

**Teratogenic Effect of Chlorpyrifos and Glyphosate on Pregnant Rats:  
Biochemical and Morphological Evaluations**

*Running Head:* Teratogenic Effect of Chlorpyrifos and Glyphosate

**Abstract**

The impact of pesticides on the environment and human health is a serious matter of concern. The present study focusses on the teratogenic effect of pesticide chlorpyrifos (CPF) and glyphosate (GLY) on the pregnant rats and their offspring during gestation and lactation period. The female rats were exposed to these pesticides (CPF and GLY) throughout their pregnancy at a dose of 10 mg/kg. The biochemical markers and lipid profile of pesticides exposed pregnant rats were analyzed. The maternal and reproductive outcome was also assessed followed by rat pups morphometric analysis. A significant alteration in the blood glucose level, triglycerides, total cholesterol, SGOT, and SGPT levels were observed in pesticide exposed groups. The body weight, crown-rump length, eye length, eye width, hind limb, and forelimb size of rat neonates were significantly found to be lower in the pesticide exposed group when compared with the control animals. Morphological abnormalities like microcephaly, microtia, micromelia, dysmorphogenesis, distorted axis abdominal, and brain hemorrhages were observed in pesticide exposed rat neonates. Skeletal observations of the CPF exposed group show disruptive malformations, wavy ribs, and curved spinal cord. Intraventricular and spinal cord hemorrhages were observed in 21 days old rat pups in GLY treated group. Findings of the present study indicate that exposure to pesticides during the gestation period causes the morphological abnormalities in rat fetuses by altering the mechanisms involved in growth and development. Thus, on the basis of observed results, we concluded the teratogenic effects of CPF and GLY in rats.

**Key words:** Teratogenicity, pesticide, chlorpyrifos, glyphosate, morphological abnormality

## **Introduction**

The studies related to environmental-cum-health risk assessment provide a better understanding of various health related issues. The atmosphere is changing day by day with the introduction of chemicals like pesticides, most of which have not been adequately studied for their impact on vulnerable group of population like infants, pregnant females. The exposure to these pesticides during pregnancy and lactation is an important factor because it affects the mother as well as the fetus and this exposure at different stages of growth and development i.e. pre-conception, prenatal, pubertal and adult has serious impact on health at different levels (Nurulain and Shafiullah, 2012). Some of the pesticides are the 'persistent organic pollutants' that persists in the environment for longer duration of time and gets accumulated in the living body tissues because of their high lipophilicity and ability of not getting metabolized. Pesticides provide benefit in the control of pests and disease used in various agricultural and non-agricultural settings (Aktar et al., 2009). Serious health outcomes like carcinogenicity and teratogenicity has been found to be associated with the exposure to these pesticides. Their action is endocrine disruption and also they have the capacity of inducing oxidative stress, both of these mechanisms have found to be reported in the impairment of normal embryonic growth and development processes (Ren et al., 2011).

Some of the studies reported the association between environmental pollutants i.e. pesticides as a risk factor in the fetal growth and development. Lacasan et al. (2006) shows the impact of occupational exposure (both maternal and paternal) and the risk of neural tube defect. Maternal occupational exposure in agricultural field during peri-conceptual period leads to the risk of having child born with anencephaly. Previous study reported the association between neural tube defects (NTDs) and the residential proximity of mothers near to the agricultural fields (Rull et al., 2006). They performed this study on population and uses several parameters like ethnicity, maternal educational, cigarette smoking and use of nutritional supplements. They found the risk of spina bifida or anencephaly related with the exposure of agricultural pesticides. An experimental study reported the effect of perfluoroalkyl acids on the embryonic growth and development of mice (Abd El-Nasser et al., 2009). The study also reported the reduction in body weight of fetuses, teratogenic and genotoxic effects in exposed mice (Abd El-Nasser et al., 2009). Ren et al. (2011) showed the presence of persistent organic pollutants i.e.  $\alpha$ -endosulfan,

phenanthrene, polycyclic aromatic hydrocarbons (PAH),  $\alpha$  and  $\gamma$  hexachlorocyclohexane (HCH), p,p'-isomers of dichlorodiphenyltrichloroethane (DDT) and their metabolites in the placenta and their associated risk for NTDs.

Chlorpyrifos (CPF) is an organophosphorus insecticide acts by inhibiting the enzyme acetyl cholinesterase through phosphorylation reaction (Figure 1). Acetyl cholinesterase enzyme metabolizes acetyl choline to acetyl and choline causing termination of muscular, dendritic nerve endings and motor end plates stimulation (Hancock et al., 2007). Glyphosate (GLY) [*N*-(phosphonomethyl) glycine] is a non-selective systemic, broad spectrum organophosphorus compound which is used as an herbicide in both agricultural and non-agricultural settings. Figure 2 represents that GLY acts by inhibiting the enzyme 5-enoyl pyruvyl shikimate 3-phosphate synthase (EPSPS), involved in shikimic acid pathway, synthesizing three aromatic amino acids i.e. phenylalanine, tyrosine and tryptophan (Richard et al., 2005).

The present study was designed to investigate the impact of CPF and GLY exposure on the serum biochemistry of pregnant females and growth and development of the fetus.

## **Materials and Methods**

### ***Chemicals and drugs***

Insecticide CPF with the brand name Aladdin TC (Shri Ram Agro Chemicals) and herbicide GLY with the brand name Topper 77 (Crystal Crop Protection Pvt. Ltd India) were purchased from the local market of Bhimtal, Uttarakhand India.

Diagnostic Kits for biochemical estimations were purchased from ERBA Company. Glucose estimation was performed with the help of glucometer and glucose strips.

### ***Experimental Animals***

Eighteen Wistar female rats weighing 200-250 g were used in the present study. Prior to animal experimentation, the protocol was approved by animal ethics committee of Department of Pharmaceutical Sciences, Kumaun University, Nainital, India (Registration number - KUDOPS/77). The animals were acclimatized for one week under laboratory conditions prior to

experiment. The animals were kept at 12 h light and dark cycle, maintained under controlled conditions i.e. temperature ( $22 \pm 3^{\circ}$  C) and humidity (30-70%), given synthetic pellet diet and water *ad libitum*.

### ***Dose Selection***

The dose of the pesticide was selected according to the no observed effect level (NOEL) and no observed adverse effect level (NOAEL) as per OECD guidelines (OECD, 2001). NOAEL is the maximum selected dose that causes no adverse alterations in the functional capacity, morphological parameters, growth and developmental processes and life span of the exposed organism. NOEL is the highest dose which shows no observed changes in functional capacity, morphological parameters and life span of the exposed organism in comparison with the reference group (IPCS, 2009). The NOEL dose of CPF for developmental study in rats was greater than 15 mg/kg body weight (Akhtar et al., 2006). The NOAEL dose studied for GLY exposed rats was 31 mg/kg body weight (FAO, 2006).

### ***Experimental Protocol***

Adult healthy female rats were selected and randomly divided into three groups (6 rats each). Female rats having proestrous phase were placed individually with male, for breeding and those with copulatory plug or vaginal smear sperm positive were considered under gestational day zero (Abd El-Nasser et al., 2009). All the female rats after confirmation of their gestational day were treated with the pesticides. Group 1 was served as control and administered with normal saline only. Group 2 and 3 were given CPF and GLY at a dose of 10 mg/kg, orally throughout their gestation period, **the successive dosages of pesticides were given at a time interval of 24 h**. Body weight and gestation time duration were recorded for all the animals. Animals were also observed for any clinical sign of toxicity.

On day 21 of gestation period, blood samples were collected from all the pregnant rats through retro orbital plexus puncture. Blood samples were centrifuged at 10,000X g for ten min to separate the serum. All serum samples were collected immediately and stored for the further biochemical estimations and lipid profile analysis.

### ***Body weight and gestational length Analysis***

Body weight of all the pregnant rats was recorded throughout their gestation period and gestation time duration was measured in the control as well as pesticide exposed groups.

### ***Serum Biochemical Analysis***

Blood glucose level was analyzed by glucometer. Serum glutamate oxaloacetate transferase (SGOT), serum glutamate pyruvate transferase (SGPT), bilirubin, and total protein, were estimated in serum by using commercially available diagnostic kits (BioSystems S.A., Barcelona, Spain).

### ***Lipid profile estimation***

The serum levels of total cholesterol, triglycerides (TGs), and high density lipoprotein (HDL) were analyzed using commercial assay kits (Giese Diagnostics S.r.l., Rome, Italy). Very low density lipoprotein (VLDL), low density lipoprotein (LDL-C), and atherosclerotic index (AI) were calculated by using formulas:

$$VLDL = TGs/5$$

$$LDL = TC - (VLDL + HDL)$$

$$Atherosclerotic\ Index = (TC - HDL)/HDL$$

### ***Morphological Analysis***

Maternal and reproductive outcome of pregnant rats exposed to pesticides were examined during and after gestation period. The offspring variables including total number of pups delivered, number of dead pups, live pups, male pups, female pups, sex ratio and live birth index were analyzed. For morphological analysis among live rat pups, 15 pups were selected from each group and parameters studied were body weight, brain size, crown rump length, eye size, and eye width, lengths of both limb and hind limbs and length of tail of each pup. For investigating any bone abnormality X-ray analysis of the rat neonates was performed by using semi-automatic eagle-I X-ray machine.

### ***Statistical analysis***

All the results obtained were exemplified as Mean  $\pm$  SEM. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using GraphPad Prism (GraphPad Software, San Diego, CA, USA) and the individual comparisons were obtained by Tukey's test. The level of significance was considered at  $p < 0.05$ .

## **Results**

### ***Effect of CPF and GLY on body weight and gestational length Analysis***

In case of pesticides exposed group, the mean body weight gain of female rats was found to be lower when compared with the control group throughout their gestation period. It was also observed in pesticide treated group, the gestation duration was greater when compared with the control group which was observed as 22 days. The alterations in body weight as well as gestation length were shown in Table I.

### ***Effect of CPF and GLY on maternal and reproductive outcome***

The maternal and reproductive outcomes of pregnant rats exposed to pesticides were observed. No maternal death was reported during pregnancy in pesticide exposed groups. The offspring variables including total number of pups delivered, number of dead pups, live pups, male pups, female pups, sex ratio and live birth index were shown in Table II. Live birth index as well as weaning index was found 100% in case of control group. Dead pups were observed in CPF and GLY treated pregnant rats. Percentage of live birth index and weaning index was found lesser in case of pesticide treated group as compared to the control group.

### ***Effect of CPF and GLY on serum biochemical markers***

The serum biochemical analysis of blood glucose, bilirubin, total protein, and liver marker enzymes (SGOT and SGPT) were shown in Table III.

It was observed that in CPF exposed group, the blood glucose level was significantly decreased on the day 21 of gestation period ( $84.66 \pm 1.14$  mg/dl) when compared to control group. However, no significant changes were observed in the blood glucose level of GLY exposed group when compared with the control group.

Slight increase in the level of SGOT was observed in GLY ( $18.93 \pm 0.87$  IU/L) and CPF ( $17.34 \pm 0.55$  IU/L) exposed group when compared with the control group ( $15.18 \pm 0.26$  IU/L). Same results were observed with the SGPT analysis, as there was slight increase in the SGPT level in both CPF ( $18.31 \pm 0.29$  IU/L) and GLY treated group ( $19.20 \pm 0.84$  IU/L) in comparison with control group ( $16.56 \pm 0.32$  IU/L).

Total bilirubin level was found significantly higher in CPF ( $2.08 \pm 0.14$  mg/dl), and GLY ( $1.84 \pm 0.16$  mg/dl) exposed group as compared to control group ( $1.07 \pm 0.08$  mg/dl).

The total protein value was found to be significantly higher in CPF ( $18.83 \pm 1.33$  g/dl) and GLY exposed group ( $14.14 \pm 0.74$  mg/dl) when compared with the control group ( $7.66 \pm 1.04$  g/dl) on the same gestation day 21.

#### ***Effect of CPF and GLY on lipid profile***

The serum lipid profile in control and CPF and GLY exposed groups was analyzed and shown in Table III. Serum cholesterol, triglycerides, LDL, and VLDL levels were found to be significantly ( $p < 0.001$ ) higher in the CPF and GLY exposed group with concomitant decrease in HDL levels than those in the normal control group.

High cholesterol level was observed in GLY ( $148.33 \pm 3.40$  mg/dl) and CPF ( $137.68 \pm 5.09$  mg/dl) exposed pregnant rats on the gestation day 21 when compared with the control group ( $114.68 \pm 4.25$  mg/dl).

In comparison to the control group ( $80.31 \pm 1.53$  mg/dl), the triglyceride levels were found to be significantly higher in CPF ( $136.33 \pm 2.29$  mg/dl) and GLY ( $114.43 \pm 2.35$  mg/dl) exposed groups.

LDL, and VLDL levels were also found to be significantly ( $p < 0.001$ ) higher in CPF and GLY exposed pregnant rats as compared to control rats, whereas, HDL levels were significantly ( $p < 0.001$ ) decreased in CPF and GLY exposed pregnant rats as compared to control rats.

#### ***Effect of CPF and GLY on morphological analysis***

Rat pups delivered by the control group female rats were normal and observed with great uniformity, regarding all the morphometric and morphological measurements. The morphometric observations showed significant differences between the pups whose mothers were exposed to CPF and GLY. These parameters were shown in Table IV including body weight, crown rump length, eye length, eye width, brain size, forelimb size, hind limb size and tail length. Significant decrease in the body weight of rat pups was observed those were delivered by the pesticides exposed group i.e. CPF ( $5410 \pm 125.21$  mg) and GLY ( $5844 \pm 94.44$  mg) when compared with the control group ( $6243 \pm 84.11$  mg).

Pesticides exposure also resulted in decreased crown rump length, forelimb, hind limb, brain and eye size, eye width and tail length as compared to control group.

Morphological abnormalities observed in pesticides exposed rat pups were shown in Figure 3. The CPF exposed rat pups shows morphological abnormalities like microcephaly (MCP), microtia (MTA), micromelia (MML), dysmorphogenesis (DMG), distorted axis (DA) and abdominal and brain hemorrhages. GLY exposed rat pups shows abdominal and brain hemorrhages, MCP and MML abnormalities.

X-ray of CPF exposed group shows skeletal disruptive malformations, wavy ribs, and curved fetus when compared with the control and GLY treated group (Figure 4).

In GLY exposed group, the rat pups delivered from GLY exposed group shows microphthalmia and also observed for hematoma in neck, back, abdomen, and head on day 21 after their birth as shown in Figure 5 and Figure 6 whereas in case of control and CPF treated group no such abnormalities were observed. Skeletal abnormality like distorted axis observed in all the rat pups of GLY exposed group on 10<sup>th</sup> day after birth (Figure 7). Intracranial hemorrhage as well as hemorrhage in the spinal cord was observed in 10 % of rat pups of GLY treated group on 21 days after birth (Figure 7).

## **Discussion**



This study shows that there is a significant decrease in the average body weight gain of pesticide exposed group as compared to the control group. In case of pregnant rats, body weight gain at the end of the pregnancy determines the growth of the fetus starting from day 18. Maternal weight gain on day 20 of pregnancy subsequently represents the increase in fetal weight, growth and development of extra embryonic membrane. The reduction in average body weight gain in pesticide exposed pregnant rats indicates the reduction in growth and development of fetus as well as extra-embryonic membrane like placenta. A condition like placental insufficiency develops in which the placenta fails to supply adequate oxygen and nutrients to the growing fetus. Also, the increase in gestational length indicates the placental insufficiency disorder leading to adverse pregnancy outcomes like oxygen deprivation causing cerebral palsy, behavior and learning disorder. Another associated health risk related to increased gestational length is meconium asphyxia causing breathing problem in infants, lung inflammation, persistent pulmonary hypertension, and brain damage (Sharma et al., 2015). The pesticide exposure in rats produces stress which develops anorexia and causes reduction in body weight gain.

In CPF exposed group, the blood glucose level was found significantly lower than the control group on day 21 of gestation period. It causes hypoglycemia in pregnant rats, as there is a very close relationship between the glucose level of the mother and the fetus. Maternal hypoglycemia during pregnancy not only affects mother but also the conceptus. During pregnancy placenta produces hormones which helps in the growth and development of the baby. Some researchers hypothesized that low blood glucose level during pregnancy can induce serious adverse effects which causes fetal malformations, and poor neuropsychiatric development (Rosenn and Miodovnik, 2000; Evers et al., 2002; ter Braak et al., 2002). The cholesterol level of the herbicide GLY exposed group was found significantly higher as compared to the control group on the day 21 of gestation duration. In the current obstetric practice the cholesterol level is not measured. No reference range is defined for lipid parameters during gestation period, because of the lack of evidence on the significance of increased cholesterol levels. Total Cholesterol level has been shown to be elevated during the second and the third trimesters. Elevated cholesterol level during pregnancy is due to hepatic adipose metabolism and sex steroid hormones. Cholesterol plays an important role in steroid hormone synthesis. The total cholesterol level is the most easily measured lipid and it reflects low density lipoprotein (Kaushik and Saini, 2014).

Hyperlipidemia is now linked with high risk of atherosclerosis in both mother and infant, preterm delivery, preeclampsia and gestational diabetes in mothers (Bartels and O'Donoghue, 2011). Less cholesterol in pregnant female's body may hamper the development of fetus causing low birth weight and smaller head circumference (Emond et al., 2018). Elevated levels of triglycerides during gestation has many positive effects that contribute to normal growth and development of fetus, as well as serve as a depot of energy for mothers and preparing them for lactation. Excessive increase in triglycerides level during gestation causes preeclampsia, gestational diabetes and preterm delivery (Lepercq et al., 2001). Serum total protein value in CPF and GLY treated pregnant rats was found higher when compared with the control group. The pregnancy related plasma protein level is a well-known serum marker of pathological conditions during pregnancy. Maternal serum plasma protein level is elevated in pre-eclamptic pregnancy (Hughes et al., 1980). High levels of bilirubin occur due to excessive peripheral breakdown of hemoglobin or may be due to dyserythropoiesis. Hyperbilirubinemia indicates septicemia, parenchymal disease and renal failure (Bacq et al., 1996).

The SGOT and SGPT level remain normal during uncomplicated pregnancies. The findings of our results also suggests that in the pesticides exposed group the level of SGOT, SGPT and total protein is elevated when compared with the reference. This elevation in serum biomarker responses might be associated with the preeclampsia like condition of pregnant rats which hampers the growth and development of the fetuses (Mei-Dan et al., 2013).

All the rat pups in the control group were normal and a great uniformity was observed in the rat pups regarding all the morphometric and morphological measurements. The CPF exposed rat pups shows morphological abnormality like microcephaly (MCP), microtia (MTA), micromelia (MML), dysmorphogenesis (DMG), distorted axis (DA), abdominal and brain hemorrhages. The morphological abnormalities observed following exposure to CPF was due to the inhibition of acetylcholine esterase enzyme at the nerve terminals from the central, autonomic, peripheral, somatic divisions of the nervous system. This inhibition triggers signs and symptoms involving gastrointestinal, respiratory, cardiovascular and ocular effects. In the embryo, the cholinergic receptors are broadly spread in the central and peripheral systems involving smooth muscles, and lining of epithelium of digestive, cardiovascular, central nervous system as well as urinary

system. Nicotinic and muscarinic receptor in fetus develops in amount and distribution pattern related to gestational time. Also cholinergic mechanism has been found to be functional in the vascular homeostasis control during fetus development *in utero* in the last trimester of gestation (Mao et al., 2007).

In GLY exposed group, abdominal and brain hemorrhages, MCP and MML abnormalities were observed. The exact mechanism of action of GLY in plants is inhibition of an enzyme 5-enoylpyruvylshikimate-3-phosphate synthase (EPSPS) in the biosynthesis of amino acids tyrosine, phenylalanine and tryptophan. The toxic effect of herbicide is that serum proteins binds with them and reduces their availability to cells. Some studies shows that Roundup containing GLY inhibits cytochrome P-450 aromatase enzyme activity as well as has cytotoxic effect.<sup>8</sup> Aromatase (CYP19A1, CYP19) catalyzes the conversion of androgens to estrogen in many tissues (Boon and Simpson, 2012; Haddad and Eugster, 2016). Estrogen hormone produced during pregnancy primarily in the placenta. Androgen precursor originated from adrenal glands of the mother and the fetus. Any disorder in the production of estrogen plays a major role in pre-eclampsia symptoms as they are produced by the placenta and promotes uterine artery vasodilation and placental angiogenesis (Berkane et al., 2017). Globally, preeclampsia is the main cause of maternal and fetal death (Xiong et al., 1999).

## **Conclusion**

This study correlated the association of environmental pollutants with the increase risk of birth defects. Serum biomarkers are used as an evaluation parameter for detecting the overall health of an individual especially during pregnancy and determine the normal growth and development of the growing fetus. Any exposure to environmental pollutants during prenatal development affects the growth of the fetus and leads to various physical as well as mental abnormalities in infants. Also additional research is being required to determine the etiology of birth defects and exposure to environmental toxicants.

## **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we

do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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**Table I****Effect of chlorpyrifos and glyphosate on body weight and gestational length**

S. No.	Groups	Mean body weight (g)		Percentage Gain in body weight	Gestational length (in days)
		Day 0	Day 21		
1	Control	210.33 ± 0.91	286.66 ± 2.94	36.29%	22.33 ± 0.33
2	Chlorpyrifos (10 mg/kg)	206.66 ± 1.67	240.50 ± 1.67	16.37%	26.50 ± 0.22***
3	Glyphosate (10 mg/kg)	205.50 ± 0.40	266.83 ± 2.35	29.84%	25.33 ± 0.21***

Data are presented as mean ± SEM (n=6). \*\*\*p < 0.001, compared with control group (One-way ANOVA followed by Tukey's test).

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**Table II**

<b>Parameters</b>	<b>Control</b>	<b>Chlorpyrifos (10 mg/kg)</b>	<b>Glyphosate (10 mg/kg)</b>
<b>Maternal death</b>	00	00	00
<b>Total pups delivered</b>	37	35	42
<b>No. of Dead pups</b>	00	04	04
<b>No. of Female pups</b>	16	13	21
<b>No. of Male pups</b>	21	18	17
<b>Sex ratio <sup>a</sup></b>	1.31	1.38	0.80
<b>Live birth index <sup>b</sup> (%)</b>	100	88.57	90.47
<b>Weaning index <sup>c</sup> (%)</b>	100	90.32	97.36

<sup>a</sup> Sex ratio = Number of males / Number of females

<sup>b</sup> Live birth index = (Number of live offspring / Number of offspring delivered) X100

<sup>c</sup> Weaning index = (Number of live pups on day 21 / Number of live offspring's born) X100



**Table III****Effect of chlorpyrifos and glyphosate on serum biochemical makers and lipid profile**

Parameters	Control	Chlorpyrifos (10 mg/kg)	Glyphosate (10 mg/kg)
Glucose (mg/dl)	96.83 ± 3.49	84.66 ± 2.14*	94.33 ± 3.88
SGOT(IU/L)	15.18 ± 0.26	17.34 ± 0.55*	18.93 ± 0.87**
SGPT (IU/L)	16.56 ± 0.32	18.31 ± 0.29	19.20 ± 0.84**
Bilirubin (mg/dl)	1.07 ± 0.08	2.08 ± 0.14***	1.84 ± 0.16**
Total Protein (g/dl)	7.66 ± 1.04	18.83 ± 1.33***	14.14 ± 0.74**
Cholesterol (mg/dl)	114.68 ± 4.25	137.68 ± 5.09**	148.33 ± 3.40***
Triglycerides (mg/dl)	80.31 ± 1.53	136.33 ± 2.29***	114.43 ± 2.35***
HDL (mg/dl)	51.48 ± 3.97	28.77 ± 2.20***	23.35 ± 1.26***
LDL (mg/dl)	47.13 ± 4.94	81.64 ± 4.67***	102.10 ± 3.93***
VLDL (mg/dl)	16.06 ± 0.31	27.27 ± 0.46***	22.89 ± 0.47***
AI	1.28 ± 0.16	3.91 ± 0.38***	5.46 ± 0.44***

Data are presented as mean ± SEM. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, compared with control group (One-way ANOVA followed by Tukey's test).

**Table IV****Effect of chlorpyrifos and glyphosate exposure on rat pups morphometric parameters**

Parameters	Control	Chlorpyrifos (10 mg/kg)	Glyphosate (10 mg/kg)
Body weight (mg)	6243 ± 84.11	5410 ± 125.21***	5844 ± 94.44**
Crown Rump length (mm)	53.00 ± 2.06	37.70 ± 1.08***	40.55 ± 1.32***
Eye length (mm)	29.44 ± 0.55	23.00 ± 1.33***	22.77 ± 0.32***
Eye width (mm)	16.66 ± 0.83	15.00 ± 0.48	12.22 ± 0.87**
Brain size (mm)	12.57 ± 0.81	6.55 ± 0.33***	8.55 ± 0.24***
Fore Limb size (mm)	12.43 ± 0.68	7.15 ± 0.31***	9.88 ± 0.92*
Hind Limb size (mm)	11.86 ± 0.55	9.81 ± 0.41**	10.55 ± 0.17
Tail length (mm)	15.43 ± 0.48	12.30 ± 0.42***	14.44 ± 0.44

Data are presented as mean ± SEM. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, compared with control group (One-way ANOVA followed by Tukey's test).

## Figure Legends

**Figure 1.** Mechanism of action of Chlorpyrifos (Acetyl cholinesterase enzyme inhibitor)

**Figure 2.** Mechanism of action of Glyphosate (5-enoyl pyruvyl shikimate 3-phosphate synthase inhibitor)

**Figure 3.** Morphological abnormalities observed in rat pups exposed to (B) Chlorpyrifos and (C) Glyphosate. Chlorpyrifos exposed group shows abdominal and brain hemorrhages, microcephaly (MCP), microtia (MTA), distorted axis (DA), dysmorphogenesis (DMG) and micromelia (MML) abnormalities. Glyphosate exposed group shows abdominal and brain hemorrhages, microcephaly (MCP) and micromelia (MML) abnormalities.

**Figure 4.** Skeletal system of Control group (A) shows no abnormality. Chlorpyrifos (B) and glyphosate (C) exposed group shows skeletal malformations as disruptive malformations, wavy ribs, and curved fetus. Figure (D, E and F) represents the X-ray of control, chlorpyrifos and glyphosate exposed group, respectively.

**Figure 5.** Microphthalmia and hematoma observed in rat neonates exposed to glyphosate during prenatal development.

**Figure 6.** Skeletal abnormality like distorted axis observed in rat pups of glyphosate exposed group only on day 10 after their birth. In control and Chlorpyrifos exposed group, skeletal abnormality was not observed.

**Figure 7.** Intraventricular as well as spinal cord hemorrhage observed in rat pups of glyphosate treated group on day 21 after their birth. In control and chlorpyrifos exposed group, no hemorrhage was observed.

Figure 1

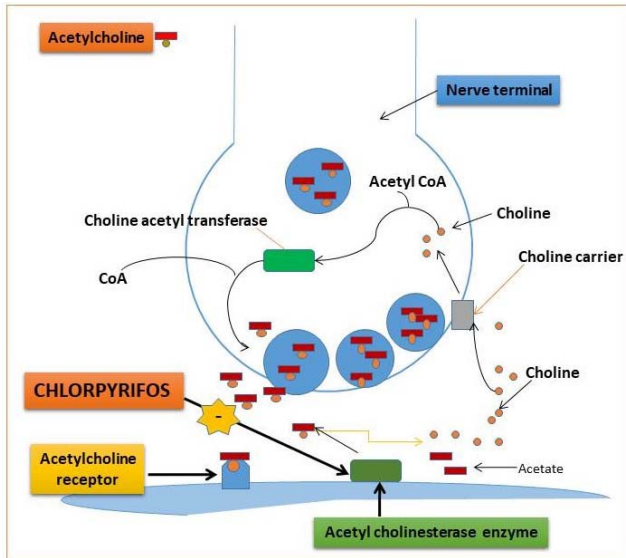
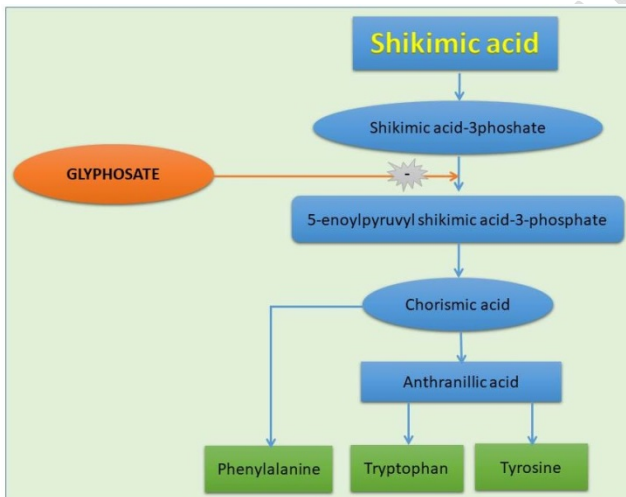
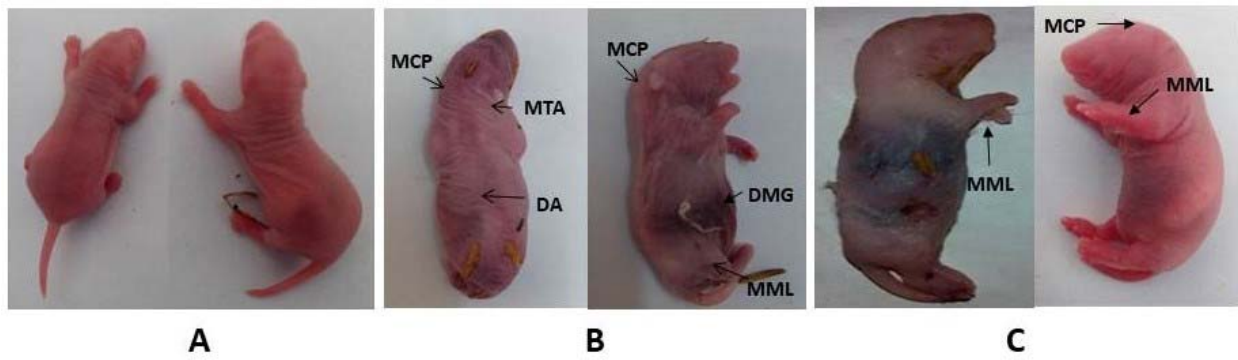


Figure 2

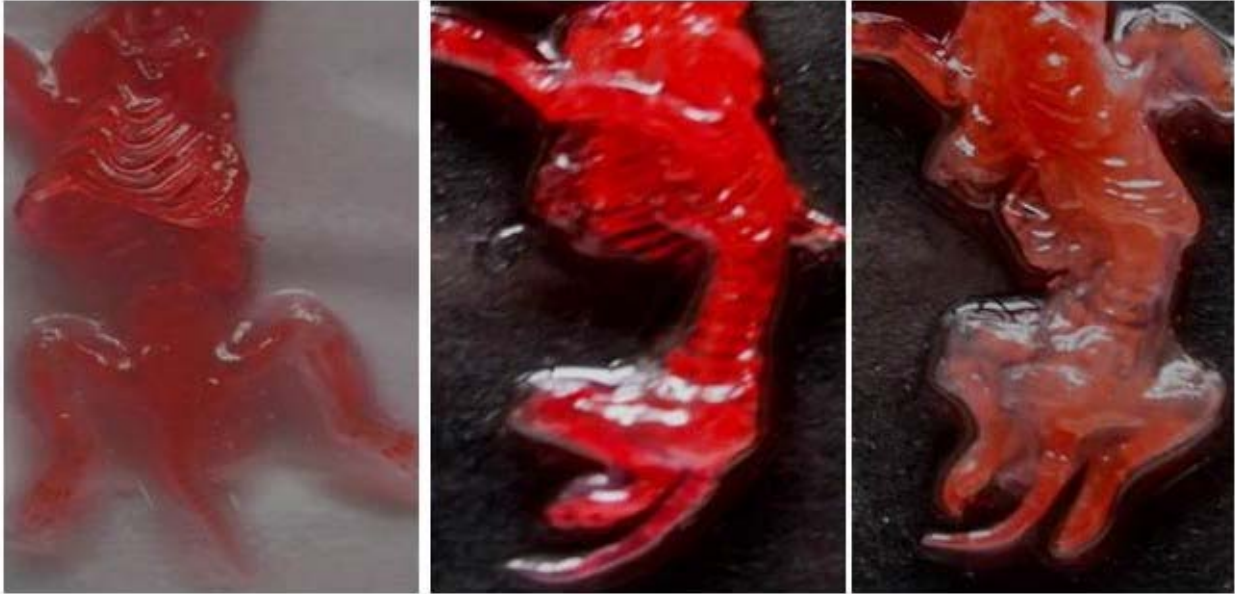


**Figure 3**



UNDER PEER REVIEW

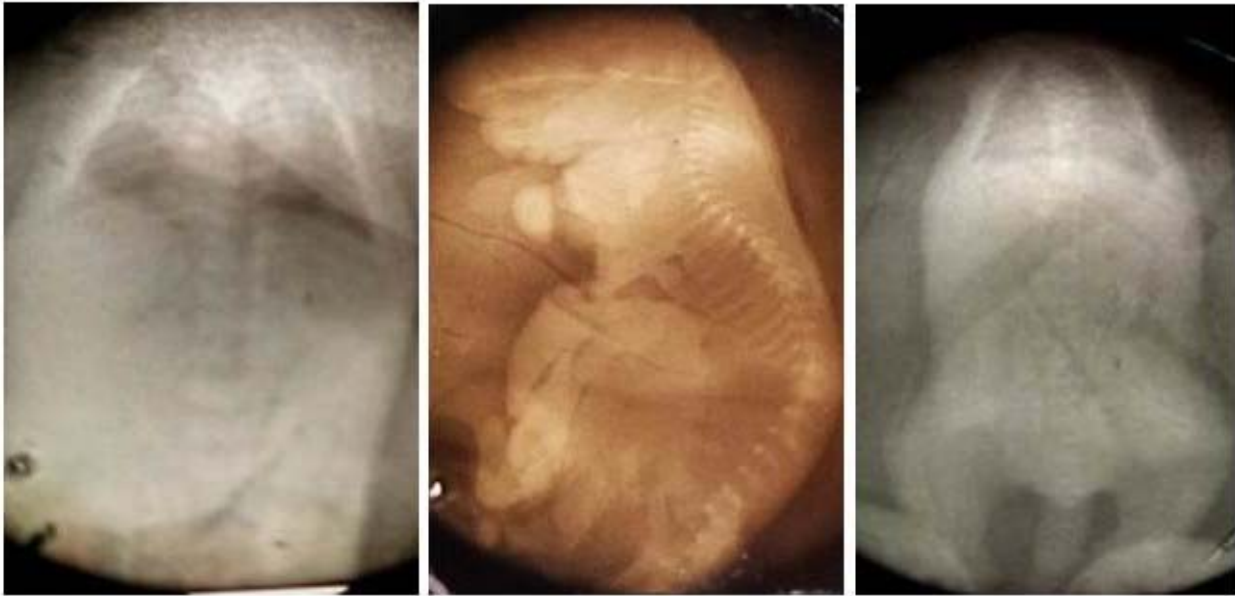
Figure 4



A

B

C



D

E

F

**Figure 5**



Figure 6

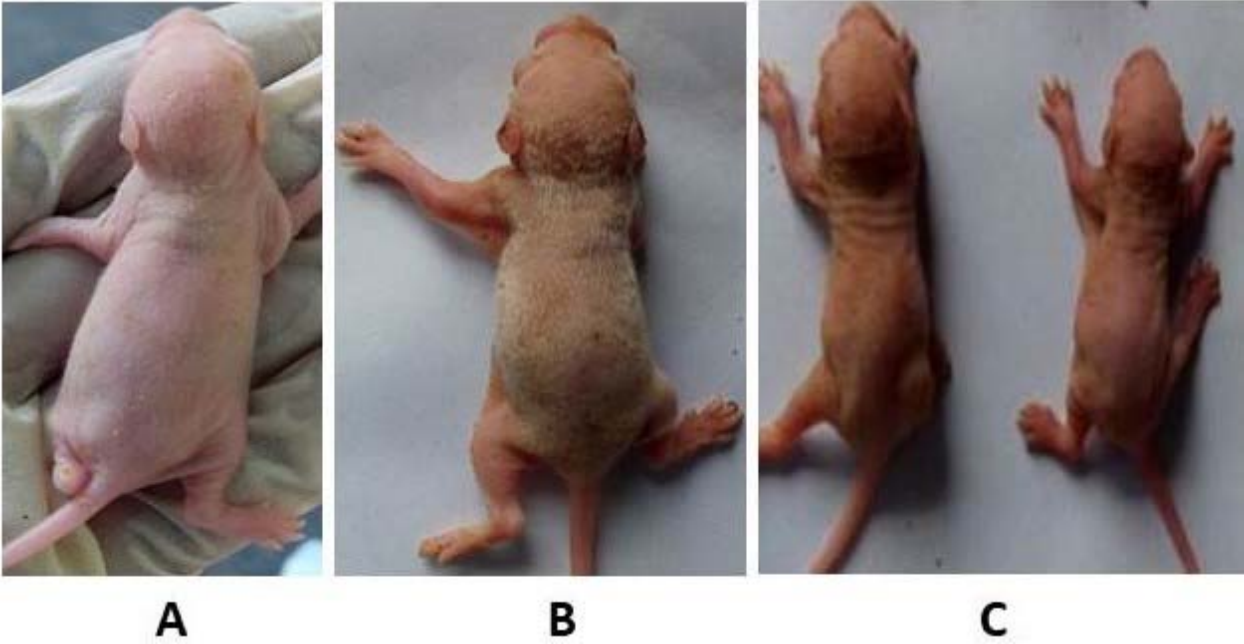


Figure 7

