

BEHAVIOR OF RATS AFTER PERINATAL EXPOSURE TO PLASTIC COLLAR IMPREGNATED WITH DICHLORVOS

COMPORTAMENTO DE RATOS APÓS EXPOSIÇÃO PERINATAL À COLEIRA PLÁSTICA IMPREGNADA COM DICLORVÓS

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SUMMARY

The use of plastic collars impregnated with the organophosphate dichlorvos is a measure taken in veterinary medicine to control ectoparasites in dogs and cats. The aim of this study was to determine the effects of the use of plastic collars impregnated with this product (8.37%) in Wistar rats during the periods of pregnancy and lactation, as a cause of behavioral alterations in the offspring. No statistically significant differences were detected in the variables examined in open-field behavior for pups whose mothers were exposed to dichlorvos during pregnancy. However, when exposure to the pesticide was during the lactation period, the pups showed prolonged immobility and decrease in the frequencies of grooming and urination, suggesting alterations in behavior. Although tests with the elevated T maze did not demonstrate any alteration suggesting an effect of the treatments on the anxiety of the animals, the results obtained in the evaluation of learning and memory, in the Morris water maze, revealed alterations in the female pups whose mothers were exposed to dichlorvos during the periods of pregnancy and lactation. This study showed that perinatal exposure of mothers to dichlorvos caused alterations in the behavior of offspring in open field and Morris water maze tests.

KEY WORDS: 2,2 - Dichlorovinyl dimethyl phosphate. Flea collar. Open field. Elevated T maze. Morris water maze.

RESUMO

O organofosforado diclorvós impregnado em coleiras plásticas é um recurso utilizado em medicina veterinária para o controle de ectoparasitas de cães e gatos. O objetivo deste trabalho foi avaliar os efeitos do uso de coleiras plásticas impregnadas com este produto (8,37%) em ratos Wistar durante os períodos de gestação ou lactação, como fonte de alterações comportamentais nos filhotes. Não se detectou diferenças estatisticamente significativas nas variáveis avaliadas no campo aberto, quando se analisou filhotes cujas mães foram expostas ao diclorvós durante o período de gestação. No entanto, quando a exposição ao praguicida ocorreu durante a fase de lactação, observou-se nos filhotes aumento do tempo de imobilidade e diminuição nas frequências de auto-limpeza e micção, sugerindo alterações de caráter emocional. Embora a avaliação realizada com o labirinto em cruz elevado não ter demonstrado qualquer alteração que sugerisse efeito dos tratamentos sobre a ansiedade dos animais, os resultados obtidos na avaliação do aprendizado e memória, no labirinto aquático de Morris, revelaram alterações nos filhotes fêmeas em virtude da exposição das mães ao diclorvós durante os períodos de gestação e lactação.

Estes estudos sugerem que a exposição perinatal das mães ao diclorvós causou alterações no comportamento dos filhotes testados no campo aberto e no labirinto aquático de Morris.

PALAVRAS-CHAVE: 2,2 - Diclorovinil dimetil fosfato. Coleira antipulga. Campo aberto. Labirinto em cruz elevado. Labirinto aquático de Morris.

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INTRODUCTION

2,2-Dichlorovinyl dimethyl phosphate (DDVP), known as dichlorvos, is an organophosphate with strong pesticide activity, which is utilized as an insecticide in prolonged release formulations, such as in impregnated polyvinyl chloride (PVC) collars. Dichlorvos has been produced commercially since 1961 and utilized in the control of flies, mosquitoes, cockroaches, fleas and ticks, among other pests (WHO/IPCS, 1989). Controlled experiments with collars impregnated with dichlorvos have demonstrated the effectiveness of this preparation in combating fleas in dogs and cats (KIBBLE, 1968).

Although there have been scientific reports of the efficacy of dichlorvos as an anti-parasite, little is known about its potential to cause neurobehavioral deficits due to its interference with cerebral development. Experiments examining behavioral variables in the offspring of female rats given dichlorvos orally, with 8.0 mg/kg DDVP or its vehicle (1 ml/kg) from the 6th to the 15th day of pregnancy, have demonstrated motor alterations in juvenile and adult offspring, concluding that pre-natal exposure, even at low doses of DDVP, reduce exploratory activity in young animals and motor activity in adult animals (LAZARINI et al., 2004).

There are reports of the effects of experimental dichlorvos on the behavior of rats after acute exposures to high concentrations of the product. But little has been reported about the behavioural effects before a chronic exposure to sublethal concentrations of insecticide. The central nervous system is very sensitive to chemical products, especially in the initial stages of development (SPYKER, 1975). This sensitivity of the developing cerebrum to toxic insult is manifested as behavioral disturbances which are determined by means of behavioral psychology techniques capable of recording and quantifying seemingly immeasurable phenomena such as intelligence, anxiety, learning and memory.

Mazes are widely utilized animal behavior tests. They can be arenas such as in the case of the open field test or in the form of a cross. Generally, mazes suggest that the animals learn particular localization which offers them safety, food or some other desired situation, and that this information is stored for varying periods of time (XAVIER, 1999). Acetylcholinesterase, the enzyme target of dichlorvos, has an active role in neuronal development in both cholinergic and non-cholinergic systems (Robertson & YU, 1993). Blocking the function of this enzyme can interfere in some way with sensory functions, important to animals. Some functions are especially important in the open field paradigm, elevated T maze and Morris water maze, since animals need to incorporate a maximum of information during the exploration of a new environment.

Considering that flea collars are products that emit vapors of dichlorvos in a prolonged manner and that they are used indiscriminately in pregnant or lactating dogs, there is a possibility of toxic risk to the offspring. In addition, in view of the very frequent close contact

between people and their pets, there can also be a risk of intoxication, especially for children. Thus, the aim of this study was to determine if exposure of female Wistar rats to collars impregnated with dichlorvos during pregnancy or lactation cause behavioral alterations in offspring, and to see if there a period of development in which rats are more sensitive to this pesticide.

MATERIAL AND METHODS

For this study, 40 female Wistar rats, sexually mature and nulliparous (average weight of 200 g), were obtained from the Bioterio Central of Unesp, Botucatu Campus, S.P. Brazil. The animals were housed in the experimental animal facility of the Toxicology Laboratory, Department of Morphology and Animal Physiology of the School of Agricultural and Veterinary Sciences of Unesp, Jaboticabal Campus, S.P. Brazil. The rats were kept in plastic cages measuring 16 x 30 x 18 cm (4 animals/cage) which were placed in rooms with controlled temperature ($23 \pm 2^\circ\text{C}$), 40-70% relative humidity and a 12-h photoperiod. The animals were given water and food ad libitum.

The rats were examined daily by means of vaginal washes to determine estrus cycle. They were caged with males (1 male per 2 females), and after testing positive for pregnancy, the animals of the treated group were fitted with plastic collars impregnated with 8.37% dichlorvos^a. The collars had a standard length of 27.5 cm and liquid weight of 8.35 g, which went around the neck of the animal two times. The animals of the control group were fitted with inert collars. The rats that wore the collars during pregnancy were separated from those that were exposed to the collars during the lactation period. The rats were kept in separate compartments and submitted to equivalent conditions of temperature and humidity. The possible routes of exposure to the pesticide for the mother rats were dermal, oral (due to grooming behavior) and inhalatory, and for the offspring these were transplacental, oral (from mother's milk during lactation), dermal and inhalatory.

Behavior was evaluated using equipment for open field, elevated T maze and Morris water maze studies. The animals were observed by closed-circuit TV in an adjacent room. For evaluations, 20 pups were selected randomly from the control group (10 males and 10 females) and from the treated group (10 males and 10 females).

Exploratory activity was evaluated by means of the open field test at 21 days after birth. The test consisted of measuring the behavioral variables of the experimental individuals which were placed in an arena that was 70 cm in diameter surrounded by a circular wall 32.5 cm in height and located 45 cm from the floor (BROADHURST, 1960). The arena was painted white and the bottom was subdivided into 19 approximately equal regions, outlined by three inner circles of different diameters radii (8, 14 and 20cm) which were intersected by radial lines painted dark blue. Each animal was placed individually in the center

of the arena and observed with respect to the following behavioral categories: ambulation, standing, grooming, defecation, urination and period of immobility. The test was carried out in three different sessions of 5 min each, with between-session intervals of approximately 85 min.

The level of anxiety was measured using the elevated T maze at 22 days after birth. The apparatus made of wood consisted of two open, opposite arms measuring 50 x 10 cm each, crossed perpendicularly by two other closed arms of the same size. The closed arms had opaque walls that were 40 cm in height, while the open arms had sides of transparent acrylic 1 cm in height to prevent animals from falling. The maze was in the form a cross, elevated 50 cm from the floor. Each animal was placed individually in the center of the maze in front of one of the closed arms and observed for 5 min. The percentage of time that the animals stayed in the open and close arms of the maze was determined.

Learning and memory were evaluated using the Morris water maze, at 35 days after birth. The apparatus consisted of a black circular pool (diameter of 200 cm, depth of 40 cm) filled with 30 cm of water which was approximately 26°C and made opaque by the addition of approximately 150 g of powdered milk. In one quadrant of the pool, there was a platform (diameter of 10 cm) submersed 1 cm below the water level (XAVIER, 1999). The animal was placed on the side of the pool opposite the platform, and was allowed a period of 120 seconds to find it. When it was not able to find it, the experimenter placed it on the platform and left it there for 30 seconds. After this session, the animal was removed from the pool and submitted to new training in the next period of the day. The position of the platform was not changed during the experiment. The animals were submitted to two daily training sessions (morning and afternoon periods) totaling 11 sessions. The wall of the room in which the test was performed contained geometric figures differing in brightness to facilitate spatial mapping of the location of the platform. The latency to reach the platform was determined for each animal.

Student's t-test was used to compare the mean weights of the pups, exploratory activity, anxiety measure and learning and memory. Significance was set at the 5% level ($p < 0.05$) and 1% level ($p < 0.01$) in the evaluation of differences.

RESULTS AND DISCUSSION

The open field experiments showed that exposure of the mother rats to the collar impregnated with dichlorvos during pregnancy (Table 1) did not induce statistically significant alterations ($p > 0.05$) in any of the behavioral variables examined in their offspring at 21 days of age.

On the other hand, when the collars were placed on the mother rats during the lactation period (Table 1), a longer time of immobility ($p = 0.003$) was observed in pups whose mothers wore the collars impregnated with dichlorvos. The open field test consists of a circular arena in which effects of an unfamiliar environment on

emotionality in rats are determined. The parameters to evaluate emotional state include levels of ambulation and of defecation of the animal during its performance in the test. According to Denenberg (1969), these two parameters can define an animal's heightened emotional condition by lower level of ambulation and higher rate of defecation. This test asserts that an unfamiliar environment markedly different from its usual surroundings causes fear and/or anxiety in the animal. This fear produces a reaction in the central nervous system that leads to defecation and paralysis.

The results obtained from open field experiments demonstrated that the use of the collar impregnated with dichlorvos (8.37%) by mother rats during pregnancy, was not capable of inducing statistically significant alterations in any of the behavioral variables examined. Conversely, when the collars were placed on rats during the lactating period, there was a significant increase in the immobility time of pups. It is interesting to note that despite the longer immobility time, no significant alteration was observed in locomotor activity, which could be explained by a more rapid movement of the animals through the different quadrants of the open field and then stopping for a long time, denoting possible emotional alteration such as discomfort, fear or excitability.

Another behavioral change that was observed only in animals that had contact with the pesticide during the lactation period was with regard to grooming behavior. A statistically significant decrease ($p = 0.026$) was seen in this variable, particularly in female pups (data not shown, $p = 0.028$). In addition, the open field experiments showed a significant reduction in the frequency of urination in animals from exposed mothers ($p = 0.013$), mainly seen in male pups (data not shown, $p = 0.015$), compared to those of the control group with very high levels. This result suggests a territory marking behavior more evident in males.

In relation to experiments performed with the elevated T maze, this test was not sensitive enough to detect any significant difference between the control and treated groups (data not shown). In the Morris water maze test, pups exposed to the collar during the pre-natal period (Figure 1) showed a significant difference between sessions 1 and 11, for both the control group ($p = 0.0045$) and treated group ($p = 0.0152$). In both groups, the time required to locate the submersed platform decreased significantly between the first and last session, demonstrating that all the animals was capable of learning with training (sessions).

On the other hand, for the animals whose mothers wore the collars during the lactation period, it was observed that the pups of the control group had a significantly shorter latency in finding the platform ($p = 0.0103$). However, the same was not observed in the group that was exposed to dichlorvos, which did not show a decrease in latency ($p = 0.0935$) to achieve the task, between the first and last training session, suggesting that learning was impaired in this group.

The purpose of the Morris water maze is test for the capacity of learning and memory in animals (XAVIER, 1999). Experiments have been conducted to determine

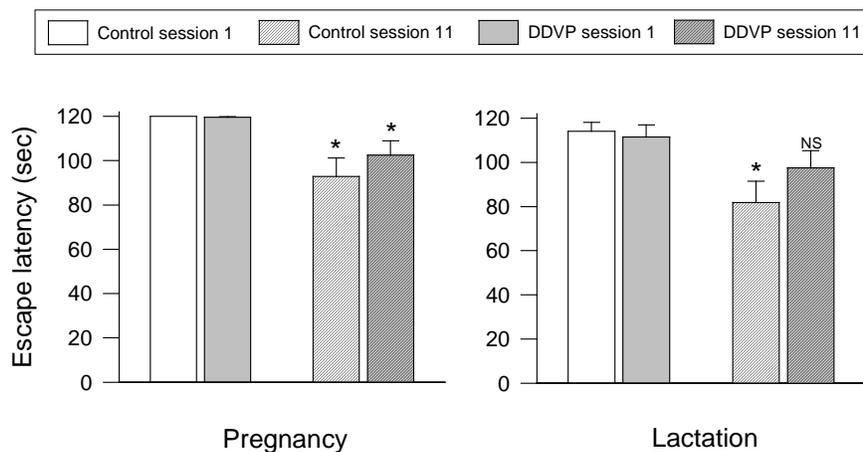
the effects of metrifonate, dichlorvos and other organophosphates on the capacity to perform the task in the Morris water model, in young and adult rats exposed by oral administration 60 min before the test (VAN DER STAAY et al., 1996). The latter study demonstrated that treatment with metrifonate caused shorter latency by locating the platform in the first training sessions, in a animals submitted to 10 and 30 mg/kg of product, while treatment with dichlorvos also led to a diminution of latency of escape in the last training session for animals that were given 0.03 mg/kg dichlorvos. Thus, both metrifonate and dichlorvos were shown to be able to enhance the capacity to perform the task in the Morris water maze.

On the other hand, other studies examining the effect of exposure of adult male rats to organophosphates on behavior in the Morris water maze, demonstrated that subcutaneous injection of 2mg/kg de methyl parathion made it difficult for the animals to perform the maze task, thereby being capable of impairing learning in the animals (CASTILLO et al., 2002).

The results obtained in this study suggest that the exposure of mother rats to dichlorvos during pregnancy and the lactation period causes harm to learning in female pups.

Table 1- Mean exploratory activity of pups whose mothers wore or did not wear collars impregnated with dichlorvos (8.37%) during pregnancy or lactation. The values between parentheses indicate the standard error of the mean.

Variable	Pregnancy			Lactation		
	Control	Dichlorvos	p value	Control	Dichlorvos	p value
Locomotion	69.5 (4.9)	57.8 (6.8)	> 0.05	72.0 (4.9)	75.0 (6.6)	> 0.05
Immobility	42.6 (5.6)	60.6 (10.2)	> 0.05	32.8 (5.5)	75.9 (9.5)	0.0003
Hearing	10.6 (1.0)	9.1 (1.4)	> 0.05	16.5 (2.0)	15.2 (2.1)	> 0.05
Grooming	2.1 (0.4)	1.2 (0.3)	> 0.05	1.9 (0.2)	1.2 (0.2)	0.0268
Defecation	1.5 (0.3)	1.9 (0.6)	> 0.05	1.2 (0.3)	2.2 (0.3)	0.0449
Urination	0.5 (0.1)	0.4 (0.1)	> 0.05	1.0 (0.2)	0.4 (0.1)	0.0144



CAPTION TO THE FIGURE

Figure 1 - Graphic representation of the mean time of localization of the platform, in the first and last training session in the Morris water maze for offspring of females exposed to a collar impregnated with dichlorvos (DDVP) during pregnancy or lactation. The vertical bars indicate standard error of the mean

CONCLUSION

Taken together, the results of this study lead to the conclusion that perinatal exposure to dichlorvos in rats can interfere with behavior evaluated by the open field test, as well as with capacity for learning in females assessed with the Morris water maze. It can also be concluded that there was no perinatal period where exposure to the pesticide was capable of causing greater adverse effects. Therefore, precautions should be taken when using flea collars impregnated with dichlorvos during the perinatal period, since they can cause alterations in behavioral parameters in offspring. Because of exposure, further studies are warranted that focus on the capacity of flea collars to cause adverse effects in humans, due to the close contact of children with pets, considering the indiscriminate use of this product.

SOURCES AND MANUFACTURE

^a Dog flea collar – DDVP Purina Tratto, Nestlê Purina Pet Care Unit. Ribeirão Preto, SP; batch number 009/2, date of manufacture, Jan/2002; expiration date, Jan/2005.

ANIMAL WELFARE APPROVAL

The present study is in accordance with Ethical Principles of Animal Experimentation, adopted by the COBEA (Brazilian College of Animal Experimentation) and was approved by the CEBEA e

(Ethical and Animal Welfare Commission) of the Faculdade de Ciências Agrárias e Veterinárias (FCAV/UNESP – Jaboticabal, SP, Brazil).

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