Eosinophil (%)	4.00 ± 0.91	2.50 ± 0.29	1.50 ± 0.29*	5.50 ± 0.29
Mean corpuscular volume (fL)	85.00 ± 3.54	61.25 ± 2.29*	62.50 ± 1.50*	74.75 ± 4.21
Mean Corpuscular Haemoglobin (pg)	26.75 ± 0.85	18.75 ± 0.25*	19.25 ± 0.63*	24.25 ± 1.25
Mean corpuscular haemoglobin concentration (g/dl)	31.50 ± 0.29	31.00 ± 0.91	31.00 ± 0.41	32.25 ± 0.63

Adult male rat (n = 3), Values represent means ± SEMs, *$P \leq 0.1$ compared to Group V

hypertrophy with elevation in liver markers[39]. Similarly, intraperitoneal administration (0.75 and 8 µg/day) had reported early steatotic and degenerative changes in hepatocytes at higher doses[38]. The structural changes of tissues can be related to dioxin-induced AHR activation, which alters gene expression to promote inflammation and metabolic dysfunction[40-44]. Prolonged exposure to TCDD can cause persistent activation of the AHR and disrupt signal transduction homeostasis through cytochrome P450 genes, leading to oxidative stress[45,46]. These effects are also observed through changes in histology and serum biomarkers, linking dioxin toxicity at environmentally relevant levels to liver damage.

The altered liver function tests, along with parameters of oxidative stress and secretory activity, suggest the presence of inflammation or tissue damage in the liver, as evidenced by the levels of pro-inflammatory cytokine IL-6 and anti-inflammatory cytokines. IL-6 serves as an early-response biomarker, rather than a lesion-specific endpoint[47,48]. IL-6 generally exerts a protective and regenerative effect in the liver, whereas IL-11 is associated with fibrosis and tissue damage[49-51]. To the best of our

knowledge, IL-11 has no direct evidence from toxicology studies utilising rats as an experimental model for dioxins. However, previous research suggests that concurrent elevations in IL-6 and IL-11 levels are more indicative of hepatocellular toxicity than of whole-body toxicity[47-49,52]. The decline in the activity of glutathione reductase (GR) in serum across all three sub-chronic exposure groups suggests an inadequate reversal of glutathione disulfide (GSSG) to glutathione (GSH).

Sub-acute exposure has resulted in elevated TSH levels in group IV. Previous research indicates a correlation between dioxin exposure and increased TSH levels[53,54]. Additionally, this group has demonstrated decreased levels of luteinizing hormone (LH). The observed increase in TSH levels, coupled with the decline in LH levels, suggests a possible case of hypothyroidism[54]. The meta-analysis conducted on rodents exposed to TCDD has revealed significant reductions in testis size, sperm count, and serum testosterone levels[55]. Peritoneal administration of dioxin (50 µg/kg body weight) has resulted in a significant decrease in serum testosterone levels [56]. Sub-chronic exposure to dioxins at low doses in the present study also resulted in a decline in levels of testosterone in groups VI and VII.

In conditions such as colour variation, inconsistency, and difficulty in cutting during tissue grossing, pathological investigation in tissues cannot be performed due to inadequate sectional characteristics and loss of tissue integrity and architecture[57]. This may be considered a limitation of the present study.

The observed alterations in haematological profiles, lipid parameters, and clinical chemistry markers during sub-acute and sub-chronic exposure underscore the critical need for systematic monitoring and rigorous risk assessment of dioxin concentrations across commercial and industrial AOF sources. Although necropsy findings did not reveal structural sub-acute hepatic or renal damage, the significant fluctuations in liver enzymes and kidney function indicators strongly warrant further investigation. Future research must prioritise elucidating the mechanisms driving dioxin-induced hepatotoxicity, particularly focusing on the roles of oxidative stress and inflammation at environmentally relevant concentrations. Furthermore, expanding future acute and chronic toxicity studies to include larger sample sizes will be essential to validate these biochemical trends and comprehensively map toxicological variations across contaminated and market zones.

Conclusion

Dietary exposure to environmentally relevant dioxin concentrations in AOFs induced systemic changes in hematological, immunological, biochemical, and oxidative stress profiles during sub-acute and sub-chronic phases. These findings provide critical insights into the potential human health risks associated with contaminated food intake, highlighting a need for long-term epidemiological surveillance and robust dietary risk-assessment frameworks.

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Conflict of interest

All authors declare no conflict of interest.

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