

STUDIES ON CLINICAL SIGNS AND SOME
HAEMATOLOGICAL PARAMETERS IN EXPERIMENTAL
ACUTE INTOXICATION WITH THE TRIAZOLE
FUNGICIDE TRITICONAZOLE IN SWINE

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Summary

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Experiments for evaluation of the toxic effects of the triazole fungicide triticonazole in one control and three experimental groups of pigs, treated orally via a gastic tube at increasing doses of 200 mg/kg, 1000 mg/kg and 2000 mg/kg (0.1LD₅₀, 0.5LD₅₀ and LD₅₀ respectively) were performed.

The clinical status of animals (body temperature, heart and respiratory rates, colour of mucosae, appetite, thirst, general condition, locomotion, sensitivity, urination, defecation) and haematological parameters (haemoglobin content, red blood cell counts, haematocrit and mean corpuscular volume) were determined at hours –48, –24 and 0 prior to the treatment and hours 2, 4, 6, 8, 10, 12, 24, 48 and 72 after the treatment.

It was observed that the tested triazole fungicide had a toxic effect manifested by hypothermia, tachycardia, polypnea, oligochromaemia, erythropenia, lacrimation, salivation, tremor, seizures, clonic spasms, paresis and paralysis of limbs.

The observed changes were most obvious between post treatment hours 2 and 12 and afterwards the analysed parameters restored their initial values.

Key words: triazole fungicides, triticonazole, pesticides, pesticide toxicity, swine

INTRODUCTION

Triazole fungicides are among the most commonly used pesticides against fungal pathogens in cereals, leguminous plants, technical plants and fruit trees. They consist about 35% of all chemical agents used for plant protection. One of the routes of their applying is for decontamination of seeds. The wide use of those pesticides is determined by their low acute toxicity and high activity in fungi destruction in soil and during plant vegetation via inhibition of ergosterol synthesis and impairment of fungal membrane formation. Thus, the

development of fungi stops and they die (Sengalevich *et al.*, 1998).

From the other hand, the extensive use of triazole compounds (ketoconazole, intraconazole, clotrimazole, econazole, fluconazole, enilconazole etc.) as fungicides in both veterinary and human medicine creates prerequisites for intoxication in men and animals following overdosing or improper use (Rochette *et al.*, 2002; Van den Bossche *et al.*, 2002).

The triazole fungicide triticonazole – C₁₇H₂₀ClN₃O; 5-(4-chlorobenzylidene)-2,

2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol – is intended for decontamination of wheat seeds in prophylaxis of *Tilletia tritici* and *Ustilago tritici* infections. The risk of intoxication in domestic animals is present when triticonazole-treated wheat is used for forage (Sengalevich *et al.*, 1998).

In previous studies of ours (Binev, 2003) on acute triticonazole intoxication in swine, leukocytosis, neutrophilia and lymphocytopenia were observed. Such changes have not been present in hens (Binev, 2000a; 2000b) and rabbits (Binev, 2001) after experimental acute intoxication with triazole fungicides in analogous doses. The acute intoxication with diniconazole and triadimefon in hens (Binev, 2000a; 2000b) and rabbits (Binev, 2001; 2002) resulted in a dose-dependent hypothermia, tachycardia, polypnea, dyspnea, weight loss, incoordinated movements, tremor, seizures, convulsions, paresis and paralysis. The associated haematological changes consisted in oligochromaemia, erythropenia and retarded erythrocyte sedimentation rates.

Similar changes have been observed in mice and rats, treated with bitertanol (Allen and Mac Rhail, 1993), in female and male rats treated with triadimefon (Walker *et al.*, 1990; Moser *et al.*, 2001) and in rats intoxicated with triadimefon and triadimenol (Walker and Mailman, 1996). The authors reported that the increase in challenging doses resulted in better manifested clinical effects characterized with increasing agitation, turning of the head, frequent vomiting, self-mutilation, convulsions, spasms, failing of reflexes and the sensory perception, hypothermia, tachycardia, polypnea, loss in weight.

The data for triticonazole intoxication in farm animals, including pigs are however few. This motivated the performance

of the present experiments in order to elucidate the clinical changes occurring after acute triticonazole intoxication in pigs with respect to facilitate its diagnostics, treatment and prophylaxis.

MATERIALS AND METHODS

Experimental animals

The experiments were performed on 22 castrated pigs from the Danube White breed, aged 3–4 months and weighing 18–26 kg. Thirty days prior to and during the experiments all pigs were housed in individual boxes under uniform standard hygienic conditions, fed with standard combined forage and had free access to water. At the beginning of the 30-day adaptation period, the pigs were treated s.c. against parasites with 0.3 mg/kg Ivermectin (Alfamec inj. sol. – Alfasan, Woerden, Holland) containing 10 mg Ivermectin in 1 mL.

Tested substance and treatment

An acute intoxication was provoked with the commercial preparation Real 200 FS (Rhone-Poulenc Agro, Lyon, France) containing 200 mg triticonazole in 1 mL oral LD₅₀ for rats – 2000 mg/kg; Sengalevich *et al.*, 1998). The fungicide was administered to experimental animals internally, using a gastric tube, 2 h prior to feeding (6:00 AM) during the spring.

Experimental design

The pigs were divided into 4 groups – one control (n=4) and three experimental groups (n=6 each) with equal number of animals from both genders in each. The control group was left untreated while the experimental ones received triticonazole as followed:

- Group I: 200 mg/kg (= 0.1 oral LD₅₀ for rats).
- Group II: 1000 mg/kg (= 0.5 oral LD₅₀ for rats).
- Group III: 2000 mg/kg (= oral LD₅₀ for rats).

The clinical status of all groups was followed out three consecutive days prior to the treatment (hours -48, -24 and 0) and 2, 4, 6, 8, 10, 12, 24, 48 and 72 hours thereafter. The body temperature (BT), heart and respiratory rates (HR, RR), colour of mucosae, appetite, general condition, locomotion, skin sensitivity, urination, defecation were monitored. Blood was sampled from *sinus ophthalmicus* for determination of haemoglobin content (Hgb), red blood cell counts (RBC), haematocrit (Hct) and the mean corpuscular volume (MCV) with an automated analyser (Serono+ System 150, USA).

Statistical analysis

The statistical significance of results was determined using the Student t-test at a

level of significance of $p \leq 0.05$. Comparisons between control values and the values in experimental groups for each experimental interval were performed. All p-values, mentioned thereafter are referring to those differences.

RESULTS

Prior to and during the experiment, no significant variations in monitored parameters occurred in controls (group I).

In group II, BT decreased and was the lowest by hour 8 vs controls (38.2 ± 0.2 °C and 39.1 ± 0.2 °C respectively; Fig. 1). Normalization of BT values occurred between post treatment hours 10 and 12. In group III, the decrease was significant as early as by hour 2 (38.4 ± 0.1 °C; $p < 0.05$), the lowest by hour 6 when it reached 37.8 ± 0.1 °C ($p < 0.01$ vs controls – 39.1 ± 0.2 °C). The initial BT values were attained again after post treatment hour 12. In group IV, the peak decrease was by

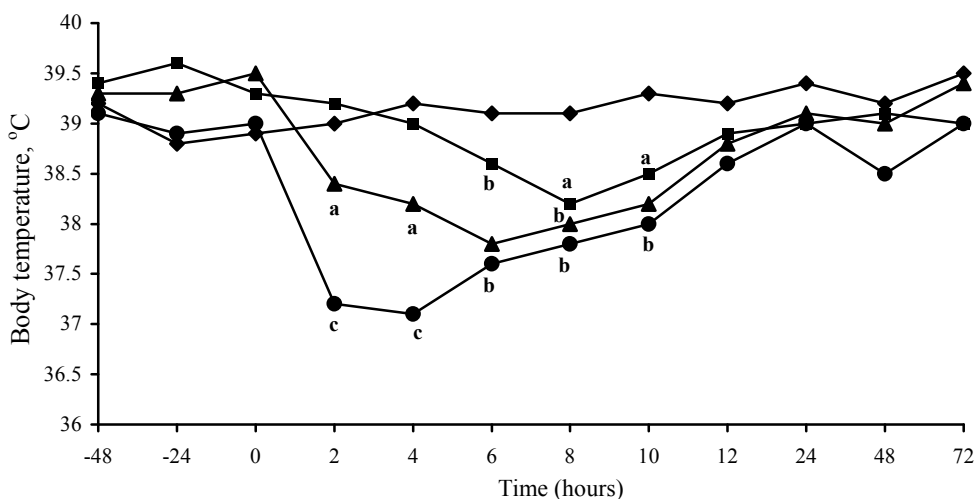


Fig. 1. Changes in body temperature in pigs – untreated (group I, controls) and treated orally with triticonazole at doses of 200 mg/kg (0.1×LD₅₀, group II); 1000 mg/kg (0.5×LD₅₀, group III) and 2000 mg/kg (LD₅₀, group IV). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ group I; ■ group II; ▲ group III; ● group IV.

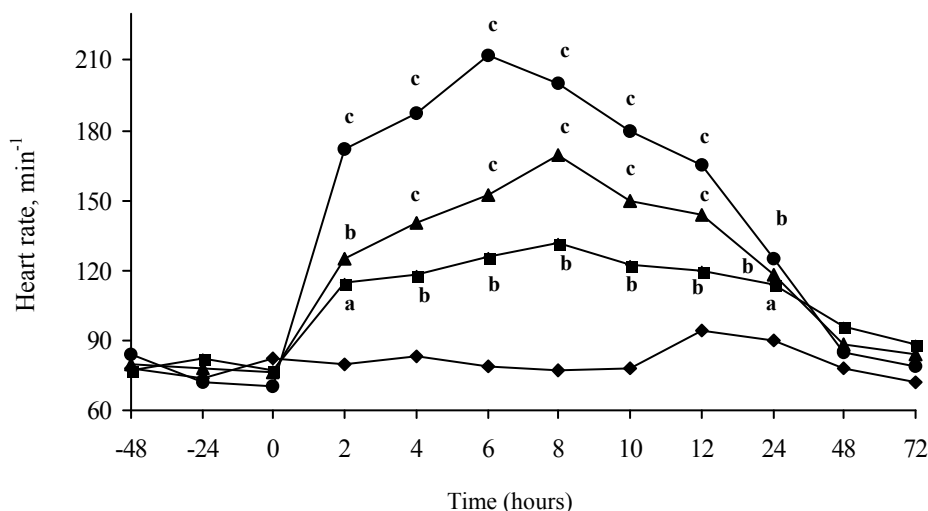


Fig. 2. Changes in heart rate in pigs – untreated (group I, controls) and treated orally with triticonazole at doses of 200 mg/kg ($0.1 \times LD_{50}$, group II); 1000 mg/kg ($0.5 \times LD_{50}$, group III) and 2000 mg/kg (LD_{50} , group IV). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ group I; ■ group II; ▲ group III; ● group IV.

hour 2 (37.2 ± 0.2 °C) and hour 4 (37.1 ± 0.2 °C) at $p < 0.001$ compared to control values (39.0 ± 0.2 °C and 39.2 ± 0.1 °C respectively). BT returned to normal between hours 12 and 24.

The administration of triticonazole resulted in enhanced HR (Fig. 2) in all three experimental groups between post treatment hours 12 and 24. The highest heart rates in groups II and III were detected by hour 8 (132 ± 12 min⁻¹ at $p < 0.01$ and 169 ± 15 min⁻¹ at $p < 0.001$ vs controls: 77 ± 6 min⁻¹). In group IV, the peak values were measured by hour 6 (212 ± 18 min⁻¹, $p < 0.001$ vs controls; 79 ± 7 min⁻¹). Heart rates regained initial values after post treatment hour 24.

RR changes (Fig. 3) were similar. In group II by hour 4 RR was significantly higher than that in controls (39 ± 5 min⁻¹ and 15 ± 5 min⁻¹ respectively; $p < 0.01$). The most enhanced RR was observed by hour 6 (48 ± 4 min⁻¹ vs 15 ± 5 min⁻¹ in con-

trols). In groups III and IV RR increased 2 hours after the treatment but the peak values were noticed by hour 4 (56 ± 6 min⁻¹ and 62 ± 5 min⁻¹ respectively). The differences vs control estimates (19 ± 5 min⁻¹) were statistically significant ($p < 0.001$). RR was recovered by post treatment hours 8, 10 and 12 in groups II, III and IV respectively.

The acute fungicide intoxication resulted in changes in the general condition and behaviour of treated pigs. They refused water and food, their conjunctives were pale, corneal opacification, lacrimation, salivation and dyspnea were also observed. In group II all those changes disappeared 6 hours after the intoxication. In group III, tremor, decreased skin sensitivity and motor activity, clonic spasms of skeletal muscles were further observed. They were present up to post treatment hour 8. Between hours 4 and 6, spontaneous defecation and urinary incontinence, seizures, convulsions, paresis and paraly-

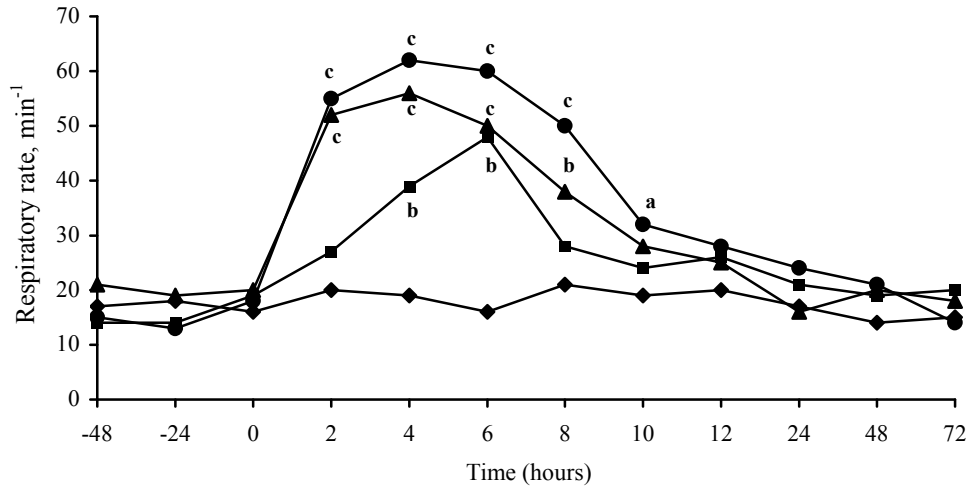


Fig. 3. Changes in respiratory rate in pigs – untreated (group I, controls) and treated orally with triticonazole at doses of 200 mg/kg ($0.1 \times LD_{50}$, group II); 1000 mg/kg ($0.5 \times LD_{50}$, group III) and 2000 mg/kg (LD_{50} , group IV). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$. Legend: ♦ group I; ■ group II; ▲ group III; ● group IV.

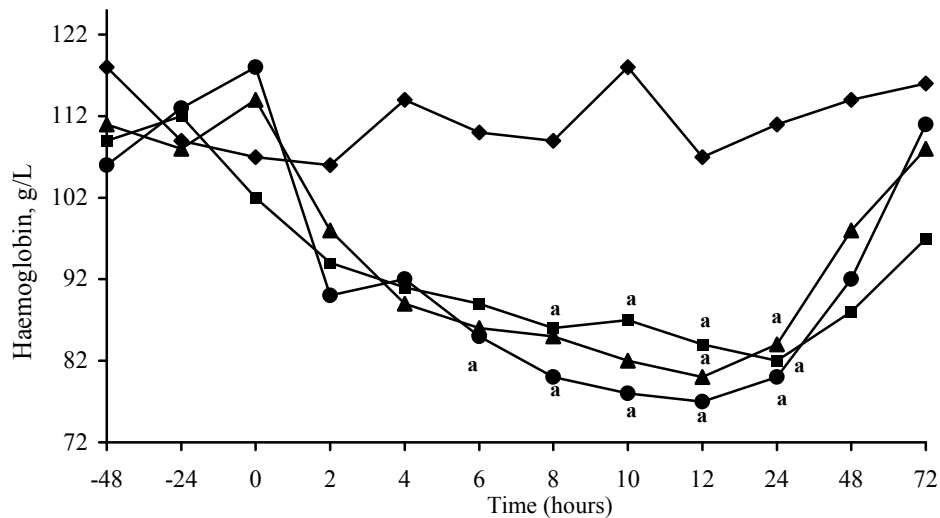


Fig. 4. Changes in blood haemoglobin in pigs – untreated (group I, controls) and treated orally with triticonazole at doses of 200 mg/kg ($0.1 \times LD_{50}$, group II); 1000 mg/kg ($0.5 \times LD_{50}$, group III) and 2000 mg/kg (LD_{50} , group IV). Level of significance vs controls: ^a $p < 0.05$. Legend: ♦ group I; ■ group II; ▲ group III; ● group IV.

sis of limbs were also present in group IV persisting up to hours 8–10.

Changes occurred in Hgb levels as well (Fig. 4). After treatment, Hgb content decreased. In group II the oligochromae-

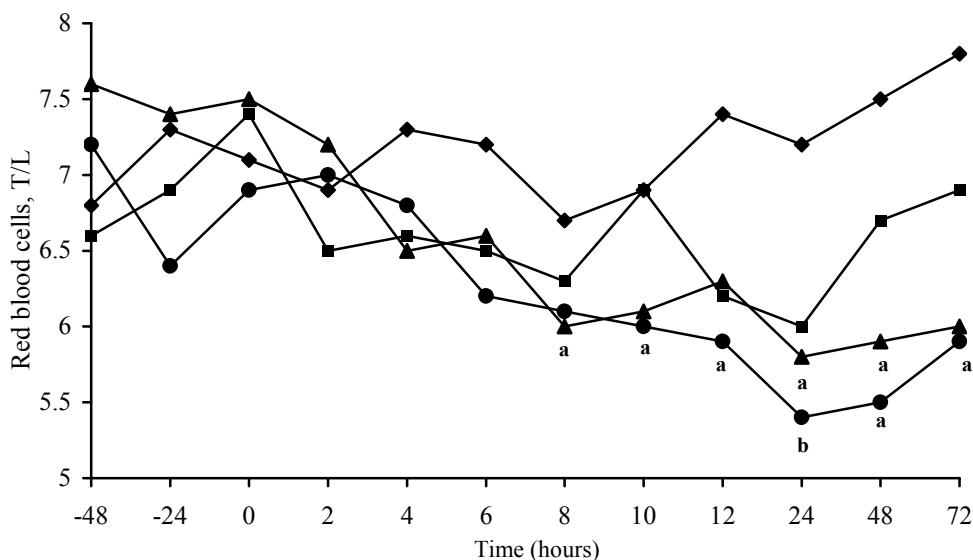


Fig. 5. Changes in red blood cell counts in pigs – untreated (group I, controls) and treated orally with triticonazole at doses of 200 mg/kg ($0.1 \times LD_{50}$, group II); 1000 mg/kg ($0.5 \times LD_{50}$, group III) and 2000 mg/kg (LD_{50} , group IV). Level of significance vs controls: ^a $p < 0.05$; ^b $p < 0.01$. Legend: ♦ group I; ■ group II; ▲ group III; ● group IV.

mia was the most evident by hour 24 – 82.1 ± 6.4 g/L ($p < 0.05$ vs controls – 111.8 ± 8.8 g/L). In groups III and IV, the peak Hgb decrease occurred by hour 12 (80.1 ± 7.7 g/L and 77.2 ± 6.7 g/L respectively; $p < 0.05$ vs group I: 107.6 ± 9.2 g/L). Within hours 48–72, the parameter reached control values.

RBC counts (Fig. 5) in controls and treated animals were about 7.24 ± 0.48 T/L prior to the intoxication. After that, in group II no significant changes were observed. In groups III and IV, a statistically significant decrease occurred with lowest counts by hour 24 – 5.86 ± 0.32 T/L at $p < 0.05$ and 5.42 ± 0.28 T/L at $p < 0.01$ respectively compared to controls (7.27 ± 0.36 T/L). At post treatment hour 72 RBC counts in group III were similar to initial ones whereas in group IV – still lower but tended to normalize.

Hct and MCV values in controls varied from 29 and 43 % and from 48 fL to 54 fL respectively. The differences between experimental and control animals were not statistically significant.

DISCUSSION

The clinical and experimental studied in pigs intoxicated with increasing doses of triticonazole showed that that fungicide can produce significant variations in clinical and some laboratory parameters, depending on the dose.

The BT changes were indicative that low doses ($=0.1 LD_{50}$) did not have any significant effect on this parameter. The higher doses ($=0.5 LD_{50}$ and LD_{50}) led to significant BT decrease between post treatment hours 4 and 12. The hypothermia, observed also in other investigations

(Moser and Mac Phail, 1989; Allen and Mac Phail, 1993; Crofton, 1996; Shandrenko and Dmitrenko, 1996; Binev, 2000a; 2001; Filipov and Lawrence, 2001; Binev, 2002) was most probably correlated to the decreased metabolism rate ensuing from hypothyroidosis, which was evidenced after treatment with 3-amino-1, 2, 4,-triazole (Mayberry, 1968).

The erythropenia in experimental groups could be related to the multiple haemorrhages in parenchymal organs on one hand (Markelov and Shormanov, 1994; Filipov and Lawrence, 2001) or to depletion of liver glutathione, preserving RBC from haemolysis (Celic *et al.*, 1996; Shandrenko and Dmitrenko, 1996; Filipov and Lawrence, 2001) on the other.

Our data (Binev, 2000a; 2000b; 2001) as well those of other authors (Machera, 1995; Celic *et al.*, 1996; Shandrenko and Dmitrenko, 1996; Filipov and Lawrence, 2001) about lowering of RBC counts could explain the observed oligochromaemia.

It is accepted that the systemic reaction to oligochromaemia is HR acceleration (Markelov and Shormanov, 1994). The tachycardia observed in our studies was probably a compensatory mechanism of reported oligochromaemia (Celic *et al.*, 1996; Shandrenko and Dmitrenko, 1996) and erythropenia (Markelov and Shormanov, 1994; Shandrenko and Dmitrenko, 1996), that impeded the process of oxygenation, clinically manifested with HR acceleration (Machera, 1995; Markelov and Shormanov, 1994; Kurumbaev *et al.*, 1996; Filipov and Lawrence, 2001).

The causes for the observed polypnea and dyspnea were probably similar to those resulting in HR changes (oligochromaemia and erythropenia), i.e. these are a compensatory reaction. From the other side, they could be a functional

effect of morphological changes in the pulmonary parenchyma such as hyperaemia, haemorrhages, peribronchial lymphocytic proliferation etc., observed in cattle (Markelov and Shormanov, 1994), in cats (Kurumbaev *et al.*, 1996) and in mice, rats and humans (Machera, 1995).

The changes in the behaviour and the general condition – tremor, clonic seizures, spasms, convulsions, incoordinated movements, ataxia, limb paresis and paralysis, observed in the present study and by others (Moser and Mac Rhail, 1989; Walker *et al.*, 1990; Machera, 1995; Moser *et al.*, 2001; Filipov and Lawrence, 2001) in triazole fungicides-treated animals could be interpreted as a functional response to the pesticide toxic effect on brain substance (vascular hyperaemia, dystrophy of glial cells etc.) (Moser and Mac Phail, 1989; Walker *et al.*, 1990; Machera, 1995; Kurumbaev *et al.*, 1996; Moser *et al.*, 2001). At the same time, the observed neurotoxicological syndrome could be due to the indirect effect of triazole compounds upon dopamine via inhibition of dopamine and noradrenaline degradation in cortical synaptosomes (Walker *et al.*, 1990; Allen and Mac Phail, 1993; Walker and Mailman, 1996). The described neurological signs correspond with those in rabbits treated with diniconazole (Binev, 2001) and triadimenol (Binev, 2002), in rats (Moser and Mac Phail, 1989; Walker *et al.*, 1990; Allen and Mac Phail, 1993; Machera, 1995; Walker and Mailman, 1996; Moser *et al.*, 2001) and in cattle (Markelov and Shormanov, 1994).

The lack of changes in Hct values provides an evidence for the independent character of observed haematological changes – erythropenia and oligochromaemia.

In conclusion, it could be summarized that the application of the triazole fungicide triticonazole (the preparation Real 200 FS) in toxic doses (equal to 0.1LD₅₀, 0.5LD₅₀ and LD₅₀ oral doses for rats) in pigs resulted in clinical alterations manifested by hypothermia, tachycardia and polypnea as well as in haematological parameters variations such as oligochromaemia and erythropenia. The clinical signs were also characterized by lacrimation, salivation, tremor, seizures, clonic spasms, paresis and paralysis of limbs.

REFERENCES

- Allen, A. and R. MacPhail, 1993. Bitertanol, a triazole fungicide, increases operant responding but not motor activity. *Neurotoxicology and Teratology*, **15**, No 4, 237–242.
- Binev, 2003. Influence of acute poisoning with the triazole fungicide triticonazole upon the leukocyte profile in swine. In: Proceedings of the Scientific Conference with an International Participation “Stara Zagora 2003” Union of Scientists, Stara Zagora, **3**, 311–316 (BG).
- Binev, R., 2000a. Clinical and experimental studies on the acute intoxication with the triazole fungicide diniconazole in birds. I. Clinical and paraclinical studies. *Veterinary Medicine (Sofia)*, No 1, 44–48 (BG).
- Binev, R., 2000b. Clinical and experimental studies on acute intoxication with the triazole fungicide diniconazole in birds. II. Biochemical studies. *Veterinary Medicine (Sofia)*, No 1, 49–52 (BG).
- Binev, R., 2001. Clinical and experimental studies on acute poisoning with the triazole fungicide diniconazole (Sumi 8 2WP) in rabbits. I. Clinical and haematological studies. *Bulgarian Journal of Veterinary Medicine*, **4**, No 2, 103–114.
- Binev, R., 2002. Influence of acute poisoning with the triazole fungicide triadimenol upon the clinical indices in rabbits. In: Proceedings of the Scientific Conference with an International Participation “Stara Zagora 2002” Union of Scientists, Stara Zagora, **3**, 99–102 (BG).
- Celic, I., H. Camas, O. Arslan, E. Yegin and I. Kutrevioglu, 1996. The effects of some pesticides on the activity of liver and erythrocyte enzymes (*in vitro*). *Journal of Environmental Science and Health*, **7-A**, 1645–1649.
- Crofton, K., 1996. A structure-activity relationship for the neurotoxicity of triazole fungicides. *Toxicology Letters*, **84**, No 3, 155–159.
- Filipov, N. and D. Lawrence, 2001. Developmental toxicity of a triazole fungicide: Consideration of interorgan communication. *Toxicology Science*, **62**, No 2, 339–352.
- Kurumbaev, Y. K., S. O. Kulyakova, N. V. Sanotskaya and S. O. Aleynikov, 1996. Pathomorphological study of lungs in feline pneumonia model. *Bulletin of Experimental Biology and Medicine*, No 1, 103–104 (Ru).
- Machera, K., 1995. The studies on the effects of triazole fungicides: Cyproconazole, flusilazole, 1,2,4-triazole and propiconazole. *Bulletin of Environmental Contamination Toxicology*, **54**, 363–369.
- Markelov, M. Y. and V. K. Shormanov, 1994. Distribution of the pesticide Baytan and its primary metabolite (DTChB) in warm-blooded animals. *Pharmacia*, **6**, 16–19 (RU).
- Mayberry, W. E., 1968. Antithyroid effects of 3-amino-1,2,4-triazole. *Experimental Biology and Medicine*, **129**, 551–556.
- Moser, V. and R. Mac Phail, 1989. Neurobehavioural effect of triadimefon, a triazole fungicide, in male and female rats. *Neurotoxicology and Teratology*, **11**, No 3, 285–293.
- Moser, V.C., S. Barone, R. Smialowicz, M. Harris, B. Davis, D. Overstreet, M. Mauney and R. Chapin, 2001. The effects of perinatal tebuconazole exposure on adult neurological, immunological, and repro-

- ductive function in rats. *Toxicological Sciences*, **62**, No 2, 339–352.
- Rochette, G., M. Engelen and H. Van den Bossche, 2002. Antifungal agents of use in animal health – practical applications. *Journal of Veterinary Pharmacology and Therapeutics*, **26**, 31–53.
- Sengalevich, G., T. Tonev and A. Nikolov, 1998. Manual for Application of Chemicals in Plant Protection. Higher Agricultural Institute, Plovdiv (BG).
- Shandrenko, S. G. and N. P. Dmitrenko, 1996. Haemoglobin oxidation as an index of the harmful influence of xenobiotics in erythrocytes: In: 2nd World Congress Alternat. and Animal Use (Abstracts), Utrecht, 20–24. Oct., *Life Science*, ATLA 24 Spec. Issue, 284.
- Van den Bossche, H., M. Engelen and F. Rochette, 2002. Antifungal agents of use in animal health – chemical, biochemical and pharmacological aspects. *Journal of Veterinary Pharmacology and Therapeutics*, **26**, 5–29.
- Walker, Q. and R. Mailman, 1996. Triadimefon and triadimenol effects on monoamine uptake and release. *Toxicology and Applied Pharmacology*, **139**, No 2, 227–233.
- Walker, Q., M. Lewis, K. Crofton and R. Maiman, 1990. Triadimefon, a triazole fungicide, induces stereotyped behaviour and alters monoamine metabolism in rats. *Toxicology and Applied Pharmacology*, **102**, No 3, 474–485.

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