Bull. Environ. Contam. Toxicol. (1990) 44:819–825

Bulletin of Environmental Contamination and Toxicology

Mechanism of Cell Destructive Action of Organophosphorus Insecticide Phosalone in *Clamydomonas reinhardtii* Algal Cells

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Effects of insecticides do not remain restricted to target organisms but also extend to nontarget organisms which play an important role in the ecosystem and the food chain by way of their involvement in many biological processes such as biogeochemical cycling, decomposition reactions, energy production, transformation of energy through tropic levels, and also numerous other microbe-microbe, microbe-plant and microbe-animal interactions (Hurlbert 1975; Lal and Saxena 1980; Ware 1980; Subba-Rao and Alexander 1980). A major portion of the nontarget organisms is constituted by algal cells. In studies on the effects of insecticides on these organisms, the alga Chlamydomonas reinhardtii can be used as a model since the laboratory conditions for the sexual and the asexual growth of this organism are well established (Sueoka 1960; Chiany et al. 1970; Cain and Cain 1984; Netrawali et al. 1986; Pednekar et al. 1987). This paper describes the mechanism of cell destructive action of widely used organophosphorous insecticide phosalone in C. reinhardtii cells in stationary phase condition.

MATERIALS AND METHODS

Chlamydomonas reinhardtii WT cells (a kind gift from Dr. P.E. Brayant, Institute of Biology, Frankfurt, Main, FRG) were grown vegetatively in the synthetic medium under continuous light from a band of fluorescent tubes (3750 lux) for 72 hr at 25°C (Sagar and Granic 1953). At the end of incubation period, the cell-growth was usually in the range of 3-5 x 10^6 cells/ml.

Cells grown vegetatively for 72 hr (3-5 x 10^6 cells/ml) were isolated by centrifugation, washed and resuspended in the fresh growth medium to the cell density of 5-6 x 10^7 cells/ml. Cells at this density in suspension remained in stationary phase condition for 80 hr without any change in cell-number and cellular protein and chlorophyll contents (Gandhi et al. 1988). Cells thus maintained were termed as 'cells in stationary phase condition'.

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Technical grade phosalone [O,O-diethyl-S-(6-chloro-2-oxobenzoxazoline-3-yl) methyl phosphorodithioate] was obtained from Volrho Ltd., Hyderabad, India, and its concentration used in the experiments was expressed on the basis of its purity (97%). The insecticide was dissolved in DMSO (Dimethyl sulfoxide, AR Grade, Sigma Chemical Co., USA) and was diluted to the desired concentration (1×10^{-3} M) with the filter sterilized C. reinhardtii growth medium. The concentration of DMSO in the growth medium containing the desired concentration of the insecticide (1×10^{-3} M) was 0.025%. That the presence of DMSO in the medium up to the concentration of 2% did not affect C. reinhardtii in stationary phase cell condition and also its vegetative cell-growth was reported earlier from this laboratory (Gandhi et al. 1988). Additionally, the report showed that phosalone up to the concentration of 1.2×10^{-5} M did not affect the vegetative growth of the organism. However, the insecticide inhibited the growth from 0 to 99% in a dose dependent manner in the concentration range of 1.2×10^{-5} M to 1×10^{-3} M.

The experiments described below were carried out with C. reinhardtii cells in stationary phase condition and the treatment of phosalone in the growth medium containing 0.025% DMSO was accorded at the concentration of 1×10^{-3} M. Corresponding control groups consisting of cells treated with and without 0.025% of DMSO in the medium were included.

In the first set of experiments (Fig. 1a), stationary phase cells in the growth medium were incubated in the presence of phosalone in light for 66 hr at 25°C. At different periods, aliquots were withdrawn for the measurements of the cell count.

In the second set of experiments (Fig. 1b), stationary phase cells were treated with phosalone for 2 hr, washed, resuspended to the original cell density in the fresh growth medium (5-6 x 10^7 cells/ml) and post-treatment incubated in light for 64 hr at 25°C. At different time intervals, aliquots were removed for determinations of cell count and protein content of the undestroyed cells.

The leakage of alkaline phosphatase enzyme (EC 3.1.3.1) and cellular protein in the cell suspension medium of phosalone treated cells during the early post-treatment period was examined as follows. Cells in stationary phase condition were treated with phosalone for 2 hr, washed, resuspended to the original cell density in ice cold 0.05 Tris-HCl buffer (pH 7.0) and post-treatment incubated in light for 4 hr at 4°C. At the different periods, aliquots were withdrawn for measurements of cell number and the levels of protein and alkaline phosphatase enzyme activity in the cells and the cell suspension medium.

Alkaline phosphatase enzyme activity was measured at pH 9.0 according to the procedure of Sulkowski et al. (1963) using p-nitrophenylphosphate as a substrate. One unit of the enzyme activity was expressed as mM of p-nitrophenol formed/min/mg protein (1 µm p-nitrophenol = 1.0 OD A 410).

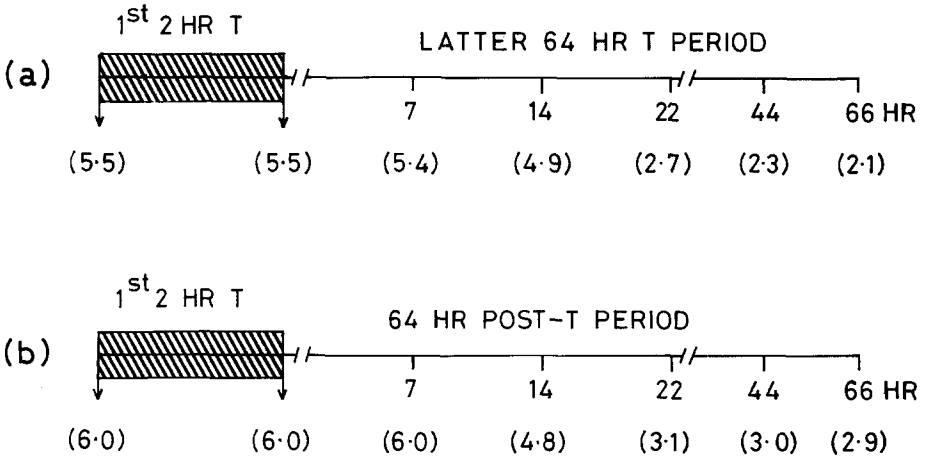

Figure 1. Effect of phosalone treatment on stationary phase C. reinhardtii cells. T = Treatment. The figures in parenthesis represent cell number x 10^7 cells/ml. Each value is an average of 4 replicates of 5 independent experiments.

Cells were counted in haemocytometer after fixing in formaldehyde solution (5%, v/v).

Protein was estimated according to the procedure of Lowry et al. (1951).

RESULTS AND DISCUSSION

Results from Fig. 1a show that the treatment of phosalone (1×10^{-3} M) to C. reinhardtii cells in stationary phase condition did not produce cell-loss in the first 2 hr of the treatment period, but caused significant loss in cells ($\approx$ 50%) in the next 22 hr treatment period. In the following 44 hr treatment period (from 22 to 66 hr), the loss increased by about 10% only.

As seen in Fig. 1a (first 2 hr treatment period), the treatment of phosalone for 2 hr to stationary phase cell population did not give rise to cell-loss during the treatment period (Fig. 1b). However, significant loss in cell population ($\approx$ 50%) took place in the first 22 hr post-treatment period. The magnitude of the loss did not increase in the following 44 hr post-treatment period (from 22 hr to 66 hr).

The results (Fig. 1a,b) demonstrated that the presence or the absence of phosalone in the suspension medium of the cells pretreated with phosalone for 2 hr did not exert much influence on the magnitude and the time-pattern of the cell-loss. Thus the cellular injury induced in 2 hr treatment period of phosalone appeared to be the main factor responsible for causing significant cell-loss in the 22 hr post-treatment period.

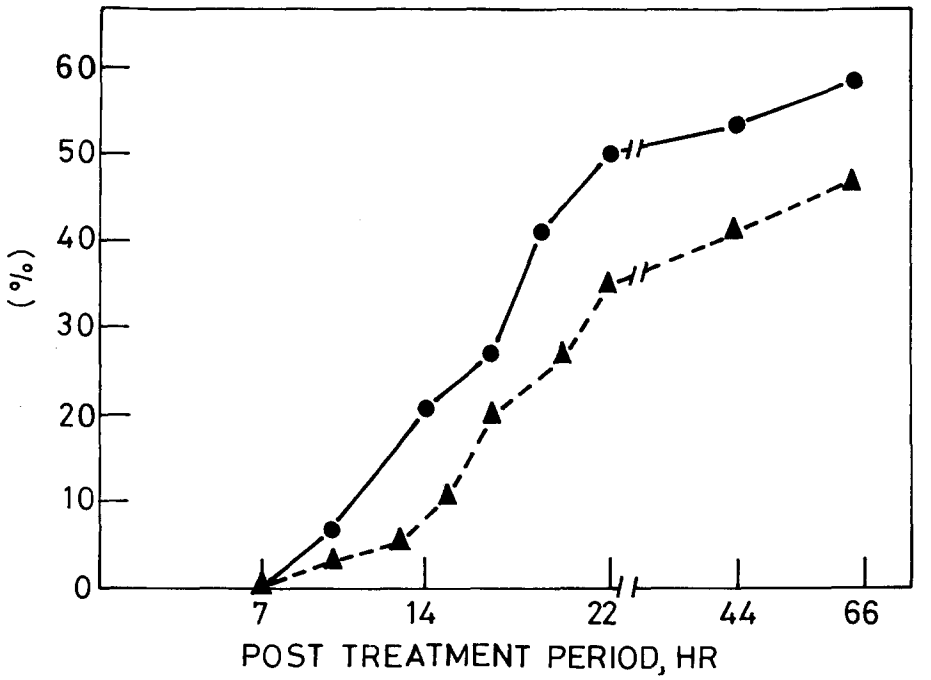

Figure 2. Post-treatment effect of phosalone on stationary phase C. reinhardtii cells. ●——●, cell-loss, ▲-----▲, decrease in level of protein in undestroyed cells. Each value is an average of 4 replicates of 5 independent experiments.

The treatment of phosalone to the cells in stationary phase condition caused significant cell-loss and reduction in the protein content of undestroyed cells (Fig. 2). The magnitudes of these impairments increased rapidly in 7 hr to 22 hr post-treatment period as compared to that in 22 hr to 66 hr post-treatment period. Results that the loss in cells appeared around 7 hr post-treatment period suggested that the cellular injury produced in 2 hr treatment period of phosalone, which did not cause loss in cells during the treatment period, may require additional time for its enlargement before giving rise to depletion in cellular protein content and destruction of cells.

The data from Fig. 3 showed that the reduction in the level of alkaline phosphatase activity (a marker enzyme of cell membrane) in phosalone treated cells, which began around 30 min of the post-treatment period, increased with the extension in the post-treatment period (from 30 min to 240 min). The decrease in the enzyme activity of the treated cells, accompanied by the increase in its activity in the cell-suspension medium, indicated damage to cell membrane of phosalone treated cells. The leakage of cellular protein in the insecticide treated cells began to appear at about 2.5 hr post-treatment period.

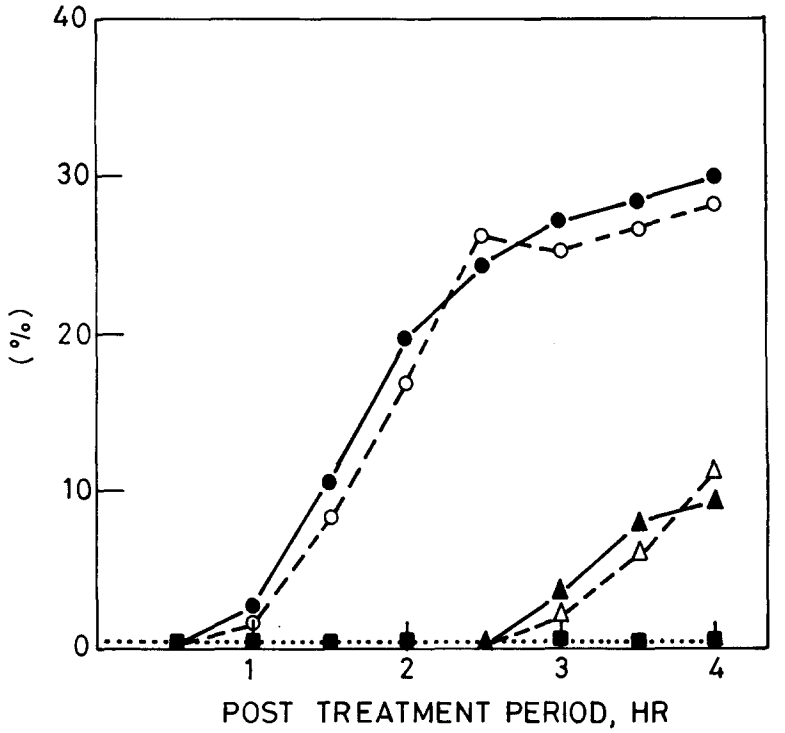

Figure 3. Leakage of alkaline phosphatase enzyme and cellular protein during post-treatment period of phosalone treated C. reinhardtii cells. ●——●, decrease in the enzyme activity in cells; o-----o, increase in the enzyme activity in cell suspension medium; ▲——▲, decrease in level of protein in cells; △——△, increase in level of protein in cell suspension medium; ■——■, cell-loss. Each value is an average of 4 replicates of 5 independent experiments.

The results of our experiments demonstrated that phosalone at the concentration of 1 x 10^{-3} M produced lesions in the membrane of C. reinhardtii cells in 2 hr treatment period. These lesions enlarged in the first 2 to 3 hr post-treatment period and gave rise to the leakage of cellular protein. The impairments finally caused destruction of significant number of cells in 22 hr post-treatment period.

Phosalone induced lesions in the cell membrane and their enlargement in the post-treatment period can be explained as follows. Antunes-Medeira et al. (1980) have reported that organophosphorous insecticides, parathion and azinphos, interact with lipid bylayer and produce disordering effects. These authors have indicated that this effect is probably related to an enhancement of disorder in the lipid packing with increased motion of the lipid hydrocarbon chains of phospholipids. The increased disorder in the hydrophobic core can weaken the interactions of the fatty acid chains by Van der Waals attractions (Lenaz 1979) and decrease lipid adhesion. The action of phosalone

on cell membrane may involve both chemical and physical changes which alter the native properties of these membranes (Lee and Wilkinson 1973; Antunes-Madeira and Victor-Madeira 1979; Antunes-Madeira et al. 1980). Antunes-Madeira et al. (1980) have shown that organophosphorous insecticides, parathion and azinphos, interact better with more fluid phases composed of phospholipids and shorter chains. Thompson et al. (1977) have shown that phase separations provide a way for introducing lateral heterogeneities into the plane of bio-membranes at the fluid solid boundries. Such effect may produce lateral compressibilities and extensibilities (Linden et al. 1973; Phillips et al. 1975; Shimshick and McCounell 1973), which, in turn, may affect the physiological functions of biomembranes, through modulation of permeability (Phillips et al. 1975; Marcelja and Wolfe 1979), enzymes and carrier activities (Linden et al. 1973; Phillips et al. 1975; Shimshick and McConnell 1973; Marcelja and Wolfe 1979) and membrane fusion processes (Papahadjopoulous and Portis 1978). Our results that the lowering in the levels of alkaline phosphatase (a cell membrane marker enzyme) in phosalone treated cells began in the post-treatment period suggest the possibility of such mechanism of action of phosalone on cell membrane of the algal cell.

The studies demonstrate the modes of toxic action of phosalone in the C. reinhardtii algal cells. Such studies with other insecticides in use employing nontarget organisms would eventually help to understand the possible impact of the contamination of insecticides on the ecosystem and would also give better insights for the development of new synthetic insecticides.

DEDICATION

MSN dedicates this paper with reverence to Prof. A. Sreenivasan on the occasion of his 80th birthday.

REFERENCES

Antunes-Madeira MC, Madeira VMC (1979) Interaction of insecticides with lipid membrane. Biochim Biophys Acta 550: 384-392

Antunes-Madeira MC, Carvalho AP, Madeira VMC (1980) Effect of insecticides on thermotropic lipid phase transition. Pesticide Biochem Physiol 14: 161-169

Cain JR, Calin RK (1984) Effects of five insecticides on zygospore germination of the green alga Chlamydomonas moewassi. Bull Environ Contam Toxicol 33: 571-574

Chiang KS, Kates JR, Jones RP, Sueoka N (1970) On the formation of homozygous zygotic population in Chlamydomonas reinhardtii. Develop Biol 22: 655-669

Gandhi SR, Kulkarni SB, Netrawali MS (1988) Comparative effects of synthetic insecticides, endosulfan, phosalone and permethrin on Chlamydomonas reinhardtii algal cells. Acta Microbiologica Hungarica 35: 93-100

Hurlberts ST (1975) Secondary effects of pesticides on aquatic ecosystem. Residue Rev 57: 81-148
Lal R, Saxena DM (1980) Cytological and biochemical effects of pesticides on microorganisms. Residue Rev 73: 49-86
Lee TT, Wilkinson CE (1973) Differential response of plant cell membranes to some vinyl organophosphorous insecticides. Pesticide Biochem Physiol 3: 341-350
Lenaz G (1979) The role of lipids in the structure and function of membranes. Subcellular Biochem 6: 233-238
Linden CD, Wright KL, McConnel HM, Fox CF (1973) Lateral phase separation in membrane lipids and the mechanism of sugar transport in Escherichia coli. Proc Natl Acad Sci (USA) 70: 2271-2275
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193: 265-275
Marcelja S, Wolfe J (1979) Properties of bilayer membranes in the phase transition or phase separation region. Biochim Biophys Acta 557: 24-31
Netrawali MS, Gandhi SR, Pednekar MD (1986) Effect of endosulfan malathion and permethrin on sexual life cycle of Chlamydomonas reinhardtii. Bull Environ Contam Toxicol 36: 412-420
Papahadjopoulous D, Portis A (1978) Calcium induced lipid phase transitions and membrane fusion. Ann N Y Acad Sci (USA) 308: 50-63
Pednekar MD, Gandhi SR, Netrawali MS (1987) Effect of a single exposure of organophosphorous insecticide, phosalone on sexual life cycle of alga Chlamydomonas reinhardtii. International Environment 13: 219-223
Phillips MC, Graham DE, Hauser H (1975) Lateral compressibility and penetration into phospholipid monolayers and bilayer membranes. Nature (London) 254: 154-156
Sagar R, Granic S (1953) Nutritional studies with Chlamydomonas reinhardtii. Ann N Y Acad Sci (USA) 56: 831-838
Shimshick EJ, McConnel HM (1973) Lateral phase separation in phospholipid membrane. Biochemistry 12: 2351-2360
Subba-Rao RV, Alexander M (1980) Effects of DDT metabolites on soil respiration and on aquatic alga. Bull Environ Contam Toxicol 25: 215-220
Sueoka N (1960) Mitotic replication of deoxyribonucleic acid in Chlamydomonas reinhardtii. Proc Natl Acad Sci (USA) 46: 83-91
Sulkowski E, Biork W, Laskowski M (1963) A specific and non-specific alkaline monophosphate in the venom of Bothrops atrox and their occurrence in the purified venom phosphodiesterase. J Biol Chem 238: 2477-2486
Thompson TE, Lentz BR, Barenholz Y (1977) A calorimetric and fluorescent probe study of phase transitions in phosphatidyl-choline liposomes. In: Semenza G, Carafoli E (Eds), Biochemistry of membrane transport, Springer-Verlag, Berlin, pp 47
Ware GW (1980) Effects of pesticides on nontarget organisms. Residue Rev 76: 173-202

Received February 6, 1989; accepted October 18, 1989.