

PATHOMORPHOLOGICAL STUDIES FOLLOWING  
EXPERIMENTAL ACUTE INTOXICATION WITH THE  
TRIAZOLE FUNGICIDE TRITICONAZOLE IN PIGS

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**Summary**

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The gross and microscopic pathomorphological changes in internal organs were investigated for evaluation of the toxic effect of the triazole fungicide triticonazole. One control and three experimental groups of pigs, treated orally via a gastric tube at doses of 0.2 g/kg (group I), 1 g/kg (group II) and 2 g/kg (group III) (0.1LD<sub>50</sub>, 0.5LD<sub>50</sub> and LD<sub>50</sub> respectively) were used. The pigs were euthanized 72 h after the treatment, a complete necropsy was performed and material for histological study was obtained. It was found out that the toxic effect of the triazole fungicide resulted in morphological changes in the liver (mononuclear proliferation, granular and initial toxic dystrophy), in lungs (peribronchial mononuclear proliferates), in lymph nodes (haemorrhages and lymphatic follicle activation), in pituitary gland (hyperaemia), in medulla oblongata (ballooned dystrophy of glial cells), in stomach (hyperaemia of gastric mucosa) and in kidneys (mononuclear proliferations among tubules and among nephrons).

**Key words:** macro- and microstructural changes, pesticide toxicity, swine, triazole fungicides, triticonazole

INTRODUCTION

The triazole fungicide triticonazole is intended for decontamination of seeds from fungal pathogens. A possible risk of intoxication occurs when treated seeds are used for animal forage. The oral LD<sub>50</sub> of triticonazole for albino rats is 2000 mg/kg (Sengalevich *et al.*, 1998). The pathomorphological changes after treatment with other triazole fungicides are reported in laboratory animals – mice (Filipov & Lawrence, 2001) and rats (Mayberry, 1968), challenged with 3-amino-1,2,4-triazole and tebuconazole (Moser *et al.*, 2001). The pathomorphological altera-

tions in cattle under the influence of triadimenol (Markelov & Shormanov, 1994) and in cats following treatment with tebuconazole (Kurumbaev *et al.*, 1996) are also reported. In these communications, depending on the toxic dose, the morphological changes consisted in carcinogenic, teratogenic and mutagenic effects on the thyroid gland (Mayberry, 1968; Machera, 1995), on sexual glands (Moser *et al.*, 2001), dystrophic changes of liver and lungs (Markelov & Shormanov, 1994; Kurumbaev *et al.*, 1996; Filipov & Lawrence, 2001), kidneys (Ronis *et al.*, 1998)

hyperaemia and oedema of brain tissue and meninges (Moser *et al.*, 2001; Filipov & Lawrence, 2001). These reports as well as the data from previous studies of ours upon the clinical and laboratory changes following acute intoxications with triazole fungicides diniconazole and triadimenol in birds (Binev, 2000a; Binev, 2000b; Binev, 2000c) and rabbits (Binev, 2001; Binev, 2002a; Binev, 2002b) motivated us to perform morphological studies for determination of pathomorphological changes in experimental acute intoxication with triticonazole in pigs in order to elucidate some aspects of the toxicodynamics and pathogenesis of this intoxication in pigs and to facilitate its diagnostics, treatment and prophylaxis.

## MATERIALS AND METHODS

### *Experimental animals*

The experiments were performed on 8 pigs from the Danube White breed, both genders, aged 3–4 months and weighing 18–26 kg. Thirty days prior to and during the experiments all pigs were housed in individual boxes conforming to standard hygienic and housing conditions. Before the intoxication (by days –30 and –15), the pigs were treated twice against parasites with ivermectin (Alfamec – Woerden, Holland). Prior to and during the experiments, the animals received a standard combined forage and had free access to water.

### *Treatment*

The acute intoxication was provoked with the commercial preparation Real 200 FS (Rhône-Poulenc Agro, Lyon, France) administered to experimental animals internally, using a gastric tube, as a suspension containing 200 g triticonazole in 1 L.

### *Experimental design*

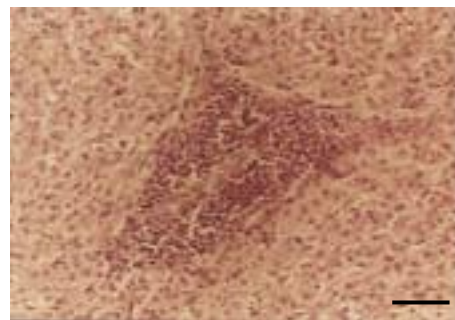
The pigs were divided into 4 groups with equal number of animals from both genders in each: one control – untreated (n=2) and three experimental groups (n=2 each). At baseline (hour 0), the first experimental group received 1 mL Real 200 FS (equivalent to 200 mg triticonazole /kg) (= 0.1 oral LD<sub>50</sub> for rats); the second experimental group was treated with 5 mL Real 200 FS /kg or 1000 mg triticonazole /kg (= 0.5 oral LD<sub>50</sub> for rats) and the third experimental group: with 10 mL Real 200 FS/kg or 2000 mg triticonazole /kg (= oral LD<sub>50</sub> for rats). By the post treatment hour 72, the two animals from each group were euthanized by intravenous administration of 10 mL of the combination: procaine hydrochloride 40,0; potassium chloride 5,0 and excipients ad 100 mL (Nicovet, Sofia, Bulgaria). A complete necropsy to each animal was performed. Material for histological study was obtained from the internal organs. The materials were fixed in 10% neutral formalin, processed through an ethanol series, embedded in paraffin, cut on a microtome (5 µm cross-sections) and stained with haematoxylin-eosin (H/E).

## RESULTS

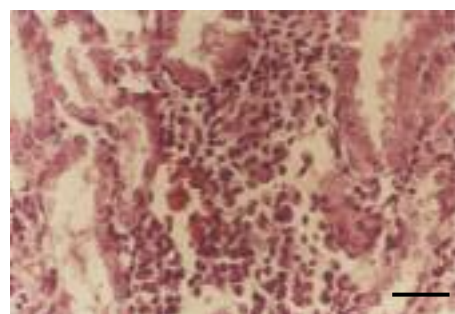
In pigs that received 200 mg/kg triticonazole, a moderate subcutaneous oedema of the ventral abdominal wall was present. The liver was pale yellow, with a friable consistency and dark red areas. Petechial haemorrhages were observed on the serosa over the pancreas. The inguinal and perirenal lymph nodes were almost twice enlarged and reddened. The renal core was also reddened. The adrenals were enlarged. The heart was with a pale grey colour and a dry cross-sectional surface.

The thyroid gland was slightly larger and lungs – with dough-like consistency and red-bluish colour. The brain meningeal blood vessels were hyperaemic. In the other organs, no gross changes were observed. The histopathological study of the liver revealed a granular dystrophy of liver epithelial cells and haemorrhages in liver parenchyma. Sinusoidal capillaries were distended and at some places, mononuclear cell proliferates could be observed (Fig. 1). The Kupffer cells were activated. In the spleen, subcapsular haemorrhages were present. The mesenteric and perirenal lymph nodes were hyperaemic. Hyperaemia was present in kidneys (better manifested in the core), adrenal glands, ovaries (accompanied by interfollicular oedema), in testes (among the seminiferous tubules). In the lungs, focal lymphocytic peribronchial proliferates were found out. The blood vessels in all pituitary gland lobes were hyperaemic. In the medulla oblongata, a ballooned dystrophy of glial cells occurred.

The gross findings in animals, treated with triticonazole at 1000 mg/kg consisted in petechial haemorrhages in liver parenchyma, a medium-degree enlargement and reddening of mesenteric, inguinal, portal and perirenal lymph nodes. The lungs were oedematous. The gastric mucosa was hyperaemic. The histopathological study revealed liver granular dystrophy and vascular hyperaemia. The Kupffer cells were activated. The sinusoidal capillaries were distended and at some sites, there was a mononuclear cell proliferation of liver parenchyma. In the spleen, slight subcapsular haemorrhages were observed. In the core and the cortex of inguinal lymph nodes, haemorrhages and serous-cellular exudate in lymphatic sinuses were present. Among the renal parenchyma, focal lymphocytic prolifera-



**Fig. 1.** Liver from pig, treated with triticonazole at 200 mg/kg ( $=0.1LD_{50}$ , group I). Mononuclear proliferation; granular dystrophy of hepatocytes; H/E, bar  $\approx 30 \mu\text{m}$ .

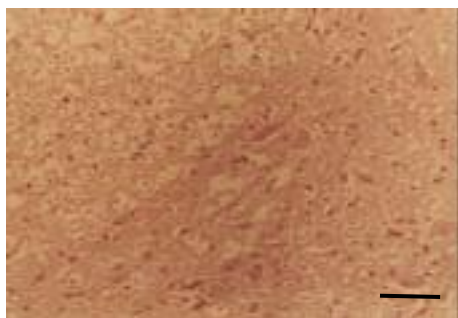


**Fig. 2.** Kidney from pig, treated with triticonazole at 1000 mg/kg ( $=0.5LD_{50}$ , group II). Mononuclear proliferation among the renal tubules; congestion of the peritubular capillary endothelium; proliferation of connective tissue cells; granular dystrophy of proximal tubules; H/E, bar  $\approx 50 \mu\text{m}$ .

tions, edematous endothelium of peritubular capillaries, proliferation of connective tissue cells and granular dystrophy of epithelial cells of proximal tubules were noticed (Fig. 2). Pulmonary alveoli were filled with a transudate and a moderate vascular hyperaemia and peribronchial lymphocytic proliferates were present. The pituitary gland, the ovaries and the testes were with hyperaemic blood vessels.



**Fig. 3.** Lung from pig, treated with triticonazole at 2000 mg/kg (LD<sub>50</sub>, group III). Peribronchial mononuclear proliferate; H/E, bar ≈ 10 μm.



**Fig. 4.** Medulla oblongata from pig, treated with triticonazole at 2000 mg/kg (LD<sub>50</sub>, group III). Ballooned dystrophy of glial cells; H/E, bar ≈ 30 μm.

Macroscopically, in pigs treated at 2000 mg/kg triticonazole, a moderate icterus of fascia and aponeuroses was present. The liver was icteric, with a friable consistency. The portal and mesenteric lymph nodes were fairly enlarged. The degree of renal icterus was medium and meninges – hyperaemic. The ovaries were atrophied (small and soft).

Histopathologically, a liver granular dystrophy was observed. The mesenteric lymph nodes showed signs of serous lymphadenitis. In inguinal lymph nodes, a strong vascular hyperaemia and petechial

haemorrhages were observed. The renal blood vessels were hyperaemic. Petechial haemorrhages and dystrophic necrobiotic changes in epithelial renal tubular cells were visible. In lungs, focal peribronchial lymphocytic proliferates were encountered (Fig. 3). In the medulla oblongata, ballooned dystrophy of glial cells was present (Fig. 4).

## DISCUSSION

The performed morphological observations in pigs treated with increased doses of triticonazole (equivalent to 0.1LD<sub>50</sub>; 0.5LD<sub>50</sub> and LD<sub>50</sub>, oral doses for albino rats) evidenced that the tested triazole fungicide exerted a toxic effect on almost all internal organs. The changes in the liver (granular and initial stage of toxic dystrophy) were indicative about a medium-degree injury of liver parenchyma. Similar results were communicated in cattle (Markelov & Shormanov, 1994), after treatment with triadimenol as well as in rats (Chhabra, *et al.*, 1999). The liver dystrophy and the observed subcutaneous tissue icterus correlated to observed blood biochemical alterations (bilirubinaemia, hyperglycaemia, elevated pyruvate, ASAT, ALAT, LDH concentrations etc.) in birds and rabbits, treated with diniconazole (Binev, 2000a; Binev, 2002b) and triadimenol (Binev, 2000c). The observed changes in the brain (vascular hyperaemia and ballooned dystrophy of glial cells) were probably the cause of observed nervous signs (convulsions, uncoordinated movements, ataxia, paresis, paralysis etc.) reported in rats (Moser, *et al.*, 2001; Filipov & Lawrence, 2001), birds (Binev, 2000a) and rabbits (Binev, 2001).

The morphological investigations of renal parenchyma showed that low doses (0.1LD<sub>50</sub>) provoked only a hyperaemia.

The increases in toxic substance amounts ( $0.5LD_{50}$  and  $LD_{50}$ ) resulted in a more severe hyperaemia together with appearance of lymphocytic proliferates, petechial haemorrhages and dystrophic changes in renal tubular epithelial cells. These changes were similar to the ones found out in Japanese quails (Ronis *et al.*, 1998). The damage of renal parenchyma was probably responsible for the observed functional changes (increased blood urea, creatinine and uric acid) in Japanese quails (Ronis *et al.*, 1998), birds (Binev, 2000b) and rabbits (Binev, 2002a).

The changes in the lung parenchyma were indicative of a clear toxic effect of the tested preparation (hyperaemia, oedema, haemorrhages, peribronchial lymphocytic proliferation etc.), clinically manifested with polypnea and dyspnea as reported in cattle (Markelov & Shormanov, 1994), birds (Binev, 2000a), rabbits (Binev, 2001;), mice, rats and humans (Machera, 1995).

The observed hyperaemic gastric mucosa was presumably due to the direct irritating effect of the studied preparation, that was also manifested by leukocytosis and neutrophilia (Binev, 2001) and that was also correlating to lymph node hyperplasia.

The toxic effect of the triazole fungicide triticonazole resulted in considerable morphological changes in parotid, pituitary, adrenal and sexual glands, displayed through hyperaemia, haemorrhages, lymphocytic proliferation and oedema, found out also in rats (Filipov & Lawrence, 2001). In our experiments, haemorrhages were present in most internal organs (heart, spleen, lymph nodes etc.) that were probably causing some of the observed blood laboratory changes (oligochromemia, erythropenia, decreased haematocrit values and delayed erythrocyte sedimenta-

tion rate), registered in birds (Binev, 2000a), cattle (Markelov & Shormanov, 1994), rats (Filipov & Lawrence, 2001) and rabbits (Binev, 2001).

The performed pathomorphological investigations in pigs treated with increasing doses ( $0.1LD_{50}$ ;  $0.5LD_{50}$  and  $LD_{50}$ , oral doses for albino rats) of the triazole fungicide triticonazole showed that the degree of observed morphological changes was not strictly dose-dependent. Evaluated from a pathogenetic and patho-anatomical points of view, the leading pathology was that observed in liver, lungs, kidneys, lymph nodes, parotid, pituitary, adrenal and sexual glands. The changes in liver, kidneys, lymph nodes and brain substance are essential with regard to the diagnostics of the intoxication.

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