BIOCHEMICAL TARGETS AFFECTED BY SUBACUTE DOSES OF NEW PESTICIDE MIXTURES TESTED ON ALBINO RATS

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Abstract: Few studies have characterize the toxicological effects of exposure to pesticide mixtures. For this reason, the present study aimed to estimate the median lethal doses (LD_{50}) of some new pesticide mixtures; chlorosan, feroban, cygron, engeo and kingbo on albino rats. The *in vivo* effect of these compounds on some biochemical targets were also investigated. The estimated median lethal doses (LD_{50}) of chlorosan, feroban, engeo and cygron were 140.8, 264.0, 281.5 and 352.0 mg/kg body weight (b.w.), respectively. The estimated median lethal dose was more than 160.0 mg/kg b.w. in the case of kingbo. The symptoms of the affected animals included salivation, bleeding, activity increase and the chlorosan treated rats closed their eyes. Some animals died after doses of chlorosan, feroban and engeo administered at different intervals. The results showed a significant increase in the activities of plasma transaminases [Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT)] and glutathione S-transferase (GST). It was found that the tested pesticides significantly inhibited acetylcholinesterase (AChE) activity 1 h after the last dose. Also, there was a significant increase in creatinine and urea levels. The obtained data concluded that chlorosan was the most effective against albino rats followed by feroban and engeo, while kingbo was the least effective. By the end of the experiment, the enzyme activities and kidney functions of animals treated with chlorosan, feroban and engeo did not return to normal.

Key words: pesticide mixtures, toxicity, biochemical targets, albino rats

INTRODUCTION

Pesticide applications have increased dramatically since the mid 1960s, and the related adverse health effects in human as well as in wild/domestic animals have become a serious public concern (Crumpton 2001). Pesticide usage is beneficial for increasing agricultural productivity, and reducing insect-borne diseases. But, human exposure to these toxic chemicals is virtually unavoidable due to contamination of air, water, soil, and food (Ecobichon 2000). The potential toxicity of most of these chemicals has been studied extensively and several databases have been developed. Despite this fact, more than 95% of all pesticide toxicity studies were conducted on individual environmental pollutants (Yang 1994; Groten *et al*. 1999). Recently, more emphasis has been placed on chemical mixture studies because people are actually exposed to countless mixtures of chemicals daily.

There is an increased commercial interest in developing insecticide mixtures. The mixtures may work quickly, as in the case of virus vectors. The mixtures also work in the case of longer lasting residual activity. Both of these examples may allow for fewer applications. It is important to remember that for any mixed active product which suggests additive or synergistic effects between the active agents, the effects must be highlighted (or cross-referenced) in each of the efficacy, toxicological, and ecotoxicological submissions. The combined effect across the different classes of pesticides is more difficult to predict and understand (Lydy *et al*. 2004). Few studies have begun to characterize the toxicological effects of exposure to pesticide mixtures (Richardson *et al*. 2001; Schuler *et al*. 2005; Moser *et al*. 2006).

Therefore, the present study was conducted to estimate the acute toxicity of some new pesticide mixtures on male albino rats, and determined the possible effects on some biochemical targets.

MATERIALS AND METHODS

Tested compounds

1. Chlorosan 29% EC (chloropyrifos 24% + cypermethrin 5%), was obtained from the Kafer El-Ziat Pesticides and Chemicals Co., Egypt.
2. Feroban 50% EC (chloropyrifos 47.5% + lufenuron 2.5%), was obtained from the Agrochem Co., Egypt.
3. Cygron 10% EC (flufenoxuron 3% + alpha-cypermethrin 7%), was obtained from the Agrochem Co., Egypt.
4. Engeo 24.7% SC (thiomethoxam 14.1% + lambdacyholothrin 10.6%), was obtained from the Syngenta Co.
5. Kingbo 0.6% SL (oxymatrine 0.2% + prosuler 0.4%), was prepared by the Egypt Group Development Co. This

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compound is extracted, refined and produced from several Chinese wild medicinal plants, such as Sophora, flavescenst and others. Also, this insecticide was used in inorganic farming according to Anonymous (1991).

Animal Model

Male albino rats (wistar strain) weighing 150±20 gm were procured from the animal breeding house of the National Research Centre (NRC), Dokki, Cairo, Egypt. Animals were acclimatized for laboratory conditions, fed on standard diet as per formula of NRC animals breeding house. Water was supplied *ad libitum*.

Determination of the median lethal dose (LD_{50})

According to the method of Finney (1971) for determination of the median lethal dose (LD_{50}), exploratory trials were performed in five groups with two rats in each group. Feroban, cygron, chlorosan, engeo and kingbo were administered orally at five gradual doses given equally to the five groups. This was done to find the smallest toxic dose to start with. The dose 100, 200, 80 and 100 mg/kg body weight (b.w.) of feroban, cygron, chlorosan and engeo, respectively, were was the first doses to cause signs of toxicity multiplied by the constant factor (1.5) for each succeeding groups of rats. On the other hand, rats were orally administered kingbo at a dose of 160.0 mg/kg b.w. (OECD 1992) given in multiple doses within 24 hrs. To achieve the total dose, animals received four equally divided doses separated by approximately 6-h intervals. Mortality of rats was recorded after 24 h. The toxicity index of each compound was determined according to Sun (1950).

Acute toxicity

To study the toxicity of tested compounds on some vital biochemical parameters, the animals were divided into six groups (10 rats each). The 1st, 2nd, 3rd and 4th groups were treated with one – fourth of the median lethal dose (¼ LD_{50}) of chlorosan, feroban, cygron and engeo. The 5th group was treated by 6.0 mg (1 ml formulation)/rat [40 mg active substance (a.s.)/kg b.w. approximately] of kingbo. The 6th group of rats served as a control. Toxicants were dissolved in corn oil and administered orally by, convenient use of a stomach tube, for four successive days. All animals were observed daily for signs of pharmacological or toxicological effects.

For clinical studies, blood samples were collected at 1, 24, 48, 96 and 192 hours after the last dose of toxicants. Serum was collected after centrifugation at 3 000 rpm for fifteen minutes and kept at –20°C until used. For determination of liver functions, the activities of serum esterases including glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) enzymes were estimated calorimetrically (Reitman and Frankel 1957). The kidney functions were evaluated by measuring urea and creatinine concentration according to Coulombe and Farreau (1963) and Henry (1974), respectively. Serum cholinesterase (AChE) activity was determined by the spectrophometric method of Ellman *et al*. (1961). Also, the glutathione S-transferase (GST) activity was determined in plasma by the method described by Habig and Jakpby (1981).

Statistical analysis

The experimental design was a factorial Complete Randomized Design (CRD) with ten replicates. Statistical analysis of data collected was carried out using a computer program (Cohort Software 1986).

RESULTS AND DISCUSSION

Determination of the median lethal doses (LD_{50})

Data in tables 1 and 2 and figure 1 showed that the median lethal doses of chlorosan, feroban, engeo and cygron were 140.8, 264.0, 281.5 and 352.0 mg/kg b.w., respectively. In other words, chlorosan acute toxicity was much higher compared with the other compounds. The toxicity index being 53.33, 50.02 and 40.00 % for feroban, engeo and cygron (Based on LD_{50} of chlorosan 100.00%), respectively. Rats were orally administered kingbo at a dose of 160.0 mg/kg b.w. in multiple doses within 24 h, to achieve the total dose. Animals received four equally divided doses of kingbo in separate 6-h intervals, but kingbo produced neither signs of toxicity nor death in the animals. The LD_{50} value of kingbo, therefore, is more than 160.0 mg/kg b.w. for male albino rats. We could not estimate the LD_{50} of kingbo because this compound may have low toxicity on rats. Our results revealed also, the estimated median lethal doses (LD_{50}) of feroban, cygron and engeo were more than the pamphlet LD_{50} of one of its components and less than the other, except chlorosan treated rats.

Table 1. The pamphlet and the estimated acute toxicity (LD_{50}) of tested compounds

Compounds	Components	*Pamphlet LD_{50} mg/kg b.w.	Estimated LD_{50} mg/kg b.w.
Chlorosan 29% EC	chloropyrifos 24%	135–163	140.8
	cypermethrin 5%	250	
Feroban 50% EC	chloropyrifos 47.5%	135–163	264.0
	lufenuron 2.5%	> 2000.0	
Cygron 10% EC	flufenoxuron 3%	> 3000.0	352.0
	alpha-cypermethrin 7%	57.0	
Engeo 24.7% SC	thiomethoxam 14.1%	1563.0	281.5
	lambdacyholothrin 10.6%	79.0	
Kingbo 0.6% SL	oxymatrine 0.2%	4000.0**	more than 160.0
	prosuler 0.4%		

*pamphlet LD_{50} according to Anonymous (2005); **according to Sineria

Table 2. Toxicity index of tested compounds against albino rats

Compounds	LD_{50} [mg/kg b.w] its limits at 95%	LD_{95} [mg/kg b.w] its limits at 95%	Slope	Toxicity index [%]	
				LD_{50}	LD_{95}
Chlorosan	140.8 126.6 158.2	619.2 467.7 932.7	2.56±0.26	100.00	100.00
Feroban	264.0 237.2 296.5	1165.2 879.3 1757.7	2.55±0.26	53.33	53.14
Engeo	281.5 203.9 470.5	1376.8 1337.3 10174.0	2.55±0.26	50.02	44.97
Cygron	352.0 316.3 395.4	1553.5 1172.4 2343.4	2.39±0.25	40.00	39.86

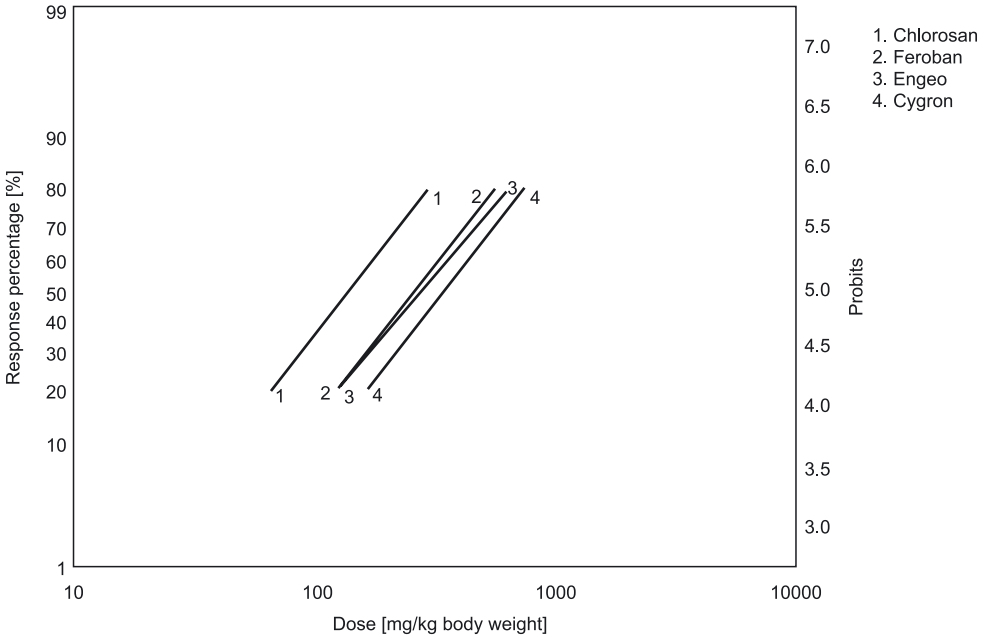

Fig. 1. Log Dose Probit Lines of albino rats to tested compounds

Elhalwagy and Zaki (2009) also reported that the commercial preparation of the pesticide mixture durasin, which contains 60% diazinon and 0.5% deltamethrin, has potentially greater toxic impact for rats than the components alone.

Clinical signs

The clinical signs in chlorosan treated rats (Table 3) included salivation; bleeding and closed eyes. Three chlorosan treated rats died; two at 24 hrs and one at 72 hrs. Also, feroban treated rats caused salivation, diarrhea and two animals died at 24 and 48 h., but, increase activity and lacrimation was observed in cygron treated rats. Engeo treated rats produced bleeding, diarrhea, lacrimation and two animals died at 24 h. Kingbo did not produce any signs of poisoning except aggressiveness.

Enzymatic studies

The results of the activities of serum GOT, GPT, GST and AChE on treated and control rats are shown in tables 4 and 5. The obtained data revealed that there was a significant increase in the activity of serum transaminases enzymes (GPT and GOT) in rats treated rats with the different compounds as compared with the untreated rats. The two enzyme activities reached their peaks at 24 h after the last dose in the case of GPT , GOT and cygron treated rats and then gradually decreased after 48, 96 and 192 h, but did not return to the normal level except in kingbo treated rats. Moreover, chlorosan followed by engeo and feroban induced severe hepatic damage as represented by markedly elevated levels of GOT and GPT activity coupled with a marked hepatic tissue injury. In the other means, the disruption of transaminases from normal values denotes biochemical impairment and lesions of tissues and cellular function. This is because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential functions (Tordior and van Heemstra Lequin 1980). Previous study has shown that insecticides cause serum aspartate aminotransferase (AST and ALT) elevation (Lowenstein *et al*. 1996). Elevation of AST, a cytosolic enzyme of the hepatocytes, reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes (Plaa and Hewitt 1982) and is used to detect liver damage (Klaassen and Eaton 1991). Our results agree with those obtained by Tos-Luty *et al*. (2003) and Kerem *et al*. (2007).

Table 3. Toxic symptoms in male albino rats which received tested compounds during the experiment period

Compound	Signs of toxicity	Animal mortality
Control	nil	nil
Chlorosan	salivation, bleeding and closed eyes	three animals died; two at 24 h and 1 at 72 h
Feroban	salivation and diarrhea	two animal died; one at 24 h and 1 at 48 h
Cygron	increased activity and lacrimation	nil
Engeo	diarrhea, lacrimation and bleeding	two animals died at 24 h
Kingbo	increase activity and aggressiveness	nil

Table 4. Changes of glutamic pyruvic transaminase and glutamic oxaloacetic transaminase activities in rats exposed to subacute doses of tested compounds

Treatment	GPT activity [U/l]					GOT activity [U/l]				
	1 h	24 h	48 h	96 h	192 h	1 h	24 h	48 h	96 h	192 h
Control	40.3 f	39.7 f	43.0 e	40.7 e	39.7 d	31.7 d	29.7 d	31.0 e	31.7 e	29.0 d
Chlorosan	71.7 b (78.0)	75.3 a (89.7)	77.3 a (79.7)	67.3 a (65.3)	62.7 a (58.0)	57.7 a (82.0)	61.3 a (106.4)	62.3 a (101.0)	51.7 a (63.1)	45.0 a (55.2)
Feroban	61.7 c (53.1)	63.3 c (59.4)	60.7 c (41.1)	52.0 c (27.7)	44.3 c (11.6)	49.0 b (54.6)	52.7 b (77.4)	53.0 b (71.0)	46.7 b (47.3)	32.3 c (11.4)
Cygron	56.3 d (39.7)	60.3 d (51.8)	59.0 c (37.2)	54.0 c (32.6)	49.3 b (24.2)	46.3 b (46.1)	51.3 b (72.7)	48.3 c (55.8)	41.0 c (29.3)	31.3 cd (8.0)
Engeo	74.0 a (83.6)	70.7 b (78.0)	71.3 b (65.8)	57.7 b (41.7)	61.3 a (54.4)	59.7 a (88.3)	62.7 a (111.1)	55.0 b (77.4)	50.7 a (60.0)	39.7 b (36.9)
Kingbo	50.7 e (25.8)	48.7 e (22.7)	52.3 d (21.6)	45.7 d (12.3)	39.0 d (-1.8)	40.7 c (28.4)	39.0 c (31.3)	39.7 d (28.1)	36.0 d (13.6)	33.7 c (16.2)
LSD = 5%	1.75	2.88	2.317	2.22	2.48	2.93	2.0	2.5	2.61	2.418

Letters mean the significant differences between treatments according to Duncan's test; Each figure between brackets represents the percentage of content as check; GOT – Glutamic Oxaloacetic Transaminase; GPT – Glutamic Pyruvic Transaminase

A remarkable significant increase in GST activity (Table 5) was observed after 1, 24 and 48 h in all treated groups as compared with the untreated animals ($p < 0.05$). At the end of the experiment (192 h), rats treated with cygron and kingbo returned to normal (0.0 and –2.7%, respectively), but chlorosan, feroban and engeo treated rats did not (70.6, 10.6 and 63.7% above normal level). Glutathione S-transferase (GST) plays an important role in the detoxification and excretion of xenobiotics by catalyzing the conjugation of the tripeptide glutathione (GSH) with the xenobiotic in phase II of the biotransformation process, promoting its elimination from the organism (Leaver *et al*. 1992). Also, many studies analyzing GST activity in experimental animals exposed to different insecticides showed enzymatic induction (El-Gendy *et al*. 1999; Khan and Kour 2007; Chao *et al*. 2009; Elhalwagy and Zaki 2009). This response is usually expected once the GST plays an important role on detoxification and elimination of electrophilic compounds.

On the other hand, there was a significant inhibition in the activity of acetyl cholinesterase (AChE) after 1 hr of the last dose (Table 5). The inhibition of the activity increased at 24 h, except in kingbo treated rats where AChE returned to normal levels (+2.1%). Chlorosan caused more inhibition in AChE at 1 and 24 h (41.5 and 46.6% below normal level) followed by feroban treated rats (–29.7 and 38.9%) then engeo (–19.4 and 23.9%) and cygron (–11.2 and –14.7%). At 192 h, AChE activity did not returned to normal levels in rats treated with chlorosan, feroban and engeo (–30.1, –22.5 and –17.5%). There was no significant differences in cygron and kingbo treated rats as compared with the control. Our results also indicated that chlorosan and feroban showed much higher anticholinesterase activity than other compounds. The activity was higher because both compounds contains organophosphorus insecticide (chloropyrifos). These insecticides bind to the enzyme, leading to the accumulation of acetylcholine in the synapse, resulting in the disruption of normal nervous system functioning (Morifusa 1979; Habig and Di Giulio 1991; Grue *et al*. 1997).

In addition, according to Rawi (1984) the pyrothroid decamethrin caused a prolonged decrease in AChE activity in rats after a single administrated dose. Our results are in agreement with Chao *et al*. (2009) who reported that AChE activity was significantly inhibited in both brain and muscle samples of juvenile goldfish exposed to propoxur (alone, in a binary mixture with isoprocarb, and in a ternary mixture with isoprocarb and chlorpyrifos). However, the significant decrease of AChE activity recorded in our results contradict those recoded by Yavasoglu *et al*. (2006) who showed that liver AChE activities increase in rats treated with cypermethrin compared with control values but these inductions were not statistically significant.

Table 5. Changes in glutathione S-transferase and acetyl cholinesterase activities in rats exposed to subacute doses of tested compounds

Treatments	Glutathione S-transferase (GST)					Acetyl Cholinesterase (ChE)				
	1 h	24 h	48 h	96 h	192 h	1 h	24 h	48 h	96 h	192 h
Control	40.0 e	43.7 d	41.7 e	42.7 c	37.7 d	115.7 a	111.7 a	120.7 a	112.3 a	118.3 a
Chlorosa	65.3 a (63.3)	71.3 a (63.3)	70.7 a (69.5)	65.7 a (53.9)	64.3 a (70.6)	67.7 f (–41.5)	59.7 e (–46.6)	73.0 d (–39.5)	60.7 d (–45.9)	82.7 d (–30.1)
Feroba	48.7 d (21.8)	52.7 c (20.6)	51.7 c (24.0)	46.7 b (9.7)	41.7 c (10.6)	81.3 e (–29.7)	68.3 d (–38.9)	60.7 e (–49.7)	60.3 d (–46.3)	91.7 c (–22.5)
Cygro	51.7 c (29.3)	55.3 c (26.5)	49.3 cd (18.2)	43.7 c (2.3)	37.7 d (0.0)	102.7 b (–11.2)	95.3 b (–14.7)	110.0 b (–8.9)	103.3 b (–8.0)	120.7 a (+2.0)
Enge	57.0 b (42.5)	61.7 b (41.2)	62.0 b (48.7)	64.3 a (50.6)	61.7 b (63.7)	93.3 d (–19.4)	85.0 c (–23.9)	103.0 c (–14.7)	91.7 c (–18.3)	97.7 b (–17.4)
Kingbo	50.7 cd (26.8)	54.7 c (25.2)	48.0 d (15.1)	40.0 d (–6.3)	36.7 d (–2.7)	97.7 c (–15.6)	114.0 a (+2.1)	104.0 c (–12.7)	109.3 a (–2.7)	117.3 a (–0.85)
LSD = 5%	2.34	2.54	2.85	1.85	2.43	3.69	3.74	5.85	4.33	3.35

Letters mean the significant differences between treatments according to Duncan's test;
Each figure between brackets represents the percentage of content as check

Table 6. Changes on creatinine and urea concentration in rats exposed to subacute doses of tested compounds

Treatments	Creatinine concentration [mg/dl]					Urea concentration [mg/dl]				
	1 h	24 h	48 h	96 h	192 h	1 h	24 h	48 h	96 h	192 h
Control	0.93 d	1.03 c	1.2 c	0.9 e	1.0 d	23.0 e	21.7 d	24.7 e	22.7 e	23.3 d
Chlorosan	1.83 a (96.8)	2.03 a (97.1)	2.17 a (80.8)	1.67 b (85.6)	1.43 b (43.0)	37.3 b (62.2)	40.3 a (85.7)	44.7 a (81.0)	42.3 a (86.3)	38.3 a (64.4)
Feroban	1.4 bc (50.5)	1.53 b (48.5)	1.43 c (19.2)	1.3 c (44.4)	0.93 d (-7.0)	30.7 c (33.5)	32.3 b (48.8)	28.7 d (16.2)	31.3 c (38.0)	25.3 c (8.6)
Cygron	1.57 b (68.8)	1.5 b (45.6)	1.4 c (16.7)	1.43 c (58.9)	1.23 c (23.0)	27.7 d (20.4)	31.3 bc (44.2)	34.3 b (38.9)	28.3 d (24.7)	22.7 d (–2.6)
Engeo	1.6 ab (72.0)	1.93 a (87.4)	1.8 b (50.0)	1.9 a (111.1)	1.6 a (60.0)	40.7 a (77.0)	40.0 a (84.3)	45.3 a (83.4)	39.3 b (73.1)	32.0 b (27.2)
Kingbo	1.2 c (29.0)	1.27 bc (23.3)	1.3 c (8.3)	1.1 d (22.2)	1.03 d (3.0)	28.7 cd (24.8)	30.0 c (38.2)	31.3 c (27.0)	27.3 d (20.3)	24.0 cd (3.0)
LSD = 5%	0.241	0.257	0.259	0.173	0.147	2.37	1.65	2.23	1.98	1.87

Letters mean the significant differences between treatments according to Duncan's test; Each figure between brackets represents the percentage of content as check

The toxic effect of tested compounds on kidney functions

The *in vivo* effects of ¼ LD_{50} of tested chemicals on kidney functions are presented in table 6. Creatinine levels were significantly increased in all treated animals after 1, 24 and 96 h of the last dose. But, there were no significant differences between feroban, cygron, and kingbo treated rats and untreated rats at 48 h. There was also no significant differences at 192 h in feroban, and kingbo treated rats and the control. The results show that there was significant elevation in urea concentration in all treated rats, except in treated animals by cygron and kingbo at 192 h, where animals returned to the normal level.

Another finding in this study was that chlorosan and engeo exposure in rats led to an acute renal failure as represented by markedly increased levels of urea and creatinine in both treatments. The kidney, the major detoxification organ for many xenobiotics, is frequently susceptible to their nephrotoxic effects (Kerem *et al*. 2007). In other words, the level of creatinine and urea of the treated animals was increased significantly. This increase may indicate renal function impairment due to tested pesticide mixtures toxicities. Significant increase in the level of creatinine may occur in renal damage and excessive muscular catabolism and during pregnancy in young humans or those with less skeletal muscle caused by trauma, atrophy, necrosis or starvation (Kelly 1984; Metwalli 1987; El-Grieb 1990).

To sum up, this study shows that tested pesticide mixtures in subacute doses (¼ LD_{50}) induces significant effects on liver and kidney functions and caused marked increase in GST activity after 1 and 24 h of the last dose, while, regarding ChE activity inhibition was observed in treated rats after 1 h of treatment. The data obtained concluded that chlorosan was the most effective against albino rats followed by feroban and engeo, while kingbo was the least effective. Animals treated with chlorosan, feroban and engeo did not return to their normal case of enzyme activities and kidney functions by the end of experiment.

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POLISH SUMMARY

WPŁYW PODOSTRYCH DAWEK NOWYCH MIESZANIN PESTYCYDÓW NA PRZEMIANY BIOCHEMICZNE U SZCZURÓW ALBINOSÓW

Z przeglądu literatury wynika, że doniesienia na temat zagrożenia toksycznym działaniem pestycydów są na ogół sporadyczne. Celem prezentowanych badań było ustalenie średniej dawki letalnej (LD_{50}) wybranych nowych mieszanin pestycydów: chlorosan, feroban, cytron, engeo i kingbo dla szczura białego. Prowadzono również badania *in vivo* nad wpływem pestycydów na niektóre przemiany biochemiczne w organizmie testowanych zwierząt. Ustalone średnie dawki letalne następujących pestycydów: chlorosan, feroban, engeo i cytron wynosiły odpowiednio 140,8, 264,0, 281,5 i 352,0 mg/kg masy ciała. W przypadku preparatu kingbo ustalona średnia dawka letalna przekraczała 160 mg/kg masy ciała szczura. U zwierząt poddanych działaniu pestycydów stwierdzono symptomy nadmiernego wydzielania śliny, krwawienie wzmożoną aktywność ruchową, a szczury potraktowane pestycydem kingbo miały zamknięte oczy. Odnotowano również zgon niektórych zwierząt po zastosowaniu dawek pestycydów: chlorosan, feroban, i engeo w różnych odstępach czasu. Wyniki badań wykazały istotny wzrost aktywności transaminaz cytoplazmy (GOT &GTP) i S-transferazy glutationu (GTS). Stwierdzono, że badane pestycydy wyraźnie hamowały acetylocholinesterazy (AChE) po opływie 1 godziny od zastosowania ostatniej dawki. Ponadto u testowanych szczurów wystąpił silny wzrost poziomu kreatyniny i mocznika. Na podstawie uzyskanych wyników wyciągnięto wniosek, że pestycyd chlorosan w największym stopniu wpływał na białe szczury, a dalszej kolejności preparaty feroban i engeo, natomiast pestycyd Kingo wykazał najsłabsze działanie. Do czasu zakończenia doświadczenia aktywność enzymów i czynności nerek u zwierząt potraktowanych pestycydami chlorosan, feroban i engeo nie powróciły do normalnego stanu.