ISSN : 0974 - 7435 Volume 7 Issue 6

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 7(6), 2013 [225-230]

Impact of Diflubenzuron on mortality level, biochemical parameters of embryonic (*in ovo*) and neo-hatched chicks of *Gallus domesticus*

Rouabhi Rachid[1]*, Djebar-Berrebbah Houria[2], Djebar Mohammed-Réda[2]

[1]Department of SNV, Tébessa University, 12000, (ALGERIA)

[2]Cellular Toxicology Laboratory, Department of Biology, Faculty of Sciences, Annaba University, 23000, (ALGERIA)

E-mail : r_rouabhi@yahoo.fr

ABSTRACT

The struggle against the agricultural devastating is increased in few last years and many products are created to kill the harmful insects and bug, without controlling their effect on ecosystem and non-target animals.

In present work, we try to investigate the effects of the Diflubenzuron (DFB), which is an Insect growth Regulator (IGR) on biochemical and embryonic development of hen's eggs as a non-target for this pesticide. The pesticide is tested *in ovo* by injection in the egg air cell of three concentrations in strictly controlled conditions, to show the impact on some biochemical parameters.

The treatment with 1, 10 and 20µg/egg of the pesticide induced some blood parameters perturbations, especially in 10 and 20µg according to the controls.

The 20µg concentration induces the blood serum proteins decreasing, increasing of triglycerides, decreasing of cholesterol and increasing of Alanine amino transferase (GPT) and GOT. This concentration induced a great level of embryonic mortality. The 01 and 10µg concentrations perturb (increase/decrease these parameters).

KEYWORDS

Diflubenzuron;
IGR;
POPs;
Gallus domesticus;
Embryonic development;
Biochemical parameters;
Triglycerides;
GPT;
GOT;
Toxicity;
Cholesterol.

INTRODUCTION

The struggle against the agricultural ravagers is increased in few last years; many products are appeared to kill the harmful insects, without we control their effects on ecosystem and non-target animals. Pesticides and other Persistent organic pollutants (POPs) harm human health and the environment. POPs are produced and released to the environment predominantly because of human activity. They are long lasting and can travel great distances on air and water currents. Some POPs are produced for use as pesticides, some for use as industrial chemicals, and others as unwanted by products of combustion or chemical processes that take place in the presence of chlorine compounds[1]. Today, POPs are widely present as contaminants in the environment and food in all regions of the world. Humans everywhere carry a POPs body burden that contributes to disease and health problems[4].

Free-range chicken eggs collected in Vikuge vil-

FULL PAPER

lage and its surroundings 56 km northeast of Dar Es-Salaam City showed elevated levels of dioxins and high levels of hexachlorobenzene (HCB). HCB levels were 2-fold higher than the newly proposed limit for HCB as a pesticide residue and were very close to the existing limit for this chemical in eggs. Dioxin levels exceeded background levels by almost 2.5-fold and were slightly higher than the European Union (EU) dioxin limit for eggs[6].

Insect growth regulators are substances destined for killing pests and bugs[15], they act on chitin synthesis causing an inhibition of insect moulting[19], also they have an undesirable effects on non-target organisms, by accumulation and magnification in food chain[12,16].

The Diflubenzuron or Dimilin (1-chlorophenyl)-3-(2,6-difluorobenzoyl) urea) is a pesticide of third generation to large spectre, it is used mainly in agriculture: forests, cereal cultures, it is the more used between the Benzoylphenyl ureas. It is considered like a poison of contact and ingestion; it inhibits the synthesis of the chitin and interferes with the formation of the cuticle (exoskeleton), probably by the inhibition of the N-acétylglucosamine incorporation in the chitin. It is shown that the Diflubenzuron could modify the metabolism of the moulting hormones[17], translated by cuticle deformation; Figure 1 shows the metabolism of DFB in animals.

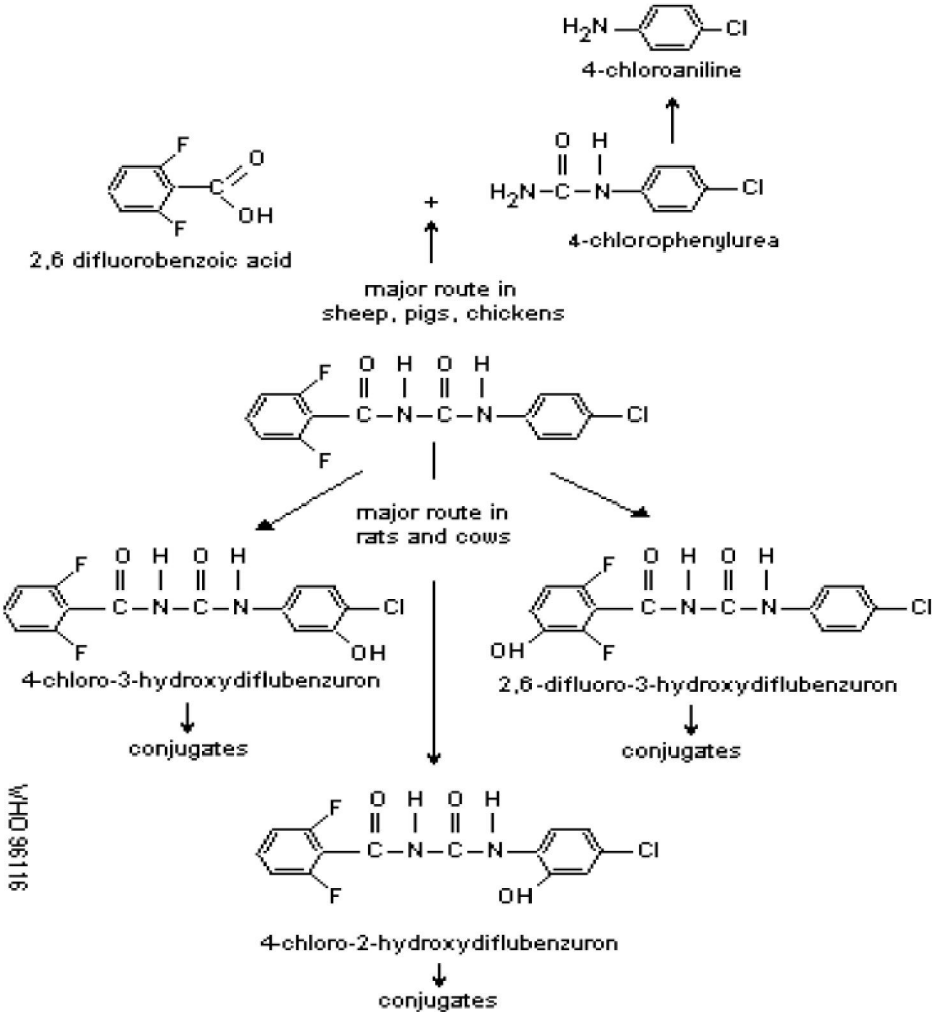

Figure 1 : Animal metabolism of DFB (FAO/WHO, 1982b)

So, for these reasons, we hypothesis that DFB may enter in eggs by adsorption or feeding of hens and causes a perturbation in embryonic development, these effects may be extrapolated and transferred to human being by chain food.

MATERIALS AND METHODS

Culture and treatment

The fertilized 50 eggs (55-60g) were collected and

numerated. The concentrations (1µg, 10µg and 20µg/ 5µl acetone/ egg) of Diflubenzuron were injected in air cell of eggs with sterile syringe (because of insolubility of Diflubenzuron in water), in sterilized conditions. The control was received only acetone. The 10 control eggs and 10 of each treatment (n=10) were incubated in 38°C and 65% of relative humidity in artificial incubator.

Weight mobility of eggs was followed every day, and the development stage by candling of eggs (to estimate mortality).

After eggs hatching, the new chicks were sacrificed and the blood is collected for biochemical analysis using a technikon autoanalyser, and the skull is measured using a slide gage (9.1mm graduation). After opening up, all the body cavities were inspected *in situ*. The liver and heart were removed to determinate their weights using precision balance.

All the experiments were repeated ten times or more, and the results were expressed as mean and standard deviation (SD) values. We use Minitab 16.1 software to make simple two-way ANOVA and the test of Dunnett for comparison between the control and treated eggs.

RESULTS AND DISCUSSION

The impact of Diflubenzuron on mortality level

Results in the TABLE 1 show the impact of Diflubenzuron on embryonic lethality; it observes that at 20µg/egg the mortality is about 50%. This percentage is decreased at 10µg/egg to 20%. The 1µg/egg seems do not influence the embryonic mortality.

TABLE 1 : Impact of Diflubenzuron on embryonic mortality level.

Group	n	n.f	I	1-7 Day	8-12 Day	13-17 Day	18-21 Day	Total
Control	10	1	-	-	-	-	-	1/10
Acetone-control	10	-	-	1	-	-	-	1/10
DFB 1µg/egg	10	-	-	-	1	-	-	1/10
DFB 10µg/egg**	10	1	-	-	1	-	1	3/10
DFB 20µg/egg***	10	-	-	1	1	2	2	6/10

Control-acetone: acetone (5µl/egg); n: eggs number; n.f: not fertilized; I: infection; DFB : Diflubenzuron; **: p<0.01; ***: p<0.001.

The high level of embryonic mortality was caused by the intervention in metabolic process, and enzymatic inhibition as it will see after, these is in concordance with Rouabhi et al[12,16]. Results where he found an increasing mortality of chicks treated by Flucycloxuron due by errors in transformation of nutriments causing a perturbation in eggs weight kinetic. Also Soltani et *al*[18]. found a big mortality level of *Tenebrio molitor* treated with growth regulators. Klitschika et al[8]. Reported that these types of pesticides might incorporate with DNA or RNA of cells causing some malformations and mortality of embryos.

Impact of flufenoxuron on blood serum biochemical parameters

The effect of Diflubenzuron on blood proteins, triglycerides, cholesterol and Alanine aminotransferase (GPT) level is illustrated in Figure 1, 2, 3 and 4.

The effect of Diflubenzuron on blood proteins level in 0 day hatched chicks is illustrated in Figure 1. Whenever, the concentrations of Diflubenzuron reduced the protein level significantly, especially at the highest dose where the diminution is about 0.1 g/dL. This result is confirmed by Khabbeb *et al*[7]. when they found a significant perturbation of proteins and lipid levels in crustaceans. This may due to the metabolism affects. Soltani *et al*[17]. found also a perturbation in metabolism of *T. molitor* treated by Benzoyl Phenyl ureas (BPUs).

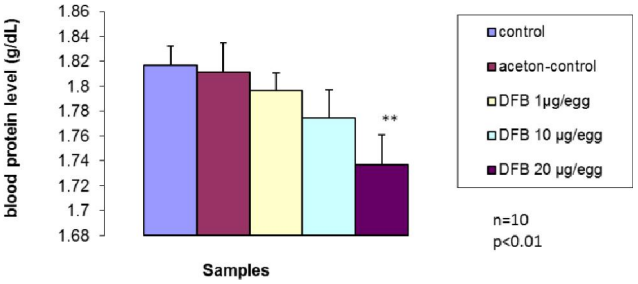

Figure 2 : Impact of Diflubenzuron on blood protein level in 0 day hatched chicks.

The Figure 3 shows the impact of Diflubenzuron on triglycerides level in neo-hatched chicks. Results showed an increasing level of triglyceride (TG) in chicks treated with 10 and 20µg/egg of DFB ($p<0.001$), contrarily to the treated with 1µg/egg where it shows a little decrease of Triglycerides (TG) level ($p>0.05$) dunnett test show that there is a difference between control and treated with 10 and 20µg/egg.

It is intelligible that the effect on triglycerides level due to the impact on general metabolism and on cells

FULL PAPER

themselves. Indeed Khabbeb et *al*[7]. found a perturbation of lipids level and some other parameters. The increased level of TG due probably to the cells apoptosis, because of the TG are the main components of cell membrane[12,16]. The same case to the cholesterol level Figure 4, results shows a decrease of cholesterol level in eggs treated with 10 and 20µg/egg of DFB ($p<0.001$), contrarily to eggs treated with 1µg/egg where it shows an increased level ($p<0.05$). The acetone didn't affect the level of cholesterol, because of the perturbation of metabolism the level of cholesterol is decreased. In addition, the detoxification needs energy so the cholesterol here may be the spring for the process. All these results are confirmed and investigated by[12,16].

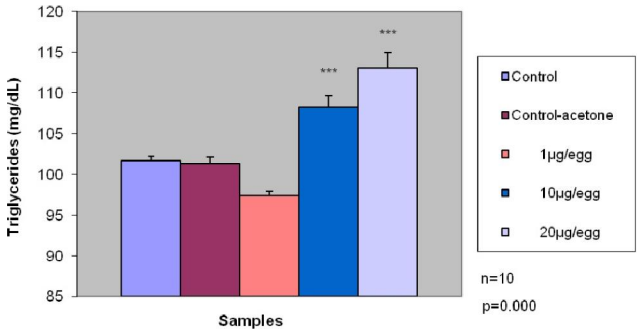

Figure 3 : Impact of DFB on triglycerides level in neo-hatched chicks.

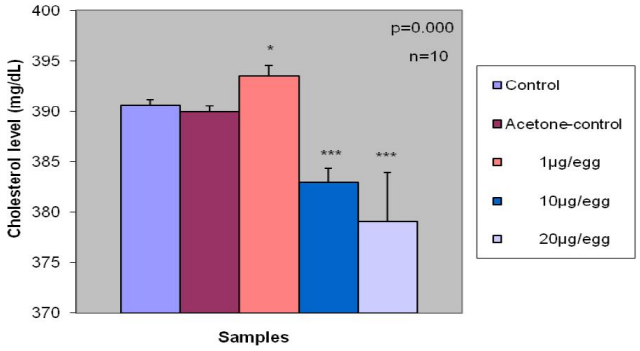

Figure 4 : Effect of Diflubenzuron on cholesterol level in neo-hatched chicks.

The Figure 5 shows the impact of DFB on Alanine Amino transferase (GPT). Indeed, 01 and 10µg/egg of DFB increase the level of enzyme, especially at the lowest concentration ($p<0.001$). The highest concentration of DFB did not cause a statistical effect on GPT level. This result is explained if based on the detoxification process and the enzymatic level. Indeed, the high level of GPT means that the liver is affected[11]. Mauchamp[10]; Marbury and Grosby[9] showed that BPUs effect the level of hormones and enzymes of insect by interfering with metabolism process. The high level of GPT translated by the activity of liver (site of detoxification). There is a non-coupling phenomenon for the highest concentration (20µg/egg)[12,16].

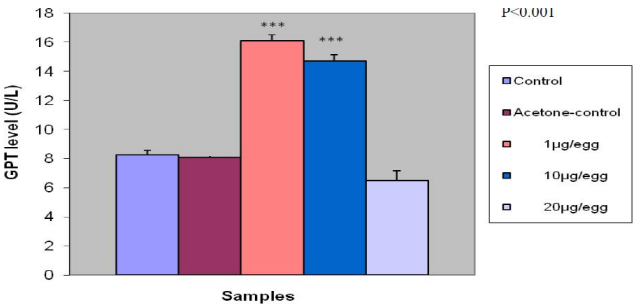

Figure 5 : Effect of DFB on GPT level of neo hatched chicks of *Gallus domesticus*

Figure 6 shows the effect of DFB at different concentrations on the rate of GOT. We are seeing a general disruption of GOT rate compared to controls especially at low concentrations of DFB ($p<0.001$) where there is an increase of this factor. Dunnett's test showed that the Treaties 1 and 10ìg/egg are not identical to the control and in the concentrations of 20ig/egg, DFB significantly affect the rate of GOT compared to the controls. This result can be explained by the fact that this organ is the primary site of metabolism / detoxification and metabolic disturbance affects preferentially on organ and biochemical assays have confirmed that this toxicity which is in agreement with the work of Mauchamp et al[10], Marbury and Grosby[9] Khebbeb et al[7]. Indeed, our results showed a significant increase in GOT and GPT are biomarkers of liver injury, this disturbance of liver function is accompanied by a dose-dependent decrease of protein levels and general disruption of blood parameters.

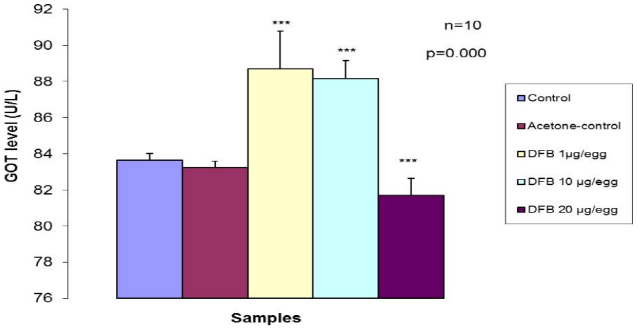

Figure 6 : Effect of DFB on GOT level of neo hatched chicks of *Gallus domesticus*.

In conclusion, diflubenzuron has a toxic effects on embnryonic devlopment of chicks, these results are

confirmed by several works on protozoa of marine and fresh water, earthworms and some insect predators, the impact is now generalized for all ecosystem. It is to note that all trials confirm biotransformation and bioaccumulation throught the trophic chain, and it is necessary to estimate the hole metabolists in non-target organisms.

REFERENCES

[1] T.Apiwat, T.Usavadee, B.Payu, Ch.Jakkrawarn, S.Padet, A.Preecha, S.Mulla; Field evaluation of Novaluron, a chitin synthesis inhibitor larvicide, against mosquito larvae in polluted water in urban areas of Bangkok, Thailand, Insect.Sci., **38(3)**, 434-440 **(2007)**.

[2] G.C.Cutler, C.D.Scott-Dupree, J.H.Tolman, C.R.Harris; Toxicity of Novaluron to the non-target predatory bug *Podisus maculiventris* (Heteroptera: Pentatomidae), Biological Control, **38**, 196-204 **(2006)**.

[3] G.C.Cutler, C.D.Scott-Dupree, J.H.Tolman, C.R.Harris; Field efficacy of Novaluron for control of Colorado potato beetle (Coleoptera: Chrysomelidae) on potato, Crop Protection, **26**, 760-767 **(2007)**.

[4] Eu-Scoop; European Commission, Scientific cooperation on questions relating to food, Assessment of dietary intake of dioxins and related PCBs by the population of the EU member states, Task 3.2.5, final report Scoop/Diox/report/1. 7 June 2000. Brussels, Belgium, **(2000)**.

[5] FAO/WHO, Pesticide residues in food - 1981, Evaluations, The monographs, *Rome*, Food and Agriculture Organization of the *United Nations*, (FAO Plant Production and Protection Paper 42), **(1982b)**.

[6] S.Greet, H.Ron; Contamination of free-range chicken eggs with dioxins and dioxin-like polychlorinated biphenyls, Mol.Nutr.Food Res., **50**, 120-126 **(2006)**.

[7] M.E.H.Khebbeb, J.Delachambre, N.Soltani; Lipid metabolism during the sexual maturation of the mealworm (*Tenebrio molitor*), Effect of ingested Diflubenzuron, Pest.Biochem.Physiol., **58**, 209-217 **(1997)**.

[8] G.E.Klitschika, R.T.Mayer, R.E.Droleskey, J.O.Norman, A.C.Chen; Effects of chitin synthesis inhibitors on incorporation of nucleosides into DNA and RNA in a cell line from *Manduca sexta* (L.), Toxicol., **39**, 307-315 **(1986)**.

[9] A.S.Marbury, G.D.Grosby; Fate and disposition of Diflubenzuron in Rice fields, Environmental, Toxicology and Chemistry. University of California, Davis, California 95616, USA, **15(11)**, 1908-1913 **(1996)**.

[10] B.Mauchamp; Endocrinologie du développement, les régulateurs de la croissance, les analogues d'hormones et les anti-hormones, Colloque Int. Mode d'action et utilisation des insecticides, ANGERS, France, 18-19 Mai**(1985)**.

[11] F.X.Reichl, R.Perraud, E.Krahe; Guide pratique de Toxicologie, Traduction de la 2ème Edition Allemande, 348 **(2004)**.

[12] R.Rouabhi; Impact de deux pesticides le Diflubenzuron et le Flucycloxuron sur trois modèles cellulaires alternatives, Paramecium sp., Tetrahymena pyriformis, Tetraselmis suecica et sur le développement embryonnaire de la poule domestique (Gallus domesticus). Thèse de Doctorat de l'université d'Annaba, Option, Toxicologie appliquée, Université de Annaba, Algérie, 200 **(2007)**.

[13] R.Rouabhi, H.Djebar, M.R.Djebar; Toxicity evaluation of flucycloxuron and diflubenzuron on the cellular model, *Paramecium sp*, Afr.J.Biotechnol., **5(1)**, 045-048 **(2006a)**.

[14] R.Rouabhi, H.Djebar-Berrebbah, M.R.Djebar; Toxic Effect of a Pesticide, Diflubenzuron on Freshwater Microinvertebrate (*Tetrahymena pyriformis*), Chin.J.App.Environ.Biol., **12(4)**, 514-517 **(2006b)**.

[15] R.Rouabhi, H.Berrebbah, M.R.Djebar; Evaluation of toxicity of two pesticides, Flucycloxuron and Diflubenzuron on a cellular model, *Paramecium sp.*, Comm.Appl.Biol.Sci.Ghent University, **71/2a**, 83-90 **(2006c)**.

[16] R.Rouabhi, H.Djebar-Berrebbah, M.R.Djebar; The impact of Diflubenzuron (DFB) Feeding on Glycosaminoglycan and Sulfhemoglobin Biosynthesis in 1 day hatched chicks *Gallus domesticus (L)*, Sci.Res.Essays., **3(2)**, 079-083 **(2007)**.

[17] N.Soltani, J.P.Delbecque, J.Delachambre, B.Mauchamp; Inhibition of ecdysteroïd increase by Diflubenzuron in *Tenebrio molitor* pupae and compensation of Diflubenzuron effect on cuticule secretion by 20-hydroxyecdysone, Intern.J.Invert.Reprod.Develop, **7**, 323-332 **(1984)**.

[18] N.Soltani, S.Chebira, N.Pitoizet, J.P.Delbecoque, J.Delachambre; Effect of Flucycloxuron, a novel benzoylphenylurea derivate, on the *in vivo* and *in*

vitro, production of ecdysteroids in *Tenebrio molitor*, Med.Fac.Landbowi.Univ.Gent., **607(3b)**, 1017-1022 **(1995)**.

[19] N.Soltani-Mazouni, N.Soltani, B.Quennedey, J.Delachambre; Protein synthesis in developing ovaries of mealworm under in vivo and in vitro conditions, effects of Diflubezuron, J.Stored.Prod.Res., **32(3)**, 205-212 **(1996)**.

[20] N.Van Larebeke, L.Hens, P.Schepens, A.Covaci; Dioxins in animal nutrition from soil.Environ.Health Perspect., **109**, 265-273 **(2001)**.