RESEARCH PUBLICATIONS
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6. *Prasad A Deshpande and Katti Pancharatna. 2012. Withdrawal of meiotic arrest in preovulatory oocytes of zebrafish (Danio rerio) is mediated by IGF1 signalling? (Communicated)

*From thesis*
Developmental disruptions induced by insect growth regulator (Novaluron) in *Bufo melanostictus* tadpoles

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**ABSTRACT:** Novaluron is an insect growth regulator (IGR) used against fruit-borers and domestic pests. In this study, effects of different concentrations (0.5, 0.75, 1.0, 1.5 μg L⁻¹) of novaluron on the tail regeneration, limb development and metamorphosis were examined in *Bufo melanostictus*. Thyroxine (1.0, 2.0, 3.5, 5.0 μg L⁻¹), which promotes amphibian development/metamorphosis, and vitamin A (5, 20, 40, 60 IU L⁻¹), which disrupts development and induce polymelia, were used for comparison. *Bufo melanostictus* tadpoles were raised in the laboratory from eggs collected around Dharwad in August 2007. The tail of tadpoles was amputated at limb-bud stage under ether anesthesia. Ten tadpoles were exposed to each concentration of chemicals in triplicate for 5 days and then reared in conditioned water. Tadpoles were fed on boiled spinach. In controls (tadpoles exposed to ring solution), the tail regenerated on the 5th day, hind-limbs and fore-limbs appeared on days 15 and 24, respectively; metamorphosis was complete on the 30th day. In 1 and 2 pg L⁻¹ thyroxine exposed tadpoles, regeneration of the tail, development of limbs and metamorphosis were preponeed to the 4th, 7th, 11th and 23rd day respectively. Exposure to all the concentrations used. Novaluron at lower dose (0.5 pg L⁻¹) was not effective, but at 0.75 pg and higher concentrations it elicited results comparable to those of vitamin A. The study indicates that novaluron interferes with amphibian development if found as contaminant in the water bodies where amphibians live and reproduce. Copyright © 2010 John Wiley & Sons, Ltd.

**Keywords:** novaluron; tail regeneration; limb development; metamorphosis; *Bufo melanostictus*; developmental disruption

**INTRODUCTION**

Many agrochemicals, which are commonly used as pesticides, weedicides, insecticides or fungicides, found as contaminants in the water bodies are known to be potential endocrine disrupters for non-target aquatic organisms, affecting their growth, reproduction and immune system (Pickford and Morris, 1999; Sparling et al., 2001; Hayes et al., 2002; Bevan et al., 2003). The exposure effects are more severe on developing organisms than adults (Park and Kild, 2005; Marquis et al., 2006; Sparling and Fellers, 2007; Allran and Karasov, 2000; Carr et al., 2003). Amphibians are considered to be indicator species for environmental, and in particular aquatic, health as they typically reproduce and pass through all the developmental stages in fresh water, and are highly vulnerable to chemical contaminants owing to their permeable skin; therefore, any change in the surrounding medium may significantly affect the Individuals and populations of amphibians (Hayes et al., 2006; Sayim and Kaya, 2007). In recent years, owing to water pollution, there has been an increase in the production of amphibian fauna with gross malformations affecting survival ability, leading to overall decline in populations (Wake, 1991; Hayes et al., 2003). These have prompted serious concern regarding the biological status of many anuran species (Green, 1999; Cohen, 2001; Daniels, 2003; Hayes et al., 2006). During metamorphosis of amphibians, the tail of the tadpole degenerates completely only when the alternative means of locomotion (limbs) appear (Saxen et al., 1957; Kollros, 1961).

Thyroid hormone is known to promote the amphibian metamorphosis in general (Hayes and Wu 1995; Opitz et al., 2006; Degitz et al., 2005; Oka et al., 2009) while retinoids (vitamin A/retinoic acid/palmitic acid) interfere with limb development and are known to induce homeotic transformation, leading to the appearance of ectopic limbs at the amputated end of the tail (Mahapatra and Mohanty-Hejmadi, 1994; Das and Mohanty-Hejmadi, 1998, 1999, 2003; Hejmadi and Crawford, 2003). It has been reported that exposure of *Xenopus laevis* and *Bufo bufo* larvae to methoprene (an insect growth regulator) and its derivatives results in similar effects to those reported for vitamin A when the tail is amputated (Paulov, 1976; Degitz et al., 2003). Although very few chemicals have been tested, there may be several chemicals/insecticides/pesticides which are routinely in use and may have similar, i.e. developmental disrupting/endoctrine disrupting actions, exerting harmful effects on amphibian populations (Daniels, 2003).

Novaluron, an insecticide belongs to the class diflubenzuron ures used in many American, European, African and Asian countries against fruit-borers for crops like cotton, soybean, fruits and...
vegetables and domestic pests, belongs to a class of insect growth regulators (IGRs). It is known to inhibit chitin synthesis, affecting the moultling stages of insect development (Tawatsin et al., 2007).

In the present study, the effects of exposure to different concentrations of novaluron on the regeneration of amputated tail, limb development and completion of metamorphosis in tadpoles of common toad Bufo melanostictus were studied. Thyroxine and vitamin A were used for comparison, and tadpoles exposed to amphibian ringer alone served as controls.

**MATERIALS AND METHODS**

**Procurement of Eggs and Rearing of Tadpoles**

Egg clutches of *B. melanostictus* obtained from breeding grounds around Karnatak University campus, Dharwad, in the early mornings of the last week of August 2007, were transferred to glass aquaria and reared in conditioned water. The fertilized eggs underwent development and hatched into tadpoles within 3 or 4 days. The tadpoles were maintained on boiled spinach under natural temperature (23 ± 1°C) and photoperiod (11.5-12.5 h). The rearing medium (conditioned water) was changed every alternate day.

**Amputation of Tail and Treatment**

In order to study the abnormality or delay in tail regeneration, emergence of limbs and induction of polymelia, if any, owing to exposure to chemicals, tail amputation was carried out in all the tadpoles before starting the treatment. The tadpoles at hind-limb bud stage (stage 26 of Gosner, 1960) were selected. The tail was amputated approximately at the middle of its length using a sterilized blade under ether anesthesia (Das and Mohanty-Hejmadi, 1999). Immediately after tail amputation, the tadpoles were transferred to trays (30 x 25 x 10 cm) containing treatment media (1 l). Ten tadpoles were accommodated in each tray and three trays were used for each concentration of chemicals. All the doses of vitamin A and novaluron chosen were sub-mortality concentrations as the objectives of the study were to observe the developmental disruptions, if any, induced by the presence of low concentrations in the insecticide in comparison with vitamin A and thyroxine.

The following treatment groups were made:

- **group 1** - controls: tadpoles exposed to only amphibian ringer (AR);
- **group 2** - thyroxine (Sigma, USA) (tadpoles exposed to 1.0, 2.0, 3.5 and 5.0 μg thyroxine I⁻ of AR);
- **group 3** - vitamin A (Nicholas Piramat, India) (tadpoles exposed to 5, 20, 40 and 60 IU vitamin A I⁻ of AR);
- **group 4** - novaluron (Rimon, Indofii Chemical Co. India) (tadpoles exposed to 0.5, 0.75, 1.0 and 1.5 μg novaluron I⁻ of AR).

**RESULTS**

**Controls**

In all the 10 tadpoles per tray exposed to ringer solution and subsequently reared in conditioned water, the tail regenerated on the 5th day after amputation, hind-limbs appeared on the 15th day followed by the development of fore-limbs on the 24th day. The metamorphosis was completed by the 30th day (Fig. 1). The tadpoles were active, exhibited normal movements and fed voraciously.

**Thyroxine**

In the tadpoles exposed to 1 and 2 μg thyroxine I⁻ the development proceeded significantly (P < 0.05) faster compared with the controls, i.e., the tail regeneration was observed on the 4th day, hind limbs appeared on the 11th/12th day, fore-limbs between days 17 and 18 and overall metamorphosis was completed between days 23 and 24 (Fig. 1). In higher dose (3.5 and 5 μg) thyroxine-exposed tadpoles the development was slower and was comparable to controls (Fig. 1). All the tadpoles exposed to different concentrations of thyroxine were healthy, fed on spinach voraciously and exhibited active swimming movement.

**Vitamin A**

In the majority of the tadpoles exposed to different concentrations of vitamin A the tail regeneration was abnormal, i.e., regenerated portion of the tail was twisted in a lateral direction, resulting in a drooped/crooked tail in 40-60% of tadpoles (Figs 2 and 4). In such tadpoles hind limb developed normally on the 15th day, while the appearance of fore-limbs was hindered and the metamorphosis was delayed (Fig. 2). The movement and feeding rate of tadpoles decreased with the increasing concentration of vitamin A.

**Novaluron**

No mortality was observed for the exposed doses of pesticide. In lower dose (0.5 μg I⁻) exposed tadpoles, the regeneration of tail, development of hind limbs and fore-limbs and completion of metamorphosis were comparable to control tadpoles (Fig. 3), but in tadpoles exposed to 0.75-1.0 μg I⁻ of novaluron, a laterally drooped/crooked tail was regenerated (Fig. 4). In such tadpoles, hind limbs developed normally but their appearance was delayed compared with controls (Fig. 3). Fore-limbs failed to develop and metamorphosis was hindered. Thus, with the increase in the concentration of novaluron the rate of metamorphosis was delayed. Feeding and movement were normal in all the novaluron exposed tadpoles.

**DISCUSSION**

Although the widespread decline of amphibian populations in recent years has been attributed to habitat loss, UV radiation,
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Figure 1. Effects of different concentrations of thyroxine (1, 2, 3.5 and 5 μg l⁻¹) on the regeneration of amputated tail, appearance of limbs and completion of metamorphosis in B. melanostictus tadpoles. Values mean ± standard error. *Significant at 5% level compared with corresponding controls.

Figure 2. Effects of various concentrations (5, 20, 40 and 60 IU l⁻¹) of vitamin A on the regeneration of amputated tail, appearance of limbs and completion of metamorphosis in B. melanostictus tadpoles. Values mean ± standard error. *Significant at 5% level compared with corresponding controls.

Figure 3. Effects of various concentrations (0.5, 0.75, 1.0 and 1.5 μg l⁻¹) of novaluron on the regeneration of amputated tail, appearance of limbs and completion of metamorphosis in B. melanostictus tadpoles. Values mean ± standard error. *Significant at 5% level compared with corresponding controls.

global warming and diseases, the major cause appears to be chemical pollution owing to the increased contamination of natural water bodies by agrochemicals (Green, 1999; Cohen 2001; Daniels, 2003; Hayes et al., 2006; Wake, 2007). Field studies have indicated that there is a correlation between the use of chemical pesticides and emergence of the amphibian larvae with developmental deformities in Northern America (Glare and O'Callaghan, 1999). Experiments in the laboratory have confirmed that a mixture of pesticides has greater inhibitory effects than individual pesticides on growth and development of X. laevis and Rana pipiens larvae (Hayes et al., 2006). The developmental disruptions/anomalies induced by herbicide (atrazine), organophosphate insecticides and environmental estrogens in amphibians are well documented (Bevan et al., 2003; Hayes et al., 2003, 2006; Ezemonye and Ilechie, 2007). However, the impact of insect growth regulators (which are also in widespread use) on amphibian development and metamorphosis is rarely reported, except for methoprene.

In the present study, the tadpoles reared in amphibian ringer alone showed normal regeneration of tail and development of limbs and completion of metamorphosis. When they were exposed to various concentrations of thyroxine, lower (1-2 μg l⁻¹) doses caused tail regeneration, limb development and metamorphosis faster than higher (>3 μg l⁻¹) doses. Thyroxine (T4) is known to accelerate amphibian metamorphosis by regulating the gene expression and protein synthesis (Hayes and Wu, 1995; Opitz et al., 2006; Degitz et al., 2005; Oka et al., 2009). The plastic
monomer Bisphenol A and related chemicals are known to suppress metamorphosis in *Rana rugosa* by interfering the binding of hormone to its receptors (Goto et al., 2006). Similarly corticosteroids are reported to disrupt development in *Xenopus laevis* by modulating thyroid hormone effects (Lorenz et al., 2009). In the present study, tadpoles exposed to various concentrations of vitamin A exhibited developmental deformations such as delay in tail regeneration, tails regenerated being crooked/drooped laterally to either left or right direction, followed by the abnormal fore-limbs and delay in metamorphosis. This is in agreement with the earlier studies reporting that retinoids elicit frog malformations (Gardiner et al., 2003). Experimental studies on amphibian tadpoles have shown that treatment of amputated tails with retinoids leads to inhibition of regeneration in *Bufo andersonii* (Niazi and Saxena, 1968), *Notophthalmus viridescens*, *Ambyosoma maculatum* and *X. laevis* (Scadding, 1987). Further, in addition to inhibition of tail regeneration, ectopic supernumerary limbs generated at the amputated region were demonstrated in *Upedodon systoma* (Mohanty-Hejmadi et al., 1992), *Polypedates maculatus*, *B. melanosticus*, *Microhyla ornata* and *Rana tigrina* (Mahapatra and Mohanty-Hejmadi 1994; Das and Dutta, 1996). The difference in results obtained in the present experiment compared with those of earlier workers may be attributed to difference in the vitamin A source, length of exposure and time of the exposure.

Figure 4. Shows regeneration of normal tail and completion of metamorphosis on day 30 in thyroxine-treated tadpoles (A, D and G) and laterally drooped tails and delayed metamorphosis in vitamin A (Retinol)-treated (B, E, H) and novaluron-treated (C, F, I) *B. melanosticus* tadpoles (x40).
Novaluron induced developmental disruptions in *B. Melanostictus* and the experimental design used (tadpoles were exposed to vitamin A only for first five days after amputation and then reared in conditioned water in the present study).

In *B. Melanostictus* in commonly used against fruit/stem borers and household pests. Studies involving measurement of environmental levels of this chemical reveal that residues of novaluron are present in crapejs (1-15 m³), street drains (4-6 m³) and disused wells with effective durations of 11-13, 6-17 and 33-69 days, respectively, when used at a dose of 1-10 mg m⁻³ against *Culex quinquefasciatus* for >80% inhibition of emergence; further, field studies in artificial and natural habitats have shown that novaluron (10% EC) was effective against populations of *Culex* species at application rates of 10-50 ppb (Jambulingam et al., 2009). In the present study, the exposure of tadpoles to novaluron (especially 0.75 μg and higher concentrations) elicited results comparable to the effects of vitamin A. However, polyme- last was not observed in novaluron-exposed tadpoles, unlike reports on retinoids (Hejmadi and Crawford, 2003). The synthetic Insect egg juvenile growth hormone methoprene (and its degradation products), used widely in the West, is known to mimic the actions of retinoids and induce similar developmental toxicity in *X. laevis* and *B. bufo* tadpoles by regulating the gene expression (Paulov, 1976; Degitz et al., 2003). It is well known that pesticides, Insecticides and endocrine disrupter chemicals interfere with the reproduction, i.e. egg production and oocyte maturation, of amphibians (Pickford and Morris, 1999; Sower et al., 2000).

In conclusion, the study reveals the first time that novaluron, an IGR used widely in agriculture, disrupts the normal amphibian development when present in minute concentrations In the aquatic medium where amphibians live and reproduce.

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REFERENCES


OOGONIAL MITOSIS IN THE OVARY OF ZEBRAFISH (DANIO RERIO)

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ABSTRACT: This study aims to understand the oogonial mitosis and its nutritional regulation in the ovary of adult zebrafish. Adult female zebrafishes were maintained in the laboratory for 30 days under natural temperature and photoperiod conditions. Fishes were fed on commercial pellets ad libitum. Different feeding regimes (i) thrice a day (ii) twice a day (iii) once a day (iv) every alternate day and (v) once after three days were maintained. On 31st day, fishes were autopsied and the ovaries were fixed in Boulin's fluid and processed for paraffin embedding. Oogonia were quantified from serial histological sections. The results reveal that the number of oogonia increased significantly (P<0.05) in once a day, alternate day and once after third day fed fishes over initial controls. In daily twice and daily thrice fed fishes although there was increase (P<0.05) in body weight, the oogonial number remained comparable to initial controls. It is suggested that, overfeeding may lead to increase in body weight but, not in the rate of oogonial mitosis. The nutritive regulatory mechanism may involve a signaling molecule in controlling oogonial mitosis in fish ovary.

KEYWORDS: Proliferation, stem cells, zebrafish, nutritive signaling.

INTRODUCTION

Germline stem cells (GSCs) divide continuously throughout development and adulthood, initially to increase the pool size and subsequently to provide a constant supply of differentiating germ cells1. Growth factors such as Insulin-like growth factor (IGF) are known to play key role in embryonic germline development in zebrafish2. Gonadotropins are also known to influence oogonial proliferation3-4. Although a number of signaling pathways underlying germ cell development have been identified in zebrafish5-10, these studies are restricted to embryonic development and the understanding of this process remains largely incomplete owing to the paucity of studies on adult animals. Zebrafish is considered as an excellent model for in vivo studies of vertebrate germline development2.

Present investigation is an attempt to elucidate the influence of different feeding regimes on proliferative activity of oogonia, in the ovary of adult zebrafish Danio rerio.

MATERIALS AND METHODS

Animals:

Adult female zebrafish (Body length: 26 ± 0.5mm, body mass 200 ± 21mg) were obtained from commercial suppliers and were maintained in the laboratory under natural temperature (26 ± 1°C) and photoperiod (11.30-12.30) conditions for 30 days. The water in aquaria was replaced by conditioned water for every alternate day.

Experimental Design:

Each group consisted 10 fishes. Following groups with different feeding regimes were maintained: Group I: thrice a day, Group II: twice a day, Group III: once a day, Group IV: every alternate day and Group V: once after three days. Commercial food pellets were used...
on specific growth factors and other undetermined compounds\textsuperscript{15}.

The present study clearly reveals that frequency of feeding has an influence on body mass and oogonial mitosis of adult zebrafish. Overfeeding lead to increase in body weight, but did not alter the rate of oogonial mitosis indicating the significance of nutritional regulation of proliferative activity of ovarian stem cells. Whether this nutritive regulatory mechanism is mediated through any signaling molecule needs further elucidation.

**REFERENCES**


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Dear Dr Pancharatna

Sub: Acceptance Letter

Title of the paper:

In vitro Induction of Germinal Vesicle Breakdown (GVBD) in Zebrafish (Danio rerio) oocytes by Environmental Estrogenic Compounds

Prasad A. Deshpande, Basavaraj Goundadkar and Katti Pancharatna.

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In vitro Induction of Germinal Vesicle Breakdown (GVBD) in Zebrafish (Danio rerio) oocytes by Environmental Estrogenic Compounds

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Page Title: In vitro induction of GVBD in zebrafish oocytes

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Abstract

In the present study, environmental estrogenic chemicals, Fenvalerate and Dimethoate, which are commonly used for crop protection in agriculture were tested for the induction of germinal vesicle break down (GVBD) in the oocytes of zebrafish (Danio rerio) in vitro. Diethylstilbestrol (DES) which is an established GVBD inducer in zebrafish was used to compare the potency of chemicals. Fully grown oocytes (500-690 µm) mechanically dissected from the ovaries of gravid females were exposed to graded (5, 10, 15 and 20 µg/ml) concentrations of chemicals in triplicate sets using 24-well culture plates. Per cent GVBD was scored every hour for 6 hours. To assess the GVBD oocytes were exposed to clearing solution (4% acetic acid + 5% paraformaldehyde in ringer). DES (2 µM/ml) caused 70 ± 0.5% GVBD in all the three sets. Fenvalerate induced 27 ± 0.1, 50 ± 0.3, 70 ± 0.4 and 60 ± 0.3 % GVBD, and dimethoate induced 80 ± 0.8, 60 ± 0.6, 30 ± 0.3, 60 ± 0.6 % and at 5, 10, 15 and 20 µg/ml respectively. Results suggest that pesticides and insecticides with estrogenic activity induce oocyte maturation preternaturally and interfere with the normal egg production and fecundity of fish fauna if found as contaminant in the natural aquatic system.

Key words: oocyte maturation; in vitro; zebrafish; xenoestrogen; aquatic contaminants; reproduction.
Introduction

Environmental estrogenic chemicals or xenoestrogens have become the topic of significant current interest and a major public concern in recent years owing to their potential endocrine disrupter activity (Mc Lachlan and Arnold, 1996; Lynn, 2001; Wagner and Oehlmann, 2009; Yang et al., 2011). These exogenous compounds when present in the surroundings have an ability to disrupt the normal endocrine homeostasis by interfering the molecular mechanisms regulating reproduction, development and behavior of non-target organisms inhabiting the niche (Zhou et al 2009; Mc Lachlan and Arnold, 1996). Pharmaceuticals estrogens (ethynyl estradiol), synthetic estrogens (diethylstilbestrol), plant estrogens (genestein and coumestein), pesticides/herbicides (organochlorines, organophosphates, pyrethroids) and plasticizers (phthalate esters, bisphenol, octylphenol) are commonly detected xenoestrogens in the environment (Wagner and Oehlmann 2009).

In the last few decades, the use of pesticides/insecticides has been increased tremendously throughout the world to protect crops and/or to store the food grains. These pesticides reach aquatic ecosystems in considerable amounts from agricultural runoff from land, contaminated ground water, bottom sediments, urban runoff and outputs from municipal water treatment and manufacturing plants (Goksoyr, 2006; Lal, 2007). Although, the application of these chemicals is targeted on their toxicity to selective pests, the effects are found to be largely non-specific. Investigations focusing on the reproductive toxicity of pesticides are very few and do not encompass the diverse range of events involved in female reproduction, such as, oogonial proliferation, oogenesis, folliculogenesis, steroidogenesis, oocyte maturation, ovulation, spawning,
fertilization and developmental events (Lai, 2007). In the present study, pyrethroid insecticide, fenvalerate and organophosphate dimethoate which are commonly used for crop protection were tested for their ability for induction of germinal vesicle break down (GVBD) in the oocytes of zebrafish (*Danio rerio*) *in vitro*. The estrogenic potency of fenvalerate has been confirmed previously on breast cancer cell lines using an *in vitro*-E-screen test (Xiao et al 2003). While, organophosphorus pesticide Dimethoate is reported to mimic estrogenic activity in the fish *Onchorhynchus mykiss* (Dogan and Can, 2011).

Materials and Methods

I. Procurement and Maintenance of adult zebrafish

Zebrafish (*D. rerio*) were obtained from commercial suppliers (Aquastar aquarists, Chennai) and maintained in the laboratory under natural temperature (26 ± 1°C) and photoperiod (11.30 – 12.30 hrs), in glass aquaria with well-aerated conditioned water (Rajapurohit and Pancharatna, 2007). They were fed on commercial fish pellets *ad libitum*. The water in aquaria was replaced by conditioned water every alternate day (Anuradha and Pancharatna 2009ab).

II. In vitro GVBD Assay

All the glass wares/instruments used for the *in vitro* experiments were thoroughly cleaned, sterilized and autoclaved. Ovaries dissected from gravid females were placed in a petri dish containing incubation medium [Ringer Solution -116 mM NaCl, 2.9 mM KCl, 1.8 mM CaCl₂ and 5 mM HEPES in distilled water, pH 7.2] (Tokumoto et al., 2004). Oocytes were then separated manually with the help of a pair of fine watchmaker’s forceps under a dissecting microscope. Stage III oocytes (500-690 µm in diameter) were selected as they are known to readily respond to *in vitro* assays (Selman
et al., 1993). These oocytes had centrally a located prominent germinal vesicle (GV). They were then transferred to a 24-well culture plate (Axygen Life Sciences, California, USA), each well containing 2 ml of incubation medium. Four different concentrations of test chemicals, a single concentration (2 μM/ml, at which dose it elicits maximal GVBD in this fish) of DES and a control group (Ringer’s solution only) were used. Each concentration was tested in triplicate sets, each set containing 10 fully-grown oocytes. Oocytes were incubated at room temperature (26 ± 1° C) and observed for the occurrence of GVBD for every hour for 6.00 hours.

III. Diethylstilbestrol (DES)

2 μM/ml DES was prepared by dissolving 2.6 mgs of DES (Sigma, USA) in 100 ml of Ringer Solution to make a stock solution of 100 mM/ml and then 2ml of Stock Solution was further diluted with 98ml of Ringer solution to obtain the required concentration.

IV. Test chemicals

The test chemicals, Fenvalerate [Isagro (Asia) Agrochemicals Pvt. Ltd., India], [(R,S)-a-cyano-3-phenoxylbenzyl (R,S)-2-(4-chlorophenyl)-3-methyl butyrate – a pyrethroid insecticide] and Dimethoate (Hyderabad Chemical Supplies Ltd., India) {[O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] dithiophosphate, an organophosphate}] were used in four graded concentrations (5, 10, 15 and 20μg/ml of ringer solution). Oocyte maturation process was assessed by observing the oocytes under a stereozoom, after placing them in clearing solution (4% acetic acid + 5% paraformaldehyde in ringer) and the GVBD was ascertained by dissolution of GV (Lessman and Kavumpurath, 1984; Tokumoto et al, 2004).
chemical varied thus, in case of Fenvalerate, highest percentage of GVBD (70 ± 0.4%) was found in 15 μg/ml concentration (Fig. 2) while, Dimethoate, induced 80 ± 0.8% GVBD in 5 μg/ml concentration (Fig. 2).

Discussion

Recent research based on the experiments employing animal models indicate that exposure to xenoestrogens is linked with perturbation in reproductive processes. The present study reveals that organophosphorus and pyrethroid insecticides (with estrogenic activity) tested induced GVBD in vitro in the fully grown oocytes of zebrafish. Other estrogenic compounds such as, DES and ethynyl estradiol are reported to induce GVBD in this fish (Tokumoto et al., 2005). Organophosphorus compounds, malathion, mevinphos, chlorovinphos, tetravinphos and organochlorine endosulphan are also known to induce GVBD in common carp (Haider and Upadhyaya, 1986; Inbaraj and Haider, 1988). Ghosh et al., (1999) demonstrated that final oocyte maturation was induced in fresh water perch Anabas testudineus by metacid-50. On the contrary, several endocrine disrupting chemicals (EDCs), such as Kepon and o, p-DDD, have been reported to antagonize induction of meiotic maturation of fish oocytes in vitro (Thomas, 1999). EDCs such as methoxychlor and ethynyl estradiol also antagonize frog oocyte maturation (Pickford and Morris, 1999).

Although the adverse effects of pesticides on fish reproduction have been documented previously, studies involving analysis at cellular and molecular level are virtually lacking. The present study uses in vitro system and shows that the xenoestrogens (Fenvalerate and Dimethoate) tested have induced GVBD preterminally and thus interfere with normal reproduction of fish fauna. In conclusion, the study emphasizes
and cautions on the impact of excessive use of agro-chemicals on the reproductive health of aquatic fauna.

Acknowledgements

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(a) Fully grown oocytes of *Danio rerio*

(b) GVBD in the oocytes of *Danio rerio*

Fig. 1
Fig. 2

% GVBD

Fenvalerate  Dimethoate

Treatment Groups

Control  DES 2μM/ml  5μg/ml  10μg/ml  15μg/ml  20μg/ml

*