

### Oral Toxicity of Agro-Fungicides: Tilt (Propiconazole), Bayleton (Triadimefon) And Their Mixture to Nubian Goats

Tahir Y.F.<sup>1</sup> and Nour S. M.<sup>2</sup>

#### Abstract

##### Introduction:

The hazard use of pesticides, emergence of many diseases with high prevalence e.g (cancer, kidney failure and hepatic problems) urged the need for research on fungicides which are continuously received by human in Sudan via fruit and vegetables.

##### Objective:

To detect the toxicity of these fungicides in experimental animals.

##### Methods:

Twelve Nubian goats were used in these experiments; they were grouped into three groups (and one control group) and dosed orally with two fungicides [Propiconazole (100mg/kg/day), Triadimefon (100mg/kg/day)] and their mixture (50:50 mg/kg/day). Animals were closely observed for clinical signs and behavior. Dead or slaughtered animals underwent postmortem examination and lesions were recorded. Samples from different organs were preserved for histopathological studies. Fresh blood was collected for heamatological and Serobiochemical analysis.

##### Results:

Five minutes post-dosing, the animals showed some clinical signs which recovered after four hours. Death occurred in days 12- 25 in the animals dosed with the mixture. The most prominent feature in postmortem lesions was the congestion in different organs. Histopathological changes were the fatty change of liver and kidneys. In Triadimefon dosed group, the values of PCV, Hb and MCHC decreased significantly ( $p < 0.001$ ). The serum urea concentration and GOT activity were high ( $p < 0.001$ ) in both of them. Animals dosed with mixture had significantly ( $p < 0.05-0.001$ ) higher PCV, MCV, MCH and WBC than the control. Significantly high values of serum urea concentration ( $p < 0.01$ ) and GOT activity ( $p < 0.001$ ) were reported in goats dosed with the mixture.

##### Conclusion:

Both fungicides and their mixture showed toxicological and pathological effects in dosed animals.

**Keywords:** pesticides, fungicides, preneoplastic, hepatectomy, hepatocarcinogenic.

**H**uman in Sudan continuously receive agro-fungicides via misuse and residues in fruits and vegetables. The two fungicides which are used in this work have a potential for carcinogenicity in rodents. Eight pesticides were tested in medium-term bioassay uptake induction of preneoplastic lesions in the liver of rats. After application of 200mg/kg of triadimefon for six weeks, the rats were subjected to partial hepatectomy.

After three weeks hepatocarcinogenic potential was assessed by computing the number and areas of glutathione transferase, placental form positive foci in the liver with those of control given diethylnitrosamine alone. Because triadimefon and DDT gave positive results, it is claimed that their findings provided experimental evidence that the compounds in their assay have a potential for liver carcinogenicity in rodents<sup>1</sup>. Because of the cytotoxic effects of Propiconazole and its metabolites on the tumorigenesis process in mouse and human hepatoma cells and primary mouse hepatocytes resulted in formation of tumor cells; it was suggested that

1. Dept. of Archaeology, University of Khartoum

2. Dept. of Microbiology, Faculty of Medicine and Health Sciences, Upper Nile University

Correspondence E-mail: [yahiasd@yahoo.com](mailto:yahiasd@yahoo.com)

tumor cells would be more susceptible to the cytotoxic effects of Propiconazole<sup>2</sup>.

Propiconazole (25% WP, Ciba Geigy, Switzerland) is a fungicide of many crops. Its chemical formula is {1-(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl(methyl)-1H-1,2,4-triazole)}. Triadimefon (25% WP, Bayer, Germany) is a fungicide of many crops. its chemical formula is {1-(4-chlorophenoxy)-3,3-dimethyl-1H-1,2,4-triazol-1-yl)-butanone}. The two fungicides were registered in Sudan since 1980<sup>3</sup>.

In Sudan, Triadimefon and Propiconazole are used in control of fungi of cotton, tomatoes, cucumbers, melons, potato, pepper, cereals and mango. Oral toxicities are LD<sub>50</sub> rat 750-1200 mg / Kg and LD<sub>50</sub> rat 1517mg / Kg for Triadimefon and Propiconazole respectively<sup>4</sup>. It is reported that the poisoning with Triadimefon causes nausea, excitation and drowsiness in human<sup>5</sup>. The ability of Triadimefon to induce hyperactivity in male long Evans rats was discussed<sup>6</sup>. In addition; reversible induction of hepatic microsomal activity can be accompanied by compensatory hepatic hypertrophy. Female American Dutch rabbits were given Triadimefon at a dose 120mg/kg / day; this resulted in reduction of food consumption, a loss in body weight and increased spleen and adrenal weights.

Propiconazole belongs to chemicals that inhibit enzyme responsible for steroid hormone biosynthesis. This specific effect has led to the usage of some of these chemicals (imidazole-derivatives) as a male contraceptive in human. Propiconazole has the potential to impair reproductive success of chronically exposed animals. The fungicide is defined to be slightly / moderately toxic to all aquatic animals and mammals<sup>7</sup> (LD<sub>50</sub> rats 1517 mg/kg). Propiconazole was classified as a possible human carcinogen (group c)<sup>4</sup>. According to<sup>8</sup> ketoconazole, a systemic azole drug, at high dose inhibits adrenocortical steroid and testosterone synthesis, the later may results in gynae comastia in male human.

## Materials and Methods

Twelve healthy Nubian goats were used in these experiments (Table 1). Tested animals were closely observed for clinical signs and behavior. Dead or slaughtered animals underwent postmortem examination. Samples from heart, lungs, liver and kidneys were immediately preserved for histopathological studies. Fresh blood was collected for Hb, PCV, RBC count, WBC count, MCV, MCH and MCHC. Serobiochemical analysis included Glutamic - Oxaloacetic Transaminase (GOT), total protein and total urea determination using commercial kit, (Plasmatec laboratory products Ltd., England). Serum concentrations of Ca, Mg, Na, K, Cu, Fe, Mn, and Zn were determined using atomic absorption spectrophotometer (PERKIN - ELMERT 2380, Germany). Tissue samples for histopathological studies were prepared according to conventional methods<sup>9</sup>. Data of treated and non-treated groups of animals were analyzed using Student t- test<sup>10</sup>.

## Results

Five minutes post-dosing dosed animals showed clinical signs. Group1 showed salivation, grinding of teeth, frothing, muscle tremor at hind limbs, tail fagging, frequent urination and defecation and knocking their heads against objects, crying, moaning, depression and inappetent. They recovered after 4 hours. These clinical signs were same during the subsequent dosages. The animals were slaughtered on week 12. Group 2 showed mild clinical signs. Goats died on week 8, 8 and 11 respectively. Group 3 which had been given a mixture of Bayleton and Tilt showed mild clinical signs. Towards the third dose they showed lacrimation and became inappetent. After 10 days they became uncoordinated and recumbent. Death occurred in days 12, 19 and 25 respectively (Table 1).

The loss in body weight was significant ( $p < 0.01$ ).

The most prominent feature in postmortem lesions of goats poisoned with Bayleton and Tilt were slight congestion in different body organs.

Table 1: Propiconazole, Triadimefon and their combination dosing schedule and animals fates.

	No	Sex	Initial wt (kg)	Final wt (kg)	Dose (mg/kg/day)	Fate of animals
Group 1	1	M	7	7	100 (Tri)	Slaughtered after 12 weeks
	2	F	7.5	7	100(Tri)	Slaughtered after 12 weeks
	3	F	7.5	6.5	100(Tri)	Slaughtered after 12 weeks
Group 2	4	M	10	9	100(Pro)	Died after 8 week.
	5	F	10	7	100(Pro)	Died after 8 week.
	6	F	10	7.5	100(Pro)	Died after 11 weeks.
Group 3	7	M	10	9	100(50:50) (Tri :Pro)	Died after 25 days.
	8	F	9	9	100(50:50) (Tri: Pro)	Died after 19 days.
	9	M	7.5	7	100(50:50) (Tri: Pro)	Died after 12 days.
Group 4	10	M	8	10	0.00	Slaughtered after 12 weeks
	11	M	9	11	0.00	Slaughtered after 12 weeks
	12	F	10	11	0.00	Slaughtered after 12 weeks

M = Male, F = Female, Tri = Triadimefon, Pro = Propiconazole

The trachea was frothy and the lungs showed emphysema (Fig 1). The liver and kidneys were fatty infiltrated. The digestive tract was inflamed while the heart was flabby with more prominent haemorrhage (Fig 2). It was noticed that goats of group 3 showed mild lesion than those recorded by any fungicide given alone, but haemorrhage in the heart was more prominent.

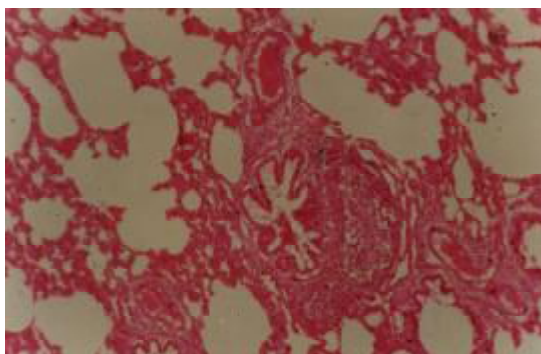


Fig. 1: Section through lungs of Nubian goat treated with tilt showing emphysema, peribronchiolar lymphocytic infiltration (40XH&E).

In all dosed groups lungs, some of the alveoli contained RBCs, acidophilic albuminous exudate and lymphocytes.

In group1, the bronchioles were dilated or contained eosinophilic materials and detached epithelial cells. Group 2 showed severe pulmonary oedema.

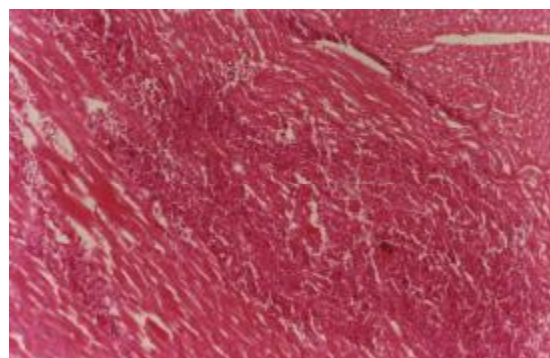


Fig. 2: Section through heart of Nubian goat treated with bayleton, showing RBC and inflammatory cells between the cardiac muscle bundles (10XH&E).

The liver tissue showed centrilobular hepatocytic necrosis and the sinusoids were congested and dilated, also slight bile ductless hyperplasia was observed. In goats of group 2, the liver also showed centrilobular hepatocytic necrosis. Both groups showed more liver fatty infiltration (Fig.3).

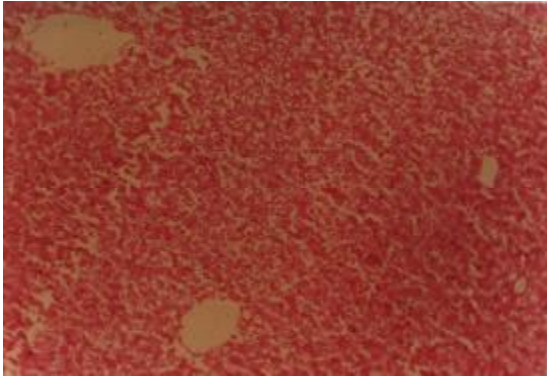


Fig. 3: Section through the liver showing necrosis and/or fatty cytoplasmic hepatocytic vacuolations (10XH&E).

In group 1, the proximal renal tubular epithelial cells showed vacuolations. Some of these underwent coagulative necrosis, with some detached into the lumen. Congestion in renal blood vessels and medulla rays was observed. Scattered RBC in interstitial tissue sometimes formed foci. Some of the collecting ducts exhibited individual cell necrosis (Fig. 4).

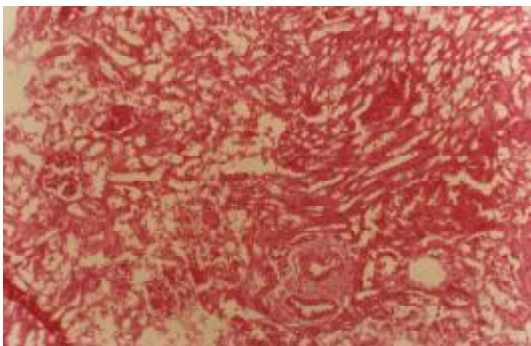


Fig. 4: Section through the kidney of Nubian goat treated with bayleton showing proximal convoluted tubules undergone hydrophilic vacuolation and the glomerular tuft were lobulated in addition congestion of the medullary rays(40XH&E).

Some of the renal tubules and the interstitial tissue contained inflammatory cells.

In group 2 animals' moderate dilation of proximal convoluted tubules and some glomeruli disappeared and the renal parenchyma contained scattered haemorrhage foci (Fig. 5).

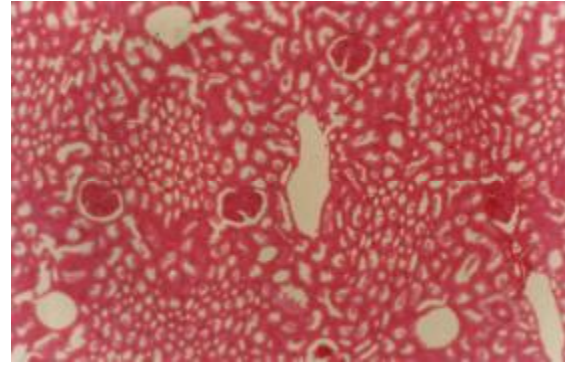


Fig. 5: Section through kidney of Nubian goat treated with tilt showing some glomerular disappearance, shrinkage and lobulation and some tubular dilation (40XH&E).

In group 3 animals, some of the glomeruli disappeared. The most prominent lesion in the renal tissue was the severe congestion of the renal blood vessels, medullary rays and interstitial tissue (Fig. 6).

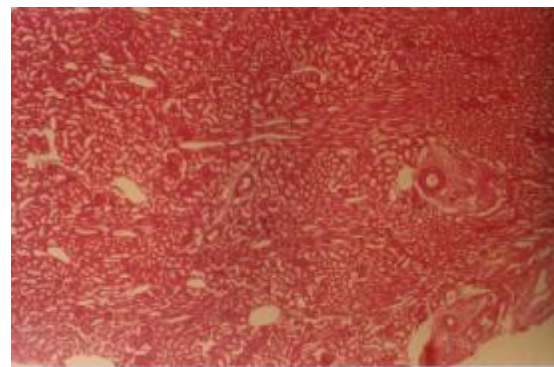


Fig. 6: Section through kidney of Nubian goat treated with mixture of Triadimefon and Propiconazole, showing lobulation of the glomeruli, dilation of renal tubules and congestion of medullary rays (25XH&E).

Some of the renal tubules contained acidophilic albuminous casts and RBCs.

Small foci of haemorrhages were also noticed in the parenchyma of the kidneys (Fig. 7).

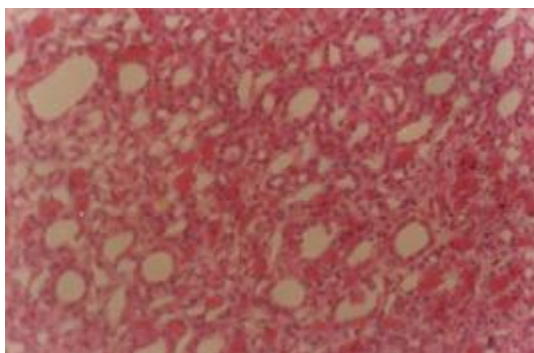


Fig.7: Section through kidney of Nubian goat treated with mixture of Triadimefon and Propiconazole, showing thickening of the interstitial tissue and scattered RBC and inflammatory cells (40XH&E).

Their liver showed hepatocytic cytoplasmic vacoulation at the portal zone, lymphocytic

infiltration in the portal zone and congestion and haemorrhage of the sinusoids (Fig. 8).

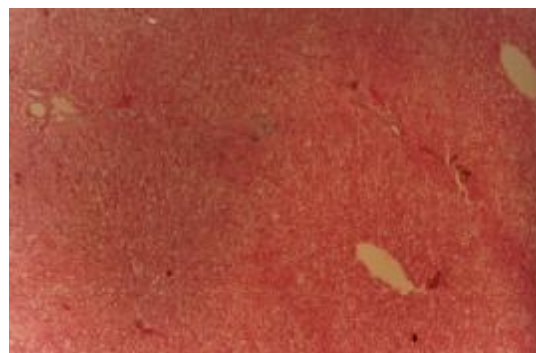


Fig. 8: Section through liver of Nubian goat treated with mixture of Triadimefon and Propiconazole, showing hepatocytic cytoplasmic vacoulation at the portal zone, lymphocytic infiltration in the portal zone and congestion and haemorrhage of the sinusoids (10XH&E).

Hematological and serobiochemical findings are summarized in Table 2 and 3.

Table 2. Haematological findings for Nubian goats dosed with Bayleton, Propiconazole and their combination.

Group	Parameter						
	RBC (X10 <sup>3</sup> ml)	PCV (%)	HB (gm/dl)	MCV (fl)	MCH (pg)	MCHC (gm/dl)	WBC (ml)
Group 1 T (100 mg )	10.235± 1.699 †	22.11± 1.99 †	8.06± 0.40 ‡	21.65± 1.59 †	7.40± 0.26 Δ	36.45± 2.67 †	9.771 ± 2.781 Δ
Group 2 P (100 mg )	12.569± 1.251*	25.10± 2.11 Δ.	9.61± 0.72 Δ	19.96± 0.81 *	7.60± 0.60 Δ	38.29± 3.30 Δ	11.004 ± 1.780 ‡
Group 3 T: P (50 :50) mg	11.938± 0.610 †	27.88± 1.69 †	10.37 ± 0.83 Δ	23.35± 1.94 †	8.70 ± 0.71 †	37.20± 2.93 Δ	10.200 ± 1.536 ‡
Group 4 (control)	13.536± 1.281	25.69± 1.61	10.01± 0.82	18.98± 1.11	7.40± 0.45	39.31± 1.90	9.403 ± 0.953

T = Triadimefon, P = Propiconazole, \* = p<0.05; ‡ = p<0.01; † = p<0.001; Δ = not significant.

Table 3. Serobiochemical constituents for Nubian goats dosed with Triadimefon, Propiconazole and their combination

Group	Parameter										
	GOT I.U.	Total protein g/100ml	Urea mg 100 ml	Ca mg 100 ml	Mg mg 100 ml	Na mg 100 ml	K mg 100 ml	Cu mg/ml	Fe mg/ml	Mn mg/ml	Zn mg/ml
Group 1 T (100 mg )	57.80 ± 6.30 †	7.60 ± 2.30 ‡	39.20 ± 5.70 ‡	10.80 ± 0.59 Δ	0.10 ± 0.01*	361.10 ± 10.0Δ	10.20 ± 0.51 *	0.98 ± 0.13 Δ	1.60 ± 0.09Δ	0.15 ± 0.03Δ	1.12 ± 0.01Δ
Group 2 P (100 mg )	51.60 ± 6.57†	5.70 ± 0.66 ‡	41.38 ± 2.11 †	11.12 ± 1.10Δ	0.88 ± 0.09 †	370.0 ± ±9.5Δ	9.30 ± 0.50 †	0.98 ± 0.09Δ	1.55 ± 0.04Δ	0.17 ± 0.02Δ	1.00 ± 0.01 Δ
Group 3 T: P (50 :50 mg )	94.75 ± 7.75†	5.46 ± 0.88 ‡	34.64 ± 5.44 ‡	10.34 ± 1.01Δ	0.92 ± 0.09 †	353.7 ± 17.2 *	9.13 ± 0.68†	0.92 ± 0.12 Δ	0.87 ± 0.05 ‡	0.19 ± 0.03 ‡	1.06 ± 0.09 Δ
Group 4 (control)	17.92 ± 4.87	6.29 ± 0.63	22.56 ± 4.16	10.94 ± 1.30	0.11 ± 0.08	371.0 ± 14.0	11.10 ± 0.91	0.96 ± 0.13	1.48 ± 0.03	0.14 ± 0.03	1.11 ± 0.01

T = Triadimefon, P = Propiconazole, \* =  $p < 0.05$ ; ‡ =  $p < 0.01$ ; † =  $p < 0.001$ ; Δ = not significant.

### Discussion

Dosed animals showed different clinical signs. A group of scientists discussed the role of Triadimefon and Triadimenol action on neurobehavioral activity and found their acute administration inhibited dopamine uptake, which may play an important role in neurobehavioral activity<sup>11, 12, 13</sup>. The effect of Triadimefon on the behavior of rats is similar to that of psychomotor stimulus<sup>14</sup>. In current study hearts of animals treated with Bayleton and Tilt exhibited haemorrhagic foci and flabbiness. These lesions were reported before<sup>15</sup>. The hepatic lesions were same in all experimental animals and were similar to that in animals treated with Tilt and Milcurb super<sup>16</sup>. and similar to that reported by Silva-M.do et al. who studied the pathological effect of ketoconazole in albino mice<sup>17</sup>.

In this study both fungicides and their mixture showed histopathological effects on livers and kidneys of animals.

Toxicity effect of hydropericardium was a common feature in groups exposed to repeated and long timed doses of fungicides. Lifetime feeding in both sexes of mice and rats produced an increased incidence of liver tumors in male mice at the level of (2500 ppm), consequently Propiconazole was classified as a possible human carcinogen (group c)<sup>4</sup>. Propiconazole was suggested to have a potential for carcinogenicity in rodents<sup>2</sup>. Human triazoles exert haemolysis effect in human<sup>17</sup>. The oral dosing of Triadimefon was associated with a decrease in values of PCV, Hb and MCHC. However, PCV, Hb were increased with mixture dosage

while the MCV was increased in both. The leucocytosis observed at low and repeated doses of fungicide is attributed to the presence of inflammatory lesions in many tissues such as lungs, liver and kidneys. The significant increase in serum urea and GOT levels and the decrease in total protein are indicative of hepatorenal disorder caused by these fungicides. Chronic feeding in mice, rats and dogs with Triadimefon produced a dose related increase in liver weights accompanied with elevation of serum hepatic alkaline phosphatase and transaminase activities<sup>19</sup>. The hypomagnesaemia observed might be attributed to the loss of the appetite, which results in rumen dysfunction<sup>20</sup>. The hyponatremia observed in the experiment might be attributed to loss of the cations in profuse saliva. Azoles, used as human drugs, inflict toxicity. For example; ketoconazole causes gastrointestinal disturbance, anaemia and hyponatraemia and fluconazole causes liver toxicity<sup>21, 22</sup>. According to Hyes<sup>8</sup> ketoconazole - a systemic azole drug- at high dose inhibits adrenocortical steroid and testosterone synthesis, the later may result in gynaecomastia in human males.

The results of the study have shown that combined dose of Triadimefon (50mg/kg) and Propiconazole (50mg/kg) produced toxic manifestations and rapid death more than either of the individual fungicide (100mg/kg). This may be attributed to drug interaction.

As a result of this study we could conclude that the human drugs Azoles which are used frequently in Sudanese patients and the pesticides -which are continuously but unintentionally taken by human via fruits and vegetables- may affect the liver.

Experiments to test carcinogenicity, teratogenicity and/or embryotoxicity of these fungicides has to be undertaken. It is evident that a great deal of work is needed in the area of metabolism and pharmacokinetic in ruminants in order to understand clearly the fate of these chemicals in the body.

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