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Bismi Antony and C. P. Anitha Devi

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Research Review

New Phthalic Acid Diamide Flubendiamide - Its Effects on Non-Target Species: A Review

Bismi Antony[1] and C. P. Anitha Devi*[2]

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ABSTRACT

Pesticides are used to protect food crops, but can also threaten organisms unrelated to their intended targets. With population growth and pesticide use increasing, food is becoming more scarce and pesticides are used to combat them. Flubendiamide is an insecticide that is toxic to lepidopterans and other non-target organisms. The objective of our review is to identify how the insecticide affects non-target organisms. Several recent studies are systematically reviewed and analyzed. A review of a number of different studies undertaken on different organisms exposed to the chemical showed that the pesticide is capable of causing morphological, biochemical, hematological, enzymatic, histopathological, and behavioral changes.

***Key words*:** Biochemical changes, Enzyme profile, Hematological changes, Histopathological alterations, Lepidopteran insecticide, Morphological changes

Crop protection is very important in sustainable agriculture. In order to meet the increasing need for food, growing as well as stored grains should be protected. For this purpose, we use pesticides [1]. Pesticides are a group of chemicals which are used to kill insects, weeds, fungi, bacteria. They are generally called insecticides, herbicides, fungicides, bactericides and rodenticides. Most of these chemicals are designed in such a way that they can act as broad spectrum. But some of them have a specific action [2]. Besides target, pesticides have a serious impact on non-target species also [3]. Most of the pesticides used in the agricultural fields reach the soil. From there they reach the water bodies by surface runoff and leaching. The persistence of these pesticide residues in soil and water affects the soil as well as aquatic life [1]. The present review focuses on the effect of Flubendiamide, a new phthalic acid diamide, a new generation insecticide on various non-target organisms. It registered in India on 2007 under the trademark Fame [4]. Phthalic acid diamides used as a substitute for organochlorine and organophosphate pesticides due to their high specificity towards lepidoptera and low persistence in the soil.

Flubendiamide (N2- [1,1- dimethyl-2-(methyl sulphonyl ethyl]-3-iodo-N1-[2-methyl-4-[1,2,2,2-tetrafluro-1 (trifluromethyl) ethyl] phenyl]-1,2-benzene dicarboxamide) is registered in 2008 for sale distribution and use under the federal insecticide, fungicide, rodenticide act (FIFRA). It is a new stomach poison insecticide which is used to control important lepidopteran pests. Unlike all the other insecticides which act on the nervous system of the target pest, Flubendiamide act on the ryanodine sensitive receptors in the muscles of insects and causes the cessation of feeding thereby killing them. The insecticide resistance action committee has classified the diamides into a new group (Ryanodine receptor modulators) [4]. Its insecticidal activities are very relevant. It is fast acting and long lasting. Even though no mammalian toxicity of Flubendiamide is reported not so far, most of other non-target organisms are affected by the same. Pesticide usage which is desirable for pest control ultimately results in pollution and will affect non-target organisms also [5-6]. The present review focuses on the effect of Flubendiamide on its various non-target organisms.

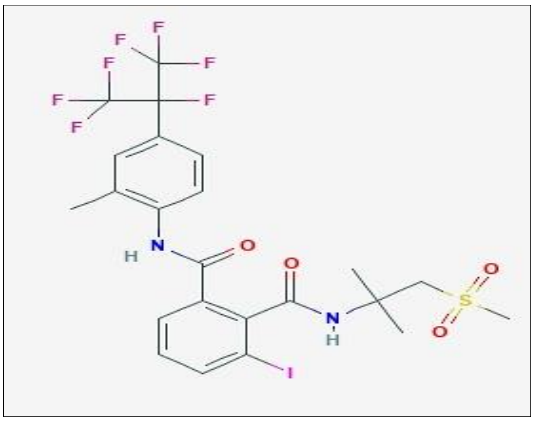

Fig 1 Chemical structure of Flubendiamide
Source: pubchem.ncbi.nlm.nih.gov

* **C. P. Anitha Devi**
cpanitha@gmail.com

1-2 P. G. and Research Department of Zoology, N. S. S. Hindu College, Changanacherry - 686 102, District Kottayam, Kerala, India

Overview about Flubendiamide

Flubendiamide is a novel class of insecticide, the phthalic acid diamide. It has a unique structure. The uniqueness is due to the presence of three structural components.

- A heptafluroisopropyl unit in the anilide moiety
- A sulphonyl alkyl group in the aliphatic amide moiety
- An iodine atom in the 3rd position in the phthalic acid moiety [7]

The chemical structure of Flubendiamide is given in (Fig 1).

Table 1 Physico-chemical properties of Flubendiamide

Common name: Flubendiamide
Chemical name: (N2- [1,1- dimethyl-2-(methyl sulphonyl ethyl]-3-iodo-N1-[2-methyl-4-[1,2,2,2-tetrafluro-1-(trifluromethyl) ethyl] phenyl]-1,2-benzene dicarboxamide
Molecular formula: C23 H22 F7 IN2 O4 S
Appearance: Colorless crystal
Molecular weight: 682.39
Odour: No characteristic odour
Melting point: 217°C
Density (at 20.8°C): 1.659g/cm3

Mode of action

Flubendiamide activates ryanodine sensitive intracellular calcium release channels (RyR receptors) in insects. Rather than affecting the sodium-potassium ion balance in the cell, it affects the calcium ion balance which causes the contraction of insect muscles. Flubendiamide keeps the RyR Receptors in an open state causing massive calcium release leading to the rapid contraction of muscles followed by death [4]. It will induce cessation of feeding by the insects [1]. It is effective against a broad range of pests belonging to the order lepidoptera [7]. It is considered to be a new stomach poison, Oral intoxicant, long lasting and fast acting insecticide [1]. It is marketed with different trade names including Belt®, Fame™, Tenos™ and Synapse®.

Review of recent findings

The concentration of toxicant when less than the lethal dose, the exposed organisms might show changes in morphological, behavioral, physiological and biochemical changes. Few of such studies are listed below:

Earthworm Eudrilus eugeniae

The avoidance behavior and neurotoxicity of earthworm *Eudrilus eugeniae* studied by [8]. The neurotoxicity was studied based on the production of marker enzyme acetyl choline esterase. Toxicity signs such as increased mucus secretion and bloody lesions in the body were observed. The earthworms tend to avoid the Flubendiamide contaminated soil. Apparent changes in the body pigmentation were also there in exposed group compared to the control as noted by [8]. Morphological and physiological alteration in earthworms were observed in response to decreased acetyl choline esterase levels. kinetic study of the enzyme in the presence and absence of the inhibitor was studied and the results showed that it acted as a competitive inhibitor. The effects of Flubendiamide on*Eudrilus eugeniae* is given in (Table 2).

Table 2 The effects of Flubendiamide on *Eudrilus eugeniae*

Dose of Flubendiamide	Parameters studied	Results
54-94µg/cm-2	Determination of median lethal concentration	LC50- 94.4 µg cm -2 (48 hr. paper contact test) 332.21 and 238.31 mg kg-1 respectively, for 7 and 14 d of artificial soil test
54-94µg/cm-2	Avoidance test	Animals tend to avoid regions where higher concentrations of Flubendiamide were present
54-94µg/cm-2	Morphological changes	Sluggish movement, excessive mucous secretion, coiling and swelling of clitellum, extrusion of coelomic fluid, formation of bloody lesions
54-94µg/cm-2	Acetyl choline Esterase activity	As the concentration of Flubendiamide increased, the enzyme inhibition also increased drastically. Concluded that the inhibition is competitive in nature

Table 3 Effects of Flubendiamide on *Daphnia magna*

Dose of Flubendiamide	Parameters studied	Results
EC50 -63.5µ/L (Acute toxicity study)	ROS	Significant increase
	Antioxidant status	Significant decrease in the activity of enzymes such as superoxide dismutase and glutathione peroxidase, which is consistent with the downregulated transcription of their genes. Increased catalase activity as a result of increased transcription of *cat* genes
EC50-36.8µg/L (chronic toxicity)	Mobility	Decreased
Chronic LOEC19.36µg/L	Survival growth and reproduction	Affected
48 hr. EC50- 30.8µg/L (acute exposure)	Developmental abnormalities	Embryos had underdeveloped second antennae, curved tail spine and abnormal body region

Daphnia magna

The effect of three diamides (Chlorantraniliprole, Cyantraniliprole and Flubendiamide) on life history, embryonic development and oxidative stress biomarkers of *Daphnia magna* was experimented by [9]. They observed increased ROS, decreased activities of antioxidant enzymes (SOD and Glutathione peroxidase) which was consistent with the downregulated transcription of the corresponding genes. An increased catalase activity was noted but there was no change in the gene expression [9]. Most xenobiotic compounds like pesticides can lead to the production of reactive oxygen species in response to oxidative stress via several mechanisms such as inhibition of electron transport chain in the mitochondrial membrane and subsequent accumulation of its reactive intermediates, inactivation of antioxidant enzymes and decrease in the non-enzymatic antioxidants and membrane lipid peroxidation. The adverse effects of exposure to Flubendiamide in *Daphnia magna*are given on (Table 3).

Table 4 Effect of Flubendiamide on locomotory activities of *Drosophila melanogaster*

Dose of Flubendiamide	Parameters studied	Results
0.25-20 µg/ml	Phototactic behavior	Reduction in light seeking behavior (P, F_1 and F_2)
0.25-20 µg/ml	Climbing or negative geotaxis behavior	Decline in climbing behavior. P, F_1 and F_2 flies failed to reach the top of the glass tube
0.25-20 µg/ml	Adult acetylcholine esterase activity	Showed dose dependent decrease in the choline esterase activity
0.25-20 µg/ml	Adult cytochrome P450 activity	Increased resorufin production indicating the increased enzyme activity

Drosophila melanogaster

The studies on the effect of Flubendiamide on locomotory activities of non-target species *Drosophila melanogaster* for three generations was reported by [10]. Their studies suggest that chronic Flubendiamide exposure might induce alteration in neurotransmission leading to inconsistent behavioral responses (vision and flight) in other beneficial insects and insect dependent organisms [10]. The results of the study are consolidated in (Table 4).

Toxicological evaluation of Flubendiamide in non-target *Drosophila melanogaster* for different developmental stages showed that the acute LC50 values for both larvae and adult are higher than that of the chronic ones. The study revealed that the acute LC50 value for adult was 28 folds higher than the LC50 for the larvae. Similarly, the chronic LC50 for adults was 32 folds higher than for larvae. This showed that the chemical is more efficient against larvae than adult (larvicidal activity) [11]. The results are given in (Table 5).

Table 5 Toxicological evaluation of Flubendiamide for different developmental stages of *Drosophila melanogaster*

Dose of Flubendiamide	Parameters studied	Result
5000, 7500, 10000 and 15000 µg/mL	LC 50 values for larvae	After 24 hr- 12011.64 µg/mL After 48 hr- 8317.64 µg/mL After 72 hr- 4897.79 µg/mL
100000, 200000, 300000 and 500000 µg/mL	Acute LC50 values for adult	After 24 hr- 338844.26µg/ml After 48 hr- 257038.58µg/ml After 72 hrs- 154881.66µg/ml
10000, 25000, 50000 and 75000 µg/mL	Chronic LC50 determination	For larvae - 562.34µg/mL For pupae - 676.08µg/mL For adult flies - 17782.79µg/mL
250, 500, 1000 and 1500 µg/mL	Adult emergence	As the concentration of the Flubendiamide increases, there was a significant reduction in percentage of adult emergence

Trichogrammachilonis

The effect of Flubendiamide against egg parasitoid *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) under laboratory conditions was noted by [12]. The highest percentage parasitation was noted in Flubendiamide W.G at 50 g.a.i./h. and maximum adult emergence (89.70) was shown at Flubendiamide W.G. at 50 g.a.i./h. (g.a.i/h – grams of active ingredient per hectare)

The effect of Flubendiamide on parasitation of *Trichogramma chilonis is* given on (Table 6).

Table 6 The effect of Flubendiamide on parasitation of *Trichogramma chilonis*

Dose of Flubendiamide	Parameters studied	Results
a)50 g.a.i. ha^-1 b)60 g.a.i. ha ^-1 c)70 g.a.i. ha^-1	Parasitation	little effect on the parasitation. Untreated group showed greatest parasitation. Among Flubendiamide treated groups, the highest percentage parasitation was found to be at 50 g.a.i./ ha followed by 60 and 70
	Adult emergence	Maximum adult emergence was found at the 50 g.a.i./ ha followed by 60 and 70 at 72 hours after treatment

Fishes

Pesticide pollution in water affects the fish and other aquatic organisms, relatively sensitive to the changes in the surrounding environment [13]. The greatest hazard is to the aquatic organisms, especially fish, which are more susceptible to them as they got concentrated into their organs more readily than terrestrial organisms [14]. The toxicants are accumulated in fish through food chain or by absorption through general body surface which affect severely their life support systems at molecular and biochemical levels [15].

The nutritional value of different species of fish and shellfish depend on their biochemical components such as proteins carbohydrates and lipids. It is important to study the effect of specific concentrations of pesticides on various systems of aquatic organisms. When exposed to certain chemicals, organisms show alterations in their biochemical parameters. Animals uses their muscle protein as an energy source during acute respiratory stress [16]. It is reported that many of the toxicants alter the protein metabolism in fishes such as bleached craft pulp mill effluent [17], textile mill effluent [18], DDT, malathion and mercury [19- 21].

Changes in the blood parameters of fish is an indication of its pathophysiological status and their determination is essential for the diagnosis of structural and functional status of fish when exposed to toxicants [22]. Genotoxic compounds will induce alterations of hematological parameters of fish and this is always associated with their physiological state. The biochemical parameters in fish are validated for evaluating their physio pathological conditions and they are sensitive for detecting potential adverse effects and relatively early events of pollutant damage [23]. Alterations in the blood parameters of organisms are due to the damage caused to their hemopoietic organs by the water born pollutants [24-28]. Hematological studies in fishes revealed that the alterations in the blood parameters are indicators of a stressed environment and it gives details about the health conditions of fishes [29]. Studies on the impact of pesticides on fishes revealed that they will interfere with their ability to resist diseases and increases mortality rates [30]. The effect of Flubendiamide on protein metabolism of freshwater fish *Labeo rohita*was studied by [31]. They exposed the fishes with sublethal concentrations of Flubendiamide and found that the exposed ones showed decrease in protein content in various organs. They concluded that the protein depletion may be due to the increased pesticide stress when compared with the control group [31]. The reduction in the protein content is due to the increase use of protein to meet the high energy demand caused by the toxins such as detoxification reactions, tissue repair, homeostasis and stress metabolism [20].

The assessment of the health status of wild fish inhabiting a cotton basin, heavily impacted by pesticides, including Flubendiamide in Benin (West Africa) was done by [32]. Their findings revealed that the pesticides disrupt the endocrine regulation of fish living in the basin and also it causes severe damage to the liver and gonads. Many significant changes were observed in the biochemical profile of *Heteropneustes fossilis*such as decrease in glycogen and total protein content and increased cholesterol content when exposed to Flubendiamide as reported by [33]. Biochemical analysis of Flubendiamide toxicity on gill of *Heteropneustes fossilis* showed that it is not only the gill, but other organs such as liver, kidney, intestine and blood also got affected [34-35] determined the consequences of Flubendiamide exposure on hematological parameters of the fish *Oreochromis mossambicus*. The results of the experiment showed that all the blood parameters except WBC count showed reduction. The concentration of the leucocytes was high throughout the experiment. The enzyme profile also showed significant increase. This is an indication of the impact of the toxin to the organism. (Table 7) gives a summary of the effect of Flubendiamide on various biological parameters of different fishes.

Table 7 Effects of Flubendiamide on various parameters of different fishes.

Name of fishes	Dose of Flubendiamide	Parameters studied	Results	References
Labeo rohita	11µg/L	Total protein content	Depletion of protein content in all types of tissues were observed when compared to the control	[31]
Heteropneustes fossilis	0.1ml/L	Biochemical parameters of the intestine: 1. Total glucose 2. Total protein 3. Total cholesterol	Reduces as exposure period increases Lower than the control Higher than the control	[33]
Heteropneustes fossilis	0.1 ml/L	Biochemical analysis of gill 1. Total protein 2. Glycogen 3. Cholesterol	decreased as the exposure time increases Showed decline in the glycogen concentration as exposure time increases Declined initially but after 14th day, there was a sudden increase	[34]
Oreochromis mossambicus	10 ppm	Heamatological parameters		[35]
		1. TEC (Total Erythrocyte Count)	Decreased	
		2. TLC (Total Leucocyte Count)	Increased	
		3. Hb (Hemoglobin)	Decreased	
		4. PCV (Packed cell volume)	Decreased	
		5. MCV (Mean Corpuscular Volume)	Increased	
		6. MCH (Mean Corpuscular Hemoglobin)	Decreased	
		7. MCHC (Mean Corpuscular Hemoglobin Concentration)	Decreased	

Table 8 In-vitro studies of wistar rats when exposed to Flubendiamide and the observed effects

Dose of Flubendiamide	Parameters studied	Results
1-80µM	Median lethal concentration	40 µM was selected as the median lethal concentration Flubendiamide treated cells showed 71.88 non-viable cells when compared to control
40µM	Viability of splenocytes	The combined treatment of Flubendiamide with antioxidants and xenobiotics decreased the number of non- viable cells
40µM	Tunnel assay	Showed higher number of tunnel positive cells when compared to control
40µM	Micronuclei formation	7.86% cells show micronuclei formation
40µM	DNA fragmentation	Flubendiamide treated splenocytes showed more DNA fragmentation
40µM	Comet formation	Flubendiamide alone treatment in spleenocytes produced 22.90% comet formation when compared to control

Wistar rats (Rattus norvegicuz)

When an organism is in toxic stress, the study of histochemistry and pathology is very important. Studies on the in-vitro and in-vivo effects of Flubendiamide and copper in cytogenotoxicity, oxidative stress and spleen histology of rats showed that they induce oxidative stress, cytogenetic effects along with histoarchitectural changes in spleen [36]. In- vitro studies on Wistar rats and the results are given in table VIII and In-vivo studies are given in (Table 9).

Table 9 In-vivo studies in Wistar rats when exposed to Flubendiamide and the observed effects

Dose of Flubendiamide	Parameters studied (In-vivo)	Results
40µM	Oxidative stress biomarkers: 1. Lipid peroxidation levels	Higher when compared to control
	2. Reduced glutathione	Lower when compared to the control
	3. Glutathione peroxidase	Significant decrease when compared to control
	4. Glutathione-S-transferase	Significantly lower
	5. Total protein content	No significant decrease
	6. Catalase activity	No change when comparing to control group
	Histology	Flubendiamide and copper treated group showed separation of splenocytes and small sized splenic parenchyma. Treatment along with antioxidants showed normal histoarchitecture of spleen

Table 10 Effects of Flubendiamide on various hematological parameters of Sprague Dawley rats

Dose of Flubendiamide	Parameters studied	Results
Between 1-2 ml/ 100gm body weight	1. RBC (Red Blood Cells) 2. Hb (Hemoglobin) 3. PCV (Packed Cell Volume) 4. CHCM (Cellular hemoglobin concentration mean) 5. CM (Cellular Hemoglobin Concentration) 6. MCV (Mean Corpuscular Volume) 7. MCH (Mean Corpuscular Hemoglobin) 8. MCHC (Mean corpuscular hemoglobin concentration) 9. RDW (Red Cell Distribution Width) 10. HDW (Hemoglobin Distribution Width) 11. WBC (White Blood Cells) 12. PCT (Procalcitonin) 13. MPC (Mean platelet Component) 14. LPT (Lymphocyte Proliferation test) 15. PLT (Platelet count) 16. MPV (Mean Platelet Volume) 17. PDW (Platelet Distribution Width)	• In females, Significant decrease in RBC count with respect to dose rate. Rest of the parameters had no significant decrease when compared to control. • In males, Significant difference was exhibited by various parameters. WBC, LPT, PCT, PDW, MPV and PCs showed significant decrease whereas parameters such as RBC, PCV, HDW showed significant increase. For some parameters there was bidirectional response. • Significant differences in some parameters such as RBC, Hb, PCV, RDW, MCHC CHCM, CM and LPTs were observed in males and female rats of same groups. • Sex dependent differences were consistent in Hb and PCV • In males' dose dependent increase in RBC, Hb and PCV • In females, a dose dependent decrease of RBC, Hb and PCV was observed.

Sprague dawley rats (Rattus norvegicus)

Vemu and Dumka [4] studied the hematological alterations on sub-acute exposure to Flubendiamide in Sprague Dawley Rats. They observed significant changes in the female rats than male rats. Thus, concluded that many of those changes were dose independent, but sex specific [4]. The various heamatogical parameters studied and the obtained results are consolidated on (Table 10).

CONCLUSION

Flubendiamide is a new generation insecticide belonging to a new chemical class, the phthalic acid diamide. They are used against lepidopteran pests. They have a greater larvicidal activity too. It has a novel action- it affects the muscular system rather than the nervous system. It acts on the ryanodine sensitive calcium channels and causes the contraction of insect muscles. It will result in the rapid cessation of feeding thereby crop damage is prevented. Although it is designed against lepidopteran pests, they have a greater impact on the non-target species also. They affect different organisms differently. No mammalian toxicity is reported so far, there were reports of Flubendiamide poisoning in buffalos in response to consumption of fodder recently sprayed with pesticide. Morphological, biochemical, histopathological, hematological parameters of various organisms towards sub-lethal concentrations of Flubendiamide was studied. The results of the study revealed that Flubendiamide has a significant impact on all these parameters. The decrease in the antioxidant enzymes and production of ROS reveals the oxidative stress caused by the chemical. Inhibition of acetyl choline esterase activity showed the ability of the chemical for competitive inhibition. The studies on *D. melanogaster* showed that the insecticide have larvicidal activity on non-target insects also. Since water bodies are the ultimate destination of the pesticides from agricultural fields, fishes were studied mostly. The effects of this insecticide should be monitored properly for protecting the non-target organisms, which accidently comes in contact with the chemical.

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