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Haematology and Blood Chemistry Changes in Mice Treated with Terbuthylazine and its Formulation Radazin TZ-50

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Abstract TBA is an herbicide in general low acute toxicity and placed into a third category of toxicity. The aim of this study was to determine the effect of TBA and its formulation Radazin TZ-50 in doses of ADI values and 1/100 LD 50 on haematological and biochemical blood parameters in mice. The number of leukocytes was significantly decreased ($p < 0.05$) in all treated groups compared to non-treated mice ($8.81 \pm 3.23 \times 10^9$/L). The lowest value $3.90 \pm 0.74 \times 10^9$/L was observed in group treated with TBA (1/100 LD 50) followed by TBA (ADI) $4.49 \pm 0.98 \times 10^9$/L, Radazin TZ-50 (1/100 LD 50) $4.67 \pm 1.24 \times 10^9$/L and Radazin TZ-50 (ADI) $4.73 \pm 1.15 \times 10^9$/L. The values of the enzyme AST was increased from 190.00 ± 26.46–270.00 ± 147.30 U/L in serum of all treated groups as compared to non-treated mice (110.00 ± 20.00). LDH values showed significant increase (3236.67 ± 56.86–$4054.33.5 \pm 837.16$ U/L) as compared to non-treated mice (1010.00 ± 222.71 U/L). Total protein value was significantly ($p < 0.05$) increased in TBA 1/100 LD50 (63.00 ± 7.48 g/L) and Radazin TZ-50 1/100 LD50 (60.00 ± 2.00 g/L) compared to non-treated mice 52.00 ± 4.00 g/L. Increased serum concentrations of urea and creatinine obtained in mice treated with TBA and Radazin TZ-50 indicates a greater degree of dysfunction of the nephron. TBA and its formulation of Radazin TZ-50 in applied doses demonstrate changes in the number of leukocytes and limited hepatotoxic effects.

Keywords Terbuthylazine · Radazin TZ-50 · Haematology · Biochemistry · Mice

TBA (TBA) (2-(tert-butylamino)-4-chloro-6-(ethylamino)-s triazine) is an herbicide that belongs to the chloro-triazine pesticides. TBA has similar effect as atrazine and is used as its substitute because atrazine is banned in the EU countries by end of 2006. TBA is an herbicide generally low acute toxicity and placed into a third category of toxicity. It is very toxic to aquatic organisms (USEPA 1995). TBA is classified in Group D carcinogen due to inadequate evidence of carcinogenic effects in humans. Increased incidence of mammary adenocarcinomas in rats at the dose level of 7.6 mg/kg bw/day, classify TBA in R40 "limited evidence of carcinogenic effect" (EC 2002). Formulation Radazin TZ-50 (Herbos) is an herbicide containing TBA as an active substance (500 mg/L). It is used to non-treated annual broadleaf weeds such as white goosefoot, pigweed, ragweed, etc. and reduction annual grass weeds. Although human toxicity and exposure data are limited there are evidence that significant population is exposed to it especially agricultural workers and rural residence but urban population is also threatened (Otto et al. 2007; Tsatsakis et al. 2008; Mercadante et al. 2011).

Since the reports of TBA toxicity is scarce, the aim of this study was to determine the effect of TBA on haematological and biochemical blood parameters of Swiss albino mice, and the identification of possible differences toxicity of TBA as pure substance or its formulation Radazin TZ-50.

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Materials and Methods

In this study we used male Swiss albino mice, aged 2–3 months, weighing 20–25 g from the breeding of the Department of Biology, Faculty of Science, University of Zagreb, Croatia. Five animals were kept in the same cage and feed standard diet for laboratory animals (Standard Diet GLP, 4RF 1, Mucedola, Settimo Milanese MI, Italy), with access to water ad libitum. We used 12 h regime changes of day and night. The research was conducted in accordance with the Law on the Protection of laboratory animals (NN #135/06) and the Guide for the keeping and use of laboratory animals (Guide for the Care and Use of Laboratory Animals, DHHS (NIH) Publ # 86–23). All used methods are proposed by OECD protocol for Pesticide Assessment and Testing (2002).

Herbicide TBA (N2-tert-butyl-6-chloro-N4-ethyl-1,3,5-triazine-2,4-diamino) (Supelco) in the form of pure substances and formulation Radazin TZ-50 (Herbos) was used.

Mice were divided into 5 groups composed of 5 individuals. Animals were treated with pesticide solutions intraperitoneally (*ip*) daily for 14 days injecting 0.5 mL of tested solution. TBA was applied in the form of pure active substance in the value of the acceptable daily intake (ADI – 0.0001 mg/day) and 1/100 LD50 (0.001 mg/day). Radazin TZ-50 was applied in dose containing the active substance TBA in the ADI values and 1/100 LD50. At the same time non-treated group was given 0.5 mL saline (0.9 % NaCl).

Analysis of blood samples was performed after 14-day of treatment. Blood was taken into heparinised vacuteiner (BD Microtainer Becton, Dickinson & Co., USA) from axillary plexus vessels of anesthetised mice (Isofluran). Haematological parameters: number of erythrocytes (RBC), number of leukocytes (WBC), haematocrit (HCT), haemoglobin (Hb), mean corpuscular volume (MCV), mean haemoglobin concentration in the volume of erythrocytes (MCHC), red cell distribution by volume (RDW), the mean amount of haemoglobin in erythrocytes (MCH) and platelet index (PLT) were determined in blood cell counter Cell-Dyn® 3200 (Abbott, USA).

Analysis of bone marrow of the thigh bone (femur) was purified from muscle tissue, and cut off both epiphyses. Contents was washed out from bone marrow and fragmented. The cells were counted on Bürker-Turk chamber.

Blood biochemical parameters were determinate in serum of treated animals. We determinate alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD), total protein (TP), creatinine (Creator), glucose (GLU) and urea were done on the machine Alcylon 300 (Abbott, USA). The experiment and biochemistry analysis were conducted according to the recommendations of the IFCC methods in enzymology with commercial kits (Herbos dd, Sisak) on the Hitachi 717 automatic analyzer (Hitachi, Japan) as previously described by Đikić et al. (2009).

Statistical analysis of numerical data obtained was performed by means of descriptive statistics, *t* test, ANOVA, analysis of variance using the Statistica, Excel and SPSS for Windows. The level of statistical significance was set at $p < 0.05$.

Results and Discussion

EFSA (2010) published data on the toxicity of TBA although scarce, showed significant toxicity of TBA. Rats acutely exposed to oral doses of TBA showed piloerection, exhaustion, fatigue and diarrhoea. General autopsy showed lung congestion and autolysis of the digestive canal. Haematology revealed effects on red blood cells in females. The pathology showed an increased incidence of non-neoplastic lesions in the liver, lung, thyroid, and testes. (Werner 1996).

After 14-day treatment the number of leukocytes in all treated groups was significantly decreased ($p \leq 0.05$) compared to non-treated (Table 1). Total count of cell in bone marrow was significantly higher ($p \leq 0.05$) in mice

Table 1 The number and percentage of leukocytes in blood of mice treated with terbuthylazin and Radazin TZ-50

Group	Leukocytes		
	Total no. ($\times 10^9$/L)	Granulocytes (%)	Agranulocytes (%)
Non-treated	8.81 ± 3.23	22.20 ± 8.08	77.81 ± 8.08
Terbuthylazin (ADI)	4.49 ± 0.98*	20.75 ± 11.07	79.26 ± 11.07
Terbuthylazin (1/100 LD50)	3.90 ± 0.74*	30.45 ± 8.19	69.53 ± 8.11
Radazin TZ-50 (ADI)	4.73 ± 1.15*	26.93 ± 9.27	73.08 ± 9.69
Radazin TZ-50 (1/100 LD50)	4.67 ± 1.24*	31.04 ± 4.22	68.97 ± 4.20

* Significantly different ($p < 0.05$) from non-treated

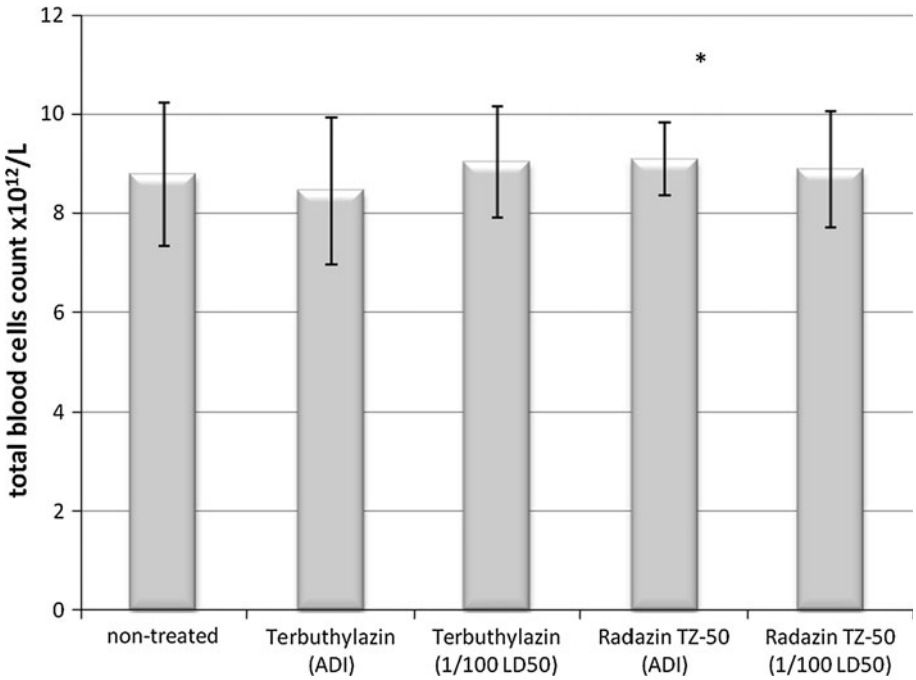

Fig. 1 Total cell count in bone marrow of mice treated with Terbuthylazin and Radazin TZ-50. * significantly different ($p < 0.05$) from non-treated animals

treated with Radazin TZ-50 in ADI dose while in other groups a slight increase compared to the non-treated group was observed (Fig. 1). Since the total number of cells present in bone marrow was not significantly disturbed we assume that the decrease of leukocyte number was result of atrophy of thymus, lymph nodes and spleen. Similar effects of higher dose were observed by Werner (1996) on rabbits. The results indicate that TBA and its formulation Radazin TZ-50 in applied doses have unsignificant, effect on number of erythrocytes, and values of haemoglobin and haematocrit (Table 2). Werner (1996) showed changes in haematology and clinical chemistry parameters but applied doses of pesticide were higher (up to 300 mg/kg) and exposure was longer (90 days).

The values of the enzyme AST and LDH in serum of all treated groups showed significant increase ($p \leq 0.05$) compared to the non-treated animals (Table 3). Observed increased activity of AST and ALT in blood serum is probably the result of hepatotoxic effects TBA and its formulations Radazin TZ-50. Higher levels and increase of serum enzyme activities, as is the case in our study, especially in animals exposed to Radazin TZ-50, occur as a result of hepatic toxicity of pesticides (Andrew 2007). Total protein were significantly ($p < 0.05$) increased in mice treated with TBA in dose 1/100 LD50 and Radazin TZ-50 in dose 1/100 LD50 compared to the non-treated group (Table 3). Statistically significant increase ($p < 0.05$) blood glucose levels in mice compared to non-treated was observed in all groups except TBA ADI compared to non-treated animals (Table 3). This observation can be associated with liver injury and is further evidence of a possible adverse effect of TBA and its formulations Radazin TZ-50 on the activity of the liver (Lang et al. 1997). Increased serum concentrations of urea and creatinine under the influence of TBA and Radazin TZ-50 in particular dose 1/100 LD50 obtained in our study indicates a greater degree of dysfunction of the nephron (Table 3). Elevated urea and creatinine is a sign of impaired renal function whose role is to cleanse the blood of these harmful products. The level of serum creatinine is not sensitive to early renal damage and reacts more slowly than urea in the blood. Since the metabolism of urea and creatinine occurs in the liver, their high values may indicate a dysfunction of the liver (Everds 2004).

The results of the assessment of toxic effects of the active substance and its formulation of TBA Radazin TZ-50 using analysis of haematological and biochemical parameters in blood of mice show characteristic changes in the number of leukocytes and limited hepatotoxic effects.

Table 2 Haematological parameters in blood of mice treated with terbuthylazin and Radazin TZ-50

Group	Erythrocyte ($\times 10^{12}$/L)	Haemoglobin (g/L)	Haematocrit	*MCV* (fL)	*MCH* (pg)	*MCHC* (g/L)	*RDW*	Platelets ($\times 10^{12}$L)
Non-treated	8.77 ± 0.37	127.90 ± 6.54	0.42 ± 0.02	94.05 ± 0.47	29.20 ± 0.47	620.00 ± 9.52	20.70 ± 0.70	1130.00 ± 133.9
Terbuthylazin (ADI)	8.44 ± 0.51	125.07 ± 3.81	0.39 ± 0.02	93.13 ± 1.51	29.67 ± 1.63	637.33 ± 25.72	21.20 ± 1.00	1375.33 ± 169.17
Terbuthylazin (1/100 LD50)	9.03 ± 0.39	128.60 ± 6.63	0.42 ± 0.01	92.80 ± 1.64	28.47 ± 0.64	614.00 ± 17.78	22.00 ± 1.04	1262.8 ± 308.52
Radazin TZ-50(ADI)	9.085 ± 0.59	135.00 ± 13.45	0.42 ± 0.03	92.80 ± 1.52	29.65 ± 1.05	639.50 ± 21.99	20.25 ± 0.67	1322.5 ± 449.02
Radazin TZ-50 (1/100 LD50)	8.87 ± 0.28	125.90 ± 4.44	0.41 ± 0.02	92.35 ± 1.98	28.45 ± 0.10	615.00 ± 12.70	21.55 ± 1.14	1293.5 ± 149.41

No significant difference between treated and non-treated animals was observed

MCV mean corpuscular volume, *MCH* the mean amount of haemoglobin in erythrocytes, *MCHC* mean haemoglobin concentration in the volume of erythrocytes, *RDW* red cell distribution by volume

Table 3 Biochemical parameters (ALP, AST, ALT, LDH, TP, CREAT, GLU, Urea) in blood of mice treated with Terbuthylazin and Radazin TZ-50

Group	ALP (U/L)	AST (U/L)	ALT (U/L)	LDH (U/L)	TP (g/L)	CREAT (mmol/L)	GLU (mmol/L)	Urea (mmol/L)
Non-treated	83.33 ± 15.28	110.00 ± 20.00	70.00 ± 10.00	1010.00 ± 222.71	52.00 ± 4.00	90.00 ± 17.32	3.33 ± 0.15	6.67 ± 1.53
Terbuthylazin (ADI)	82.50 ± 17.32	270.00 ± 147.30*	76.66 ± 11.54	4054.33.5 ± 837.16*	52.33 ± 3.40	96.67 ± 5.77	4.23 ± 0.61	7.33 ± 1.52
Terbuthylazin (1/100 LD50)	65.00 ± 12.91	222.50 ± 46.46*	77.50 ± 12.58	3765.00 ± 500.17*	63.00 ± 7.48*	110.00 ± 14.14	4.80 ± 1.75*	8.75 ± 2.87
Radazin TZ-50 (ADI)	78.00 ± 8.37	192.50 ± 75.88*	72.05 ± 9.57	3290.00 ± 575.09*	58.00 ± 6.63	104.00 ± 11.40	4.95 ± 0.17*	8.25 ± 1.58
Radazin TZ-50 (1/100 LD50)	70.00 ± 10.00	190.00 ± 26.46*	80.00 ± 10.00	3236.67 ± 56.86*	60.00 ± 2.00*	110.00 ± 10.00	5.50 ± 1.44*	8.00 ± 1.00

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *LHD* lactate dehydrogenase, *TP* total protein, *CREAT* creatinine, *GLU* glucose

* Significantly different ($p < 0.05$) from non treated animals; *ALP* alkaline phosphatise

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