

TERATOGENETIC EFFECTS OF IGR, LUFENURON ON THE EMBRYONIC DEVELOPMENT OF *GALLUS DOMESTICUS***Wagh Pinakin¹, Salokhe S.G.*¹ and Deshpande S.G.²**¹B. Gholap College, Sangvi, Pune-411027, India,²Entomology Section, National Chemical Laboratory, Pune-411008, India.Article Received on
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Accepted on 29 Jan 2015***Correspondence for****Author****Salokhe S.G.**B. Gholap College,
Sangvi, Pune-411027,
India,**ABSTRACT**

Chick embryos, Hamburger Hamilton (HH)⁽¹²⁾ stage 4,5 cultured by New's single ring technique when treated with insect growth regulator (IGR), Lufenuron at concentration of 54 ppm with different doses showed severe teratogenesis. At the dose 54ppm (10ul) Lufenuron, embryonic development is normal but it lags behind to that of control embryo. However, at the dose of 54ppm (100ul), Lufenuron induces teratogenetic effects, such as neural tube defects (NTDs), anophthalmia, cardiomegaly, fan shaped large somites and absence of somites were observed. Subsequently, it affects the development of embryonic blood vessels. This indicates that IGRs exert their teratogenetic effect by

targeting mesodermal and ectodermal tissues and key events in morphogenesis.

KEYWORDS: New's culture, Chick embryo, IGR, Lufenuron, PC (Panette Compton saline, HH staging, somites.

INTRODUCTION

The domestic fowl *Gallus gallus* is an important biological model species for evolutionary and developmental biology, immunology, genetics, as well as for agricultural sciences. The completion of a draft sequence of the chicken genome^[1] is a landmark event in avian genomics and has opened new possibilities to understand gene function and its relationship to physiology. Extensive work has been done to correlate gene expression and morphogenesis.^[2, 3, 4]

Lufenuron, a benzoylurea pesticide, inhibits chitin synthesis, is widely used as an agricultural pesticide. Lufenuron is the chief component of the veterinary flea control medication

program, and one of the key ingredients in flea control veterinary studies, heartworm protection, and also a powerful anti helminthic medicine.^[5, 6] The special property of Lufenuron to inhibit chitin synthesis, makes it an effective remedy against fungal infections, such as dermatophytes (ringworm). Since it is found to be lethal to zooplankton, Lufenuron was included in a biocide ban (Swedish Chemicals Agency & European Parliament, 2009).

As per Pesticide Properties DataBase, Lufenuron has high bioaccumulation potential, high bio-concentration factor (BCF) of 5300, and is moderately toxic in fishes, birds (Acute LD₅₀ of 2000 (mg kg⁻¹)) and mammals but highly toxic to aquatic invertebrates. The EC Risk classification of Lufenuron shows that it is Xn - Harmful: R43, R48/22, and N - Dangerous for the environment: R50 (Very toxic to aquatic organisms), R53 (May cause long-term adverse effects in the aquatic environment). It was found to be toxic to the earthworm, *Aporrectodea caliginosa*.^[7]

It was found that dietary Lufenuron reduces egg hatching and influences protein expression in the fruit fly *Bactrocera latifrons*.^[8] Further they discovered that expression of two genes encoding chitin synthase2, and chitin binding protein, was altered in adults exposed to dietary Lufenuron. Lufenuron treatments also led to increased expression of two odorant binding proteins one in females and one in males.^[8] It was found that Lufenuron causes prevalence of mosquito larvae and induces abnormal metamorphosis.^[9] It was also found that Lufenuron was found to reduce and suppress the resistance of Formosan subterranean termites (Isoptera: Rhinotermitidae) to entomopathogenic bacteria.^[10] We also found that Lufenuron induces limb defects and skeletal abnormalities in developing chick embryos.^[11]

MATERIALS AND METHODS

Freshly laid, fertilized *Gallus sp.* (White Leghorn strain) eggs were obtained from Venkys, Pune, washed with distilled water and allowed to air dry and wiped with 70% ethanol. The eggs were incubated at 37.5°C, with a relative humidity of 70-80% for 18 hours in BOD incubator (Bio-Technics India, BTI-06) and manually rotated periodically.

Preincubated chick embryos (HH stage 4, 5), (n=10), were cultured by New's single ring technique and treated with 10 and 100ul of 54 ppm of Lufenuron (dissolved in PC saline). The test compound was released from a height of 1.0 cm directly over the embryo (blastoderm) with a micropipette. The treated embryos were immediately placed in BOD incubator along with vehicle control embryos. The treated and control embryos were

incubated for 24 h post treatment, following which they were isolated, washed in 1X PBS and analyzed by stereomicroscopy.

RESULTS

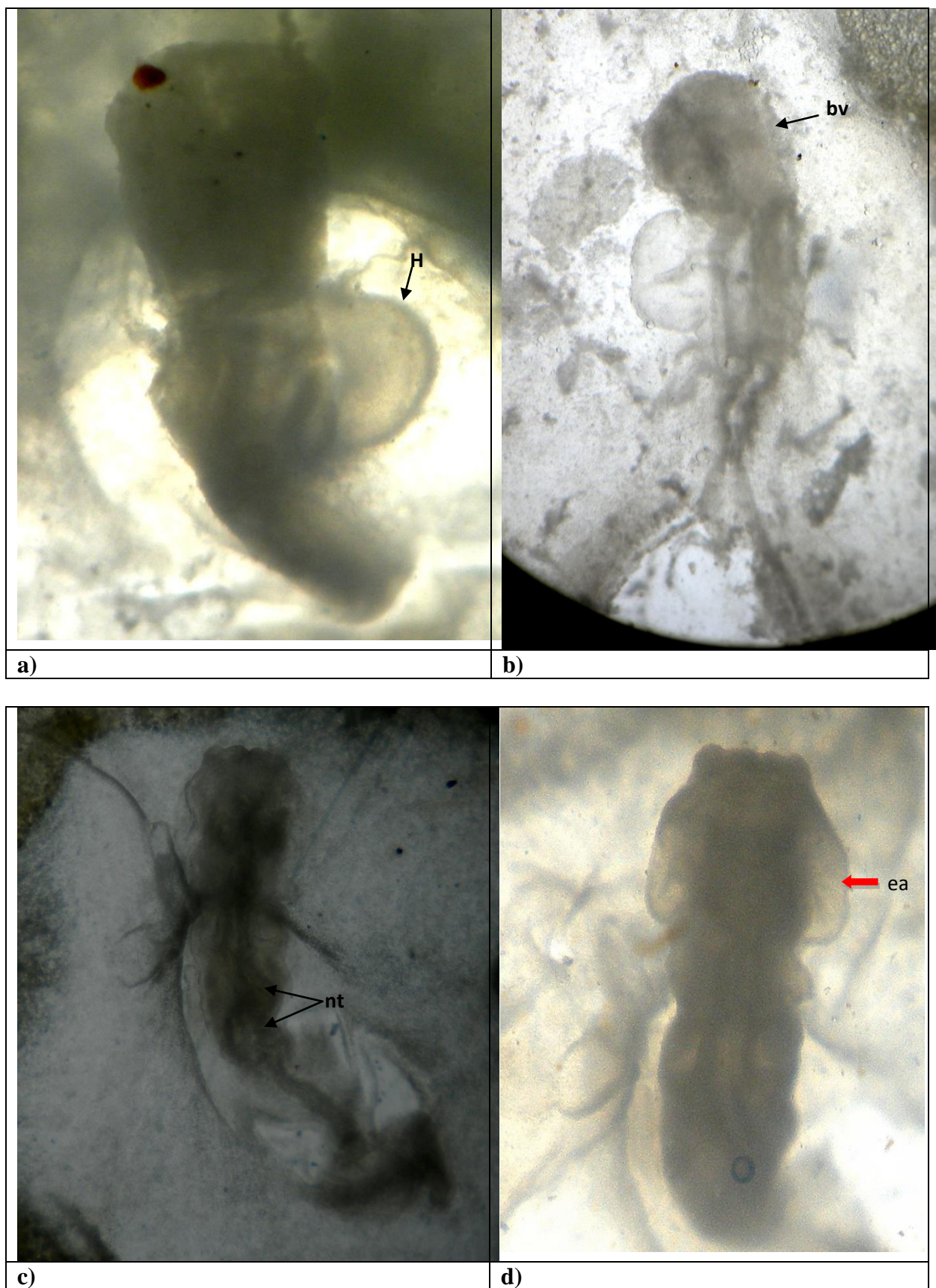
Lufenuron treated embryos showed severe developmental defects like cardiomegaly (Fig. **a** H, Fig. **f h**), fused and undifferentiated brain vesicles (Fig. **b bv**), wavy neural tube (Fig. **c nt**), indicating the ability of Lufenuron to induce NTDs. Enlarged bilateral vesicles possibly formed by fusion of neuromeres and anophthalmia, acardia, stunted and antero-posteriorly compressed embryonic axis were observed (Fig. **d ea**).

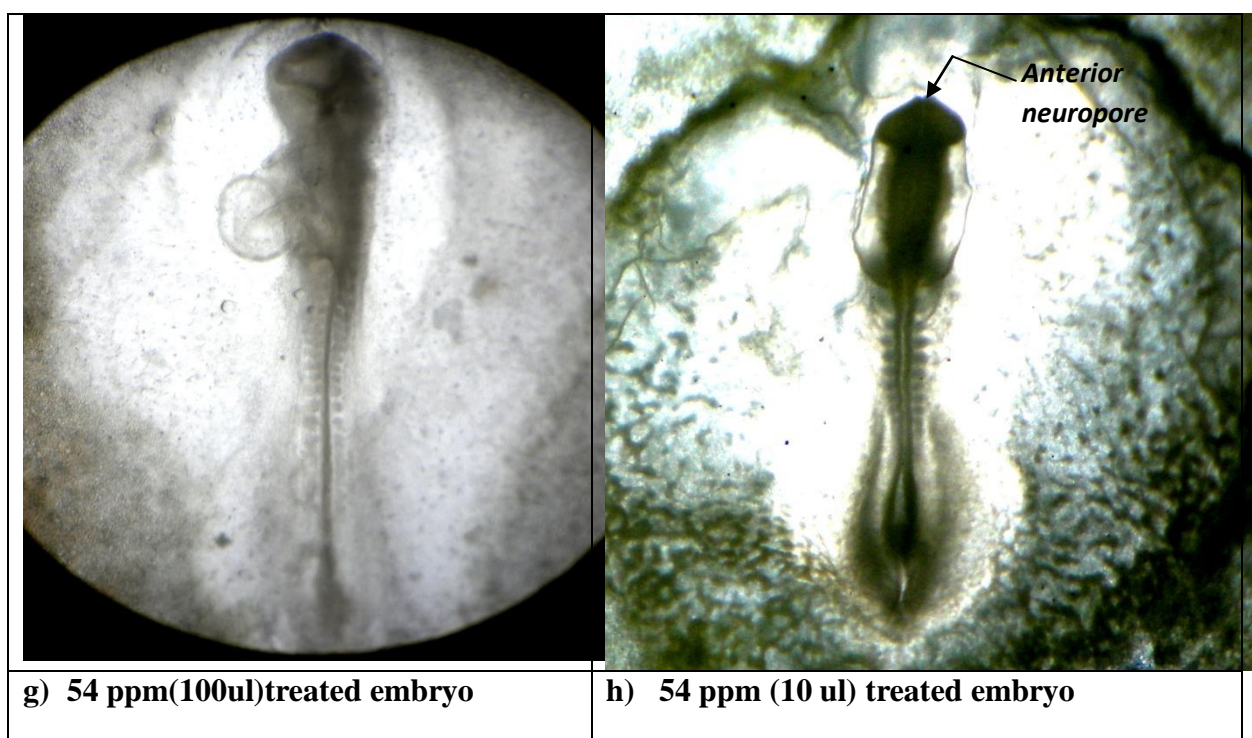
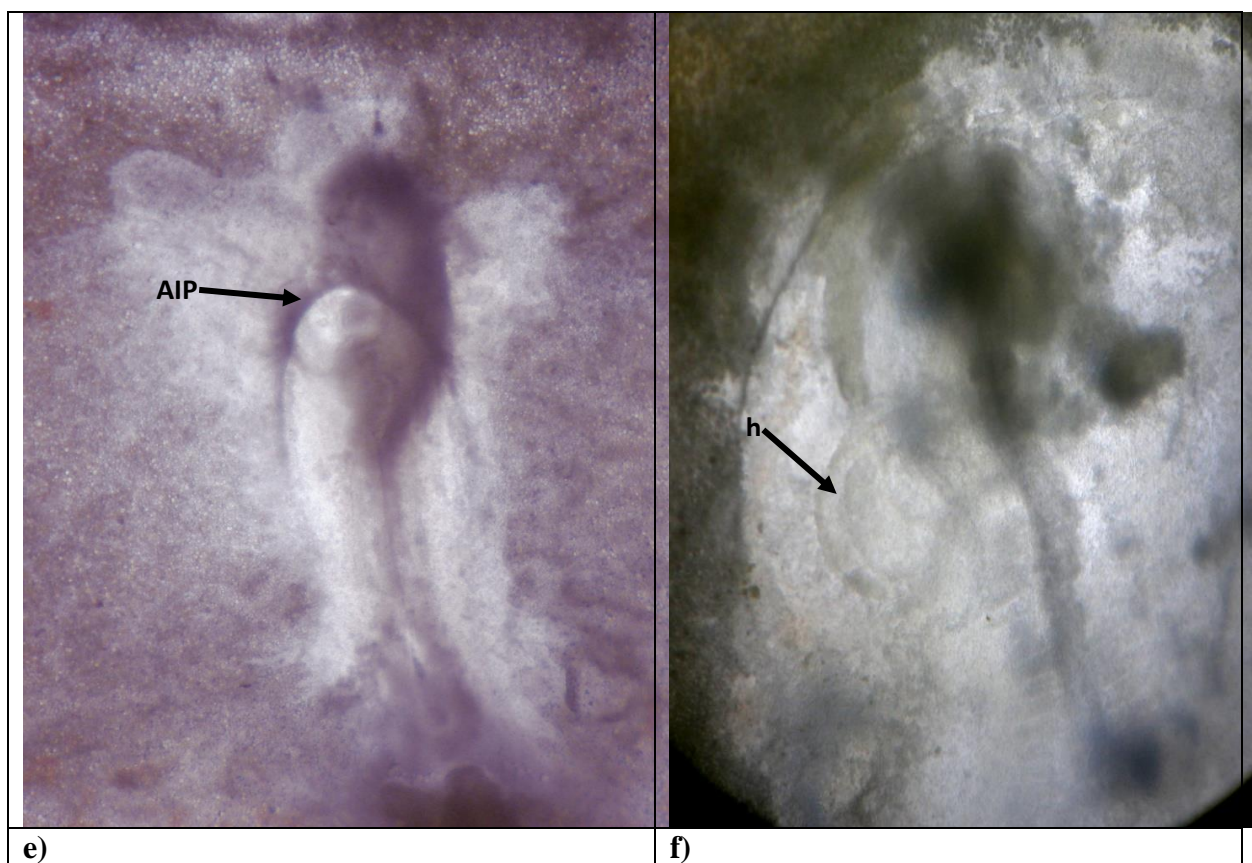
At 45-50 hrs of development (24 hrs post treatment), the embryos showed reduced degree of torsion and flexion, as compared to the control embryos, large tunnel like Anterior Intestinal Portal (Fig. **e AIP**) and absence of somites, while fan shaped large somites in the anterior region extending from last rhombomere (Fig. **l s**) were also seen.

In case of lowering the volume of dose at the same concentration (10ul; 54ppm), the embryos showed normal but reduced development as compared to the higher dose volume (100 ul) and control embryos (Fig. **h**). As seen in all the photographs (Fig. **a** to Fig. **h**), the treated embryos showed hypertrophied, massive, head ectoderm, while the anterior margin of the foregut was inconspicuous. Also, the anterior neuropore was reduced (Fig. **h**) or totally absent (Fig. **g,j**) in all embryos who received Lufenuron treatment.

The most common anomaly in all the Lufenuron treated embryos was the lack of normal vascularisation, though some embryos showed blood islands in Area Opaca, but Area Vasculosa was reduced. Sinus terminalis (**st**) was distinctly seen (Fig. **k**) indicating normal haematopoiesis but vitelline plexus and vitelline blood vessels were lacking, even at 45- 50 hours of development. Right and Left Vitelline arteries, Left Anterior Vitelline Vein which is well marked at 50 hours of development was absent in all the Lufenuron treated embryos.

Figures

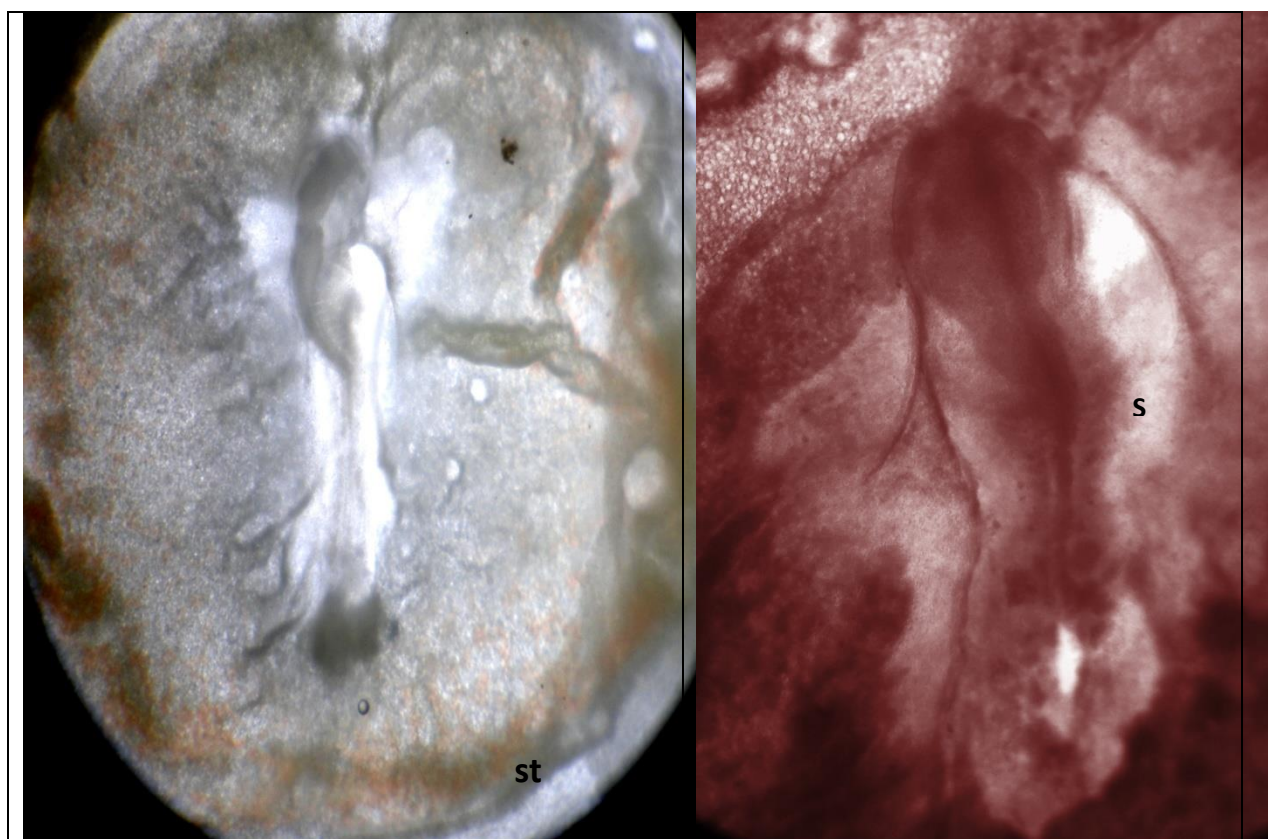






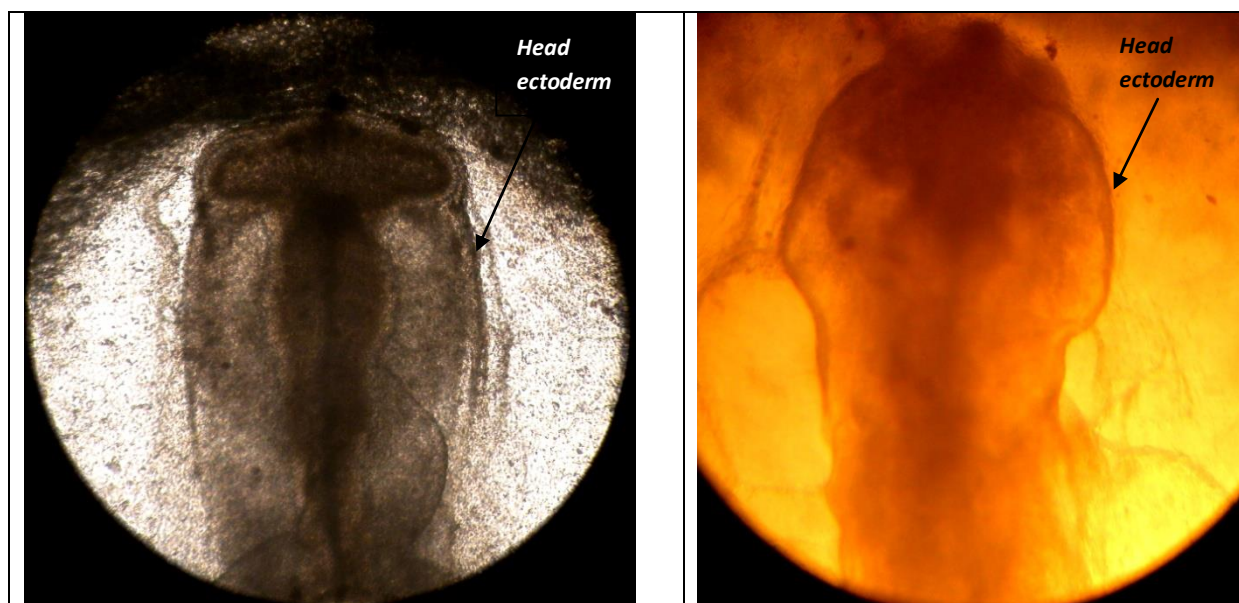
i) Ctrl embryo

j) Treated embryo at 40-45 h(HH Stage 11)



k) 54 ppm 100 ul treated embryo

l) 54 ppm 100 ul treated embryo



m) Ctrl embryo at 40-45 h(HH Stage 11) n) Treated embryo at 40-45 h(HH Stage 11)

DISCUSSION

Lufenuron induces teratogenesis in non target vertebrate organisms, in a dose dependent manner.^[15] It causes severe dysmorphogenesis in developing chick embryos as confirmed by New's culture technique. Teratogenesis is seen along the entire embryonic axis, most predominantly affecting somitogenesis and brain development. As earlier reported by us,^[15] lower dose(< 54ppm), doesn't induce detectable anomalies in the developing embryo. However at sub-lethal dose of 54 ppm, Lufenuron was found to induce severe Neural Tube Defects (NTDs), cardiomegaly, swelling of brain vesicles and fan shaped somites in the anterior region.

Complete absence of somites, lack of optic vesicles (Anophthalmia), wavy neural tube, fused and indistinct neuromeres, abnormal torsion and flexion were the most remarkable abnormalities in all the treated embryos. Heart looping in all the treated embryos was normal but cardiomegaly was seen. Some embryos showed accessory extra-embryonic vesicle in the pericardial region of the coelom (**Fig.j pr,**) indicative of prospective cardiac defects.

All the above observations possibly indicate that IGR, Lufenuron targets ectodermal and mesodermal derivatives predominantly by disturbing the normal developmental processes like gastrulation and pattern formation. Head and trunk formation in vertebrates is controlled by a cascade reaction and interaction of several genes and transcription factors like Wnt (At least six different Wnt genes are expressed in the developing CNS of the chick embryo.)⁽¹⁷⁾, Cerberus (anterior endoderm specification), Dickkopf, Chordin, Noggin, Activin, Follistatin

and Frz-b are key neuralizing and dorsalizing factors, which possibly may have been dysregulated by Lufenuron.

The ability of Lufenuron to perturb the normal pattern formation, by altering the domains of developmentally regulated genes requires further studies involving molecular genetics approaches.

REFERENCES

1. Haisheng Nie, Richard P. M. A. Crooijmans, Aart Lammers, Evert M. van Schothorst, Jaap Keijer, Pieter B. T. Neerincx, Jack A. M. Leunissen, Hendrik-Jan Megens, Martien A. M. Groenen, 'Gene Expression in Chicken Reveals Correlation with Structural Genomic Features and Conserved Patterns of Transcription in the Terrestrial Vertebrates.', PLOS ONE , August 05, 2010.
2. Denise J. Montell, Morphogenetic Cell Movements: Diversity from Modular Mechanical Properties, Science 322, Dec 2008; 1502–1505.
3. Duester G., Retinoic acid synthesis and signaling during early organogenesis, Cell 134 (6), Sep 2008; 921–31.
4. Scott F. Gilbert, Developmental Biology, Tenth Edition, Sinauer Associates, Inc., Sunderland, MA, USA.
5. <http://www.sentinelpet.com/heartworms>
6. http://en.wikipedia.org/wiki/Milbemycin_oxime/lufenuron
7. Mohamed E. I. Badawy, Anter Kenawy, and Ahmed F. El-Aswad, Toxicity Assessment of Buprofezin, Lufenuron, and Triflumuron to the Earthworm *Aporrectodea caliginosa*, International Journal of Zoology Volume 2013 (2013), Article ID 174523, 9 pages.
8. Chang Cl, Geib S, Cho Ik, Li Qx, Stanley D., Dietary lufenuron reduces egg hatch and influences protein expression in the fruit fly *Bactrocera latifrons* (Hendel), Arch Insect Biochem Physiol, Aug 2014; 86(4): 193-208.
9. Salokhe SG, Deshpande SG, Mukherjee SN., Evaluation of the insect growth regulator Lufenuron (Match®) for control of *Aedes aegypti* by simulated field trials., Parasitol Res, Sep 2012; 111(3): 1325-9.
10. Wang C, Henderson G, Gautam BK., Lufenuron suppresses the resistance of Formosan subterranean termites (Isoptera: Rhinotermitidae) to entomopathogenic bacteria, J Econ Entomol, Aug 2013; 106(4): 1812-8.

11. Wagh Pinakin, Deshpande S.G., Salokhe S.G., Studies on the effect of the insect growth regulator lufenuron on embryogenesis of chick *Gallus domesticus* (white leghorn strain), IJPBS |Volume 1| Issue 2 |APRIL- JUNE |2011.
12. Hamburger V, Hamilton HL. A series of normal stages in the development of chick embryos. J Morphol, 1951; 88: 49-92.