

Subacute neurobehavioral effects of dermally-applied alphacypermethrin in rats

O. YAVUZ^{1*}, H. KAYIR², I. YILMAZ³, G. ULUSOY², G. CAKIR⁴, I. TAYFUN UZBAY²

¹Department of Pharmacology-Toxicology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, TURKEY.

²Department of Medical Pharmacology, Psychopharmacology Research Unit, Gulhane Military Medical Academy, Ankara, TURKEY.

³Izmir Education and Research Hospital, Izmir, TURKEY.

⁴Department of Biology, Faculty of Science and Literature, University of Ankara, Ankara, TURKEY.

*Corresponding author: oguzhany@omu.edu.tr

SUMMARY

Because of the common usage for the synthetic pyrethroid alphacypermethrin in agriculture, public health and veterinary medicine, the objective of this study was to determine the potential neurobehavioral effects of dermally-applied alphacypermethrin in rats for 28 days. A total of 40 adult Wistar rats were randomly divided into four groups (5 females and 5 males in each group): only vehicle (70% alcohol) was applied to the control group whereas the 3 other groups were treated with 100, 200 and 300 mg/kg b.w pyrethroid, respectively. Mortality and clinical signs were observed daily while locomotor activities, pain sensitivity, motor coordination, catatonia, body weights, food consumptions and open-field assessments (counts of urination and defecation, agitation, stereotypy) were measured weekly. At this end of experimental period, haematological parameters were assessed and all animals were necropsied. Rats dermally treated with 100 or 200 mg/kg α -cypermethrin exhibited neither clinical and behavioural alteration nor haematological change or macroscopic lesion. However, alphacypermethrin at the highest dose (300 mg/kg) induced motor incoordination in males and decrease of pain sensitivity in females. These data emphasizes the interest of neurobehavioral tests for risk assessment of dermal alphacypermethrin application.

Keywords: Alphacypermethrin, neurobehavioral changes, dermal application, rat.

RÉSUMÉ

Induction d'effets neuro-comportementaux par une application transcutanée d'alphacyperméthrine chez le rat

En raison de l'usage très répandu de l' α -cyperméthrine (pyréthroïde de synthèse) en agriculture, santé publique et médecine vétérinaire, l'objectif de cette étude a été de déterminer l'éventuelle induction d'effets neuro-comportementaux par des applications transcutanées d' α -cyperméthrine pendant 28 jours chez le rat. Quarante rats adultes Wistar ont été aléatoirement répartis en 4 groupes de 5 femelles et 5 mâles chacun : l'excipient (alcool 70 %) seul a été appliqué sur les rats du groupe contrôle alors que les 3 autres groupes ont été respectivement traités par 100, 200 et 300 mg/kg d' α -cyperméthrine. En plus d'un examen clinique quotidien et de l'enregistrement journalier de la mortalité, les activités locomotrices, la sensibilité à la douleur, la coordination motrice, la catatonie, le poids corporel, l'ingéré alimentaire et les comportements de plein air (nombre de mictions et de défécations, agitation et stéréotypie) ont été mesurés toutes les semaines. A la fin de l'expérimentation, un examen hématologique a été effectué et tous les animaux ont été autopsiés. Les rats traités par voie transcutanée par 100 ou 200 mg/kg d' α -cyperméthrine n'ont montré aucun signe clinique et hématologique, aucune altération comportementale et aucune lésion macroscopique. En revanche, la plus forte dose d' α -cyperméthrine (300 mg/kg) a induit une incoordination motrice chez les mâles et une diminution de la sensibilité à la douleur chez les femelles. Ces résultats soulignent l'intérêt des tests neuro-comportementaux dans l'évaluation du risque lié à l'application transcutanée de l' α -cyperméthrine.

Mots clés : Alphacyperméthrine, changements neuro-comportementaux, application transcutanée, rat.

Introduction

Synthetic pyrethroids are valuable pesticides with differing stability to light, low volatility, high insecticidal potency and low toxicity to mammals under normal conditions [24]. Cypermethrin and alphacypermethrin are highly active synthetic pyrethroid insecticides, which are effective against a wide range of pests in agriculture, public health and animal husbandry [22, 23]. Alphacypermethrin is the most biologically-active *cis*-isomers from the eight isomers present in cypermethrin. In veterinary medicine, alphacypermethrin is applied topically as a spray or pour-on to cattle and sheep, and as a spray to poultry for the control of ectoparasites such as ticks, fleas, lice and blowflies [2, 22].

Similar to other synthetic pyrethroids, alphacypermethrin is a neurotoxic compound acting on the axons in the peripheral and central nervous system by interacting with sodium channels

in mammals and insects [23]. It is a type II synthetic pyrethroid and causes a long-lasting prolongation of the normally transient increase in sodium permeability of the nerve membrane during excitation, resulting in long-lasting trains of repetitive firing. The presence of α -cyan group is considered to be responsible for these effects [2].

Today, there are different alternatives to the determination of the effects of chemical compounds on the nervous system, but neurobehavioral tests are accepted as the first step of neurotoxicity studies. Several international groups have recommended that neurobehavioral tests may be included in the initial stages of hazard identification. Also, regulatory agencies have stipulated these tests for market approval of environmental and pharmaceutical chemicals. In addition, neurobehavioral tests have been used to set exposure limits for animals and humans in the workplace [15, 17].

Many previously studies have been conducted to evaluate the neurobehavioral effects of pyrethroids. However, cypermethrin has been used more than its other isomers. Also, insecticides have been mainly administrated by oral route in these studies [2, 11, 12, 16, 22, 23]. But, dermal absorption of pesticides is very important in human and animals. Occupational poisonings occur in humans, due to dermal exposure of alphacypermethrin in activities such as preparation of the working solution, spraying and washing of equipment [9, 10].

The aim of this study was to evaluate the neurobehavioral effects of dermally-applied alphacypermethrin in rats for 28 days. Neurobehavioral evaluations were conducted daily or weekly by analysing motor activity, body weight changes, food consumptions, motor coordination, catatonia, pain sensitivity and other clinical observations such as degree of lacrimation and salivation, changes in the skin, presence or absence of piloerection and exophthalmia, abnormal posture, incidence and severity of any convulsions, tremors, abnormal motor movements, red or crusty deposits around the eyes, nose or mouth, counts of urination and defecation, agitation and stereotypy.

Materials and Methods

ANIMALS

A total of 40 young adult (62 day old in average) Wistar rats (20 females and 20 males) were used in the study. They were housed separately in metal cages. The animal room was maintained at $22 \pm 2^\circ\text{C}$, $60 \pm 5\%$ relative humidity and 12:12 h light-dark cycle. Food and water were available *ad libitum*. The experimental protocol was approved by the Gulhane Military Medical Academy Ethical Committee of Animal Experiments.

EXPERIMENTAL DESIGN

The study was designed according to the EPA Health Effect Test Guidelines, OPPTS 870.6200, Neurotoxicity Screening Battery [3] and the European Community Toxicity Test Methods, Repeated Dose (28 Days) Toxicity (Dermal) [4] with some deviations.

Technical grade alphacypermethrin (95% pure, Heranba Industries, India) was used in the study. The animals were randomly divided into four groups ($n = 10$ (5 females and 5 males) for each group). Three doses of alphacypermethrin (100, 200 and 300 mg/kg bw) were dermally applied in three experimental groups (groups A, B and C, respectively) and only vehicle (70% ethyl alcohol) was applied in the control group (group D). The intermediate dose corresponded to the 1/10 of LD₅₀. Alphacypermethrin and vehicle were applied on the dorsal side of animals for seven days per week for a period of 28 days. The time of exposure was six hours per day.

The animals were kept under the experimental housing and feeding conditions for five days prior the test. The clinical and neurobehavioral changes were observed daily and weekly during the study. Mortality, degree of lacrimation and salivation, changes in the skin, presence or absence of piloerection and exophthalmia, abnormal posture, incidence and

severity of any convulsions, tremors, abnormal motor movements, red or crusty deposits around the eyes, nose or mouth were observed daily. Measurements of locomotor activities, pain sensitivity, motor coordination and catatonia, and determination of body weights and food consumptions were made weekly (on every Friday). Also, counts of urination and defecation, agitation, stereotypy were observed during the open-field assessments weekly. Stereotypic behaviours, abnormal posture and agitation were scored using a rating scale as previously described [19]. All behavioural experiments were carried out during the light period (at 10.00-11.00 a.m.). The experimenter, who was done all ratings, was blind to treatments.

After the treatment, the animals were anesthetized with intramuscular ketamine (60 mg/kg) (Alfamine 10%, Ege-Vet, Turkey) and heart blood was collected in tubes with anticoagulant (heparin) and red blood cell (RBC) count, white blood cell (RBC) count, hematocrit (Ht) and haemoglobin (Hb) concentrations were measured. After the decapitation, gross necropsy was performed. Brains were collected and weighted immediately.

NEUROBEHAVIOURAL TESTS

Locomotor activity

Locomotor activities of the rats were measured with an open-field activity monitoring system (MAY 9908&0107 model -Activity Monitoring System-Commat, Ankara, Turkey). This system had eight Plexiglas cages (42x42x30 cm³) equipped with infrared photocells. Interruptions of photocell beams were detected by a computer system and place of animals was calculated by the software at 0.1 s sensitivity. Locomotor activities of all the animals were recorded five minutes weekly and were calculated as the sum of horizontal, ambulatory and vertical activities [8, 20, 21].

Motor coordination

The motor performance of the rats was evaluated in the Rotarod / Accelerod apparatus (Rotamex V-EE/85, Columbus, OH, USA). The apparatus contained a cylinder 6.5 cm in diameter and was rotated with a pre-selected speed. Four animals could be tested simultaneously in this apparatus. Before the experiment, the speed of rotation was stable and set to 20 revolutions per minute (rpm). Animals remained on the rod until they fell down or after 10 min elapsed. When falling off the rod, rats came into contact with a metal grid beneath them kept under a mild electrical voltage. No measurements were taken on this day. In the experiment, the speed of the rod was linearly increased from 0 to 60 rpm within 10 min. The time at which rats remained on the rod was automatically measured in seconds by built-in timer of the apparatus [1, 21].

Pain sensitivity

The hot-plate test was used for the determination of pain sensitivity. Each rat was placed in a glass beaker on a hot-plate analgesia meter (Commat, Ankara, Turkey). The hot-plate apparatus was maintained at 52°C and latency to flinch or raise

hind paws was recorded. Cut-off time of the experiment was 180 s [21].

Catatonnia

Catatonnia was evaluated by the vertical wire test. A 15 s immobilisation on the vertical wire was regarded as catatonnia [20].

DATA ANALYSIS

Locomotor activity was evaluated by a two-way analysis of variance (ANOVA) for repeated measures (group x time), followed by Tukey's test for post-hoc comparisons. Pain sensitivity, motor coordination, catatonnia, body weight changes, food consumptions, counts of urination and defecation, agitation and stereotypy values in each week and blood parameters and relative brain weights were analysed by one-way ANOVA, followed by Tukey's and Dunnett tests. All data were expressed as mean \pm standard error (SEM). Differences were considered as significant when P values were less than 0.05.

Results

BODY WEIGHTS, FOOD CONSUMPTION AND CLINICAL OBSERVATIONS

All alphacypermethrin treated rats and controls have slightly put on weight (Table I) during the 28 days experimental period

and body weight gains were comparable between groups. Similarly, food consumption tended to weakly increase during the experimental period for all treated and untreated rats and no significant difference was evidenced between groups.

Only one animal died in group C (300 mg/kg) on Day 11 after exhibiting haematuria since the 6th day. Moreover, uroliths were evidenced in the necropsy. This animal was not evaluated in the neurological study. In addition, any important clinical finding (increased lacrimation and salivation, changes in the skin, piloerection, exophthalmia, abnormal posture, convulsion, tremor, abnormal motor movement, red or crusty deposits around the eyes, nose or mouth) was not observed in the daily clinical observations.

Furthermore, there was no statistically significant difference in red blood cell (RBC) and white blood cell (WBC) counts, in hematocrit and in haemoglobin concentrations between alphacypermethrin treated and control rats at the end of the 28 day whole experimental period (Table II).

NEUROBEHAVIOURAL FINDINGS

Locomotor activity

As revealed by a two-way ANOVA test, locomotor activities of male and female rats were decreased in course of time [For males: $F(3.48) = 47.412$; $p < 0.0001$; for females: $F(3.48) = 6.807$; $p = 0.001$; Figure 1]. But, no significant difference was determined between the experimental and

Rats	Time (week)	Groups			
		Control (vehicle)	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (300 mg/kg)
Body weights (g)					
Males	0	278.2 \pm 15.5	287.2 \pm 11.7	299.6 \pm 12.0	295.8 \pm 11.3
	1	285.4 \pm 19.2	291.2 \pm 11.5	307.2 \pm 13.7	295.2 \pm 14.4
	2	293.2 \pm 20.5	300.8 \pm 14.2	317.6 \pm 14.0	311.0 \pm 19.1
	3	297.2 \pm 20.7	306.4 \pm 14.0	323.6 \pm 12.5	306.5 \pm 22.4
	4	303.4 \pm 20.4	314.0 \pm 13.9	329.0 \pm 11.7	307.3 \pm 23.2
Females	0	237.4 \pm 4.1	220.2 \pm 8.3	235.6 \pm 3.2	212.4 \pm 7.1
	1	233.6 \pm 4.6	215.6 \pm 7.9	235.6 \pm 4.0	212.4 \pm 7.4
	2	233.2 \pm 6.7	220.4 \pm 8.8	242.8 \pm 2.7	216.8 \pm 7.7
	3	229.2 \pm 3.7	220.0 \pm 8.0	244.8 \pm 3.3	215.6 \pm 7.4
	4	239.0 \pm 3.7	223.2 \pm 7.8	247.8 \pm 3.6	218.8 \pm 7.6
Food Consumption (g)					
Males	1	153.0 \pm 15.8	144.6 \pm 6.9	154.8 \pm 5.6	151.6 \pm 10.6
	2	151.4 \pm 9.9	156.8 \pm 11.3	171.2 \pm 3.3	156.8 \pm 10.0
	3	116.2 \pm 10.9	124.0 \pm 14.6	148.4 \pm 4.7	134.3 \pm 8.4
	4	162.2 \pm 17.0	177.0 \pm 17.9	206.2 \pm 10.1	168.5 \pm 20.8
Females	1	82.8 \pm 8.1	92.4 \pm 6.3	103.8 \pm 3.5	89.4 \pm 10.0
	2	107.8 \pm 5.4	115.2 \pm 4.3	115.2 \pm 3.8	109.4 \pm 9.0
	3	82.4 \pm 4.3	69.8 \pm 5.6	99.0 \pm 7.2	91.4 \pm 14.3
	4	101.6 \pm 7.5	104.0 \pm 7.2	121.2 \pm 4.3	97.4 \pm 10.7

TABLE I : Body weights (g) and food consumption (g) of male and female rats dermally treated with various doses of alphacypermethrin (100, 200 and 300 mg/kg) or to vehicle for 28 days.

Haematological parameters	Sex	Groups			
		Control (vehicle)	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (300 mg/kg)
<i>RBC</i> ($10^{12}/l$)	Males	9.38 \pm 0.26	9.36 \pm 0.53	8.87 \pm 0.32	9.12 \pm 0.24
	Females	7.98 \pm 0.61	8.73 \pm 0.53	8.49 \pm 0.57	8.89 \pm 0.60
<i>WBC</i> ($10^9/l$)	Males	7.92 \pm 3.32	9.52 \pm 1.68	6.25 \pm 3.44	8.12 \pm 1.86
	Females	3.36 \pm 0.88	5.24 \pm 5.67	4.91 \pm 3.08	5.75 \pm 3.11
<i>Ht</i> (%)	Males	54.33 \pm 1.96	53.85 \pm 2.87	50.50 \pm 1.24	53.55 \pm 1.54
	Females	46.20 \pm 4.26	50.30 \pm 1.69	48.98 \pm 4.39	53.10 \pm 1.87
<i>Hb</i> (g/L)	Males	159.8 \pm 4.3	159.5 \pm 8.6	151.5 \pm 3.0	159.8 \pm 10.2
	Females	141.8 \pm 8.0	152.5 \pm 5.4	150.3 \pm 7.1	161.5 \pm 7.3

TABLE II : Red Blood Cell (RBC) and White Blood Cell (RBC) counts, hematocrit (Ht) and haemoglobin (Hb) concentrations in male and female rats dermally treated with various doses of alphacypermethrin (100, 200 and 300 mg/kg) or to vehicle at the end of the 28 day experimental period.

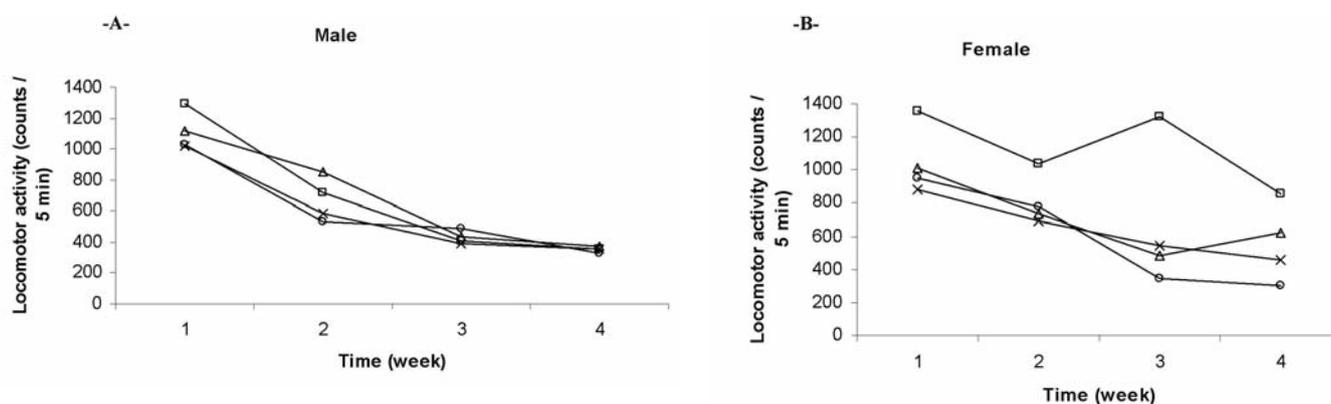


FIGURE 1 : Locomotor activities of male and female rats. Dermal alphacypermethrin administration (100, 200 and 300 mg/kg) decreased locomotor activity in all the groups, but no significant difference was determined between the groups ($p > 0.05$). [Δ : Control (vehicle); x: Group A (100 mg/kg); \square : Group B (200 mg/kg); o: Group C (300 mg/kg)].

control groups in male and female animals [For males: $F(3.16) = 0.489$; $p = 0.695$ and for females: $F(3.16) = 1.873$; $p = 0.175$]. In addition, no significant interaction between groups and time was found [For males: $F(9.48) = 0.829$; $p = 0.593$ and for females: $F(9.48) = 0.8359$; $p = 0.587$].

Motor coordination

The Rotarod / Accelerod test showed that mean latency of male rats exposed to the highest dose (300 mg/kg) of alphacypermethrin decreased in all times (Table III) and mainly in the 3rd and 4th weeks. At these times, differences with healthy controls became statistically significant ($p < 0.05$). The treated females of the group C (300 mg/kg) also presented a greater mean latency than control females but differences were not statistically different.

Pain sensitivity

Despite a great heterogeneity of the pain sensitivity in the hot plate test, a significant increase of this parameter was recorded for females dermally treated with 300 mg/kg of

alphacypermethrin compared to female controls on week 4 ($p < 0.05$) (Table IV) whereas this behavioural response remained comparable between treated and control males for all times.

Catatonnia

As an immobilisation for 15 seconds on the vertical wire was not observed in any animals during the study, all catatonnia measurements were evaluated negative in all groups of male and female rats.

Other open-field behavioural observations

Firstly, the stereotypic behaviour was similar in all the groups of male and female rats (data not shown). Secondly, counts of urination and defecation did not significantly differ between treated and untreated male and female rats for the whole experimental period.

The agitation scores significantly decreased according to time especially in control group and in group C (300 mg/kg)

Rats	Time (week)	Groups			
		Control (vehicle)	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (300 mg/kg)
Males	0	100.8 ± 10.7	114.6 ± 26.2	88.6 ± 12.6	109.0 ± 17.9
	1	110.2 ± 25.6	110.4 ± 20.2	96.4 ± 24.0	94.8 ± 26.2
	2	105.0 ± 22.9	118.2 ± 30.0	74.8 ± 20.5	67.5 ± 13.5
	3	110.8 ± 17.3	125.4 ± 20.5	127.2 ± 32.3	36.0 ± 9.5*
	4	92.0 ± 19.0	67.6 ± 15.0	122.4 ± 34.9	58.0 ± 17.6*
Females	0	137.0 ± 12.6	122.8 ± 14.0	103.0 ± 19.6	155.6 ± 32.1
	1	147.6 ± 18.8	150.0 ± 16.6	146.8 ± 17.6	117.0 ± 33.5
	2	142.2 ± 14.6	109.6 ± 12.8	103.4 ± 14.8	67.5 ± 13.5
	3	167.0 ± 24.3	166.4 ± 9.0	148.0 ± 23.7	80.6 ± 33.7
	4	176.2 ± 14.5	139.8 ± 20.2	121.4 ± 25.9	88.4 ± 28.3

*Statistically different from the control group ($p < 0.05$).

TABLE III : Mean latency (second) in the Rotarod / Accelerod apparatus of male and female rats dermally treated with various doses of alphacypermethrin (100, 200 and 300 mg/kg) or to vehicle for 28 days.

Rats	Time (week)	Groups			
		Control (vehicle)	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (300 mg/kg)
Males	0	71.16 ± 5.01	80.76 ± 15.03	63.02 ± 4.86	109.0 ± 17.9
	1	63.84 ± 5.28	67.86 ± 7.81	65.78 ± 8.74	94.8 ± 26.2
	2	74.22 ± 10.07	84.02 ± 16.78	66.42 ± 8.97	67.5 ± 13.5
	3	55.72 ± 5.80	57.98 ± 6.74	52.06 ± 3.45	36.0 ± 9.5*
	4	62.90 ± 6.64	63.12 ± 2.16	55.68 ± 8.63	58.0 ± 17.6*
Females	0	118.60 ± 18.65	99.74 ± 10.69	97.00 ± 10.63	115.82 ± 22.27
	1	87.70 ± 28.49	62.34 ± 4.71	79.16 ± 13.79	76.58 ± 15.40
	2	90.22 ± 14.60	62.80 ± 14.07	90.64 ± 20.68	108.70 ± 12.52
	3	77.74 ± 21.99	70.42 ± 10.85	76.98 ± 14.77	77.34 ± 13.43
	4	57.10 ± 7.67	83.30 ± 9.90	93.32 ± 25.50	95.22 ± 15.63*

*Statistically different from the control group ($p < 0.05$).

TABLE IV : Pain sensitivity (mean staying duration in the hot plate, second) of male and female rats dermally treated with various doses of alphacypermethrin (100, 200 and 300 mg/kg) or to vehicle for 28 days.

($p < 0.05$). At the beginning, the scores were 1.20 ± 0.84 and 2.00 ± 0.00 for male and female controls and respectively declined to 0.00 ± 0.00 for males and to 0.20 ± 0.45 for females at the end of the study. Consequently, the reduction of the agitation scores was evaluated to be habituation to the test and not as a depression finding. Similar findings were obtained for the group C: 1.60 ± 0.55 for males and 1.80 ± 0.45 for females at the beginning and 0.20 ± 0.15 and 0.10 ± 0.0 for males and females, respectively in the last observation (Week 4).

NECROPSY AND BRAIN WEIGHTS

Any morphology change of internal organs was observed during necropsy for all treated and control male and female rats. Although the relative brain weights [(absolute brain weight/body weight) x 100] tended to increase in the 3 treated

groups, the differences with male and female controls were not statistically significant (figure 2).

Discussion

The acute oral LD₅₀ values of alphacypermethrin were reported to be 79 – 400 mg/kg bw (in corn oil, value depending on concentration) in rats [18]. Alphacypermethrin-induced clinical signs are typical of α cyan-containing (Type II) pyrethroid. The clinical signs of intoxication are characterised by choreoathetosis (muscular spasm and tremors in the extremities) and increased salivation. The observed signs included ataxia, abasia (inability to walk caused by lack of muscular coordination), gait abnormalities, tip-toe walking, lacrimation, piloerection, tremor and clonic convulsions [2, 14, 23, 26], these clinical signs being observed in alphacypermethrin-exposed rats to oral doses of 20 and 40 mg/kg for 14 and 28

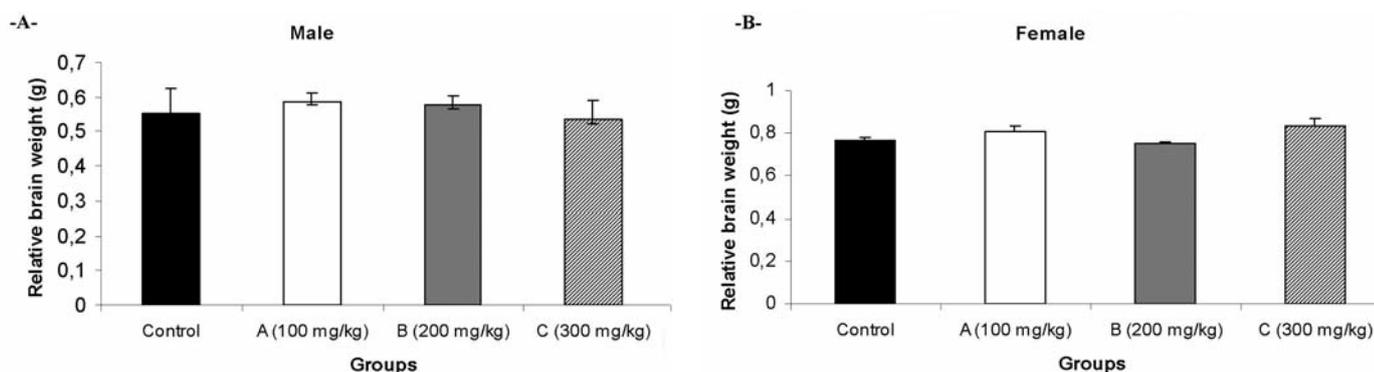


FIGURE 2 : Relative brain weights (g) [(absolute brain weight (g)/body weight (g) x100] of male (A) and female (B) rats dermally treated with various doses of alphacypermethrin (100, 200 and 300 mg/kg) or to vehicle for 28 days.

days [2, 22, 23]. MANNA *et al.* [12] reported that alphacypermethrin produced signs of CNS stimulation followed by prolonged depression at higher oral doses ranging from 125 to 225 mg/kg.

By contrast, alphacypermethrin exhibits a low dermal toxicity. The dermal LD₅₀ values in rats were reported to be generally higher than 2000 mg/kg bw [5, 18, 23]. Previous studies showed that no deaths or signs of intoxication were observed in rats and mice receiving a single 24-h dermal exposure of 500 mg/kg bw (25% in DMSO) and 100 mg/kg bw (5% in corn oil), respectively [23]. LUTY *et al.* [10] reported that no clinical sign of toxic effect was observed in rats receiving dermally 50 and 250 mg/kg/day bw alphacypermethrin during 4 weeks of observations. Also, LATUSZYŃSKA *et al.* [9] found that the dermal administration of alphacypermethrin in doses of 50 and 250 mg/kg did not lead to changes in the behaviour of the animals in the open field test after two weeks of exposure. After four weeks, only grooming was increased in those rats treated with alphacypermethrin in a dose of 250 mg/kg and slight but not significant decreases of numbers of ambulation, rearing and object exploration were noted. Similarly, in the present study, mortality was not seen (except for 1 animal) and any important clinical finding, such as increased lacrimation and salivation, changes in the skin, piloerection, exophthalmia, abnormal posture, convulsion, tremor, abnormal motor movement, red or crusty deposits around the eyes, nose or mouth were not observed in the dose of 100, 200 and 300 mg/kg/day bw during 28 days. Also, any changes for counts of urination and defecation were not observed in all the groups of male and female rats during the study. Orally administrated 800 mg/kg alphacypermethrin produced increased sensitivity to noise in both sexes of rats [23]. Although pyrethroids generally decreased acoustic startle response in experimental animals, cypermethrin increased this behavioural endpoint in some studies [16, 26]. By contrast, in the present study, any agitation and stereotypy findings were not observed in all groups of alphacypermethrin treated male and female rats.

Motor activity, like many behavioural functions, may be altered in both humans and laboratory animals by a wide variety of drugs and toxicants, and changes in motor activity can be used in risk assessment [25]. The effects of nine com-

mercial or experimental pyrethroids, (cismethrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, flucythrinate, fluvalinate, permethrin, and RU 266074), orally administered, on motor activity in rats were compared, and all these compounds caused a dose-dependent decrease in motor activity [16]. WOLANSKY *et al.* [25] determined the relative potencies of 11 pyrethroids (five from the type I: bifenthrin, S-bioallethrin, permethrin, resmethrin, tefluthrin; five from the type II: β -cyfluthrin, λ -cyhalothrin, cypermethrin, deltamethrin, esfenvalerate; and one of mixed type I/II: fenpropathrin), orally administered, in Long-Evans rats by analysing motor activity. Finally, it was found that all pyrethroids tested produced dose-dependent decreases in locomotor activity. Decreases in activity occurred at dosages producing typical pyrethroid signs (e.g., salivation, tremors, and choreathetosis) and at inferior dosages, α -cyan-compounds were determined about 10-fold more potent than non-cyan-compounds in decreasing motor activity. In addition, other laboratories have reported decreases of motor activity following acute oral exposure to permethrin, cyhalothrin, λ -cyhalothrin, fenvalerate, cypermethrin and deltamethrin, using a variety of testing devices [9, 26]. Only very few studies have demonstrated slight increases or no effects on locomotor activity following pyrethroid exposure [7, 16, 26]. In this study also, no effect was observed in motor activity following pyrethroid exposure. Locomotor activities of treated males or females have not significantly differed from those observed in healthy controls whatever the delay after alphacypermethrin exposure. Not only the applied dose but also the route of administration would explain these discrepancies: indeed, dermal application contrary to oral treatment is considered as a low-absorption route of exposure.

In addition to the effects on motor activity, pyrethroids also affect other behaviours that are dependent upon the coordinated movement of the animal. MANNA *et al.* [13] determined that alphacypermethrin at oral dose of 145 mg/kg (LD₅₀) and 14.5 mg/kg (1/10 LD₅₀) produced motor incoordination in the rotarod test in rats. In the present study, the rotarod / accelerod test showed that mean latency of male rats decreased at the highest dose (300 mg/kg) at all times and this decrease was determined to be statistically different from the control group in Weeks 3 and 4. These findings may

be included into the dose-response data about motor coordination and neurotoxicity of alphacypermethrin. Cypermethrin and its isomers caused increased tail flick or hot plate latency at different doses [16, 22, 23]. Also, neuromuscular response was evaluated using grip strength test in various studies, and it was detected that pyrethroids weaken the neuromuscular response in rats [26]. Similarly, increased latency for female rats was observed in the hot-plate test in 300 mg/kg dose in the present study.

Pyrethroids generally caused decrease of body weights and food consumptions at some doses in experimental animals [2, 6, 22, 23]. However, LUTY *et al.* [10] reported that dermally applied 50 and 250 mg/kg alphacypermethrin did not cause a clear difference in the increase of the body mass in rats during 4 weeks. In our study, in agreement to the study of LUTY *et al.* [10], no difference between groups was found in the body weight changes and food consumption patterns during the 28-days exposure period. Cypermethrin and alphacypermethrin caused some changes in haematological parameters, such as anaemia characterized by decreases of haemoglobin concentration, MCV (mean corpuscular volume) and MCHC (mean corpuscular haemoglobin concentration) [2, 11, 12, 23]. MANNA *et al.* [12] found that alphacypermethrin decreased the PCV (packed cell volume), haemoglobin concentrations and counts of erythrocytes, leukocytes and monocytes, whereas increased neutrophil count was observed after daily oral administration of 14.5 mg/kg (1/10 LD₅₀) alphacypermethrin in rats for 30 days. In the present study, dermally-applied alphacypermethrin did not significantly affect blood parameters at the administered doses.

Although MANNA *et al.* [12] reported that 4.5 mg/kg (1/10 LD₅₀) of oral alphacypermethrin administration for 30 days produced bloated stomach with severe haemorrhages in gastrointestinal tract and also in lungs, LUTY *et al.* [10] did not observe any morphological change in any internal organs after dermally applied 50 and 250 mg/kg alphacypermethrin in rats during 4 weeks. In the same way, no macroscopic anomaly consecutive to 100 – 300 mg/kg alphacypermethrin dermal application was detected in the present study. Moreover, increases of relative organ weights are generally expected in pesticide-applied animals: alphacypermethrin induced increases of the relative brain, liver and kidney weights in male and female rats at oral dose of 800 mg/kg, and brain and liver relative weights were also enhanced in males orally treated with 400 mg/kg [23]. In this study, although the relative brain weights tended to increase in all dermally exposed rats, differences with controls were not significant.

In conclusion, the present data demonstrate that dermal application of 100 and 200 mg/kg alphacypermethrin did not produce significant clinical and behavioural alterations in rats, whereas at a higher dose (300 mg/kg), this pyrethroid caused motor incoordination in males and decrease of pain sensitivity in females. These data may be used for risk assessment of dermal alphacypermethrin application. Future works will be conducted to evaluate alphacypermethrin-induced behavioural alterations accordingly the dose and the route of pyrethroid administration.

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