Eth. J. Sci & Technol. 4 (2): 1 – 12 ISSN 1816 -3378

ON THE DEVELOPMENT OF BEHAVIORAL TOLERANCE TO THE ORGANOPHOSPHATE INSECTICIDE PHOSALONE IN RATS

Sahitya Chetan, P., Ravi Kumar, R.and Murali Mohan, P.*

Department of Zoology, S.V. University, TIRUPATI - 517 502, India
*Present address: Department of Biology, Bahir Dar University, BAHIR DAR, Ethiopia. E-mail: pmuralimohan@hotmail.com

Abstract: Organophosphate pesticides exert their toxic effects by cholinesterase inhibition and the consequent prolongation of the undesirable effects of accumulation of acetylcholine. The signs of toxicity include tremors, convulsions, lachrymation, urination, defecation etc. However, prolonged cholinesterase inhibition through sustained administration of organophosphates could lead to the gradual disappearance of the initial signs of toxicity in the course of time, termed behavioral tolerance. The present study was undertaken to examine in albino rats the development of behavioral tolerance to phosalone, an organophosphate insecticide commonly used in agricultural operations. After determining the LD_{50} dose, oral doses ranging between ½ LD_{50} to ⅛ LD_{50} (both inclusive) were administered daily for a period of 15 days to six batches of rats (each batch of 12-20 animals), and changes in consummatory behavior (eating and drinking), body weight and cumulative mortality were monitored daily to assess the development of behavioral tolerance at a particular dose. The onset and disappearance and frequency of toxic signs and symptoms were also taken into consideration to arrive at a daily sublethal tolerable oral dose of phosalone. A dose of ¼ LD_{50} was finally selected for daily dosing for 15 days. The occurrence of tremors and convulsions was observed each day after dosing during a time period of 6h at intervals of ½h, 2h, 3h, and 6h. The study revealed that albino rats develop behavioral tolerance to phosalone toxicity. This may have implications in agricultural operations employing this insecticide.

Key words: Organophosphates, Phosalone, Albino rats, Behavioral tolerance.

Introduction

Organophosphate (OP) pesticides, though short-lived in terms of persistence in environment, exhibit a very high degree of toxicity in target and non-target species. Most of the organophosphate compounds, despite being used against a pest species, do normally show a wide spectrum of toxic actions in several animals including the behavioral, neurological and biochemical ones (Johnson, 1975; Davies and Richardson, 1980). Many short-term studies conducted on organophosphate poisoning enable us to understand the adverse effects of these compounds during acute exposure as a part of occupational hazard in agricultural,

industrial and public health workers. Course studies over prolonged exposure to sub-acute doses of OP compounds would reveal the development of lesions, biochemical, behavioral and neuronal, transient, incipient and permanent. These studies are more relevant and informative in providing the clues for developing management strategies in OP-poisoning. Long-term sub-acute exposure studies also bring to the fore the important concept of behavioral tolerance. Repeated administration of sub-acute doses of OP compounds has been found to produce behavioral changes, tending towards overt normalcy after the initial development of signs and symptoms of OP toxicity (Barnes and Denz, 1951; Rider *et al.*, 1952; Lim *et al.*, 1983). The development of behavioral tolerance has been shown to be dose-dependent, and there seems to be a threshold dose above which the animals may not develop any tolerance (Overstreet and Jamal, 1986). We should also take into account the possible metabolism of the administered compound causing reduction in its level in the body. Hence we may not know the exact concentration of the chemical in the body causing tolerance. It might be an area which has promise for future investigations.

It is possible to measure qualitatively and quantitatively the overt symptoms OP toxicity (Russell *et al.*, 1975; Schwab and Murphy 1981; Overstreet 1984). For the study of behavioral tolerance, selection of sub-acute dose is a very important criterion. The dose should be such that it produces initial measurable manifestations of OP toxicity, and the disappearance of symptoms could be taken as the measure of tolerance developed. Any lesser dose might over a long period of time induce tolerance, but is not useful unless it produces measurable overt behavioral symptoms.

In the present investigation, the effect of phosalone, an OP insecticide commonly used in agricultural and domestic operations, was studied on albino rats to test the possibility of phosalone-induced symptomatic behavioral tolerance during a treatment period of 15 days. Among the two types of dosing patterns, namely 1) constant daily sub-acute dose and 2) periodically altered daily sub-acute dose, the

former was selected in the present study to avoid complexities of patterns of cumulative toxicity.

Material and Methods

Male albino Wistar rats weighing 130±20 g were used. Four animals were housed per cage and allowed access to food and water *ad libitum*.

Experimental Procedure

Technical grade (98% purity) phosalone [O, O- diethyl-s- (6-chloro-1-3 ben 30XO3ol-2(3H)-O-methyl) phosphorodithiote], obtained from Volhro Ltd., India was used. Phosalone is characterized by broad spectrum action against the principal pests and immediate action and moderate toxicity to mammals (Hayes 1982). Control animals received normal saline and the experimental rats received it along with phosalone. LD_{50} values were determined by probit method (Finney, 1971). After determining the LD_{50} dose, oral doses ranging between ½ LD50 to ⅛ LD50 (both inclusive) were administered daily for a period of 16 days to six batches of rats (each batch of 12-20 animals) and changes in the consummatory behavior (eating and drinking), body weight and cumulative mortality were monitored daily to assess the development of behavioral tolerance at a particular dose. The onset and disappearance and the frequency of toxic signs and symptoms were also taken into consideration to arrive at a daily sub-lethal tolerable oral dose of phosalone. A dose of ¼ LD50 (41.35 mg) was finally selected for daily dosing for 15 days, and it was given by gavage method of daily oral intubation. The rats were fasted for 6 hours before each dosing.

Average body weights, and average amounts of food and water consumed out of the provided known quantities, were determined every day for a period of 15 days in both control and phosalone-administered rats, and the results were tabulated.

Signs and symptoms were noted at regular intervals of 3h, 6h, 12h, and 24h and pooled later. The onset of tremors and convulsions was observed each day after dosing during a time period of 6h at intervals of 1/2h, 2h, 3h and 6h. The observations pertaining to tremors and convulsions were quantified into arbitrary

units in the scale of 0 = no obvious signs; 0.2 = slight (slow tremor of the head); 0.4 = moderate (faster tremor of the head, trunk and limbs); 0.6 = high (more intense tremors) and 0.8 = severe. Similar scale was developed for convulsions, viz. 0.0 = none; 0.1 = slight; 0.2 = moderate and 0.3 = high.

Mann Whitney 'U' test was used to analyze the statistical significance for behavioral scores.

Results

The following parameters were measured daily for a period of 15 days of treatment with ¼ LD_{50} of phosalone.

i. Body Weight
ii. Amount of food consumed
iii. Amount of water consumed
iv. Tremors
v. Convulsions

Daily administration of ¼ LD50 dose did not cause any cumulative mortality.

Body Weight

Changes in the body weight of unexposed rats and experimental rats are presented in Table-1. The results show that in control rats there was an increase of 7.71% in body weight by the 7^{th} day and 18.8% by 15 days. Phosalone-administered rats did not register any increase in body weight, and after 15 days of treatment the body weight remained near pre-exposure value.

Food Consumption

Results pertaining to the amount of food consumed are given in Table-2. The total quantity of food consumed was consistently greater in control rats compared to that in phosalone-treated rats. Despite fluctuations in food consumption values in untreated rats and experimental rats, a trend was discernible. There was a greater drop in food consumption in phosalone-administered rats with time, -25.9% by day

1 and -36.5% by day 7. By the 15th day there tended to be a recovery in food consumption as the value stood at -12.7%.

Water Intake

Data pertaining to water-intake of unexposed rats and experimental rats are presented in Table-3. Untreated rats consumed more water than phosalone-treated rats throughout the experimental period. Despite fluctuations recorded up to day 7, there was a decline in water intake, -12.3% by day 1 and -36.9% by day 7. From the 7th day onwards the experimental rats exhibited a remarkable recovery, as a result of which the water intake level increased to over 20% by the 15th day.

Tremors and Convulsions

The data pertaining to tremors and convulsions are presented in Table-4. The tremors commenced 6 hours after dosing on the 2nd day, advanced to 3h on the 3rd day, and 2h on the 5th day. The onset time remained thus till the 13th day, and by the 15th day the tremors disappeared. A progressive increase in severity of tremors occurred, reaching the peak on the 9th day. There were some fluctuations between the 4th and 7th days. On the 10th day the tremors showed a downward trend with some fluctuations on the 11th day. From the 12th day onwards there was a steep fall, with normalcy returning by the 15th day.

Convulsions were found to set in only from the 5th day, 6 hours after dosing. The pattern continued up to the 11th day, and on the 12th day no convulsions were noticed. The convulsions were always slight to moderate in the scale with the peak on the 9th day.

Table-1: Changes in the mean body weights of albino rats during selected periods of exposure to phosalone

Days of Treatment	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Unexposed	100	101	101.9	103	104.1	105.5	106.4	107.7	108.4	108.8	110.1	111.4	112.5	115.4	117.2	118.8
SD	2.7	2.6	3.2	3.1	3.0	3.2	2.4	2.2	2.3	2.2	2.4	2.4	1.7	1.0	0.8	0.7
% Change	-----	1.0	1.9	3.0	4.1	5.5	6.4	7.7	8.4	8.8	10.1	11.4	12.5	15.4	17.2	18.8
Exposed	100	102.3	100.3	96.8	97.9	98.1	98.5	96.9	96.8	97.9	100	99.1	97.9	100.4	101.2	101.9
SD	1.4	2.5	3.1	3.5	3.8	4.3	3.9	4.3	3.9	4.7	4.9	5.1	5.1	5.5	5.9	5.3
% Change	-----	2.3	0.3	-3.2	-2.1	-1.9	-1.5	-3.1	-3.2	-2.1	0	-0.9	-2.1	0.4	1.2	1.9

Table-2: Changes in the mean food consumption during selected periods of exposure to phosalone.

Days of Treatment	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Unexposed	12.54	12.03	11.02	11.18	11.89	10.92	11.08	9.52	9.55	9.78	10.99	10.69	10.33	11.12	10.83	11.36
SD	1.05	0.68	0.72	0.65	0.48	0.65	0.60	0.27	0.34	0.31	0.45	0.86	0.79	0.65	0.53	0.53
% Change	-----	-4.1	-12.1	-10.9	-5.2	-12.9	-11.6	- 24.1	- 23.8	- 22.0	-12.4	-14.6	-17.6	-11.3	-13.6	-9.4
Exposed	9.74	7.22	6.48	7.55	7.51	6.61	6.58	6.19	5.45	4.96	6.34	7.01	7.37	7.86	8.35	8.5
SD	0.27	0.38	0.51	0.24	0.21	0.3	0.24	0.23	0.21	0.32	0.22	0.19	0.21	0.2	0.29	0.21
% Change	-----	-25.9	-33.5	-22.5	-22.9	-32.1	-32.4	- 36.5	- 44.0	- 49.1	-34.9	-28.0	-24.3	-19.3	-14.7	-12.7

Table-3: Changes in the mean water consumption during selected periods of exposure to phosalone.

Days of Treatment	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Unexposed	23.26	24.65	21.47	21.47	20.29	21.0	22.7	22.49	22.43	21.88	20.24	21.68	23.18	24.28	21.78	21.48
SD	1.62	2.44	1.59	1.96	1.67	1.98	1.12	1.02	0.71	0.9	0.45	1.01	1.46	2.17	1.37	1.36
% Change	-----	5.9	-7.7	-7.7	-12.8	-9.7	-2.4	-3.3	-3.6	-5.9	-12.9	-6.8	-0.3	4.4	-6.4	-7.7
Exposed	17.35	15.21	13.08	13.64	12.28	12.11	11.2	10.95	10.0	13.75	16.93	19.11	19.67	19.76	21.03	21.02
SD	0.74	0.38	0.62	0.61	0.79	0.99	1.07	0.97	0.72	1.43	1.2	1.47	1.59	1.65	1.62	1.83
% Change	-----	-12.3	-24.6	-21.4	-29.2	-30.2	-35.5	-36.9	-42.4	-20.6	-2.4	10.1	13.4	13.9	21.2	21.1

Table-4: Changes in tremors and convulsions during selected periods of exposure to phosalone.

Days of Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tremors	0	0.14	0.18	0.25	0.46	0.28	0.29	0.42	0.88	0.71	0.59	0.32	0.25	0.14	0.07
SD	0	0.07	0.07	0.09	0.13	0.03	0.03	0.17	0.22	0.23	0.09	0.08	0.17	0.1	0.07
Convulsions	0	0	0	0	0.11	0.11	0.07	0.14	0.25	0.18	0.21	0	0	0	0
SD	0	0	0	0	0.07	0.05	0.04	0.05	0.07	0.05	0.06	0	0	0	0

Other behavioral lesions such as involuntary urination, defecation, lachrymation and chromodacryorrhea were also observed but could not be quantified, and so not shown. These changes set in 6 hours after dosing on the 2nd day. While urination and defecation did not show any pattern after the 2nd day, the other parameters disappeared on the 8th day.

Discussion

It is known that repeated application of a stimulus normally leads to a process of habituation or simple learning in organismal response which will not be apparent in the course of time. While phosalone is the toxic chemical acting as the stimulus, the onset of signs and symptoms of toxicity in the animal are the responses. Behavioral tolerance implies gradual disappearance of these overt manifestations of toxicity with multiple sub-chronic (¼ LD_{50}) dosing over an extended period of time. The development of such behavioral tolerance has been reported in rats under OP treatment (Costa *et al.*, 1982; Russell *et al.*, 1986; Swamy and Murali Mohan 1991). It has also been established that in addition to tremors and convulsions, changes in several other parameters such as body weight, food consumption, water consumption and body temperature could be observed as indices of physiological lesions in the animal (Overstreet *et al.*, 1979; Russell *et al.*, 1979). The development of behavioral tolerance to OP compounds is also dose- and time-dependent (Swamy and Murali Mohan 1991). Cumulative mortality data are very useful in evaluating long-term effects of multiple sub-acute doses. Based in this, ¼ LD50 value of 41.35 mg/kg body weight has been selected for daily administration for 15 days in the present study.

It is evident that the rats did not show any addition to bodyweight during phosalone-treatment, while the untreated animals registered a 19% rise in body weight on the 15th day (Table 1). There was a very clear evidence of behavioral recovery in food and water consumption during 15 days of phosalone-exposure. Lowest food consumption value was recorded on the 9th day, and by the 15th day normal consumption was restored. Water consumption value was at its lowest on the 7th day. Recovery was evident after the 7th day, and by the 14th day the value was

nearer to the normal. These patterns clearly reveal the development of behavioral tolerance commencing between the 7th and 9th day of treatment. In regard to tremors and convulsions also, the 9th day seems to be the point from where tolerance occurred. Comparison of these trends with the observations of other workers gives an interesting picture. Bobinski and Dubois (1958) showed that rats developed behavioral tolerance to ½ LD_{50} dose of disyston over a period of 60 days. Mice treated with a daily dose of 10 mg/kg of disulfoton for 14 days developed tolerance in terms of body weight (Costa *et al.*, 1982). Swamy and Murali Mohan (1991) observed that weight losses in rats treated with OP compounds is dose-dependent. ½ LD_{50} dose did not induce tolerance, while data with ¼ and ⅛ LD_{50} doses of phosphamidon and monocrotophos revealed that the development of tolerance to OP compounds is dose-dependent (Russell *et al.*, 1969; Lim *et al.*, 1983; Ho and Hoskins 1986; Swamy and Murali Mohan 1991). In the present study, the food and water consumption tended to recover towards the control value, from the 9th day in the former and from the 7th day in the latter. Swamy and Murali Mohan (1991) reported that the recovery was faster in phosphamidon-treated rats compared to those treated with monocrotophos.

Several factors such as dosing schedules, route of administration etc determine the temporal sequence of tolerance development to OP compounds. Besides these, other factors such as binding, absorption, and avidity to lipids in the gastrointestinal tract may influence the consummatory behavior of animals.

In regard to signs and symptoms of toxicity as reflected by tremors and convulsions, which declined in phosalone-treated animals from the 9th day onwards in the present work, comparison with reports on the other OP compounds reveals that the changes are slower and milder but last longer than those which occur with other OPs, i.e. it takes longer time for the onset of tolerance in terms of tremors and convulsions. Swamy *et al.* (1993) made a detailed study of onset and disappearance of tremors and convulsions in rat after exposure to different doses of monocrotophos. The signs and symptoms appeared on the 2nd day and started

disappearing from the 11th day on exposure to ¼ LD_{50} dosing. Any dose above ¼ LD_{50} induced tolerance earlier but with greater mortality.

Different hypotheses have been proposed to explain the development of behavioral tolerance. Inhibition of cholinesterase by OP compounds is well known and the onset of tremors and convulsions is due to the inhibition of acetyl cholinesterase activity. Despite the fact that AChE remains inhibited, the organisms develop tolerance. The contributions of Russell *et al.* (1975), Wecker *et al.* (1977), Costa *et al.* (1986), Sivam *et al.* (1986) and Swamy and Murali Mohan (1991) are noteworthy in looking for explanations for the phenomenon of tolerance. Reduction in the level of OP compound in the body due to metabolism of these compounds by hepatic microsomal mixed function oxidases and hydrolases has been suggested as a mechanism for tolerance development under the caption 'metabolic tolerance.' This does not look tenable because AChE inhibition observed after exposure to OP compounds continues even after development of the so-called 'metabolic tolerance.' If the OP level in the body is reduced, AChE activity should show recovery to a greater extent.

Based on receptor-binding experiments using muscarinic antagonists and agonists, it was shown that there occurs a down-regulation of muscarinic receptors in central and peripheral nervous systems (Costa *et al.*, 1982; Yang *et al.* 1988), which helps in explaining tolerance-development despite AChE inhibition.

The third alternative seems to be the implication of non-cholinergic mechanisms to induce recovery and tolerance. Sivam *et al.* (1983) studied acute and chronic cholinesterase inhibition with DFP in muscarinic, dopamine and GABA receptors in rat striatum and showed that by the 14th day toxic symptoms disappear.

Conclusion

The present investigation on phosalone toxicity throws light on the possible changes other than those in the biochemical profiles underlying the development of behavioral tolerance.

References

Barnes, J.M. and Denz, F.A. (1951). The chronic toxicity of P-nitrophenyl diethyl thiophosphate (E.605), a long term feeding experiment with rats. *J. Hyg.* 49: 430-441.

Bobinski, T.J. and Dubois, K.P. (1958). Toxicity and mechanism of action of Di-syston. *AMA Arch. Indust. Health* 17: 192-199.

Costa, L.G , Schwab, B.W., Hand, H. and Murphy, S.D. (1986). Reduced [3H] quinuclidinyl benzilate binding to muscarinic receptors in disulfoton-tolerant mice. *Toxicol. Appl. Pharmacol.* 60: 441-450.

Costa, L.G. Schwab, B.W. and Murphy, S.D. (1982). Tolerance to Anti-cholinesterase compounds in mammals. *Toxicology* 25: 79-97.

Davies, C.S. and Richardson, R.J. (1980). In: Spencer, P.S. and Schaunburg, H.H. (eds). *Experimental and Clinical Neurotoxicology*, Williams and Williams, pp. 527-544.

Finney, D.J. (1971). *Frobit Analysis*. Third Edition. Cambridge University Press, London.

Hayes, A.W. (1982). In: Hayes, A.W. (ed). *Principles and Methods of Toxicology*, Raven Press, New York.

Ho, I.K. and Hoskins, B. (1986). Biochemical and pharmacological aspects of neurotoxicity from and tolerance to organophosphate cholinesterase inhibitors. In: Haley, T.J. and Berndt, W.O. (eds). *Handbook of Toxicology*. Hemisphere Publishing Corp., Washington.

Johnson, M.K. (1975). Organophosphorus esters causing delayed neurotoxic effects. Mechanism of action and structure/activity studies. *Arch. Toxicol.* 34: 259-288.

Lim, D.K., Hoskins, B. and Ho, I.K. (1983). Assessment of diisopropyl-fluorophosphate (DFP) toxicity and tolerance in rats. *Res. Comm. Chem. Pathol. Pharmacol.* 39: 399-418.

Overstreet, D.H., Russell R.W., Helps, H.C. and Messenger, M. (1979). Selective reading for sensitivity to the anticholinesterase DFP. *Psychopharmacol.* 65:15-20.

Overstreet, D.H. (1984). Behavioral plasticity and the cholinergic system. *Prog. Neuropsychopharmacol. Biol. Psychiat.* 8: 13-151.

Overstreet, D.H. and Jamal, O.J. (1986). Behavioral tolerance to arecoline in rats: Cross-tolerance to oxotremorine and prevention by pretreatment with atropine. *Psychopharmacol.* 89:118-120.

Rider, J.A., Ellinwood, L.Z. and Cooper, J.M. (1952). Production of tolerance in the rat to octamethyl pyrophosphoramide (OMPA). *Proc. Soc. Exp. Biol. Med.* 81: 455-459.

Russell, R.W., Warburton, D.M. and Segal, D.S. (1969). Behavioral tolerance during chronic changes in the cholinergic system. *Com. Behavior. Biol.* 4: 121-128.

Russell, R.W., Overstreet, D.H., Cotman, C.W., Carson, V.G., Churchill, L., Dalglish, F.W. and Vasquez, B.J. (1975). Experimental tests of hypotheses about neurochemical mechanisms underlying behavioral tolerance to the anticholinesterase diisopropyl fluorophosphate. *J. Pharmacol. Exp. Ther.* 192: 73-85.

Russell, R.W., Carson, U.G., Jope, R.S., Booth, R.A. and Macri, J. (1979). Development of behavioral tolerance: a search for sub-cellular mechanisms. *Psychopharmacol.* 66: 155-158.

Russell, R.W., Booth, R.A., Lauretz, S.D., Smith, C.A. and Jenden, D.J. (1986). Behavioral, neurochemical, physiological effects of repeated exposures to subsymptomatic levels of the anticholinesterase soman. *Neurobehavioral Toxicology and Teratology* 8: 675-685.

Schwab, B.W. and Murphy, S.D. (1981). Induction of anticholinesterase tolerance in rats with doses of disulfoton that produce no cholinergic signs. *J. Toxicol. Enviro. Health* 8: 199-204.

Schwab, B.W., Hand, H, Costa, L.G. and Murphy, S.D. (1981). Reduced muscarinic receptor binding in tissues of rats tolerant to the insecticide disulfoton. *Neurotoxicology* 2: 635-647.

Sivam, S.P., Norris, J.C., Lim, D.K. Hoskins, B. and Ho, I.K. (1983). Effect of acute and chronic cholinesterase inhibition with diisopropyl fluorophosphate on muscarinic, dopamine and GABA receptors of the rat striatum. *J. Neurochem.* 40:141-142.

Swamy, K.V. and Murali Mohan, P. (1991). Behavioral changes in relation to cholinesterase inhibition and tolerance during chronic sublethal dosing of three organophosphate insecticides in albino rats. *Ad. Bios.* 10: 41-52.

Swamy, K.V., Ravi Kumar, R. and Murali Mohan, P. (1993). Assessment of behavioral tolerance to monocrotophos toxicity in albino rats. *Indian J. Pharmacol.* 25: 24-29.

Wecker, L., Philip, L., Mobley and Wolf-D Dettbarn (1977). Central cholinergic mechanisms underlying adaptation to reduced cholinesterase activity. *Biochem. Pharmacol.* 26: 63-637.

Yang, C.M., Murali Mohan, P., Dwyer, T.M. and Farley, J.M. (1990). Changes in affinity states during down-regulation of muscarinic receptors in tracheal smooth muscle of organophosphate-treated swine. *J. Auton. Pharmac.* 8: 79-91.

Yudkoff, M., Zaleska, M.M., Nissim, I., Nelson, D. and Erecinska, M. (1989). Neuronal glutamine utilization: pathways of nitrogen transfer studied with [15N] glutamine. *J. Neurochem.* 53: 632-640.