Original Article

Study of the subacute toxicity of a fungicide on rabbits (Batna region)

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ABSTRACT

The present study relates to the evaluation of the subacute toxicity of a pesticide widely used in agriculture in the Batna region on a few parameters related to liver and thyroid functions in domestic rabbits. Our experimental study was performed on 12 mature male rabbits of the genus *Cuniculus lepus,* which were divided into 2 groups (n = 6): a control group (G1) and a treated group (G2) with Rivanebe®80 orally for 15 days. Analysis of the results obtained has shown that treatment with Rivanebe®80 causes harmful effects on the organism, resulting in a significant increase in body mass and consumption of food by treated animals, compared to the control group. In addition, a hepatotoxic effect by a significant increase in the mass of liver and biochemical parameters was reported including hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and an increase in bilirubin and transaminases. Finally, a disturbance of the thyroid function or a significant decrease of the thyroxin plasma levels was also reported.

Keywords: Subacute; toxicity; Hepatotoxic; Thyroid; Pesticide; *Cuniculus lepus*

Introduction

Pesticides are a group of chemicals used for the destruction of insects, weeds, fungi, and bacteria; they are generally classified in insecticides, bactericidal fungicides, herbicides, and most of them are designed to disrupt the physiological activities of the target organism causing malfunctions and even reduced vitality [1]. Pesticides are considered the most common environmental pollutants because of the high utilization in agriculture and industries [2-4]. The fungicides then make it possible to be part of a strategy to fight the most harmful fungi including rust, mildew, scab, or organoleptic alterations such as the presence of *Botrytis cinerea* on the grape [5, 6]. The Manebe under the trade name Rivanebe®80 is a sort of the non-systemic fungicides belonging to the family of Dithiocarbamates, Ethylene class Bisdithiocarbamates [7], it is used on a large scale against cryptogamic diseases, especially scab which is manifested by the appearance of brown and white spots on all the aerial parts of plants, in the control of early and late rust on potatoes and tomatoes and many other fruit diseases [8]. This compound has been carefully used for the protection of legumes; in the Batna region (Eastern Algeria), more particularly in Ngaous and Arris. Agricultural pesticides help increase agricultural productivity but at the same time pose potential risks to human health and the environment [1]. There are generally three main modes of access: inhalation (case of aerosol vapor), contact (skin, mucous membrane), and ingestion (oral) [9]. Several studies have shown that Maneb has moderately low toxicity for laboratory animals; simple toxic dose in rats induced hypotonia, slow breathing and heartbeat, functional abnormalities of the thyroid, liver, infiltration in the palms and the kidneys [9-12].

This work focused on the study of the subacute toxicity of a fungicide belonging to the Dithiocarbamate family in the domestic male rabbit *Cuniculus lepus* in the Batna region. The objective is to highlight the functional and/or pathological

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alterations consecutive to the repeated administration of Rivanebe®80 for 2 weeks. This will include determining:

- The bodyweight of the rabbits before and at the end of the experiment.
- The weight of the organs removed: liver.
- Plasma levels of biochemical factors such as cholesterol, glucose, triglycerides as well as bilirubin and transaminases.
- A hormonal dosage such as thyroxin.

Materials and Methods

Breeding

We used 12 domestic male rabbits (*Cuniculus lepus*) with a living weight between 1500 and 2500g from Batna region. They were placed and raised in metal cages of 60×54×52cm^3 lined with boxes changed daily and equipped with feeders and drinkers. These cages were cleaned by the use of detergents like bleach to avoid infections. Before setting up the experimental batches, the rabbits were acclimated for 10 days at 21°C and relative humidity of 75%. The food was well balanced and varied; it contained all the elements necessary for the natural growth of animals, based on various vegetables such as salad and carrot. Moreover, drinking water was present.

Systematic position:

Reign: Animalia
Phylum: Chordata
Class: Mammalia
Order: lagomorphs
Family: Leporidae
Genre: *Cuniculus*
Species: *lepus* [13]

Experimental protocol

At the end of the acclimatization period, the rabbits were weighed and divided into 2 batches of 6 individuals according to a daily dose of exogenous Rivaneb®80: 5mg/kg/D dissolved in distilled water administered during 2 weeks.

Constitutions of experimental groups

G1: The Control group received distilled water.
G2: The group treated with 5mg/kg/D for 2 weeks.

Blood recovery

After 2 weeks of experimentation, the rabbits were weighed, as they had been at the start of the experimentation, then they were sacrificed, the blood samples were collected in sterile heparin tubes and then centrifuged at 3000 g for 15 minutes at 4°C. The plasma obtained was aliquoted and stored at room temperature of 20°C to carry out biochemical and hormonal assays.

Organ harvesting

During the sacrifice, the dissection of the animals, the liver was removed, stripped of excess fat, washed with 0.9% saline, and then dried and weighed using a precision balance.

Biochemical and hormonal assays

The cholesterol assay was carried out by the enzymo-colorimetric method CHOD-PAP (reagents: Biomaghreb, ref: 20111-20112-20118). The glucose assay was carried out using the GOD-PAD enzymatic technique (reagents: Biomaghreb, ref: 20121- 20122- 20124- 20126- 20127). The determination of triglycerides was carried out by the enzymo-colorimetric technique GPO-POD (reagents: Spinreact, ref: 1001310-1001311-1001312-1001313-1001314). The plasma thyroxine assay was carried out by the ELISA method (reagent: free T4 ELISA, ref: 3798). The T&D bilirubin assay was carried out by DMSO (DIMETHYLSULFOXYDE) colorimetric method (reagents: Spinreact, ref: 1001044). The determination of transaminases was carried out by Colorimetric Method (reagents: Biomaghreb, ref: 20039-20041-20048-20049).

Statistical analyzes

All the numerical data were expressed in mean standard error (M ± SEM); the results were analyzed by one-way analysis of variance (ANOVA) using SPSS software for Windows (vers.18).

Results and Discussion

Average body weights:

The bodyweight of rabbits treated with Rivaneb®80 (Table1) varied significantly between the start and the end of the experimental period. Statistical analysis showed a significant increase in the weight ($p<0.05$) of rabbits.

Table 1: Average body weight (kg) of *Cuniculus lepus* rabbits (n=6)

	G1: Control	G2: Treated
Average body weight (kg)	1,751±0,116	2,103±0,211*

*Significant (p<0, 05).
** Highly significant (p<0,01).
*** Very highly significant (p<0,001).

Average liver weights:

The results obtained (Table 2) revealed that there was a significant increase ($p <0.05$) in the weight of rabbits' liver treated with 5mg/kg/D compared to the control group (G1). From the analysis results of variance, a significant dose-effect relationship ($p<0.05$) was recorded.

Table 2: Average liver weights (g) of *Cuniculus lepus* rabbits (n=6)

	G1: Control	G2: Treated
Average liver weight (g)	59,2± 13,5	88,2± 6,418*

*Significant (p<0, 05).
** Highly significant (p<0, 01).
*** Very highly significant (p<0,001).

Biochemical parameters lipemia

The cholesterol levels (Table 3) increased significantly ($p<0.05$) in treated rabbits (G2). In addition, the analysis of variance revealed that there was a highly significant dose effect ($p <0.01$). Average plasma triglyceride levels revealed a slight non-significant increase in the groups of treated rabbits (G2) with 5mg/kg/D of Rivaneb®80, by contribution to the control group (G1). The analysis of variance revealed an insignificant difference between the two groups.

Table 3: Biochemical parameters of lipemia (g/l) of *Cuniculus lepus* rabbits (n=6)

	G1: Control	G2: Treated
Cholesterol (g/l)	0,476±0,231	0,424±0,214**
Triglycerides (g/l)	1,114±0,544	1,396±0,553

*Significant (p<0, 05).
** Highly significant (p<0, 01).
*** Very highly significant (p<0,001).

Average plasma glucose levels:

The results of the plasma glucose assays (Table 4) showed a significant increase ($p <0, 05$) in this parameter in the treated rabbits (G2) by comparing with the control group (G1). Furthermore, the analysis of variance indicated a significant difference ($p<0, 05$) between the two groups.

Table 4: Average plasma glucose levels (g/l) of *Cuniculus lepus* rabbits (n=6).

	G1 : Control	G2: Treated
Glycemia (g/l)	1,158±0,008	1,36±0,95*

*Significant (p<0,05).
** Highly significant (p<0,01).
*** Very highly significant (p<0,001).

Average rate of transaminases

The average activity rate of Aspartame Aminotransferase (ASAT; Table 5) showed a significant increase ($p<0.05$) in rabbits treated with 5mg/kg/D compared to those of the control. Analysis of variance recorded a significant dose-effect relationship ($p <0.05$). The average activity rate of the Alanin Aminotransferase (ALAT) showed a significant increase ($p<0.05$) in rabbits treated with 5mg/kg/D compared to those of the control. Analysis of variance recorded a significant dose-effect relationship ($p <0.05$).

Table 5: Average rate of transaminases (U/l) *of Cuniculus lepus* rabbits (n=6)

	G1: Control	G2: Treated
ALAT(U/l)	41,046±19,75	45,416±9,77*
ASAT(U/l)	31,308±4,125	75,348±39,731*

*Significant (p<0, 05).
** Highly significant (p<0, 01).
*** Very highly significant (p<0,001).

Average rates of Bilirubin

The mean bilirubin levels (Table.6) increased significantly ($p<0.05$) in treated rabbits (G2). In addition, analysis of variance showed that there was a significant dose-effect relationship ($p<0.05$).

Table 6: Average rates of Bilirubin (mg/l) of *Cuniculus lepus* rabbits (n=6)

	G1: Control	G2: Treated
Bilirubin (mg/l)	0,678±0,291	0,81±0,166*

*Significant (p<0, 05).
** Highly significant (p<0, 01).
*** Very highly significant (p<0,001).

Average thyroxin levels

The level of plasma thyroxin showed a significant decrease ($p<0.05$) in the treated group (G2) comparing to the controls (Table 7). On the other hand, the analysis of variance revealed a highly significant dose-effect relation ($p <0.01$).

Table 7: Average thyroxin levels (Pmol/l) of *Cuniculus lepus* rabbits (n=6)

	G1: Control	G2: Treated
Thyroxin (Pmol/l)	42,775±16,03	34,884±7,261**

*Significant (p<0, 05).
** Highly significant (p<0,01).
*** Very highly significant (p<0,001).

Discussion

Today, pesticides are the major problem of public health. The diffusion of these compounds in the environment by the contamination of air, soil, water, and food products causes the continuous exposure of fauna and humans to them [10]. In order to better understand its toxicological effects, we tested a fungicide widely used in agriculture, Rivaneb® 80 on an animal model, and its effects on liver function by some biochemical and hormonal parameters.

During this study, treatment with an exogenous dose of 5mg/kg/D of Rivaneb® 80 revealed a significant increase in the bodyweight of the G2 animals by adding the control batch G1. This can be explained by the effect of Dithiocarbamates on the thyroid gland due to the decrease in the secretion of thyroxin (T4). Similarly, the results showed that treatment with a fungicide (MB) with a dose of 8mg/kg/day caused an increase in the weight of rabbits [14]. In addition, we observed a gain in body mass of rats treated with a low dose of Atrazine equivalent to 300ug/kg for 15 days [11]. Research has suggested that the disruption of thyroid hormones may be the cause of weight gain, the hypoactivity of which causes an increase in body fat. It also tends to slow down metabolic function and puts the body to rest [15].

The liver is made up of two lobes which, by its various functions, contribute to the maintenance of the homeostasis of the organism. It synthesizes various essential molecules and makes it possible to extract and metabolize the nutrients and xenobiotics

introduced into the body in part via the digestive system [16]. Occupational exposure to pesticides can cause adverse health effects [12] in general and more precisely on the various organs or tissues, including the genital tract, the hematopoietic tissue, and the liver [17].

Our results reported a remarkable increase in the hepatic weight of *Cuniculus lepus* rabbits treated with Rivanebe® 80 at a dose of 5 mg/kg/D compared to the controls. Likewise, it showed that treatment with Maneb induced an increase in rats' liver weight. This increase is due to the hepatic accumulation caused the latter [18]. Some studies [19] indicated that in rats, Mancozeb also induces an increase in liver weight.

The results during this study showed a significant increase in blood sugar in the groups treated with Rivanebe® 80 at a dose of 5 mg/kg/D for 15 days. Studies have shown, when an organism is exposed to toxic substances, an emotional reaction in the limbic system activates the hypothalamus to produce corticoliberine (CRH), and latter stimulates the pituitary gland to release ACTH (Adrenocorticotropic Hormone) which is an activator of the adrenal glands for the production and secretion of cortisol in the blood [20]. Cortisol has many actions, some of which lead to elevated blood sugar levels [21], that most likely explains this hyperglycemia. On the other hand, several studies have observed that pesticides and products containing manganese such as Maneb have effects on carbohydrate metabolism. Hepatocytes are very active in lipid metabolism. From carbohydrates to proteins, it can also produce lipids that are stored and released into the blood in the form of free fatty acids, when the body needs energy. The liver also synthesizes cholesterol, which leads to the use of lipids as a second source of energy [22, 23].

The results of our test showed a decrease in thyroxin (T4) concentrations in male rabbits treated at the dose of 5mg/kg/D compared to the control. Some studies have reported that human exposure to manganese-based ethylene bis-dithiocarbamate (EBDTC) fungicides is associated with disrupted secretion of thyroid hormones in humans and animals [24]. We found the same results which showed that treatment with Mancozeb, caused a decrease in T4 [25]. Physiologically, we can explain our result by the effect of Dithiocarbamic metabolites due to an influence on the enzymes involved in the synthesis of iodine; which induce a reduction in the absorption of the latter [26, 27] because Iodine is a mineral trace element necessary for life and the production of thyroid hormones T3 and T4. Studies have reported that human exposure to pesticides is associated with a disruption in thyroid hormone levels [28, 29], a decrease in the secretion of thyroxin which is a well-known hormone for its physiological role in the regulation of basal metabolism but also in the maintenance of stable body temperature in Vertebrates, thus causing hypothyroidism [30-32]. This explains hyperlipemia and hyperglycemia, possibly lowering the rate of catabolism [33]. This confirms our results.

The liver plays a key role in maintaining homeostasis in the body. A standard liver test provides information on the integrity of hepatocytes, such as plasma transaminases**.** ALT is considered as a standard biochemical marker for liver damage [34]. Transaminases are essential enzymes in cytolysis [35]. They are active in the liver, heart, and muscles. They pass into the serum in the event of hepatic or muscular cytolysis [36]. A significant increase is observed in the cytolysis of toxic hepatitis [37]. Our results show that treatment with Rivaneb®80 (5mg/kg/D) orally for 15 days induces an increase in hepatic enzymatic activities (ALAT and ASAT) in treated rabbits compared to the controls. These results are in agreement with [18, 34, 38]. Changes in the activities of transaminases may occur in association with liver diseases involving necrosis [7], which could potentially be attributed to hepatotoxicity, resulting in increased permeability of the liver membrane and leakage of lysosomal enzymes in mice treated with Methomyl [39]. The increase may also be due to mutations in the genes responsible for the synthesis of these enzymes or could be attributed to either hepatic or renal dysfunctions [33, 38].

Bilirubin is the product of the catabolism of hemoglobin in the reticuloendothelial system. The degradation of the heme determines the formation of unconjugated bilirubin which is then transported to the liver in conjugated bilirubin and then eliminated in the bile. The rise in conjugated bilirubin therefore more faithfully reflects liver disease caused by intrahepatic cholestasis (hepatitis, cirrhosis, etc.) or by extra hepatic biliary obstruction [40, 41]. The hepatotoxicity of methyl bromide has also been demonstrated by an increase in bilirubin levels which may result from an increase in bilirubin production resulting from hemolysis or decreased absorption by the liver and/or conjugation [42]. In accordance with previous works [39, 42] an increase was revealed in the concentration of bilirubin after exposure to pesticides.

Conclusion

This study aimed to assess the toxic potential of a fungicide (Rivaneb®80) nfor agricultural use in the domestic rabbit at a dose close to the doses used in agriculture. Rivaneb®80à acts as an endocrine disruptor which has caused severe disorders in the hormonal system of the animals. The action of this fungicide used led to:

- A remarkable increase in the body mass of the treated animals compared to the control group, associated with hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and a significant increase in liver mass in the treated group, with a significant increase in transaminases and bilirubin levels.
- A significant decrease in thyroxin level.

Health research on exposure to pesticides has so far demonstrated its harmful effects on our body. We are currently in a society where people want to know what they are consuming and their health is of greater concern to them. Existing laws and actions taken to address the problem of pesticide residues are good, but not enough. It would be interesting to consider further

studies, particularly in the field of molecular biology, on the action of these pesticides by genotoxicity (DNA) studies.

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