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 Klopmann 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 cells.

**Key words:** SCE, Chromosome aberration, Gene mutation, Organophosphorus insecticide.

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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% CO₂ 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 dimethylphosphophate, 99%, CAS 62-73-7), methamidophos (O,S-dimethyl phosphoramidiothioate, 97%, CAS 10265-92-6), monocrotophos (O,O-dimethyl-O-(2-methyl-carbomyl-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 10⁵ 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 nor-
mal 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 incub-
uation 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 solu-
tion. 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 infer-
ence for each response was also based on a trend
test similar to that for SCEs except that the per-
centage 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 $1.5 \times 10^4$ 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 fre-
cquency 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 $1 \times 10^6$ cells/100-mm petri
dish and subcultured every other day for the
expression of thioguanine-resistant mutants.
Mutants were selected by splitting $1 \times 10^6$ cells into
five 100-mm petri dishes and feeding them with
McCoy’s 5A medium containing 6-thioguanine (10
$\mu$g/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 fre-
cquency 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 associat-
ed 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 experi-
mental 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 insecti-
cides. Dose levels of dichlorvos and monocro-
tophos, respectively shown in tables 1 and 2, were
5 times higher than those of trichlorfon, methami-
dophos, and acephate, which are shown respec-
tively in tables 3-5. In addition, with treatment
using dichlorvos or monocrotophophos, the number
of mitotic cells harvested was insufficient for a signifi-
cant 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 suffi-
cient numbers of mitotic cells at a dose level up to
5 mg/ml (Tables 3-5). These facts indicate that
dichlorvos and monocrotophophos 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>
<thead>
<tr>
<th>Table 1. Dichlorvos-induced sister-chromatid exchanges (SCEs), chromosome aberrations, and hgprt gene mutations in CHO-W8 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichlorvos (μg/ml)</td>
</tr>
<tr>
<td>1% DMSO</td>
</tr>
<tr>
<td>0.32</td>
</tr>
<tr>
<td>1.6</td>
</tr>
<tr>
<td>8.0</td>
</tr>
<tr>
<td>40.0</td>
</tr>
<tr>
<td>200.0</td>
</tr>
<tr>
<td>1000.0</td>
</tr>
<tr>
<td>p value for the dose response</td>
</tr>
</tbody>
</table>

*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).

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

<table>
<thead>
<tr>
<th>Monocrotophos (μg/ml)</th>
<th>SCEs/cell</th>
<th>Percent aberrant cells</th>
<th>6TG-resistant colonies per 10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% DMSO</td>
<td>7.64 ± 2.62</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>1.6</td>
<td>8.10 ± 2.97</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>8.0</td>
<td>9.42 ± 3.24*</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>40.0</td>
<td>10.28 ± 3.13*</td>
<td>5</td>
<td>6.32</td>
</tr>
<tr>
<td>200.0</td>
<td>11.14 ± 3.17*</td>
<td>5</td>
<td>7.89*</td>
</tr>
<tr>
<td>1000.0</td>
<td>15.58 ± 3.42*</td>
<td>11*</td>
<td>1.37</td>
</tr>
<tr>
<td>p value for the dose response</td>
<td>&lt; 0.005</td>
<td>&lt; 0.001</td>
<td>&gt; 0.005</td>
</tr>
</tbody>
</table>

*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).
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.

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

<table>
<thead>
<tr>
<th>Trichlorfon (mg/ml)</th>
<th>SCEs/cell(^a)</th>
<th>Percent aberrant cells(^a)</th>
<th>6TG-resistant colonies per (10^6) cells(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% DMSO</td>
<td>7.30 ± 2.48</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.04</td>
<td>8.43 ± 2.62</td>
<td>4</td>
<td>---</td>
</tr>
<tr>
<td>0.2</td>
<td>9.16 ± 2.72*</td>
<td>5</td>
<td>0.0</td>
</tr>
<tr>
<td>1.0</td>
<td>12.57 ± 3.30*</td>
<td>5</td>
<td>0.0</td>
</tr>
<tr>
<td>5.0</td>
<td>25.86 ± 5.24*</td>
<td>6</td>
<td>0.0</td>
</tr>
<tr>
<td><em>p</em> value for the dose response</td>
<td>&lt; 0.005</td>
<td>&gt; 0.005</td>
<td>&gt; 0.005</td>
</tr>
</tbody>
</table>

\(^a\)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 \(hgp\) 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 \(hgp\) gene mutations in CHO-W8 cells

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

\(^a\)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 \(hgp\) 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 $(0-2) \times 10^{-6}$, while that of positive control group treated with 4 mM ethyl methanesulfonate was around $1 \times 10^{-4}$. The frequency of 6TG-R colonies induced by insecticides in this study was less than $1 \times 10^{-5}$, 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

<table>
<thead>
<tr>
<th>Treatment of insecticide in the 2nd BrdUrd cycle</th>
<th>SCEs/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st BrdUrd cycle</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>7.3 ± 2.55*</td>
</tr>
<tr>
<td>dichlorvos</td>
<td>14.5 ± 3.57*</td>
</tr>
<tr>
<td>dichlorvos</td>
<td>12.0 ± 3.75*</td>
</tr>
<tr>
<td>---</td>
<td>11.7 ± 3.43*</td>
</tr>
<tr>
<td>monocrotophos</td>
<td>5.9 ± 1.99</td>
</tr>
<tr>
<td>monocrotophos</td>
<td>25.4 ± 5.88*</td>
</tr>
<tr>
<td>---</td>
<td>18.5 ± 4.49*</td>
</tr>
<tr>
<td>monocrotophos</td>
<td>15.2 ± 5.25*</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Acephate (mg/ml)</th>
<th>SCEs/cell$^a$</th>
<th>Percent aberrant cells$^a$</th>
<th>6TG-resistant colonies per $10^6$ cells$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% DMSO</td>
<td>9.58 ± 3.21</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>0.04</td>
<td>10.40 ± 3.24</td>
<td>0</td>
<td>---</td>
</tr>
<tr>
<td>0.2</td>
<td>11.38 ± 4.10</td>
<td>2</td>
<td>0.98</td>
</tr>
<tr>
<td>1.0</td>
<td>14.54 ± 3.50*</td>
<td>2</td>
<td>6.2</td>
</tr>
<tr>
<td>5.0</td>
<td>25.92 ± 6.26*</td>
<td>11*</td>
<td>11.9</td>
</tr>
</tbody>
</table>

$p$ 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 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).
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 \textit{hgpR} 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-alkylation 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 \textit{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.

Acknowledgments: This study was supported by a grant from the National Science Council, R.O.C.

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