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ALDRIN AND TRICHLORFON EFFECTS ON PASSIVE AVOIDANCE BEHAVIOR OF RATS

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ABSTRACT

Rats were subjected under the influence of Aldrin (10, 20 or 30 mg/kg) or Trichlorfon (10, 30 or 50 mg/kg), to a inhibitory avoidance task. Results showed that Aldrin impaired inhibitory avoidance responses, in a dose-dependent way, 24 h after treatment but not retention along the time.

Dieldrin, the Aldrin metabolite, was detected in the plasma of all organochloride - treated animals at 9 days after the intoxications, whereas Aldrin was found only in the blood of the animals treated with the higher dosis (30 mg/kg). Both

Aldrin and Dieldrin were not detected in the blood of the animals analysed 21 days after acute administrations.

Trichlorfon administration not only decreased inhibitory avoidance response 24 h after treatment, but also disrupted the retention of the task along the test days.

Trichlorfon was able to decrease striatal, but not plasma cholinesterase activity of rats just 24 h after treatment.

These results suggest that changes observed after Aldrin administration on behavior are correlated to the organochloride plasma levels, whereas both the organophosphate effects on behavior and on cholinesterase activity were not directly related.

KEY WORDS: inhibitory avoidance, Aldrin, Trichlorfon, cholinesterase, memory.

RESUMO

Ratos que receberam uma dose única de Aldrin (10, 20 ou 30 mg/kg) ou Triclorfon (10, 30 ou 50 mg/kg) foram submetidos a esquiva inibitória. Os resultados mostraram que o Aldrin impediu as respostas de esquiva inibitória, de forma dose dependente, 24 horas após a administração do praguicida. O Dieldrin(metabolito do Aldrin) foi detectado no plasma dos animais até nove dias após a intoxicação, enquanto que o Aldrin, nesse mesmo dia, somente foi encontrado no sangue de animais tratados com a maior dose do praguicida. Além disso, aos 21 dias após a exposição ao praguicida organoclorado, a presença tanto do Aldrin como do Dieldrin não foi mais observada.

A administração do praguicida organofosforado, Triclorfon, reduziu não só as respostas de esquiva inibitória dos ratos às 24 horas após a intoxicação bem como nos testes aos 7, 14 e 21 dias após a exposição. A avaliação da atividade das colinesterases plasmática e estriatal mostrou que a atividade da enzima estriatal apresenta-se reduzida 24 horas após o tratamento enquanto que a da plasmática estava semelhante a do grupo controle.

Esses resultados sugerem que as alterações comportamentais observadas após intoxicação com o Aldrin estão correlacionadas com aquelas bioquímicas, enquanto que os efeitos do Triclorfon no comportamento parecem não estar relacionadas diretamente com as modificações bioquímicas.

UNITERMOS: esquiva inibitória, Aldrin, Triclorfon, colinesterase, memória.

PASSIVE AVOIDANCE BEHAVIOR

Introduction

In recent years behavioral toxicology has assumed a role of increasing importance in the evaluation of toxic compounds. This has not yet resulted in the development of many tests for the evaluation of behavioral toxicity, but has recquired the adaptation of psychopharmacological methods to the special demands of evaluation on the Central Nervous System (CNS) function, after exposure to toxic substances.

Learning has been defined as an adaptation to changes in the environment and memory has been defined as the retention of such adaptations over time (Cabe and Eckerman, 1982). The inhibitory avoidance task is very rapidly learned and can be applied at various times specified with respect to the time of learning in order to determine the effect of the agent on various stages of acquisition and retention processes.

Organochloride and organophosphate pesticides, such as Aldrin and Trichlorfon, might change the CNS activity (Joy, 1976; Ecobichon and Joy, 1982). Thus, they could produce changes in learning and memory processes since changes in brain activity might modify retention performances (McGaugh and Dawson, 1971; Sharma **et al.**, 1976).

In the present study, the effects of acute Aldrin and Trichlorfon administration were examined under inhibitory avoidance task. Plasma levels of both Aldrin and Dieldrin, and cholinesterase activity were also analysed in order to correlate them with the possible behavioral alterations induced by the pesticides on avoidance processes.

Material and Methods

Animals

Genetically similar male Wistar rats (200-250 g) were used. After weaning (30 days) the animals were randomly housed (groups of three) in wooden cages (26 x 20 x 23 cm) at controlled room temperature (22ºC ± 1) in a non-reversed 12 h light dark cycle (lights on at 7:30 a.m.). Animals were 120 days old at the beginning of the experiment and were provided food and water **ad libitum**.

Drugs

Trichlorfon, dissolved in destilled water and Aldrin dissolved in Tween-80 and suspended in NaCl 0,9% were injected by the i.p. route in a volume not greater than 2.0 ml/kg. Two control solutions were used: destilled water and NaCl (0,9%) plus Tween-80 (three drops).

Experiment 1 - **Inhibitory avoidance studies**

The training apparatus was a long box (80 x 15 x 30 cm) divided by a stainless steel sliding door into a dark (start) compartment (50 cm long) and well-lit (30 cm long) compartment. The floor of the apparatus was made of stainless steel grids through which footschocks could be delivered in the dark compartment.

On day 0 (zero), all animals were trained once. At the beginning of the trial, the animals were individually placed at the far end the well-lit compartment facing the door. Immediately after the door was opened, latency for the animals to enter the dark compartment was assessed; after they entered the dark compartment, a footshock of 1.6 mA was delivered for 5 sec. The maximum time allowed for each animal was 300 sec. Immediately after the footshock, the animals were randomly divided into 6 experimental and 2 control groups. Experimental animals received Aldrin (10, 20 or 30 mg/kg) or Trichlorfon

(10, 30 or 50 mg/kg); rats of control groups were retested in the same way as on the training day (day 0), on days 1, 7, 14 and 21 after the test, however the footshock was not delivered.

Experiment 2 - **Plasma level determination of organochloride pesticides**

Plasma levels of Aldrin and its metabolite Dieldrin were examined in rats treated with Aldrin (10, 20 or 30 mg/kg) or its control solution. The organochloride pesticide was extracted from plasma by the Dale and Miles (1970) method, 9 and 21 days after Aldrin injections. Both Aldrin and its metabolite were analysed by gas chromatography. The gas chromatograph was a CG model 370 provided with a tritium eletron capture detector and a glass column OV-17-1.5% + OF-1.95%. Operating conditions were: nitrogen carrier gas at 40 ml/min; column 183°C; detector 212°C; injector 212°C. Aldrin and Dieldrin recoveries were 85% and 90%, respectively.

Experiment 3 - **Striatal and plasmatic cholinesterase activity determination**

Striatal and plasmatic cholinesterases were evaluated in rats treated with Trichlorfon (50 mg/kg) or with vehicle. The animals were anesthetized with ether and their blood colected through the hepatic vein; immediately after, it was centrifuged to separate the plasma. In order to isolate the **striata**, the anesthetized rats were decapitated and their brains rapidly removed. The **striata** were dissected, frozen on dried ice, weighed and homogenized in NaCl 0,9% solution (80 mg/kg of tissue/1 ml). Both plasma and striatal cholinesterase activity were evaluated by the method of Ellman **et al**. (1961) modified by Wilhelm (1968). These determinations were made 1,9 and 21 days after Trichlorfon administrations.

Statistical Analysis: Results of the passive avoidance behavioral task were analysed by an one way analysis of

variance (ANOVA), followed by Duncan's test. Student's "t" test was used for the cholinesterase activity data. The probability of $p < 0.05$ was considered to show significant differences for all comparisions made.

Results

Experiment 1 - **Inhibitory avoidance studies**

Aldrin effects on inhibitory avoidance behavior of rats are depicted in Fig. 1 Rats of the pesticide groups presented a lowest dose-dependent avoidance response in the test day 1. Statistically significant differences ($p < 0.05$) were observed at the 30 mg/kg treated group in relation to control animals. Further more, it has been possible to observe that the latency to cross of the Aldrin-treated groups gradually increased over the time (in test days 7, 14 and 21).

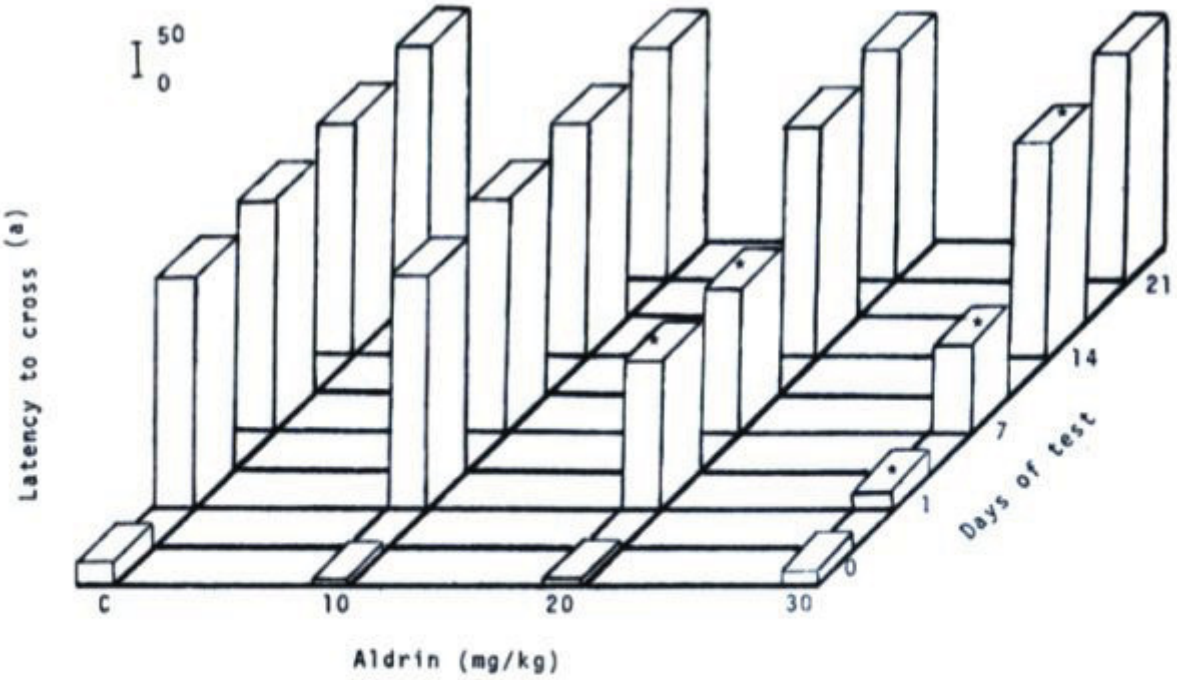

Fig. 1 - Effects of aldrin (10, 20 or 30 mg/kg) administration on passive avoidance of rats.
(*) $p < 0,05$ in relation to control group (C) - one way analysis of variance ANOVA followed by the Duncan's test.
(a) 1 cm = 100 seconds.

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Fig. 2 shows the results of animals treated with different doses of Trichlorfon. The inhibitory avoidance responses were also decreased in a dose-dependent manner on test day 1. Statistical significances ($p < 0.05$) were observed both to 30 and 50 mg/kg Trichlorfon doses. Further examination of the latency to cross on the 7^{th}, 14^{th} and 21^{st} test days, showed a persistent decrease in the responses of rats of the experimental groups ($p < 0.05$). The most pronounced effects were observed after the highest Trichlorfon dose.

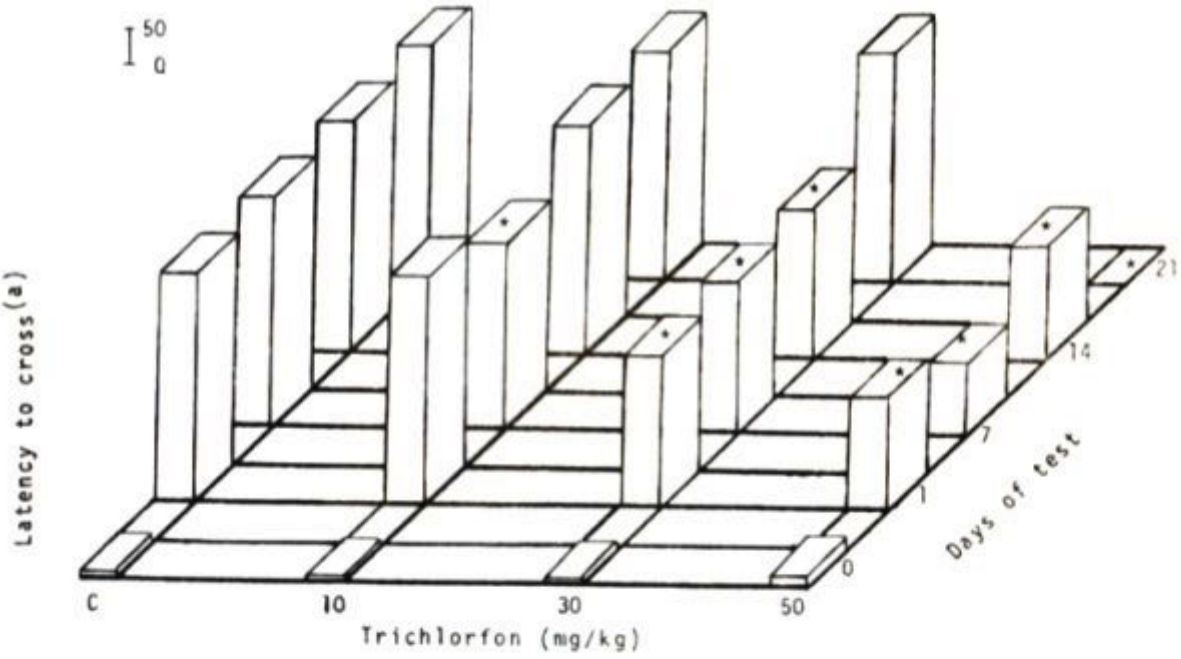

Fig. 2 - Effects of Trichlorfon (10, 30 or 50 mg/kg) administration on passive avoidance of rats.
(*) $p < 0,05$ in relation to control group (C) - one way analysis of variance ANOVA.
(a) 1 cm = seconds.

Experiment 2 - **Plasma level determination of organochloride pesticides**

Table 1 presents the plasma levels of Aldrin and Dieldrin both in control and in rats treated with the organochloride pesticide; determinations were made 9 days after the pesticide treatment. Thus, rats treated with Aldrin (10 or 20 mg/kg) showed plasma levels of only Dieldrin, whereas those treated with 30 mg/kg showed both Aldrin and Dieldrin in the plasma.

Aldrin and Dieldrin levels were not detected in the plasma of animals 21 days after the pesticide administration (data not shown).

Table 1 - Levels of Aldrin and Dieldrin in plasma of rats studied 9 days after acute Aldrin administration.

TREATMENT[a]	DOSE (mg/kg)	PLASMA LEVELS (mg/ml)
Control	-	-
Aldrin	10	-
	20	-
	30	76.60 ± 73.70
Dieldrin	10	44.09 ± 15.93
	20	40.30 ± 10.05
	30	34.89 - 17.39

[a] Aldrin was suspended in NaCl (0.9%) plus Tween-80.

Experiment 3 - Striatal and plasmatic cholinesterase activity determination

Table 2 summarizes the effects of different doses of Trichlorfon on plasmatic and striatal cholinesterase activity. The evaluation made 1 day after the pesticide administration did not show plasma enzymatic alteration; nevertheless, the activity of the striatal cholinesterase was decreased ($p < 0.05$). Further more, the evaluations of both striatal and plasmatic cholinesterase activity both 9 and 21 days after the pesticide treatmente were not different among control and experimental groups.

Table 2 - Striatal and plasmatic cholinesterase activities of rats studied 1, 9 and 21 days after acute Trichlorfon administration.

TREATMENT(b)		DAYS AFTER TREATMENT 1	2	3
Plasma activity ($\mu g/mol^{-3}min/ml$)	Control	1.89 ± 0,17 (N = 5)	0.80 ± 0.15 (N = 5)	1.10 + 0,41 (N = 5)
	Trichlorfon (50 mg/kg)	1.67 ± 0.24 (N = 6)	1.68 ± 0.93 (N = 6)	0.97 ± 0.30 (N = 5)
Striatal activity ($\mu g/mol^{-3}/min/mg$ of tissue)	Control	3.69 ± 0.82 (N = 5)	3.70 ± 1.10 (N = 5)	3.02 ± 0.93 (N = 5)
	Trichlorfon	1.53 ± 0.50* (N = 7)	3.53 ± 0.74 (N = 6)	2.87 ± 0.50 (N = 5)

(*) $p < 0.05$ - in relation to animals treated with control solution (Student "t" Test).

(b) Trichlorfon was diluted in destilled water.

Discussion

Results show that Aldrin post-training administration was able to decrease the inhibitory avoidance behaviour in rats, 24 h after the training session; this effect was dose related. In addition, the latency to cross on days 7, 14 and 21 gradually increased, being not different from that of control animals on day 21. These observed changes might be a consequence of a direct effect of the pesticide on retrieval of the inhibitory avoidance task. In fact, the acute Aldrin administration was not able to disrupt the memory storage of inhibitory avoidance behavior, since a clear recovery of animal response accompanied the decrement in organochloride plasma levels. Supporting this view is the ability of Dieldrin to eliminate the acquisition of learning task in cockroachs (Topinka **et al.**, 1984) and monkeys (Smith **et al.**, 1976) without affecting memory. These facts led Smith **et al.**, (1976) to suggest that learning acquisition is a more sensitive indicator of Dieldrin toxic effects than the maintenance of a learned task. So, it seems that high pesticide levels were able to block inhibitory avoidance retrieval but not memory storage.

As all organophosphate pesticides, Trichlorfon is able to inhibit cholinesterase activity. In this respect, the present results show that Trichlorfon (50 mg/kg) was unable to change plasmatic cholinesterase activity 24 h after the pesticide treatment in relation to that of control untreated rats; at the same time, striatal cholinesterase activity was smaller in treated rats. Moreover, plasma and striatal cholinesterase activities was similar in control and experimental rats 9 and 21 days after the pesticide administration. The inhibition of cholinesterase activity induced by Trichlorfon suggests high contents of acetylcholine not only in the **striata** but also in the whole brain. In fact, central cholinergic systems are involved in a

number of vital functions and in behavior, particularly in mechanisms of learning (DeFeudis, 1974). Indeed, lesions of direct septal cholinergic afferents on hippocampal areas cause impairment of avoidance responses, while directly applied cholinergic antagonists usually improve performance (Greene and Loman, 1970). Furthermore, cholinergic systems are implicated with arousal (Greene and Loman, 1970) and animal levels of arousal is linked to learning, memory and evocation (Belluzi and Grossman, 1969; Belluzi, 1972).

Thus, the Trichlorfon effects on inhibitory avoidance behavior might be directly or indirectly connected to the facts described above. The present data show that Trichlorfon administration was able not only to block the avoidance response retrieval but also to disrupt the memory storage of the learned task. Moreover, the present data provide the additional information that a persistent change on cholinesterase activity is not necessary to induce the observed behavioral deficit.

Taken together, the results presented here support the conclusion that inhibitory avoidance tasks are a proper method to display the toxic effects of organochlorine and organophosphate pesticides.

Moreover, the Aldrin behavioral changer was correlated with the plasma levels of the pesticide. On the other hand, it has been observed that the cholinesterase activity was decreased just 24 h after the organophosphate administration while the behavioral changes induced by this pesticide were detected untill 21 days after the treatment.

References

Belluzi, J.D. and Grossman, S.P. (1969). Avoidance learning: long-lasting deficits after temporal lobe seizures. **Science**, **166**:1435-1437.

Belluzi, J.D. (1972). Long-lasting effects of cholinergic stimulation of the amygdaloid complex in the rat. **J. Comp. Physiol. Psychol.**, **80**:269-282.

Cabe, P.A. and Eckerman, D.A. (1982). Assessment of learning and memory disfunction in agent-exposed animals. In: Mitchell, C.L. (ed.) **Nervous System Toxicology**. Raven Press: New York, pp.133-198.

Dale, W. and Miles, J. (1970). Quantitative method for determination of DDT and DDT metabolites in blood serum. **J. of AOAC**, **53**:1287-1292.

DeFeudis, F.V. (1974). **Central Cholinergic Systems and Behavior**. DeFeudis, F.V. (ed.) Academic Press: London, pp.106-117.

Ecobichon, D.J. and Joy, R.M. (1982). **Pesticides and Neurological diseases**. Ecobichon, D.J. and Roy, R.M. (eds.) CRC Press Inc.: Florida, pp.91-193.

Ellman, G.L.; Couney, K.D.; Andres, Jr. V. and Featrestone, R.A. (1961). New rapid colorimetric determination of acetylcholinesterase activity. **Biochem. Pharmacol.**, **7**:88-95.

Greene, E.G. and Lomax, O. (1970). Impairment of alternation learning in rats following microinjection of carbachol into the hippocampus. **Brain. res.**, **18**:355-358.

Joy, R.M. (1976). The alteration by Dieldrin of cortical excitability conditioned by sensory stimuli. **Toxicol. Appl. Pharmacol.**, **39**:357-368.

McGaugh, J.L. and Dawson, R.G. (1971). Modification of memory storage processes. **Behav. Sci.**, **16**:45-63.

Sharma, R.P.; Winn, D.S. and Low, J.B. (1976). Toxic neurochemical and behavioral effects of Dieldrin exposure in mallard ducks. **Arch. Environ. Contam. Toxicol.**, **5**:43-53.

Smith, R.M.; Cunningham, W.L. and Van Gelder, G.A. (1976). Dieldrin toxicity and successive discrimination reversal in squirrel monkeys (**Saimiri sciureus**). **J. Toxicol. Environ. Health**, **1**:737-747.

Topinka, M.A.; Eisenstein, E.A.; Siegel, D.; Trofeli, R. and Barraco, D. (1984). The effects of Dieldrin and Chlordimeform on learning and memory in the cockroach, **Periplaneta americana**: a study in behavioral toxicology. **J. Toxicol. Environ. Health**, **13**:705-719.

Whilhelm, K. (1968). Determination of human plasma cholinesterase activity by adapted Ellman's method. **Arch. Hig. Rad. Toksikol**, **19**:199-207.