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DEVELOPMENTAL TOXICITY OF ACETAMIPRID IN RATS

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ABSTRACT

Neonicotinoids are the most widely used group of insecticides. In the current investigation, developmental toxicity of acetamiprid as a representative of neonicotinoids was studied in rats. Acetamiprid was orally given to pregnant rats on GD 6 through 15 at a dose level of 31.4 mg/kg (equivalent to 1/10th LD_{50}). Pregnant females were sacrificed on GD 20 and the number of implantations, resorptions and live fetuses were recorded. Fetuses were subjected to morphological, soft tissue and skeletal examinations. The number of resorptions increased with reduction of the number of implantations and live fetuses. Both placental and fetal weights were reduced. Morphological and soft tissue examination revealed significant elevations in the number of fetuses with dwarfism, eye anomalies (microphthalmia and anophthalmia), intrathoracic hemorrhage, lung hypoplasia and heart hypertrophy. In addition, non-significant increases in the frequency of exencephaly, spina bifida and dilatation of nares, brain ventricles and renal pelvis were noticed. Significant skeletal anomalies were recorded in the form of widely opened fontanel, incomplete ossification of skull and sternbrae, short ribs, and absences of phalanges and sacral and caudal vertebrae. In conclusion, acetamiprid was demonstrated to be teratogenic to rats producing morphological, soft tissue and skeletal anomalies.

KEYWORDS: Acetamiprid, Rat, Developmental, Toxicity.

INTRODUCTION

Neonicotinoid insecticides were first introduced in the 1990s, and since then, they have become the most important and widely used group of insecticides in the world. They are water-soluble, and thus be taken up by the roots of the developing plant, becoming systemic

and are found in vascular tissues and foliage, providing protection against herbivorous insects.[1]

Neonicotinoids are acting as agonists of nicotinic acetylcholine receptors (nAChRs) in the postsynaptic neuron. This causes continuous activation of the receptor, leading to symptoms of neurotoxicity. These compounds have higher affinity and bind strongly to insect nAChRs than mammalian subsets of nAChRs, making them more toxic to insects than mammals.[2]

Acetamiprid is a widely used second generation neonicotinoid of outstanding systemic action and potency. It is used for control of insects on several crops and sucking insects like aphids, bees and mosquitoes.[3] Since the use of acetamiprid is increasing, it is necessary to identify its possible adverse effects on animals.

Acetamiprid produced hematological changes in poisoned rats in the form of reduced RBC, hemoglobin and HCT. It also induced liver dysfunction evidenced by elevated activities of blood AST, ALT, ALP and LDH.[4] Decrease in plasma glucose, cholesterol and low-density lipids were also reported.[5] Moreover, it increased calcium, phosphorous, sodium, potassium, chloride, zinc, copper, iron and cobalt concentrations in plasma of intoxicated rats.[6]

Subchronic administration of acetamiprid to rats caused significant reductions in lymphocyte proliferation and macrophage function[7] and suppressed both cell medicated immunity and antibody forming ability of lymphocytes.[8]

It has been reported that acetamiprid intoxication was associated with oxidative stress in rat liver manifested by reduced activities of SOD and CAT with concomitant increase in lipid peroxidation.[4] Oxidative stress was also reported in the testis of rats and mice.[9,10] Acetamiprid decreased the viability of human HEK293 cells[11] and produced cytotoxic and genotoxic effects to human peripheral lymphocytes and intestinal CaCo-2 cells *in vitro*.[12,13] The reproductive toxicity of acetamiprid was demonstrated in male rats where it induced deleterious effects on semen quality, sex organs and testosterone levels.[10] In multigeneration studies in rats, it reduced the number of implantations and fetal growth and survival.[14,15]

Although the literature available on the teratogenic potential of acetamiprid are scanty, other neonicotinoid insecticides were previously reported to produce teratogenic effects. Imidacloprid produced several teratogenic effects in rat fetuses including absence of thoracic ribs, fused ribs, wavy ribs, bifid vertebral centrum and incomplete ossification of phalangeal

cartilages.[16] In chicken eggs it induced limb deformities, beak deformities, head enlargement and ectopia viscerale[17] and malformation of heart tube.[18] Gestational exposure to thiacloprid caused reduced ossification, wavy ribs and asymmetrical vertebrae in rats[19] and retarded ossification, supernumerary 13*th* rib and forelimb arthrogryposis in rabbits.[20] Depending upon these data, it is hypothesized that acetamiprid could have teratogenic potential like other nicotinoids. Therefore, the present study was performed to explore the developmental toxicity of acetamiprid in rats.

MATERIALS AND METHODS

Animals

Animal Care and Use Committee of University of Sadat City approved the protocol for this study, and animals were cared for in accordance with the Guidelines for Animal Experiments of University of Sadat City.

Virgin female Wister rats (200-230g) were purchased from the Lab Animal House, Faculty Veterinary Medicine, Cairo University. Animals were housed in plastic cages on wood-chip bedding and kept at a temperature of 23 ± 2℃. Animals were provided with commercial rodent chow and tap water *ad libitum* for acclimatization period of 2 weeks prior to start of the experiment.

Mating was achieved by placing 2 females and 1 male in a cage overnight and successful mating was confirmed by the presence of sperm in vaginal cytology on the following morning.

Females were placed overnight with mature males of the same strain (two females to one male) and successful mating was confirmed by the presence of sperm in vaginal smears on the following morning. Sperm-positive females were considered to be in gestational day (GD) zero and singly housed in plastic cages with stainless steel wire lids and given tap water and commercial rodent chow *ad libitum*.

Test chemical

The commercial formulation cetam containing 20% acetamiprid was purchased from Al-Burj Agrivet Pesticide Manufacturing Co, Jordan. It was dissolved in corn oil and freshly prepared daily before animal treatment.

Experimental protocol

Thirty healthy pregnant rats were randomly assigned to 2 groups. The treated group (20 females) received acetamiprid orally by stomach intubation on GD 6-15 at a dose level of 31.4 mg/kg, equivalent to 1/10th LD_{50}.[21] The insecticide was given to rats at a dose volume of 5 ml/kg body weight. The control group (10 rats) received equivalent volumes of corn oil. Pregnant females were sacrificed on GD 20 by diethyl ether inhalation. Necropsies were performed and each female was examined for placental weights and numbers of implantation, resorptions and live fetuses. The uteri with no evidence of implantation were stained with 2% ammonium sulphide to identify the presence of early resorption sites.[22] The female was considered ‘‘non-pregnant” if no stained implantation sites were observed.

All fetuses were individually weighed and examined for morphological abnormalities. One third of fetuses from each litter was fixed in Bouin’s fluid for subsequent soft tissue examination with a freehand razor sectioning technique. The other two-thirds of fetuses were fixed in ethanol (95%), eviscerated, cleared in 1% KOH and then processed for skeletal staining with alizarin red S for examination of skeletal malformations.[23]

Statistical analyses

Statistical analysis was done using SPSS software (16.0 version). All morphological, visceral and skeletal anomalies were analyzed by Chi square.[24] Maternal indices and fetal weight were analyzed using T-test[25] and data were presented as mean ± SE.

RESULTS

Table 1 presents the alterations in maternal indices induced by acetamiprid. Significant decreases ($P \leq 0.05$) were recorded in the number of implantations, while number of resorptions increased "Fig. 1A". The number of live fetuses and both placental and fetal weights were significantly reduced ($P \leq 0.05$).

Morphological and soft tissue examination of fetuses "Table 2" revealed significant increases ($P \leq 0.05$) in the number of dwarfed fetuses "Fig. 1B", eye anomalies (microphthalmia and anophthalmia) (Fig. 2C), intrathoracic hemorrhage, lung hypoplasia "Fig. 2D" and heart hypertrophy. In addition, non-significant elevations ($P \leq 0.05$) in the frequency of exencephaly and spina bifida "Fig. 1B" and dilatation of nares "Fig. 2A", brain ventricles "Fig. 2B" and renal pelvis "Fig. 2E" were recorded.

The data obtained from skeletal examination of fetuses "Table 3" revealed significant elevations (P≤0.05) in the number of fetuses with widely opened fontanel "Fig. 3A", incomplete ossification of skull "Fig. 3A&C" and sternbrae "Fig. 3B", short ribs "Fig. 3C", and absences of phalanges, sacral and caudal vertebrae. Furthermore, way ribs were also noticed in some fetuses, but their frequency was not significantly different from control.

Table 1: Maternal indices in control and acetamiprid - treated rats.

Parameter	Control	Treated
No. of inseminated females	10	20
No. of pregnant females	10	18
Implantations / dam	12.6 ± 0.221	11.5 ± 0.185*
Live fetuses / dam	11.5 ± 0.307	9.3 ± 0.266*
Resorptions/ litter	1.1 ± 0.277	2.22 ± 0.339*
Total pups examined	115	167
Fetal body weights (g)	3.86 ± 0.056	3.11± 0.065*
placental weight (g)	0.56 ± 0.009	0.50 ± 0.005*

**Significant at P≤0.05 vs control.*

Table 2: Morphological and soft tissue anomalies in fetuses from control and treated dams.

		Control	Treated
NO. of fetuses examined		39	56
Dwarfism	No.	0	19
	%	0	33.9*
Exencephaly	No.	0	3
	%	0	5.4
Spina bifida	No.	0	2
	%	0	3.6
Dilated brain ventricles	No.	1	4
	%	2.6	7.1
Dilated nares	No.	0	3
	%	0	5.4
Eye anomalies	No.	0	6
	%	0	10.7*
Intrathoracic hemorrhage	No.	1	15
	%	2.6	26.8*
Lung hypoplasia	No.	0	9
	%	0	16.1*
Heart hypertrophy	No.	0	9
	%	0	16.1*
Dilated renal pelvis	No.	0	5
	%	0	8.9

**Significant at P≤0.05 vs control.*

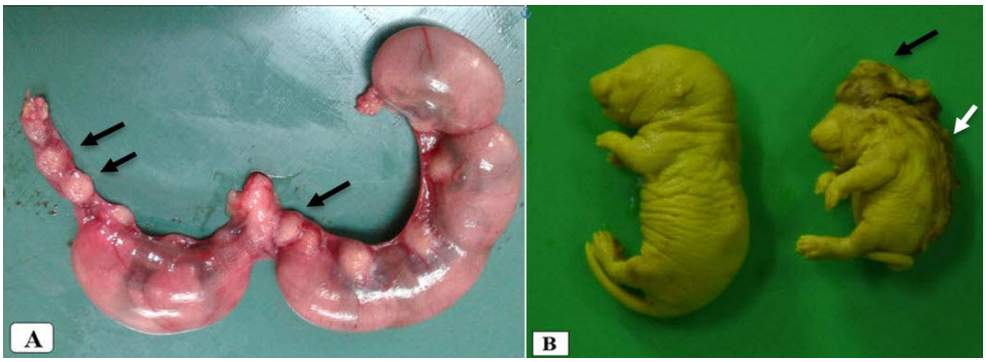

Fig. 1: Uterine findings and morphological changes induced by acetamiprid.

A) Resorption sites (arrows).

B) Left: Control fetus; Right: Treated fetus showing dwarfism, exencephaly (black arrow) & spina bifida (white arrow).

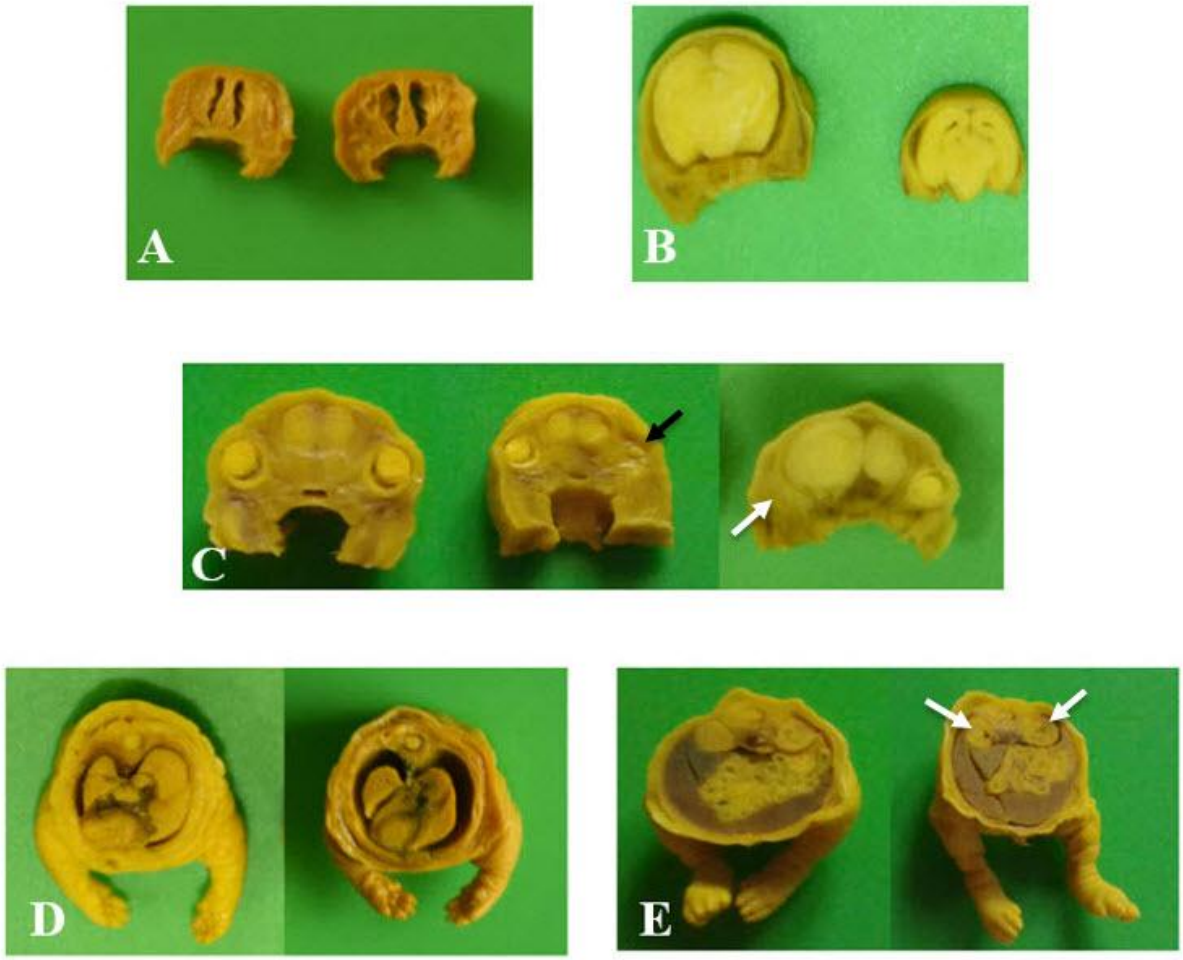

Fig. 2: Soft tissue anomalies induced by acetamiprid in rat fetuses.

A) Left: Control fetus; Right: Treated fetus with dilated nares.

B) Left: Control fetus; Right: Treated fetus showing dilated brain ventricles.

C) Left: Control fetus; Middle: Treated fetus with microphthalmia (black arrow); Right: Treated fetus with anophthalmia (white arrow).

D) Left: Control fetus; Right: Treated fetus showing lung hypoplasia.

E) Left: Control fetus; Right: Treated fetus with dilated renal pelvis (arrows).

Table 3: Skeletal anomalies induced by acetamiprid in rat fetuses.

		Control	Treated
NO. of fetuses examined		76	111
Widely opened fontanel	No.	0	12
	%	0	10.8*
Incomplete ossification of skull	No.	1	24
	%	1.3	21.6*
Incomplete ossification of sternbrae	No.	1	38
	%	1.3	34.2*
Short ribs	No.	1	18
	%	1.3	16.2*
Wavy ribs	No.	0	3
	%	0	2.7
Phalanges	No.	0	9
	%	0	8.1*
Sacral V	No.	0	6
	%	0	5.4*
Caudal V	No.	0	7
	%	0	6.3*

**Significant at P≤0.05 vs control.*

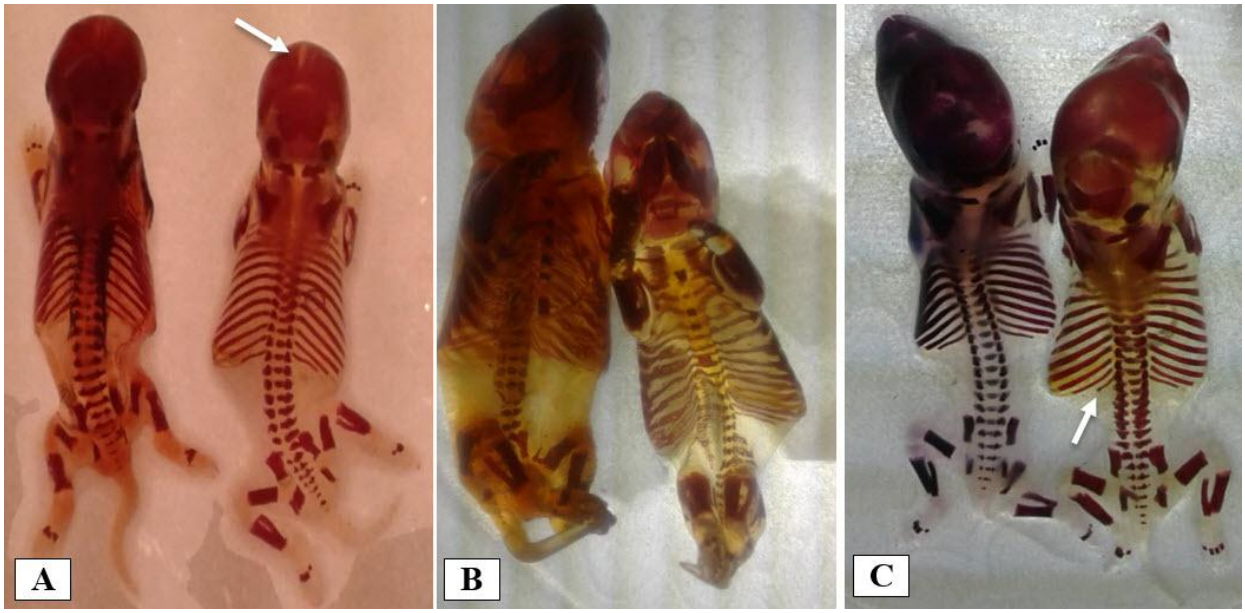

Fig. 3: Skeletal anomalies in acetamiprid-treated fetuses.

A) Left: Control fetus; Right: Treated fetus with widely opened fontanel (arrow) and incomplete ossification of skull.
B) Left: Control fetus; Right: Treated fetus with incomplete ossification of sternbrae.
C) Left: Control fetus; Right: Treated fetus with incomplete ossification of skull and short rib (arrow).

DISCUSSION

Reproductive and developmental toxicity studies are important for the safety evaluation of a variety of pesticides. In this study, we evaluated the teratogenic potential of acetamiprid administered to pregnant rats at 31.4 mg/kg on GD 6-15.

Acetamiprid induced reduction of placental weight, number of implantations and increased incidence of resorptions. The number and weight of living fetuses were reduced. Our findings agree with those of Schardein[14], Trutter[15] and Vohra and Khera[26] who reported that exposure of pregnant rats to acetamiprid and imidacloprid reduced the number of implantations and inhibited rat offspring growth and survival.

The decrease in placental weight may occur secondary to toxic effects causing disturbances in placental redox status, interference with transport of essential nutrients and substances, hormonal disturbance and interference with protein or nucleic acid synthesis or function.[27]

The decline in the total number of implants is indicator of early embryonic death. The increased incidence of resorptions might occur primarily because of the direct effect on the progeny and/or because of an indirect effect mediated through the dam such as imbalance in estrogen and/or effects on gonadotropin secretion, via central nervous mechanisms.[28-30]

The reduction in fetal body weight is indicator of intrauterine growth retardation[31] caused by adverse effects on both decidual growth and embryonic development.[32] Because delayed ossification of the fetal skeleton was recorded in this study, it is suggested that rarefaction of fetal bones may have contributed to lowered fetal weights.[28]

Acetamiprid treatment resulted in increases in the frequency of dwarfed fetuses, eye anomalies (microphthalmia and anophthalmia), intrathoracic hemorrhage, lung hypoplasia and heart hypertrophy. In addition, non-significant elevations in the frequency of exencephaly, spina bifida and dilatation of nares, brain ventricles and renal pelvis were noticed. Skeletal anomalies induced by acetamiprid included widely opened fontanel,

incomplete ossification of skull and sternbrae, short ribs, and absences of phalanges, sacral and caudal vertebrae. Furthermore, way ribs were also observed in some fetuses, but their frequency was not significantly different from control.

Similar to our results, it has been reported that acetamiprid administration to pregnant rats and rabbits produced progeny with shortening of the 13*th* rib, fused thoracic vertebral arches and fused ribs.[33,34]

The teratogenic effects of other neonicotinoids were previously demonstrated. Imidacloprid produced several teratogenic effects in rat fetuses including absence of thoracic rib, fused and wavy ribs, bifid vertebral centrum and incomplete ossification of phalangeal cartilages.[16] Exposure of chicken eggs to imidacloprid was associated with several anomalies such as limb deformities, beak deformities, head enlargement and ectopia viscerale[17] and malformation of heart tube.[18] Gestational exposure to thiacloprid produced teratogenic effects in rats including reduced ossification, wavy ribs and asymmetrical vertebrae[19] and in rabbits including, retarded ossification, supernumerary 13*th* ribs and forelimb arthrogryposis.[20]

Anophthalmia is characterized by a complete absence of ocular tissue due to a developmental lack of the optic vesicles (primary anophthalmia) or an early differentiation arrest (secondary anophthalmia). Microphthalmia is a disorder in which one or both eyes are abnormally small. The exact mechanism of eye defects is unknown, but it may be attributed to congenital problems or drug developmental toxicity on rat embryo on GD 9 and 10.[25,36] An enlarged or dilated renal pelvis could be suggestive of hydronephrosis caused by urinary stasis.[37]

The retarded ossification of skull bones and sternebrae is a good indicator of retarded development induced by acetamiprid. This may be due to maternal reduced food intake[38] and disturbed metabolism of thyroid and/or steroid hormones.[38,39] Acetamiprid was reported to induced disturbances in metabolism of important elements including calcium, phosphorous, sodium, potassium, chloride, zinc, copper, iron and cobalt in intoxicated rats.[6]

The mechanisms of acetamiprid induced developmental toxicity may include oxidative stress[4,9,10,40] and genotoxic effects causing decrease in cell proliferation.[7,13] In addition, alteration of gene expression may contribute to the teratogenic effects of acetamiprid. Kimura-Kuroda et al.[41] reported that exposure of neuron-enriched cultures from neonatal rat

cerebellum to acetamiprid altered the expression of nine genes essential for brain neurodevelopment.

CONCLUSION

Administration of acetamiprid to pregnant rats during the period of organogenesis at a dose level equivalent to 1/10th LD_{50} produced developmental toxic effects in the form of morphological, soft tissue and skeletal malformations.

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Competing interests

The author declares that she has no competing interests.

REFERENCES

1. Wood TJ, Goulson D. The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. Environ Sci Pollut Res Int, 2017; 24(21): 17285-17325
2. Tomizawa M, Casida JE. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu Rev Entomol, 2003; 48: 339–364.
3. EPA (United States Environmental Protection agency). Pesticide fact sheet: acetamiprid, 2002. Available from: http://www.epa.gov/opprd001/factsheets/ acetamiprid. pdf, 4–5.
4. Chakroun S, Ezzi L, Grissa I, Kerkeni E, Neffati F, Bhouri R, Sallem A, Najjar MF, Hassine M, Mehdi M, Haouas Z, Ben Cheikh H. Hematological, biochemical and toxicopathic effects of subchronic acetamiprid toxicity in Wistar rats. Environ Sci Pollut Res Int, 2016; 23: 25191-25199.
5. Mondal S, Ghosh RC, Karnam SS, Purohit K. Toxicopathological changes on Wistar rat after multiple exposures to acetamiprid. Veterinary World, 2014a. EISSN: 2231-0916. Available at www.veterinaryworld. org/Vol.7/December-2014/5.pdf.
6. Mondal S, Ghosh RC, Mukhopadhayay SK, Ganguly S. Effects of electrolytes, microelements and multiple exposure of Acetamiprid on wistar rats. Asian Pac J Health Sci, 2014b; 1(4): 329-334
7. Shakthi Devan RK, Prabu PC, Panchapakesan S. Immunotoxicity assessment of sub-chronic oral administration of acetamiprid in Wistar rats. Drug Chem Toxicol, 2015; 38(3): 328-36.

8. Mondal S, Ghosh RC, Mate MS, Karmakar DB. Effects of acetamiprid on immune system in female wistar rats. Proc Zool Soc, 2009; 62(2): 109–117.
9. Zhang J, Wang Y, Xiang H, Li M, Li W, Ma K, Wang X. Oxidative stress: role in acetamiprid-induced impairment of the male mice reproductive system. Agricultural Sciences in China, 2011; 10(5): 786-796.
10. Rachid M, Yousef MI, Aziez C. Nigella sativa oil protects against reproductive toxicity of acetamiprid insecticide in male rats. Toxicol Lett, 2013; 221S: 17-19.
11. Yun X, Huang Q, Rao W, Xiao C, Zhang T, Mao Z, Wan ZA. Comparative assessment of cytotoxicity of commonly used agricultural insecticides to human and insect cells. Ecotoxicol Environ Saf, 2017; 137: 179-185.
12. Kocaman AY, Topaktaş M. In vitro evaluation of the genotoxicity of acetamiprid in human peripheral blood lymphocytes. Environ Mol Mutagen, 2007; 48(6): 483-90.
13. Çavas T, Çinkılıç N, Vatan Ö, Yılmaz D, Coskun M. In vitro genotoxicity evaluation of acetamiprid in CaCo-2 cells using the micronucleus, comet and cH2AX foci assays. Pest Biochem Physiol, 2012; 104: 212–217.
14. Schardein JL. Reproduction range-finding study in mated rats, 1991. Unpublished report No. 449-018 from International Research and Development Corporation, Mattawan, MI, USA. Submitted to WHO by Nippon Soda Co., Ltd, Tokyo, Japan. In: Kanungo D, and Solecki R. 2011. Acetamiprid. In Pesticide Residues in Food. Toxicological Evaluations. Joint FAO/Who Meeting on Pesticide Residues. Geneva, Switzerland, 20-29 Sept., 2011. WHO, Pp:3-92.
15. Trutter JA. Two-generation reproduction study with NI-25 in rats (reproduction and fertility effects), 1999. Unpublished report No. Covance 6840-108 from Covance Laboratories Inc., Vienna, VA, USA. Submitted to WHO by Nippon Soda Co., Ltd, Tokyo, Japan, 1999. In: Kanungo D, and Solecki R. 2011. Acetamiprid. In Pesticide Residues in Food. Toxicological Evaluations. Joint FAO/Who Meeting on Pesticide Residues. Geneva, Switzerland, 20-29 Sept., 2011. WHO, Pp:3-92.
16. Gawade L, Dadarkar SS, Husain R, Gatne M. A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats. Food Chem Toxicol, 2013; 51: 61-70.
17. Hussein M, Singh V, Hassan MA, Gupta P, Pathak R, Singh AK. Gross morphological changes in chick embryos after exposure to neonicotinoid insecticide imidacloprid. Intern. J Innov Sci Res, 2015; 4: 206-211.

18. Gao LR, Li S, Zhang J, Liang C, Chen EN, Zhang SY, Chuai M, Bao YP, Wang G, Yang X. Excess imidacloprid exposure causes the heart tube malformation of chick embryos. J Agric Food Chem, 2016; 64(47): 9078-9088.
19. Holzum B. YRC 2894 – Developmental toxicity study in rats after oral administration (report No. 26132 of March 25, 1997) – additional information on dysplasia of limb bones in fetuses, 2000. Unpublished report No. MO-01-000450, edition No. M-031344-01-1, dated 18 December 2000, from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.
20. Holzum B. YRC 2894 – Developmental toxicity study in rabbits after oral administration, 1996. Unpublished report No. 24709, edition No. M-000780-01-1, dated 26 January 1996, from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.
21. Takaori H. Acute oral toxicity study in rats, 1997. Unpublished report No. H095 from Odawara Research Center, Nippon Soda Co., Ltd, Odawara, Kanagawa, Japan. Submitted to WHO by Nippon Soda Co., Ltd, Tokyo, Japan. In: Kanungo D, and Solecki R. 2011. Acetamiprid. In Pesticide Residues in Food. Toxicological Evaluations. Joint FAO/Who Meeting on Pesticide Residues. Geneva, Switzerland, 20-29 Sept., 2011. WHO. Pp:3-92.
22. Yamada T, Hara M, Ohba Y, Inoue T, Ohno H. Studies on implantation traces in rats. I. Size, observation period and staining. Jikken Dobutsu, 1985; 34(1): 17-22.
23. Manson JM, Zenick H, Costlow RD. Teratology test methods for laboratory animals. In Hayes AW eds.). Principles and Methods of Toxicology, New York; Raven Press: 1982; Pp: 141–184.
24. Ellis WG, Semple JL, Hoogenboom ER, Kavlock RJ, Zeman FJ. Benomyl-induced craniocerebral anomalies in fetuses of adequately nourished and protein-deprived rats. Teratog Carcinog Mutagen, 1987; 7: 357-375.
25. Gad SC. Statistics for toxicologists. In (Hayes AW eds.). Principles and Methods of Toxicology, Philadelphia USA; Taylor and Francis: 2001; Pp: 285-364.
26. Vohra P, Khera KS. A three-generation study with effect of imidacloprid in rats: biochemical and histopathological investigation. Toxicol Int, 2015; 22(1): 119–124.
27. Myllynen P, Pasanen M, Pelkonen O. Human placenta: A human organ for developmental toxicology research and biomonitoring. Placenta, 2005; 26: 361-371.
28. Mathur A., Bhatnagar P. A teratogenic study of carbaryl in Swiss albino mice. Food Chem Toxicol, 1991; 29: 629-632.

29. Goldman JM, Parrish MB, Cooper RL, Elory WK. Blocked of ovulation in the rat by systemic and ovarian intrabursal administration of the fungicide sodium dimethyl dithiocarbamate. Reprod Toxicol, 1997; 15: 185–90.
30. Bindali BB and Kaliwal BB. Anti-implantation effect of a carbamate fungicide mancozeb in albino mice. Ind Health, 2002; 40: 191-197.
31. Kim JC, Shin JY, Yang YS, Shin DH, Kim SH, Moon CJ, Park SC, Kim YB, Kim HC, Chung MK. Evaluation of developmental toxicity of amitraz in Sprague–Dawley rats. Arch Environ Contam Toxicol, 2007; 52: 137–144.
32. Cummings, AM, Harris ST, Rehnberg, GL. Effects of methyl benzimidazole carbamate during early pregnancy in the rat. Fundam Appl Toxicol, 1990; 15: 528-535.
33. Nukui T, Fujii Y. Acetamiprid teratogenicity study in rats 1994a. Unpublished report No. G-0829 submitted to WHO by Nippon Soda Co., Ltd, Tokyo, Japan. In: Kanungo D, and Solecki R. 2011. Acetamiprid. In Pesticide Residues in Food. Toxicological Evaluations. Joint FAO/Who Meeting on Pesticide Residues. Geneva, Switzerland, 20-29 Sept., 2011. WHO.Pp:3-92.
34. Nukui T, Fujii Y. Acetamiprid teratogenicity study in rabbits 1994b. Unpublished report No. G-0830 from Odawara Research Center, Nippon Soda Co., Ltd, Odawara, Kanagawa, Japan. Submitted to WHO by Nippon Soda Co., Ltd, Tokyo, Japan. In: Kanungo D, and Solecki R. 2011. Acetamiprid. In Pesticide Residues in Food. Toxicological Evaluations. Joint FAO/Who Meeting on Pesticide Residues. Geneva, Switzerland, 20-29 Sept., 2011. WHO.Pp:3-92.
35. Da Silva EO, De Sousa SS. Clinical anophthalmia. Hum Genet, 1981; 57: 115-116.
36. Ragge NK, Subak-Sharpe ID, Collin JRO. A practical guide to the management of anophthalmia and microphthalmia. Eye, 2007; 21: 1290–1300.
37. Khera KS. Common fetal aberrations and their teratologic significance: a review. Fund Appl Toxicol, 1981; 1: 13-18.
38. Carney EW, Kimmel CA. Interpretation of skeletal variations for human risk assessment: delayed ossification and wavy ribs, Birth Defects. Res B Dev Reprod Toxicol, 2007; 80: 473–496.
39. Howdeshell KL. A model of development of the brain as a construct of the thyroid system, Environ Health Perspect, 2002; 110: 337–348.
40. Ford KA, Casida JE. Unique and common metabolites of thiamethoxam, clothianidin and dinotefuran in mice. Chem Res Toxicol, 2006; 19: 1549-1556.

41. Kimura-Kuroda J, Nishito Y, Yanagisawa H, Kuroda Y, Komuta Y, Kawano H, Hayashi M. Neonicotinoid insecticides alter the gene expression profile of neuron-enriched cultures from neonatal rat cerebellum. Int J Environ Res Public Health, 2016; 4: 13(10).