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Persistence of Cypermethrin and Permethrin and their Effects on Rat Blood Hematological Characteristics

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ثبات مبيدي السيبرميثرين و البيرميثرين وتأثيرهما على بعض خواص الدم في الفئران

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خلاصة: تمت دراسة ثبات وتأثير السيبرميثرين و البيرميثرين على بعض خواص الدم في ذكور الفئران بعد سقيها مرة واحدة بأي من المبيدين بمعدل ١٠ مليجرام /كيلوجرام من وزن الجسم ، وبينت النتائج أن أعلى مستوى لمتبقيات المبيد في الدم كانت ٢,٣٤٨ ميكروجرام / مل للسيبرميثرين و ٨,٨٣ ميكروجرام / مل للبيرميثرين بعد ٢٤ ساعة من المعاملة. انخفض مستوى السيبرميثرين في الدم بعد أسبوعين من المعاملة إلى ٠,٠١٢٦ ميكروجرام / مل ولم يلاحظ وجود تركيز للبيرميثرين في الدم في نفس الفترة ، وأوضحت النتائج أيضاً انخفاض عدد كريات الدم الحمراء و حجم الخلايا المضغوطة (PCV) ، وكانت أكبر قيم للانخفاض عند الساعة ٥٠٤ و ٣٣٦ على التوالي. كان لمبيد البيرميثرين تأثيراً أكبر على عدد كريات الدم البيضاء عن السيبرميثرين خاصة بعد ٨٠ أو ١٦٨ أو ٥٠٤ ساعات من السقي. ولم يكن هناك تأثير لأي من المبيدين على تركيز الهيموجلوبين في الدم.

ABSTRACT: Persistence and biochemical characteristics of cypermethrin and permethrin in the blood of male rats were studied following oral administration of a single dose containing 10 $mg \cdot kg^{-1}$ of body weight of each pyrethroid. The highest residue concentrations of cypermethrin (2.38 $\mu g \cdot ml^{-1}$) and permethrin (8.83 $\mu g \cdot ml^{-1}$) in rat blood tissue were detected 24 h after treatment. After two weeks cypermethrin residues decreased to 0.013 $\mu g \cdot ml^{-1}$, whereas permethrin was not detected. Both pyrethroids decreased the red blood cell (RBC) count, and packed cell volume (PCV). The greatest reduction in RBC count and PCV occurred at 504 h and 336 h respectively. Moreover, the result of post treatment white blood cell (WBC) count was influenced more by permethrin than by cypermethrin, especially after 80 h, 168 h and 504 h of treatment. However, the blood haemoglobin concentration in treated rats was not affected by either pyrethroid.

Pyrethroids are broadly recognized as the fourth major class of synthetic organic insecticides. Since the commercial production of the first photostable pyrethroids in 1976, this group of compounds has achieved world wide use with widespread agricultural applications. Of the major insecticide classes, the pyrethroids as a group are amongst the most potent. However, they exhibit comparatively low toxicity to mammals [Elliott 1977]. The combination of these properties give pyrethroids a degree of selectivity that is unique among conventional insecticides. The extremely low application levels required for insecticidal activity, together with the generally low mammalian toxicity of pyrethroids when administered by oral or dermal routes, suggest that systemic poisoning with pyrethroids during spraying or by consumption of contaminated foodstuffs is unlikely.

However, as many of the pyrethroids are potent neurotoxins, misuse or abuse of some of the concentrated commercial preparations could result in poisoning. In one report, a man died three hours after ingesting beans that had been cooked in a preparation containing 10% cypermethrin instead of oil (Poulos *et al.* 1982). The stomach tissue was analyzed post mortem and was found to contain 0.7 g cypermethrin for the whole stomach.

In addition, it is well known that extensive use of pesticides especially pyrethroids, in different environmental phases and agriculture or household uses, could well affect general health. In 1991, Pasqualetti, *et al.* tried to verify the possible association between occupation, toxic substance exposure, and the risk of hematological neoplasias. They found that farmers and industrial workers have a significant risk for hematological malignancies. Moreover, they cited that exposure to pesticides as well as asbestos, aromatic hydrocarbons, fertilizers, mineral oils and radiation, is associated with a significant increase in the risk for malignant diseases. Pluth *et al.* (1996) studied the effects of malathion, a widely used pesticide with high potential for human exposure, on hematological characteristics. Their epidemiological studies suggested that individuals with chronic environmental exposure to pesticides have increased risks of various hematological

malignancies. This work presented the first evidence of an association between malathion exposure and specific mutations in humans. Moreover, Mansee *et al.* (1995) found that cypermethrin and permethrin can affect rat liver enzymes such as AChE (acetylcholienesterase), GST (glutathion-S-transferase), carboxylesterases and ATPase (total, Mg^{2+},Na^{+},K^{+}). The differences between the action of the tested pyrethroids on the activity of these enzymes were believed to be due to the variation in the former's chemical structure (i.e. cyano-group).

The present investigation aims to compare the cyano-pyrethroid, cypermethrin, with the noncyano-pyrethroid, permethrin, for persistence in rat blood using gas-liquid chromatography. Moreover, the effects of both pyrethroids on blood hematological parameters: Hb, RBC, WBC and PCV were also studied. This could be helpful in the field of human exposure to pesticides and risk assessment. Blood was chosen because of its role in partitioning many pesticides and drugs in different tissues and organs.

Materials and Methods

PYRETHROIDS: Cypermethrin[α-cyano-3-phenoxy-benzyl (±)-cis, trans-3-(2,2-dichlorovinyl)2,2-di-methyl cyclopropane-1-carboxylate] with percent purity 94.6%; and permethrin [3-phenoxybenzyl (±)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclo-propane-1-carboxylate] at a purity of 96.8% were obtained from the U.S. Environmental Protection Agency.

EXPERIMENTAL DESIGN: Fifty four male albino rats (5-6 months old) were supplied by the Home Economics Department Culture Laboratory, Faculty of Agriculture (El-Shatby), Alexandria University, Egypt. Animals were housed (3 per cage) and maintained for a 2 wk acclimatization prior to being divided into three groups. Groups 1 and 2 were given a single oral dose (10 mg kg^{-1} body weight) of either cypermethrin or permethrin mixed with corn oil (5 ml·kg^{-1} body-weight), by gastric intubation. Corn oil was used as a standard vehicle for pyrethroids because of its highly lipophilic quality (Anadon *et al.* 1991). Group 3 was injected with 5 ml kg^{-1} body weight of pyrethroid free corn oil. Animals (3 per group) were sacrificed at 3, 24, 80, 168, 336 and 504 h following dosing.

BLOOD SAMPLE EXTRACTION AND CLEAN UP: Blood samples (1 ml) were centrifuged at 14,000 rpm for 20 min. The supernatant was transferred to a separatory funnel (25 ml) and diluted with distilled water (1 ml), prior to being extracted using (3 x 6 ml) acetone-methylene chloride mixture (2:8 v/v). The organic layers were combined and rotary evaporated to dryness, and redissolved in n-hexane (2 ml). Samples of n-hexane (1 ml) were cleaned-up on a silica gel (4 g) glass chromatographic column (1 x 15 cm), pre-washed with a diethylether-n-hexane mixture (2:1, 10 ml), eluted with the same mixture (20 ml), and rotary evaporated to dryness. The residues were redissolved in n-hexane (2 ml) and injected (1-3 μL) using GLC - ECD) for quantitative determination.

GLC OPERATING CONDITIONS: Sample analysis was performed on a Varian (3700) GLC^{63}Ni-ECD equipped with an SE-30 capillary column (30 m x 0.25 mm id), under the conditions outlined in Table 1.

BLOOD HEMATOLOGICAL CHARACTERISTICS: Animal heart blood samples (4 ml) were collected using a heparinized syringe, saving a portion (2 ml) of the sample for serum preparation. Whole blood was analyzed shortly after sample collection for Hb, RBC and PCV.

TOTAL HEMOGLOBIN DETERMINATION: Hemoglobin concentration was determined (three replicates) with a Pye-Unicam (SP-8100) spectrophotometer, at λ_{540} nm, according to Eilers (1967) cyanomethaemoglobin method.

RED BLOOD CELL COUNT: Red blood cells were counted on an AO Bright line hemacytometer, according to Sieverd (1964) using a light microscope at

TABLE 1

GLC Analysis conditions and parameters

Temperature (°C)	Detector :	300
	Injector :	270
	Column :	300
Column	Dimensions :	30m x 0.25 mm
	Type :	Capillary SE-30
Gas	Type :	Free-oxygen N_2
	Flow rate (ml/min) :	1
	Pressure (kg/cm^2) :	1.6
Chart speed (cm/h)		15
Attenuation		$4x10^{-12}$
Splitting ratio		1:100
Detection limit (ng)	Cypermethrin :	0.134
	Permethrin :	0.055
Retention time (min)	Cypermethrin :	6.3
	Permethrin :	5.3
Recovery % (0.05 ppm fortification level)	Cypermethrin :	87.3 ± 5.0
	Permethrin :	84.9 ± 6.2

430x. Blood samples were diluted 200 fold with physiological saline solution (0.9% NaCl) prior to counting.

WHITE BLOOD CELL COUNT: Blood samples were diluted 20 fold with 1% aqueous acetic acid containing traces of leiohaman's stain. The white blood cell count was determined according to Sieverd (1964) with an AO Bright line hemacytometer and a light microscope (100x).

PACKED CELL VOLUME DETERMINATION: Blood samples were centrifuged (4,000 rpm, 20 min) using Wintrobe hematocrit tubes for determination of the PCV.

STATISTICAL ANALYSIS: The data were statistically analyzed according to the method of Cohort Software Inc. (1986).

Results and Discussion

CYPERMETHRIN AND PERMETHRIN PERSISTENCE IN RAT BLOOD: Blood is responsible in part for partitioning many pesticides in different mammalian tissues and organs, hence its selection for monitoring the residual persistence of pyrethroids. Blood recovery values were 87.3% ±5.0 for cypermethrin and 84.9% ±6.2 for permethrin, at a fortification level of 0.05 ppm, for both compounds (Table 1).

Statistical analysis of blood cypermethrin and permethrin residual levels over a 3 h - 14 d period indicated significant differences. Both compounds reached their highest levels (cypermethrin at 2.38 ppm and permethrin at 8.83 ppm), on the first day and were still detectable up to 336 h (cypermethrin) and 168 h (permethrin) (Figure 1). This is supported by Mansee (1992) and Saleh *et al.* (1986) who recorded high residue levels (3-4 ppm) of deltamethrin, cypermethrin and fenvalerate in chickens on the first day of oral treatment, with a sharp decrease to 0.03-0.04 ppm over 336 h. Orinak (1993) found high cypermethrin residues in sheep blood 24 h after oral administration of a single dose (1/10 or 1/20 of LD_{50}).

Shiba *et al.* (1988) studied the metabolism of the pyrethroid insecticide pallethrin in male and female rats at 2 mg kg^{-1} (orally or subcutaneously) by a ^{14}C method. They found maximum ^{14}C levels in blood and other tissues, within 3 h after oral administration. Thereafter the levels decreased rapidly. Anadon *et al.*(1991) studied permethrin toxicokinetics in male rats; after a single oral (460 mg kg^{-1}) and intravenous (46 mg kg^{-1}) doses. They concluded that the elimination half-life and the mean residence time for plasma were 8.7 and 11.3 h, respectively after intravenous; and 12.5 and 17.9 h, respectively, after oral administration.

EFFECTS OF CYPERMETHRIN AND PERMETHRIN ON HEMATOLOGICAL CHARACTERISTICS: Figure 2 presents RBC counts for cypermethrin and permethrin oral treatment (10 mg kg^{-1}) of male rats at different intervals. Statistical analysis indicated significant differences between the control and treatments. No significant difference was observed between treatments which decreased RBC counts. The results agree with those of El-Katib (1986), Qadri *et al.* (1987), Mohamed (1988) and Mansee (1992) who found that synthetic pyrethroids such as cypermethrin, deltamethrin, fenvalerate, permethrin and tralomethrin can decrease RBC in treated animals.

Results of WBC counts of male rats treated with cypermethrin and permethrin (10 mg kg^{-1}), at different intervals are shown in Figure 3. Cypermethrin

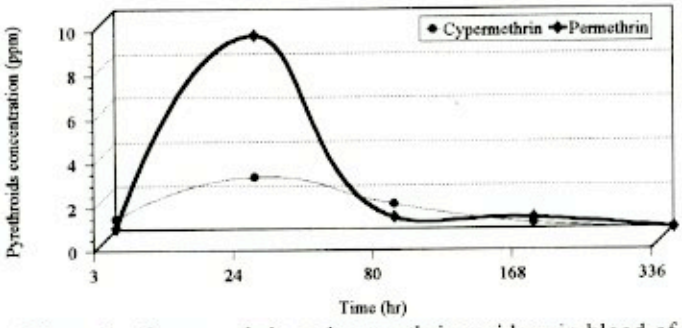

Figure 1. Cypermethrin and permethrin residues in blood of male rats.

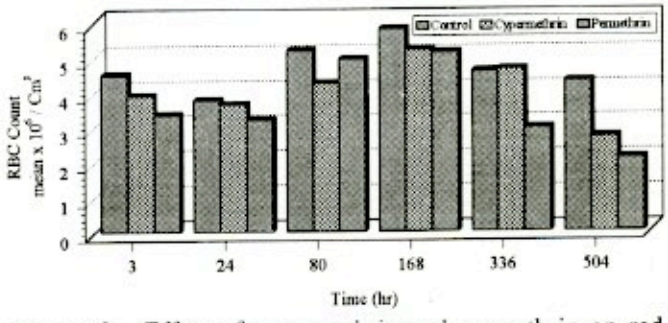

Figure 2. Effect of cypermethrin and permethrin on red blood cell count.

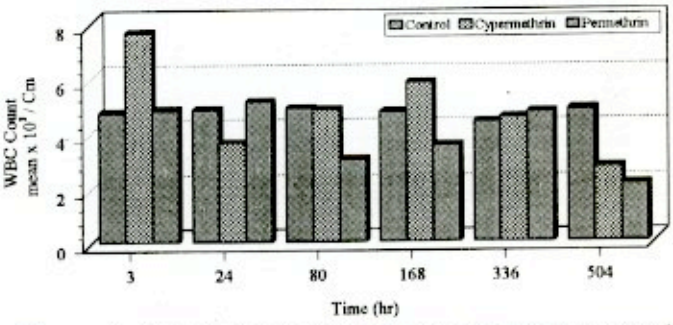

Figure 3. Effect of cypermethrin and permethrin on total white blood cell.

treatment increased WBC counts at 3 and 168 h post-treatment. Decreased levels were observed for the rest of the intervals. Permethrin treatments decreased. WBC count with time. The reduction in WBC count was very sharp for both investigated pyrethroids at the end of the experiment. Mohamed (1988) reported that fenvalerate and decamethrin can increase blood circulating leucocytes following repeated dermal application. Deltamethrin was also reported to increase male rat WBC counts after 3 h and 3 d post treatment, before decreasing (Mansee, 1992). However, another study found that two household synthetic pyrethroids decreased white blood cell counts of treated white mice El-Hendi (1986). Permethrin decreased WBC counts more significantly than cypermethrin treatments (Figure 3). This results is supported by Qadri *et al.* (1987) who found that permethrin sub-acute oral doses affects chicken biochemistry (e.g. WBC, RBC, Hb) more strongly than cypermethrin.

The effect of cypermethrin and permethrin on rat blood hemoglobin concentration (g 100 ml^{-1}), was evaluated at 10 mg kg^{-1} oral dose and at different intervals (Figure 4). Both investigated pyrethroids, slightly decreased the Hb level, with no significant differences between them. However, Mansee (1992) reported that deltamethrin and tralomethrin significantly decreased hemoglobin levels in treated male rats. Furthermore, Qadri *et al.* (1987) in hematoxicity studies on chicken treated with technical and formulated synthetic pyrethroid esters (cypermethrin and permethrin) concluded that permethrin sub-acute oral doses had the greatest effect on hemoglobin content. On the other hand, El-Hindi (1986), reported that white mice hemoglobin content was significantly affected by two synthetic household pyrethroid brands.

Packed cell volume, as a parameter for determining red blood cell volume was studied. Figure 5 shows the effect of a single dose (10 mg kg^{-1}) of cypermethrin and permethrin on male rats. Blood PCV was determined at different intervals. Both compounds significantly decreased PCV percentages as compared to the control. No significant differences between

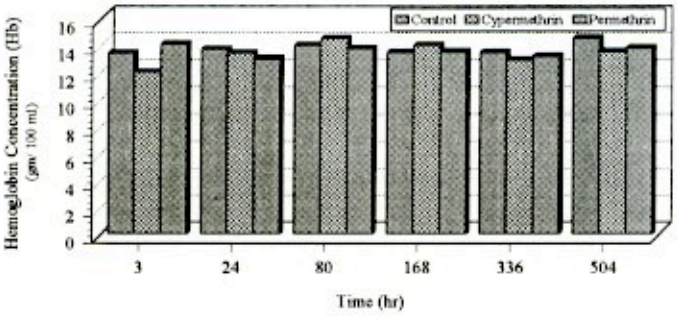

Figure 4. Effect of cypermethrin and permethrin on hemoglobin concentration.

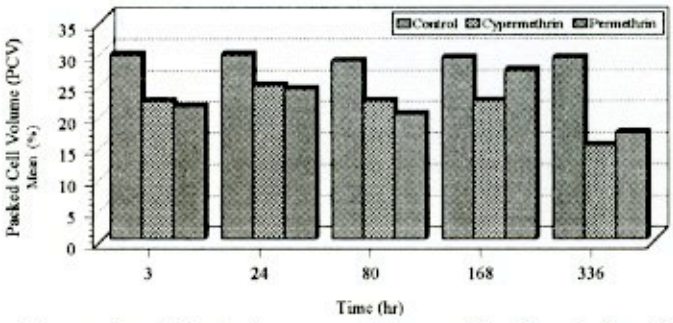

Figure 5. Effect of cypermethrin on blood packed cell volume of male rats.

cypermethrin and permethrin treatments were found. A significant decrease in PCV level on animals treated with two synthetic household pyrethroids was found in a study by El-Hendi (1986). However, Mansee (1992) found that deltamethrin and tralomethrin had no effect on PCV in male rats.

In conclusion, cypermethrin and permethrin reached their maximum concentration in blood after 24 h of treatment. Both compounds decreased RBC, WBC and PCV values up to 504 h post treatment, and had no effect on hemoglobin concentrations. Finally it is not sufficient to assay only for pesticide residues in, for example, the blood of farmers or industrial workers. Changes in blood profile such as RBC, WBC, Hb and PCV should also be investigated.

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