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DICHLORVOS POTENTIATES THE INSECTISIDE-INDUCED SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS[1]

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Tsing-Cheng Wang, Chang-Lin Wu, Ju-Hung Lin and Chung-Yiao Tarn (1988). Dichlorvos potentiates the insectiside-induced sister chromatid exchanges in Chine hamster ovary cells. *Bull. Inst. Zool., Academia Sinica* 27(2): 111-117. Anticholinesterase type insecticide, dichlorvos (DDVP) and synthetic pyrethroid insecticide, sumithrin, are positive for sister chromatid exchange (SCE) inductions in Chinese hamster ovary (CHO) cells. Our data failed to conclude that propoxur, bioallethrin, pynamin-forte and neopynamin are positive agents for SCE inductions. However, addition of DDVP at an insignificant level, i.e. 0.32 μg/ml, their abilities to induce SCE significantly increase. Genotoxicity of sumithrin, on the other hand, was reduced by the addition of DDVP at 0.32 μg/ml.

Key words: Dichlorvos, Anticholinesterase, Insecticide, Sister chromatid-exchange, Genotoxicity.

Dichlorvos (DDVP) is an organophosphorous insecticide commonly used for household insect control. It is an anticholinesterase agent capable of interacting with nucleophiles in an organism by alkylation (Wild, 1975; Ramel *et al.*, 1980). Mutagenicity of DDVP has been reported previously in microorganisms (Ashwood-Smith *et al.*, 1972: Vooged *et al.*, 1972; Bridges *et al.*, 1973; Mohn, 1973; Wild, 1973; 1975; Carerer *et al.*, 1976; Shirasu *et al.*, 1976; Bignami *et al.*, 1977; Carerer *et al.*, 1978; Griffiin III and Hill, 1978; Moriya *et al.*, 1983), *Drosophila melanogaster* (Dyer and Hanna, 1973; Hanna and Dyer, 1975) and mammals (Ramel *et al.*, 1980; Tezuka *et al.*, 1980). The risk of adverse effects on humans, however, is inconclusive (Wild, 1973; 1975; Dean and Blair, 1976; Kramers and Knaap, 1978; Nicholas *et al.*, 1978; Anonymous, 1982;). According to Klopman *et al.* (1985), the methoxyphosphinyl group in this insecticide is a good substrate for nucleophilic attack and this may cause phosphorylation of DNA. The direct methylation of DNA by DDVP itself and reaction with DNA by metabolites of the dichlor-vinyl moeity have been proposed by Ramel *et al.* (1980) as the possible mutagenic mechanisms of this insecticide. Therefore, a large segment of the population may suffer adverse health effects through DDVP residues in the environment. Several inecticides with less toxicities to humans are now used to replace DDVP for house insect control. Most of them are synthetic pyrethroids or carbamates. In many formulations, DDVP is also added to enhance the

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insecticidal activity because of its high volatility and rapid knock-down effect. The gentoxicity data of those combinations are as yet unavailable. In this paper, we report: (1) the sister chromatid exchanges (SCEs) induced by two anticholinesterase agents and four synthetic pyrethroids; (2) the synergistic effect of DDVP on the SCEs induced in Chinese hamster ovary (CHO) cells by propoxur, and three synthetic pyrethroids including bioallethrin, pynamin-forte and neopynamin; (3) the antagonistic effect of DDVP on SCEs induced by sumithrin in CHO cells.

MATERIALS ANE METHODS

Cells

CHO cells were obtained from Drs. K. Y. Jan and T. C. Lee of this Institute, recloned and designated as CHO-W line. On each experiment, the cryostored cells were thawed and cultured routinely as described previously (Lee *et al.*, 1985). To maintain karyotyplc stability, the cells for each experiment were limited to the first and second passages after thawing. Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. The growth medium was McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 0.22% sodium bicarbonate and antibioltics including penicillin (100 units/ml) and streptomycin (100 μg/ml). All materials for cell culture were purchased from Giboco,

Insceticides

dichlorvos (DDVP): 2,2-Dicholorovinyl *O*, *O*-dimethyl phosphate, 93.9% technical grade, CAS No. 62-73-7.

propoxur: 2-(1-Methylethoxy) phenol methylcarbamtae 99% technical grade, CAS No. 114-26-1.

bioallethrin: (1RS)-3-Allyl-2-methyl-4 oxocyclopent-2-enyl (1R)-trans chrysanthemate, 93% technical grade, CAS No. 584-79-2.

pynamin-forte: (1RS)-3-Allyl-2-methyl-4-oxocyclopent-2-enyl (1R)-cis, trans chrysanthemate, 92% technical grade, CAS No. 584-79-2.

neopynamin: 3,4,5,6-Tetrahydrophthalimidomethyl-trans chrysanthemate, 93.1% technical grade, CAS No. 7696-12-0.

Sumithrin: 3-phenoxybenzyl d-cis, trans-chrysanthemate, 92.8% technical grade, CAS No. 24002-80-2.

All insecticides used in this experiment were supplied by Bureau of Environmental Protection, ROC. The stock solutions of the insecticides were made according to Galloway *et al.* (1985) to obtain a maximum final concentration of 5 mg/ml in cultures by dissolving the insecticides in dimethyl sulfoxide (DMSO, E. Merck, FRG). They were then serially diluted, in the same solvent, to ten dose levels covering a range of five orders of magnitude.

Induction of SCE

3×10^5 cells were plated in a 60-mm petri dish and allowed to grow overnight. The cells were then treated with serum-free medium containing different doses of insecticide with 0.5% DMSO for two hours. The concentration of DMSO in insecticide-treated groups were kept under 0.5%. At the end of treatment, the medium was discarded and the cell were washed twice with phosphate buffered saline (PBS, Gibco). The cultures were then refilled with 5 ml medium containing BrdUrd (final concentration, 10 μM, Sigma) and incubated for another 24 hours. Two hours prior to the end of the incubation, the cultures were checked for the degree of monolayer confluence and the occurrence of mitotic cells on the surface of the cell sheet or floating in the medium. Colcemid (0.2 μg/ml, Sigma) was added to the top five doses which did not exhibit toxicity. Metaphase cells were harvested by shake-off and chromosome preparation was done by air-dried technique. The sister-chromatid-differential stain for chromosomes was prepared by a modified flourescence plus Giemsa technique (Jan *et al.*, 1982). For

each treatment 30 cells with chromosome numbers of 21±2 were randomly sampled from blindcoded slides to score the SCEs under an Olympus Vanox-S photomicroscope.

Data Analysis

Methods described by Galloway *et al.* (1985) are used for statistic analysis and final judgement of the SCE induction. An insecticide is summarized as a positive agent for SCE induction if both the trend probability (Margolin *et al.*, 1986) is smaller than 0.005 and the numbers of doses that induced SCEs over 1.2 times higher than that of control is no less than two.

RESULTS

DDVP (Fig. 1): Three doses (40, 200 and 1000 μg/ml) of DDVP induce significant SCES, The trend statistic of the induction is 21.2 indicating a trend probability of much less than 0.005. According to Galloway *et al.* (1985), our results clearly show that DDVP is a positive agent for SCE induction in CHO cells. The dose level of 0.32 μg/ml shows no apparent raise of SCE induction over the control and is therefore used for the investigations of synergistic effect of DDVP on the genotoxicities induced by other insecticides in this report.

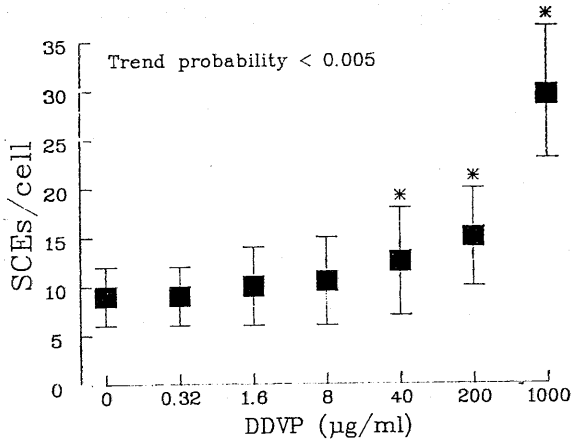

Fig. 1. Sister chromatid exchanges induced by dichlorvos (DDVP). '*' indicates that the induction is significantly raised over control according to criteria proposed by Galloway *et al.* (1985). 'p' represents the value of trend probability according to Galloway *et al.* (1985) and Margolin *et al.* (1986).

Propoxur (Fig. 2): SCEs induced by propoxur alone are dose dependent (trend statistic, 4.19 and trend probability<0.005). Significant SCE inductions, on the other hand, only observed at the dose level of 1000 μg/ml. Propoxur alone therefore is not a positive agent for SCE induction in CHO cells. However, in cultures treated with Propoxur and DDVP (0.32 μg/ml), numbers of doeses that induce significant SCEs jump from one to five. Dose response of the induction is significant (trend statistic, 12.44 and trend probability<0.005). These allow us to conclude that when used combinedly with DDVP, propoxur in a positive agent for the SCE induction in CHO cells. The genotoxicity of propoxur therefore is significantly synergized by DDVP.

Bioallethrin (Fig. 2): There is no significant SCEs induction by bioallethrin up to 200 μg/ml (trend statistic, 1.18 and trend probability, 0.12). In combination with DDVP, bioallethrin becomes a positive agent for SCE induction. The induction is dose dependent (trend statistic, 3.33 and trend probability<0.005). Numbers of dose that induce significant SCEs jump from zero to three. DDVP significantly increases the genotoxicity of bioallethrin.

Pynamin-forte (Fig. 2): This insecticide alone induces signifiicant SCE only at 200 μg/ml. The dose response of the induction is significant (trend statistic, 3.95 and trend probability<0.005). With the help of DDVP, numbers of dose that induces significant SCE increases from one to four. Trend statistic and trend probability are 8.52 and< 0.005, respectively. Pynamin-forte becomes a positive agent for SCE induction after the addition of DDVP. DDVP therefore is synergistic to the gentoxicity of this insecticide.

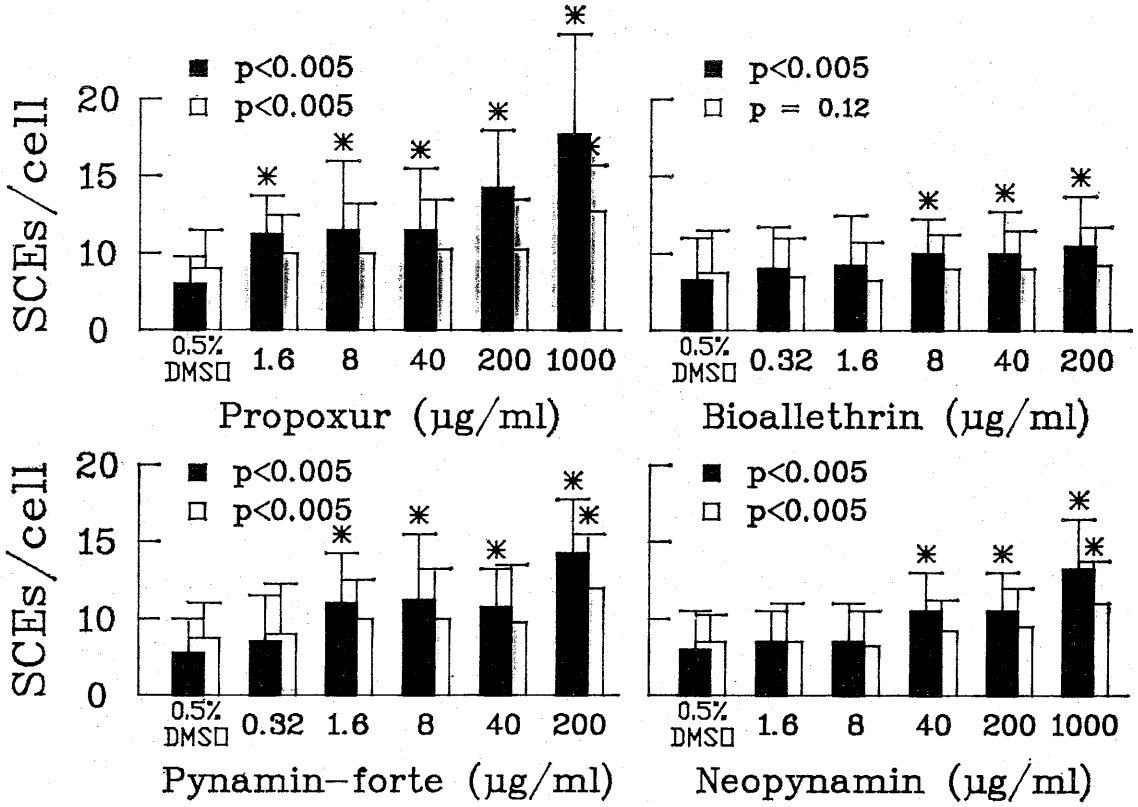

Fig. 2. Effect of dichlorvos (DDVP, 0.32 μg/ml) on the SCEs induced by propoxur, bioallethrin, pynamin-forte and neopynamin. Empty and solid bars represents the treatments without and the addition of DDVP, respectively. '*' indicates that the SCE induction is significantly raised over that of control according to Galloway *et al.* (1985). 'p' represents the value of trend probability according to Galloway *et al.* (1985) and Margolin *et al.* (1986).

Neopynamin (Fig. 2): SCE induced by necopynamin alone is dose dependent (trend statistic, 3.93 and trend probability<0,005). But there is only one dose, i. e., 1000 μg/ml, inducing significant amount of SCEs. In the presence of DDVP, three doses induced significant SCEs. Its trend statistic and trend probability are 7.60 and<0.005, respectively. The SCE induction becomes positive. Therefore, the genotoxicity of neopynamin

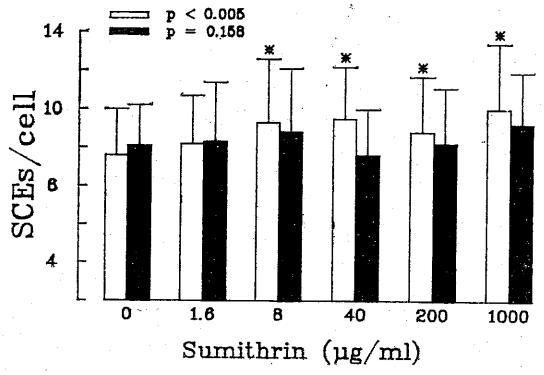

Fig. 3. Effect of dichlorvos (DDVP, 0.32 μg/ml) on the SCEs induced by sumithrin. Empty and solid bars represents the treatments without and with the addition of DDVP, respectively. '*' indicates that the SCE induction is significantly raised over that of control according to Galloway *et al.* (1985). 'p' represents the value of trend probability according to Galloway *et al.* (1985) and Margolin *et al.* (1986).

is also significantly synergized by DDVP.

Sumithrin (Fig. 3): Sumithrin alone induces significant amount of SCEs in CHO cells. Induction of SCEs was observed in four doses. The induction is also dose-dependent (trend statistic, 3.04 and trend probability <0.005). In the presence of DDVP, sumithrin does not induce any significant SCEs at dose level up to 1000 μg/ml. The dose response also becomes insignificant with trend statistic and trend probability of 1 and 0.158, respectively. Sumithrin changes its SCE induction from positive to negative with the presence of DDVP. Therefore, DDVP is antagonistic to sumithrin in the inductions of SCEs is CHO cells.

DISCUSSION

Insecticides studied in this report are categorized into two different groups according to their modes of action for killing. First group is the acetylcholinesteranse inhibitors in which DDVP is an organophosphorous and propoxur is a carbamate insecticide. Another group is the depolarizing agent for nerve cell membrane. Bioallethrin, pynamin-forte, neopynamin and sumithrin are synthetic pyrethroids. Except for DDVP, these insecticides are now very popular for the house insect control because of their low acute mammal toxicities. Mutagenicity data for DDVP in literature, however, are inconclusive. As for other insecticides included in this report, previous publication dealing with mutagenicity studies are unavailable.

Recently DDVP has been found to be mutagenic in *E. coli* (Aldrick and Rowland, 1985). Pretreatment of Syrian hamster ovary cells with DDVP also showed a significant increase in the SA7 transformation assay (Hatch *et al.*, 1986). Our results clearly indicate that DDVP is a positive agent for SCE induction in CHO cells. These confirm the previous observations in Chinese hamster cells (Sandhu *et al.*, 1985) and CHO cells (Nishio and Uyeki, 1981). Therefore, human exposure to DDVP is an important health issue.

Propoxur is probably the most popular carbamate used to replace DDVP as house insect control agent. Our data failed to prove its mutagenicity in CHO cells. It is, however, positive in SCE induction with the help of DDVP. This combination is a very popular formulation used extensively in Asia.

Synergistic effects of DDVP are also observed in the genotoxicities of synthetic pyrethroid insecticides including bioallethrin, pynamin-forte and neopynamin. Pyrethroids are considered as the most promising insecticides because of the low mammal toxicity. Recently, their application on the house insect control as wel as for plant protection in field have been increased greatly. Insecticidal activities of pyrethroid, however, are frequently handicapped by their slow killing effect and low persistence. Formulations with the additions of other insecticides therefore become necessary. This, however, could increase their adversed effects to human health. Our results in this report clearly indicate this possibility.

Sumithrin is the only pyrethroid that shows positive induction of SCEs. The data that sumithrin is antagonistic to DDVP in the induction of SCEs suggest sumithrin in combination with DDVP may be a good formulation.

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二氯松對其它殺蟲劑在中國倉鼠卵巢細胞誘引姊妹染色分體交換之增強作用

王清澄　吳彰玲　林儒宏　唐中耀

在中國倉鼠卵巢細胞中，二氯松與人工合成除蟲菊中之 Sumithrin 可以顯著地造成姊妹染色分體交換之增加，但其它殺蟲藥劑包括拜貢，Bioallethrin, pynamin-forte 與 Neopynamin 則無足够證據顯示其可顯著增加姊妹染色分體之交換，但是二氯松在一個不顯著之劑量水平下 (0.32 μg/ml)，對於拜貢，Bioallethrin, pynamin-forte 與 Neopynamin 之遺傳毒性却具有協力作用。而 Sumithrin 之遺傳毒性却反而與二氯松之加入有拮抗作用，本研究結果顯示，殺蟲藥劑之混合使用可以使本爲安全之單劑產生對生物遺傳物質之破壞。