



Evaluating Teratogenic Effects: Examination of the Impact of TCDD on Craniofacial Characteristics and Body Measurements of Isa Brown Chicken Embryos Under Controlled Experimental Conditions

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ABSTRACT

2,3,7,8-tetrachlorodibenzo-p-dioxin, sometimes referred to as TCDD is a chemical molecule that is generated via the process of burning. It poses a significant threat to both people and animals due to its toxic effects. The potential adverse effects include dermatological injury, immunosuppression, hepatotoxicity, increased risk of cancer, harm to the developing fetus, reproductive disorders, disruption of development processes, neurobehavioral impairments, and particular endocrine disturbances. Furthermore, this chemical can influence the process of embryonic development and increase the rates of embryonic mortality. Exposure to TCDD can cause higher death rates in chicken embryos, leading to a decrease in their capacity to hatch. The impact of this can have substantial economic consequences for poultry producers, as it directly influences the overall productivity and efficiency of hatcheries. This study aims to investigate the teratogenic impacts of TCDD injection on the head diameter, head shape, body length, and overall body size of Isa brown embryos. The study consisted of three treatment groups: a control group that did not receive any additional TCDD in corn oil, a placebo group that got 5 μ L of corn oil, and a treatment group that received five ng/egg of TCDD dissolved in 5 μ L of corn oil. The interventions were administered for a duration of seven days. The statistical analysis revealed that the teratogenic effect of TCDD had a substantial impact on the development of thorax circumference, but it did not affect head diameter, head circumference, overall body size, and body length.

KEYWORDS:

Embryonic development; Body length; Body-wide; Head circle; Head diameter; Seven-day chicken embryo; Malformation; TCDD.



1. Introduction

One of the most persistent environmental contaminants and the most potent poisons known is dioxins (2,3,7,8-tetrachlorodibenzo-para-dioxin or TCDD). TCDD induces a broad biological response, including induction of cytochrome P-450 1A1 (CYP1A1), disturbance of normal hormone signaling pathways, reproductive and developmental defects, immunotoxicity, liver damage, wasting syndrome, and cancer [1]. TCDD is an environmental pollutant that produces harmful biological effects, including toxicity development of the embryo (congenital malformation) and teratogenic effect [2]. Exposure to TCDD and similar chemicals causes various effects across multiple species, tissues, and developmental stages. The response ranges from relatively simple biochemical changes to highly toxic to lethal responses. The spectrum of effects of TCDD exhibits some species variability, but multiple effects are seen in several species of wildlife, domestic, and laboratory animals, from fish to birds and mammals [3].

The primary sources of TCDD exposure in poultry include soil, commercial organic feed, non-commercial organic feed, plants, and insects. The compound will be stored in egg fat after TCDD enters the hen's body through the digestive system. Exposure to TCDD was higher in free chicken-rearing systems than in caged chickens [4]. Chicken is the most sensitive species to the lethal effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [3]. The chicken embryo is also one animal model that is accessible and economically used in *in vivo* research to study embryo developmental stages [5]. The effect of TCDD in the chicken embryo can induce chick cardiotoxicity [6], decrease eye diameters and body weight [7], lack expression of AhR in the myoblast, mesenchyme, and gray matter of the brain [8], alter the development of several organs including liver [9]. This research focused on investigation into the teratogenic impacts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), specifically on the head diameter, head shape, body length, and overall body size of Isa Brown embryos. This study aims to conduct a detailed examination of the teratogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on Isa Brown embryos, primarily focusing on the influence on head diameter, head shape, body length, and total body size. This study investigates characteristics associated with the embryonic development of Isa Brown embryos in response to TCDD exposure. The focused methodology can provide significant knowledge on the precise teratogenic impacts of TCDD, particularly on the physical structure of the head and body. Such meticulous investigation can be highly valuable in comprehending the subtle effects of TCDD exposure on several facets of embryonic development within the domains of teratology and developmental biology.

2. Materials and methods

2.1 Animals and Study Design

The 39 Isa Brown chicken eggs were obtained from CV. Adiguna Sarana Mandiri, Indonesia. Three groups were created from the 52-gram average weight of the chicken eggs. The first group (19 eggs) was the control group (not injected), the second group was injected with 5 μ L corn oil - 20 eggs, and the third group was injected with five ng/egg of the TCDD standard solution in five μ L corn oil - 20 eggs. Supelco (St. Louis, MO, USA) provided a standard solution containing 1 g/mL TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin solution), which was diluted in maize oil (Mazola, Indonesia). The TCDD dosage was estimated through an examination of previously published data [8], which confirmed the harmful effects of TCDD, present at a dose of 5 μ g/kg b.w. The authors confirm that the research procedure has ethical approval obtained from the "Animal Care and Use Committee" Universitas Brawijaya with the number of ethical file clearances 064-KEP-UB-2020.

2.2 Experiment Procedure

The prescribed solution was injected into the air chamber on the 0th day of the eggs; with the egg placed horizontally, the needle of a Hamilton syringe was inserted about 0.5 cm depth into the air chamber. After removing the syringe, the hole was plugged with molten paraffin, and then the egg was oriented vertically and put in a C-82 (manual incubator). The eggs were rotated 90 degrees every hour, and the incubator was maintained at 37.6°C and 55% humidity [10]. The eggs were incubated for seven days and then harvested and measured. The embryo is then collected from the shell and cleaned from the excess embryonic membrane. The measurement of the thorax circle, head diameter, head circle, body length, and body width of Isa brown embryos was done with methods from previous research [10]. The thorax circle was measured with the string, and then the length of the string was calculated with the ruler. The head diameter of the embryo was measured by using vernier calipers on the median os frontalis to median os occipital. The body length of the embryo was measured by sticking a thread on the dorsal embryonic body from the front to the cauda. The vernier calipers measured the body width.

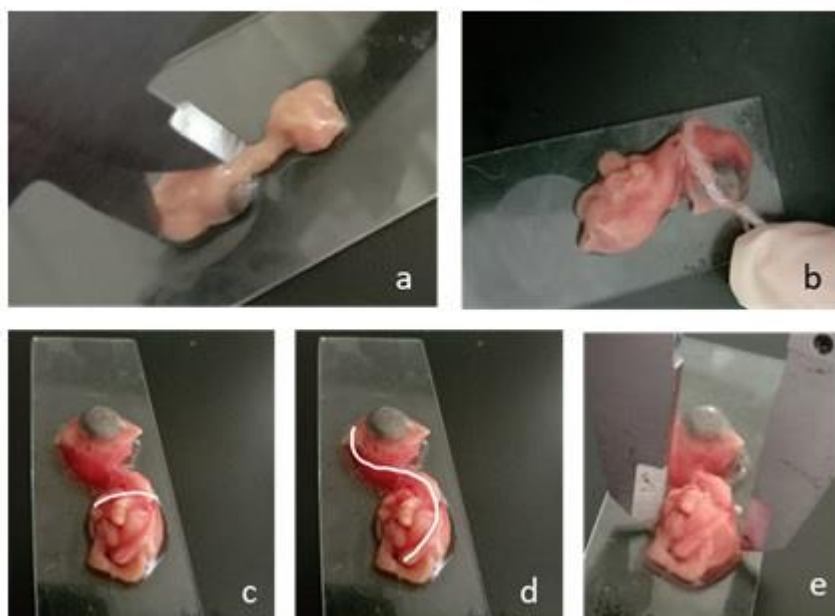


Figure 1. Measurement of chicken embryo. a. Head diameter, b. Head circle, c. Thorax circle, d. Body length and e. body-wide.

2.3 Data Analysis

The data obtained from the measurement of the thorax circle, head diameter, head circle, body length, and body-wide of Isa brown embryos were subjected to standard methods of statistical analysis using Windows-based SPSS (Version 20.0) [11]. The one-way analysis of variance was used, and the significance level was set at $p < 0.05$. To identify any notable variations in treatment, a Tukey's test was employed to ascertain the disparities among the doses.

3. Result and Discussion

Several eggs from the control, corn oil, and TCDD groups were without embryos (Table 1). The data showed that 14 of 19 eggs in the control group are embryonated; in the corn oil group, eight of 20 are embryonated; and in the TCDD group, only four eggs of 20 are embryonated.

Table 1. The result of harvesting the eggs.

| Control group | Corn Oil group | TCDD group |
|---------------|----------------|------------|
| 1 | 1 | 1 |
| 0 | 0 | 0 |
| 1 | 0 | 0 |
| 0 | 0 | 0 |
| 1 | 1 | 0 |
| 1 | 1 | 0 |
| 1 | 0 | 0 |
| 1 | 0 | 0 |
| 0 | 0 | 0 |
| 1 | 1 | 0 |
| 0 | 1 | 0 |
| 1 | 1 | 1 |
| 1 | 1 | 0 |
| 1 | 0 | 0 |
| 1 | 0 | 1 |
| 0 | 0 | 0 |
| 1 | 1 | 0 |
| 1 | 0 | 1 |
| 1 | 0 | 0 |
| 0 | 0 | 0 |

(1: embryonated egg and 0: unembryonated egg)

The embryos from the control and TCDD groups were measured in head diameter, head circle, body length, and body-wide (figure 1). The data were analyzed statistically, and the result showed that head diameter, head circle, body length, and body width had no significant difference between the control and TCDD groups. On the other hand, the thorax diameters showed a significant difference between the two groups (table 2).

Table 2. Measurement of the Embryos.

| Group | Mean control group (cm) | Mean corn oil group (cm) | Mean TCDD group (cm) |
|---------------|-------------------------|--------------------------|----------------------|
| Head Diameter | 0,60 | 0,39 | 0,43 |
| Head Circle | 1,67 | 1,42 | 1,54 |
| Body Length | 1,55 | 1,44 | 1,5 |
| Body Wide | 0,32 | 0,29 | 0,45 |
| Thorax Circle | 1,59* | 1,17* | 1,13* |

* significant different

The result in the embryonated eggs of the chicken embryo in the TCDD group was significantly different from the control group, whether the corn oil group was not. In the TCDD group, 16 of 20 eggs did not contain embryos when they were harvested. This result showed that adding five nanograms of TCDD per egg caused early embryonic death of the chicken embryo. A previous study

showed the same effect that TCDD can cause early embryonic death [8]. In ovo administration of TCDD, PeCDF, or TCDF caused a dose-related increase in embryo mortality for the White Leghorn chicken by 16% [12]. Dioxin-like chemical composition causes premature death in vertebrates by activating the aryl hydrocarbon receptor (AhR) [13]. The dioxin was inoculated on day zero of the eggs, and it caused some of the eggs to not find any embryo inside them. The seven days of chicken embryo, when taken out from the eggs, showed part of the yolk, albumen, amniotic liquid, allantoic liquid, and the embryo. The anatomy of a seven-day chicken embryo showed the formation of the brain, eye, upper limb, lower limb, and tailbud (figure 2).

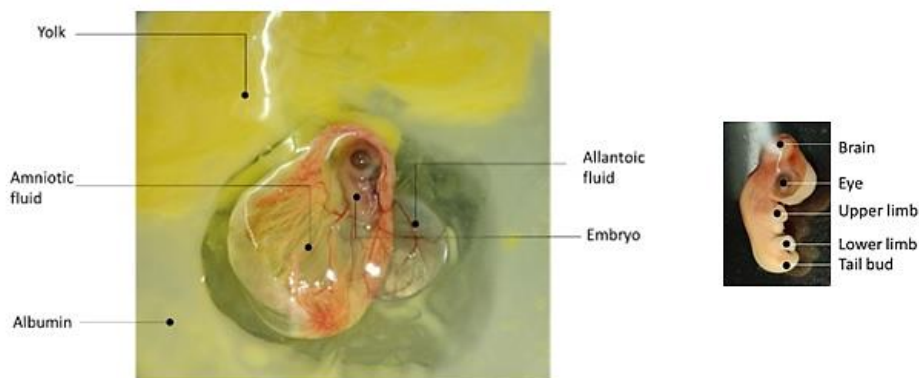


Figure 2. Chicken embryo with yolk, albumin, amniotic fluid, allantoic fluid (right), and anatomy of a seven-day chicken embryo (left).

The effect of the TCDD on head diameter, head circle, body length, and body width had no significant difference between the control, corn oil, and TCDD groups. It is suggested that this is caused by the rule of injecting TCDD in the air chamber. The previous study showed that the highest success rate is inoculation in the amniotic cavity rather than in other compartments, including the air chamber [14]. On the other hand, the administration of the TCDD affects the thorax circle. The specific rule of TCDD affecting the development of the thorax is still unknown. The organs structure inside the thoraces is Pulmo and heart. The previous study about toxicity in chicken embryos that affects the development of Pulmo is in line with the decrease of the thorax circle [15]. On the other hand, certain regions of the apex of the heart exhibited limited positive or negative responses. The heart muscle cells exhibited growth, but the vascular bed demonstrated a reduction [8]. The study investigated the cardiotoxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in chick embryos of the White Leghorn-Babcock (WLB) and Plymouth Rock-Barred (PRB) breeds. On day 0 (D0), the injection of TCDD resulted in a proportional rise in heart weight in both strains, without the presence of pericardial edema on D10. Ultimately, TCDD causes a manifestation of dilated cardiomyopathy [16]. It is suggested that there is a correlation between the thorax circle and the development of the organs on it, as well as the role of TCDD toxicity related to AhR. The role of AhR is to induce abnormality of embryo development by controlling the expansion and differentiation of stem cells [17] and dysregulation of vascular endothelial growth factor (VEGF) [18]. The AhR is bound with the growth of stem cells, and it will affect the development of the pulmo and heart [19]. The indirect suppression of VEGF by the TCDD is done by secluding the aryl hydrocarbon receptor nuclear translocator (ARNT) by AhR [20].

4. Conclusion

According to this research, the TCDD might reduce the number of embryonated eggs and affect thorax development by reducing the thorax circle size. The addition of TCDD injection in the air chamber suggested slightly decreasing the effect of the TCDD, which can make the anatomy of head diameter, head circle, body length, and body-wide have no effect. Teratogenic effects on chicken embryos may extend to other avian species and potentially impact the biodiversity of ecosystems. Research in this area is crucial for understanding the broader ecological implications of TCDD exposure.

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