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Genotoxicity of Methoxyphosphinyl Insecticide in Mammalian Cells

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T.C. Wang, Chih-Min Lin and Li-Wen Lo (2003) Genotoxicity of the methoxyphosphinyl insecticide in mammalian cells. *Zoological Studies* **42**(3): 462-469. The genotoxicity of 5 organophosphorus insecticides containing the methoxyphosphinyl subunit was assayed by examining the induction of sister-chromatid exchanges (SCEs), chromosome aberrations, and the hypoxanthine-guanine phosphoribosyl transferase (*hgprt*) gene mutations in CHO cells. Insecticides included acephate, dichlorvos, monocrotophos, methamidophos, and trichlorfon. They consistently induced significant SCEs, with the order of induction potential of acephate > trichlorfon > monocrotophos > methamidophos > dichlorvos. However, only 2 of them, dichlorvos and methamidophos, induced positive chromosome aberrations. Monocrotophos and acephate were questionable positive, while trichlorfon was negative for chromosome aberration induction. The order of chromosome aberration induction potential was dichlorvos > methamidophos > monocrotophos > acephate > trichlorfon. None of these 5 insecticides induced significant *hgprt* gene mutations compared to the concurrent negative control. The discrepancy between the results of the 2 cytogenetic endpoints, and the contradictory outcome between *hgprt* mutation and SCE are discussed, from which a possible mechanism of insecticide genotoxicity postulated.
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The chemical application of insecticides is probably the most-feasible approach for controlling pest insect populations both on crops and in household areas. Contamination of the environment by insecticides on the other hand has created many adverse effects to human health. The potentials of insecticides to induce mutagenicity and carcinogenicity are among the greatest concerns of modern society. Organophosphorus insecticides, due to their relatively nonpersistence in the environment, are some of the most extensively used insecticides in modern human history (Hodgson and Levi 1987). Many of them are electrophiles capable of interacting with nucleophiles in organisms by alkylation. The alkylating compounds are frequently mutagenic or carcinogenic to the exposed organisms, through the induction of severe cellular stress including chromosome aberrations, sister-chromatid exchanges (SCEs), gene mutations, and cell killing (Wilhelm et al. 1997). Investigations of the genotoxicity of organophosphorus insecticides are important for protecting human health.

Among organophosphorus insecticides, those characterized by a methoxyphosphinyl group especially require more extensive study. According to Klopman et al. (1985), chemicals with this structure might be mutagenic to exposed organisms, because this segment is a common structural subunit responsible for the activities detected in the battery of tests composed of the *Salmonella typhimurium* histidine reversion assay, the mouse lymphoma gene mutation assay, and recombination in the yeast *Saccharomyces cerevisiae*. Acephate, dichlorvos, trichlorfon, methamidophos, and monocrotophos are methoxyphosphinyl organophosphorus insecticides (Fig. 1), and they have been extensively used in Taiwan. In this paper, we report on SCEs, chromosome aberrations, and hypoxanthine-guanine phosphoribosyltransferase (*hgprt*) gene mutations induced by those insecticides in Chinese hamster ovary

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(CHO) cells.

MATERIALS AND METHODS

Cells

A Chinese hamster ovary (CHO) cell line, originating from Dr. Sheldon Wolff of the Univ. of California-San Francisco, was re-cloned as CHO-W8 for its karyotype stability in the Institute of Zoology, Academia Sinica by Dr. K. Y. Jan and cryostored in liquid nitrogen. Cells were thawed 2 d before each experiment and grown at 37°C in a humidified atmosphere of 5% CO_2 in air, in McCoy's 5A medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum (Hyclone, Logan, Utah, USA), 2 mM glutamine (Gibco), sodium bicarbonate (0.22%, E. Merck, Darmstadt, Germany), and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin, Gibco). Cells used for the experiment were limited to the 4th and 5th passages after re-cloning in order to maintain the karyotype stability.

Insecticide treatment

All insecticides used were purified standards purchased from Chem Service, West Chester, PA, USA. Their chemical structures are shown in figure 1. They included acephate (*O*,*S*-dimethyl acetylphosphoramidothioate, 98%, CAS 30560-19-1), dichlorvos (2,2-Dichlorovinyl dimethylphosphate, 99%, CAS 62-73-7), methamidophos (*O*,*S*-dimethyl phosphoramidothioate, 97%, CAS 10265-92-6), monocrotophos (*O*,*O*-dimethyl-*O*-(2-methylcarbomyl-1-methyl-vinyl)-phosphate, 98%, CAS 6923-22-4), and trichlorfon (Dimethyl-(2,2,2-trichloro-1-hydroxyethyl) phosphate, 98%, CAS 52-68-6). Solutions of insecticides were prepared as recommended by Galloway et al. (1985). Briefly, a 500 mg/ml stock solution was made in dimethyl sulfoxide (DMSO, E. Merck) to obtain a maximum final concentration of 5 mg/ml in culture. From the maximum concentration, a series of dilutions was made in the same solvent to achieve 10 doses in a half-log series. The treatment duration was 2 h.

SCE induction

Cells at 3 x 10^5 were plated on a 60-mm petri dish and allowed to grow overnight (for less than 24 h). Cells were then treated with test insecticides or the control chemical (1% DMSO) for 2 h. At the end of treatment, cultures were washed twice with phosphate-buffered saline (PBS) and replenished with fresh McCoy's 5A medium containing 5'-bromodeoxyuridine (BrdUrd at a final concentration of 10 μ, Sigma, St. Louis, MO, USA). Incubation of cell cultures was continued in the dark for 24 h (2 cell cycles). Two hours prior to the end of incubation, cultures were examined under an inverted microscope. The degree of confluence of the surface of the cell sheet or if cells were floating in the medium was noted as indicated by Galloway et al. (1985). The top 5 dose levels with cells likely to yield analyzable metaphases were chosen, and 0.2 μg/ml colcemid (Sigma) was added. Mitotic cells were harvested using the shake-off and air-drying methods as previously described (Jan et al. 1982). Sister-chromatids were differentially stained using the fluorescence plus Giemsa technique. At least 50 mitotic cells with 21 ± 2 chromosomes and well-differentiated sister-chromatids were randomly sampled in order to score the SCEs in each experiment. The significance of SCE induction was tested according to the method recommended by Margolin et al. (1986). Briefly, the statistical analysis of SCE data was based on an assumption of random sampling from a Poisson density for the number of SCEs scored. This involved linearly regressing the average number of SCEs per chromosome on the log-

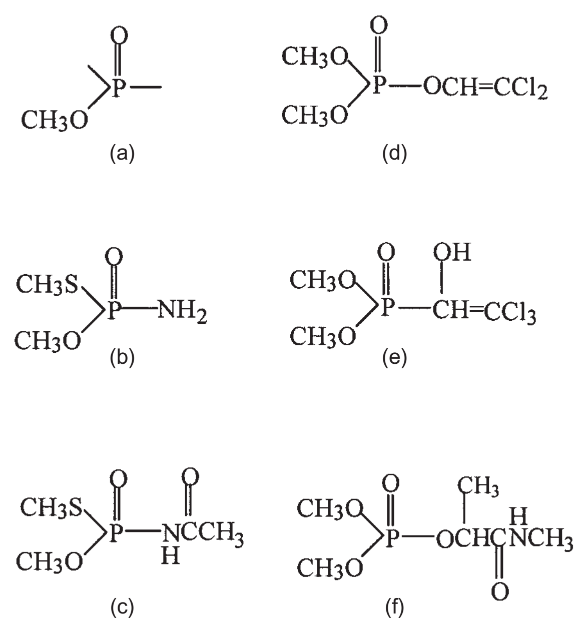

Fig. 1. Chemical structure of methoxyphosphinyl insecticides used in this investigation. (a) Basic structural fragment of the methoxyphosphinyl compound, (b) methamidophos, (c) acephate, (d) dichlorvos, (e) trichlorfon, (f) monocrotophos.

arithm of the test compound dose. The resulting trend test statistic was referenced to a table of normal probabilities from which a *p* value or an observed level of significance was read. Data are presented as the number of SCEs/cell since the cell is the biological unit of interest.

Chromosome aberration induction

The protocol for the induction of chromosome aberrations was basically the same as that for SCE induction except that the post-treatment incubation time was 18 instead of 24 h, and that no BrdUrd was added to the culture. Mitotic cells were harvested and stained in a 3% Giemsa solution. One hundred metaphases were randomly sampled from slides of each treatment to analyze chromosome aberrations. The percent of aberrant cells was used as the parameter for the analysis of the significance of induction. Metaphases with gaps only were recorded but not included in the calculation of the percent of aberrant cells. Significance of the induction was tested using methods recommended by Margolin et al. (1983, 1986). According to their analysis, a binomial sampling model was used. The statistical inference for each response was also based on a trend test similar to that for SCEs except that the percentage of cells in each category of aberration was regressed linearly on the log of the dose, and that the test statistic reflected the binomial sampling assumption. A table of normal probabilities yielded *p* values for this test. Biological concern with the number of doses whose responses were elevated over the control mean was translated into a test for each treatment-control comparison at the 0.01 level for binomial mutagenicity data from a single-dose experiment (Margolin et al. 1983).

Induction of *hgprt* gene mutations

Cells at a density of 1.5×10^4 cells/cm^2 were seeded 1 d before the experiments. The top 3 concentrations of insecticide in the induction of SCEs and chromosome aberrations were used to treat cells for 2 h. After treatment, cells were washed twice with PBS and incubated with fresh McCoy's 5A medium for 24 h. The mutation frequency at the *hgprt* locus was then determined according to the procedures described by Gupta and Singh (1982). Briefly, after a 24-h incubation in insecticide-free media, cells were trypsinized, plated at a density of 1×10^6 cells/100-mm petri dish and subcultured every other day for the expression of thioguanine-resistant mutants. Mutants were selected by splitting 1×10^6 cells into five 100-mm petri dishes and feeding them with McCoy's 5A medium containing 6-thioguanine (10 μg/ml) on the 8th day after treatment. Along with plating in selective medium, an aliquot of 100 cells was also plated in normal medium to determine the plating efficiency of the cells. Dishes were incubated for 7 d at 37°C without changing the medium, and then fixed with methanol and stained with a 10% Giemsa solution. The mutation frequency was calculated from the number of cells plated, the number of mutant colonies observed, and the plating efficiency of the cells as described by Singh and Gupta (1982). A formula developed by Margolin et al (1983) which relates the associated probability of detecting a mutagen to the mutant frequencies and the sample sizes of the 2 groups was used for the statistical analysis. A probability of < 0.05 for a binary observation from any experimental unit was accepted as positive.

RESULTS

Cytotoxicity

Each insecticide was diluted in a half-log series of 10 different doses for the induction of SCEs and chromosome aberrations. Upon mitotic cell harvest, only the top 4-5 treatments which showed no apparent cytotoxicity were selected to score the induction. Therefore, variations in dose ranges shown in the tables, to some extent, reflect the differential cytotoxicities of the tested insecticides. Dose levels of dichlorvos and monocrotophos, respectively shown in tables 1 and 2, were 5 times higher than those of trichlorfon, methamidophos, and acephate, which are shown respectively in tables 3-5. In addition, with treatment using dichlorvos or monocrotophos, the number of mitotic cells harvested was insufficient for a significant SCE analysis at a dose > 1 mg/ml, due to the cytotoxic effect (Tables 1, 2). On the contrary, with treatment using methamidophos, acephate, or trichlorfon, there was no difficulty in obtaining sufficient numbers of mitotic cells at a dose level up to 5 mg/ml (Tables 3-5). These facts indicate that dichlorvos and monocrotophos are more cytotoxic to CHO cells than are the other 3 insecticides.

SCE induction

In spite of the variation in cytotoxicity among the different methoxyphosphinyl insecticides tested, they consistently induced significant SCEs, with induction levels in at least 2 doses statistically differing from the concurrent control ($p < 0.01$, Tables 1-5). Their dose responses of overall induction were also statistically significant ($p < 0.005$). According to the criteria for SCE induction assessment proposed previously (Margolin et al. 1983 1986, Galloway et al. 1985), these 5 insecticides were positive SCE-inducing agents. In our protocol for SCE induction, logarithmically growing CHO cells were treated with insecticide for 2 h prior to the addition of BrdUrd. The insecticide was then washed off, and the cell culture was incubated with BrdUrd for another 24 h. Using this protocol, the mitotic cells sampled for the analysis of SCE induction were most likely those cells which were previously at the G1 stage during the insecticide treatment period. Therefore, it would be more precise to state that treatment of CHO cells at the G1 stage with these 5 insecticides induced significant SCEs.

Although all 5 insecticides induced significant SCEs, their potentials for SCE induction varied. Dichlorvos significantly induced SCEs at a dose level lower than any other insecticides in this study, i.e., at 8 μg/ml (Table 1). The highest SCE level induced by dichlorvos was 13.10 ± 3.58 at 200 μg/ml. At doses higher than 200 μg/ml, dichlorvos was either cytotoxic to CHO cells or showed a significant cytostatic effect, which made analysis of SCE induction impossible. Monocrotophos, although similar to dichlorvos in the trends of cytotoxicity and SCE induction, was not as cytostatic to CHO cells (Table 2). In those cells treated with monocrotophos at 1 mg/ml, sister-chromatids

Table 1. Dichlorvos-induced sister-chromatid exchanges (SCEs), chromosome aberrations, and *hgprt* gene mutations in CHO-W8 cells

Dichlorvos (μg/ml)	SCEs/cell[a]	Percent aberrant cells[a]	6TG-resistant colonies per 10^6 cells[b]
1% DMSO	7.78 ± 2.20	0	1.0
0.32	9.28 ± 2.76	-	—
1.6	9.00 ± 2.94	4	—
8.0	9.78 ± 3.11*	3	
40.0	10.02 ± 2.66*	7*	4.71
200.0	13.10 ± 3.58*	7*	2.47
1000.0	—	86*	0.0
p value for the dose response	< 0.005	< 0.001	> 0.005

[a]Significance of SCEs/cell and percent of aberrant cells in each treatment, which is indicated by an asterisk (*), and *p* values for the dose response were calculated as described by Galloway et al. (1985) and Margolin et al. (1986).
[b]Significance of *hgprt* gene mutations in each treatment, indicated by an asterisk (*), and the *p* value for the dose response were calculated as described by Margolin et al. (1983).

Table 2. Monocrotophos-induced sister-chromatid exchanges (SCEs), chromosome aberrations, and *hgprt* gene mutations in CHO-W8 cells

Monocrotophos (μg/ml)	SCEs/cell[a]	Percent aberrant cells[a]	6TG-resistant colonies per 10^6 cells[b]
1% DMSO	7.64 ± 2.62	0	0.0
1.6	8.10 ± 2.97	2	—
8.0	9.42 ± 3.24*	2	—
40.0	10.28 ± 3.13*	5	6.32
200.0	11.14 ± 3.17*	5	7.89*
1000.0	15.58 ± 3.42*	11*	1.37
p value for the dose response	< 0.005	< 0.001	> 0.005

[a]Significance of SCEs/cell and percent aberrant cells in each treatment, which is indicated by an asterisk (*), and *p* values for the dose response were calculated as described by Galloway et al. (1985) and Margolin et al. (1986).
[b]Significance of *hgprt* gene mutations in each treatment, indicated by an asterisk (*), and the *p* value for the dose response were calculated as described by Margolin et al. (1983).

were well differentiated, indicating no sign of an induction of cell cycle delay. SCEs induced at this dose level were 15.58 ± 3.42/cell, which is higher than the highest SCE level induced by dichlorvos.

Trichlorfon and acephate, although less cytotoxic than dichlorvos and monocrotophos, induced a much higher SCE level. There was no sign of significant cytotoxic or cytostatic effects with treatment using these 2 insecticides at doses up to 5 mg/ml. Acephate and trichlorfon induced SCE at levels of 25.92 ± 6.26/cell (Table 3) and 25.86 ± 5.24/cell (Table 5) respectively, which were twice as high as those of dichlorvos, monocrotophos, or methamidophos. The potency of SCE induction in methamidophos was similar to that in dichlorvos.

When we used dichlorvos or monocrotophos, both at 200 μg/ml, in the entire 1st or 2nd BrdUrd cycles for 12 h, or 1st + 2nd BrdUrd cycles for 24 h, instead of a 2-h treatment in pre-BrdUrd incubation period, monocrotophos-induced SCEs significantly increased, while dichlorvos-induced SCEs did not (Table 6). Treatment with monocrotophos in the entire BrdUrd incubation period (24 h) increased SCEs/cell to more than twice that of 2 h in the pre-BrdUrd period as shown in table 2. There were 7 and 10 more SECs/cell induced compared to treatments in the 1st and 2nd BrdUrd cycles respectively. Treatment with monocrotophos in the 1st BrdUrd cycle induced significantly more SCEs compared to the 2nd BrdUrd cycle. Dichlorvos, on the contrary, produced no difference between treatments in the 1st or 2nd BrdUrd cycles. Treatment with dichlorvos for twice as long (24 h) did not increase SCEs significantly over those with 12 h of treatment.

Chromosome aberration induction

Induction of chromosome aberrations was expressed as the percent of aberrant cells in the tables. According to the criteria proposed previously for the assessment of chromosome aberra-

Table 3. Trichlorfon-induced sister-chromatid exchanges (SCEs), chromosome aberrations, and *hgprt* gene mutations in CHO-W8 cells

Trichlorfon (mg/ml)	SCEs/cell[a]	Percent aberrant cells[a]	6TG-resistant colonies per 10^6 cells[b]
1% DMSO	7.30 ± 2.48	0	0.0
0.04	8.43 ± 2.62	4	—
0.2	9.16 ± 2.72*	5	0.0
1.0	12.57 ± 3.30*	5	0.0
5.0	25.86 ± 5.24*	6	0.0
p value for the dose response	< 0.005	> 0.005	> 0.005

[a]Significance of SCEs/cell and percent aberrant cells in each treatment, which is indicated by an asterisk (*), and *p* values for the dose response were calculated as described by Galloway et al. (1985) and Margolin et al. (1986).
[b]Significance of *hgprt* gene mutations in each treatment, indicated by an asterisk (*), and the *p* value for the dose response were calculated as described by Margolin et al. (1983).

Table 4. Methamidophos-induced sister-chromatid exchanges (SCEs), chromosome aberrations, and *hgprt* gene mutations in CHO-W8 cells

Monocrotophos (mg/ml)	SCEs/cell[a]	Percent aberrant cells[a]	6TG-resistant colonies per 10^6 cells[b]
1% DMSO	6.81 ± 2.52	0	0.0
0.04	8.07 ± 3.05	2	—
0.2	9.13 ± 3.37*	8*	0.0
1.0	11.10 ± 4.75*	10*	2.2
5.0	13.76 ± 3.54*	18*	0.76
p value for the dose response	< 0.005	< 0.001	> 0.005

[a]Significance of SCEs/cell and percent aberrant cells in each treatment, which is indicated by an asterisk (*), and *p* values for the dose response were calculated as described by Galloway et al. (1985) and Margolin et al. (1986).
[b]Significance of *hgprt* gene mutations in each treatment, indicated by an asterisk (*) and the *p* value for the dose response were calculated as described by Margolin et al. (1983).

tion induction (Margolin et al. 1983 1986, Galloway et al. 1985), dichlorvos (Table 1) and methamidophos (Table 4) were the only 2 insecticides which induced positive chromosome aberrations. Both of them induced significant percentages of aberrant cells at 3 doses and showed dose responses in overall induction ($p < 0.001$). Dichlorvos induced a very high level of chromosome aberrations, with 86% aberrant cells in treatment with 1 mg/ml, which previously showed a significant cell cycle delay in SCE induction. Monocrotophos (Table 2) and acephate (Table 5) induced a significant percentage of aberrant cells only at the highest dose, although the overall induction was dose responsive ($p < 0.001$). According to Galloway's criteria (1985), their clastogenicity was questionable positive. Although trichlorfon induced a high level of SCEs, it was negative for the induction of chromosome aberrations. Trichlorfon induced no significant percentage of aberrant cells up to 5 mg/ml, and showed no dose response ($p > 0.005$, Table 3).

Induction of *hgprt* mutations

Induction of *hgprt* mutations is expressed in the tables as the number of colonies resistant to 6-thioguanine (6TG-R) formed per 10^6 surviving cells. In this experiment, the background frequency of 6TG-R colonies in the negative control group treated with 1% DMSO was (0-2) x 10^{-6}, while that of positive control group treated with 4 mM ethyl methanesulfonate was around 1 x 10^{-4}. The frequency of 6TG-R colonies induced by insecticides in this study was less than 1 x 10^{-5}, which did not significantly differ from the negative control. Consequently, these 5 insecticides tested did not induce *hgprt* gene mutations in CHO cells.

DISCUSSION

Five insecticides containing the methoxyphosphinyl group were assayed for the induction of SCEs, chromosomal aberrations, and *hgprt* gene mutations in CHO cells. In assays for SCE, these 5 insecticides consistently produced positive induction in CHO cells. The induction of chromosomal aberrations, on the other hand, was not as consistent. Only 2 insecticides (dichlorvos and methamidophos), which induced a significant number of SCEs, showed positive results for chromosome aberration induction. The clastogenicity of these 2 insecticides has previously been documented (Amer and Sayed 1987, Lin et al. 1988,

Table 6. Induction of SCEs with dichlorvos or monocrotophos treatment in different BrdUrd cycles

1st BrdUrd cycle	Treatment of insecticide in the 2nd BrdUrd cycle	SCEs/cell
—	—	7.3 ± 2.55[a]
dichlorvos	dichlorvos	14.5 ± 3.57*
dichlorvos	—	12.0 ± 3.75*
—	dichlorvos	11.7 ± 3.43*
—	—	5.9 ± 1.99
monocrotophos	monocrotophos	25.4 ± 5.88*
monocrotophos	—	18.5 ± 4.49*
—	monocrotophos	15.2 ± 5.25*

[a]An asterisk (*) indicates a significant difference compared to the concurrent control according to the statistical analysis recommended by Margolin et al. (1985).

Table 5. Acephate-induced sister-chromatid exchanges (SCEs), chromosome aberrations, and *hgprt* gene mutations in CHO-W8 cells

Acephate (mg/ml)	SCEs/cell[a]	Percent aberrant cells[a]	6TG-resistant colonies per 10^6 cells[b]
1% DMSO	9.58 ± 3.21	0	2.0
0.04	10.40 ± 3.24	0	—
0.2	11.38 ± 4.10	2	0.98
1.0	14.54 ± 3.50*	2	6.2
5.0	25.92 ± 6.26*	11*	11.9
p value for the dose response	< 0.005	< 0.001	< 0.001

[a]Significance of SCEs/cell and percent aberrant cells in each treatment, which is indicated by and asterisk (*), and *p* values for the dose response were calculated as described by Galloway et al. (1985) and Margolin et al. (1986).

[b]Significance of *hgprt* gene mutations in each treatment, indicated by an asterisk (*) and the *p* value for the dose response were calculated as described by Margolin et al. (1983).

Tungul et al. 1991). Monocrotophos was questionable positive. Previously, in one of our reports, monocrotophos positively induced chromosome aberrations when CHO cells were treated for 18 instead of 2 h as in this study (Lin et al. 1987). In addition, acephate, on the contrary, was found to be negative for both chromosome aberration and micronuclei induction in mouse bone marrow cells (Carver et al. 1985). Although acephate has been reported to induce transformation in BALB/c 3T3 cells, this probably occurs through a non-genotoxic mechanism, such as cell proliferation (Perocco et al. 1996). Although trichlorfon induced a high SCE level, it was negative for the induction of chromosome aberrations. The results of chromosome aberration induction, however, potential for SCE induction was acephate > trichlorfon > monocrotophos > methamidophos > dichlorvos, while that for chromosome aberration was dichlorvos > methamidophos > monocrotophos > acephate > trichlorfon. Inductions of SCE and chromosome aberrations are 2 biological activities reflecting alterations in chromosomal structure of a cell. Each of these 2 cytogenetic assays responds to a different set of structural features associated with carcinogenicity and can be included in a battery of genotoxicity tests. Comparisons between structural determinants associated using computerized automated structure evaluation analysis indicated that the overlap between SCE and chromosomal aberration was 22.6%, while that between SCE and *Salmonella* mutagenicity was 54.5% (Rosenkranz et al. 1990). It seems that the induction of SCEs better fits with Klopman's structure-genotoxic activity relationships of methoxyphosphinyl insecticides than with that of chromosome aberrations (Klopman et al. 1985). Therefore, the positive induction of SCEs but not chromosome aberrations in this report implies that the electrophilic potential might be responsible for the DNA-reactive mechanism of methoxyphosphinyl insecticides.

None of the 5 methoxyphosphinyl insecticides induced *hgprt* gene mutations in CHO cells. The uncoupling of mutation and SCE induction has previously been reported to be due to either an imbalance in the nucleotide pool (Kaufman 1987) or treatments with ethylnitrosourea (Stetka et al. 1985). From their data, Stetka et al. (1985) speculated that different DNA lesions contributed to SCEs and gene mutations. Lesions from *N*-alkylations of DNA induced SCEs but not gene mutations, while those from O6-alkyl-guanine are mutagenic but do not contribute significantly to SCE induction. Similar results were also found from work on *Drosophila* (Vogel 1986). Kaina et al. (1997) found that DNA lesions produced by O6-alkyl-guanine mainly induced SCEs during the 2nd replication cycle, while those from N-methylpurine induced SCEs in the 1st replication cycle. In our studies, monocrotophos and dichlorvos induced SCEs in the 1st replication cycle, which may imply a possible role of N-alkylation in the genotoxic mechanism of methoxyphosphinyl insecticides. In our previous studies, O6-alkyl-guanine was found to be the major DNA lesion induced by carbamate insecticides through mediation of its *N*-nitrosated metabolite (Wang et al. 1998). Organophosphorus and carbamate insecticides are 2 major modern insecticides, with a common mode of action for the killing mechanism, i.e., anti-cholinesterase activity. Determining whether *N*-alkylation is the major DNA lesion induced by methoxyphosphinyl insecticides awaits further investigations. However, the possibility that these 2 closely related insecticides might have different mammalian genotoxic mechanisms is worth elucidating in order to understand their adverse effects.

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REFERENCES

Amer SM, MA Sayed. 1987. Cytogenetic effects of the insecticide methamidophos in mouse bone marrow and cultured mouse spleen cells. Z. Naturforsch. C **42:** 21-30.

Carver JH, J Bootman, MC Cimino, HJ Esber, P Kirby, B Kirkhart, ZA Wong, JA MacGregor. 1985. Genotoxic potential of acephate technical: in vitro and in vivo effects. Toxicology **35:** 125-142.

Doherty AT, S Ellard, EM Parry, JM Parry. 1996. A study of the aneugenic activity of trichlorfon detected by centromere-specific probes in human lymphoblastoid cell lines. Mutat. Res. **372:** 221-231.

Galloway SM, AD Bloom, M Resnick, BH Margolin, F Nakamura, P Archer, E Zeiger. 1985. Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. Environ. Mutagen. **7:** 1-51.

Gupta RS, B Singh. 1982. Mutagenic responses of five independent genetic loci in CHO cells to a variety of mutagens. Development and characteristics of a mutagen screening system based on selection for multiple drug-resistant markers. Mutat. Res. **94:** 449-466.

Hodgson E, PE Levi. 1987. Modern toxicology. New York: Elsevier Science Publishing.

Jan KY, S Wang-Wuu, W Wen. 1982. A simplified fluorescence method for consistent differential staining of sister chromatids. Stain Tech. **57:** 45-46.

Kaina B, A Ziouta, K Ochs, T Coquerelle. 1997. Chromosomal instability, reproductive cell death and apoptosis induced by O6-methylguanine in Mex-, Mex+ and methylation-tolerant mismatch repair compromised cells: facts and models. Mutat. Res. **381:** 227-241.

Kaufman ER 1987. Uncoupling of the induction of mutations and sister-chromatid exchanges by the replication of 5-bromouracil-substituted DNA. Mutat. Res. **176:** 133-141.

Klopman G, R Conteras, HS Rosenkranz, MD Waters. 1985. Structure-genotoxic activity relationships of pesticides: comparison of the results from several short-term assays. Mutat. Res. **147:** 341-356.

Lin MF, CL Wu, TC Wang. 1987. Pesticide clastogenicity in Chinese hamster ovary cells. Mutat. Res. **188:** 241-250.

Lin SY, TC Lee, CS Cheng, TC Wang. 1988. Cytotoxicity, sister-chromatid exchange, chromosome aberration and transformation induced by 2,2-dichlorovinyl-O,O-dimethyl phosphate. Mutat. Res. **206:** 439-445.

Margolin BH, BJ Collings, JM Mason. 1983. Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. Environ. Mutagen. **5:** 705-716.

Margolin BH, MA Resnick, JY Rimpo, P Archer, SM Galloway, AD Bloom, E Zeiger. 1986. Statistical analyses for in vitro cytogenetic assays using Chinese hamster ovary cells. Environ. Mutagen. **8:** 183-204.

Perocco P, C Del Ciello, A Colacci, L Pozzetti, M Paolini, G Cantelli Forti, S Grilli. 1996. Cytotoxic activity and transformation of BALB/c 3T3 cells in vitro by the insecticide acephate. Cancer Lett. **106:** 147-153.

Rosenkranz HS, FK Ennever, G Klopman. 1990. Relationship between carcinogenicity in rodents and the induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. Mutagenesis **5:** 559-571.

Singh B, RS Gupta. 1982. Mutagenic response to ultraviolet light and X-rays at five independent genetic loci in Chinese hamster ovary cells. Environ. Mutagen. **4:** 543-551.

Stetka DG Jr, WTJ Bleicher, JG Brewen. 1985. SCE induction is uncoupled from mutation induced in mammalian cells following exposure to ethylnitrosourea (ENU). Environ. Mutagen. **7:** 233-243.

Tungul A, AM Bonin, S He, RSU Baker. 1991. Micronuclei induction by dichlorvos in the mouse skin. Mutagenesis **6:** 405-408.

Vogel EW. 1986. O-alkylation in DNA does not correlate with the formation of chromosome breakage events in *D. melanogaster*. Mutat. Res. **162:** 201-213.

Wang TC, JM Chiou, YL Chang, MC Hu. 1998. Genotoxicity of propoxur and its N-nitroso derivative in mammalian cells. Carcinogenesis **19:** 623-629.

Wilhelm D, K Bender, A Knebel, P Angel. 1997. The level of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including Jun N-terminal protein kinases and p38 kinase by alkylating agents. Mol. Cell. Biol. **17:** 4792-4800.