

SISTER CHROMATID EXCHANGES AND CHROMOSOME ABERRATIONS INDUCED BY PESTICIDE COMBINATIONS IN CHINESE HAMSTER OVARY CELLS^{1,2}

T. C. WANG, C. L. WU, J. H. LIN and C. Y. TARN and S. Y. LIN

*Institute of Zoology, Academia Sinica,
Taipei 11529 Republic of China*

(Received August 7, 1987)

(Revision received August 10, 1987)

(Accepted August 20, 1987)

T. C. Wang, C. L. Wu, J. H. Lin, C. Y. Tarn and S. Y. Lin (1987) Sister chromatid exchanges and chromosome aberrations induced by pesticide combinations in Chinese hamster ovary cells. *Bull. Inst. Zool., Academia Sinica* 26(4): 317-329. Totally 12 pesticide combinations were assayed for their genotoxicities to Chinese hamster ovary (CHO) cells using sister chromatid exchanges (SCEs) and chromosome aberration inductions. Major findings included: (1) Combination of Kasugamycin and carbendazim is negative for SCE but positive for chromosome aberration induction. Further investigation indicated that carbendazim is clastogenic to CHO cells. (2) Combination of captafol and polyoxin is positive in SCE induction. Captafol is responsible for the positive induction. Captafol alone also is clastogenic although its combination with polyoxin is not. Application of pesticides containing isoindole derivatives as active ingredients are significant concern for human health. (3) Combination of butachlor and chlomethoxynil is positive for SCE induction. Butachlor is probably the major factor for the positive induction. (4) Combination of probanasol and isoprothislam is positive for SCE induction. When assayed individually, probanasol showed positive effect on the induction. Isoprothislam, on the other hand, is questionable positive. (5) Other combinations of pesticide although failed to give the positive results of the assay showed significant dose response of the induction in most of the cases.

Chemical control of agricultural pests using pesticides is the only method practical in Taiwan. Most of the individual pesticides commercialized have been routinely examined for their safety to human health. Farmers, however, used to mix several different kinds of pesticide on application in order to save time and manpower. Recently, several combinations of pesticides were formulated for commercialization in this country. There are

no toxicological data yet available. New formula of a combination is not necessarily safe only because their individual mother compounds have been proved to be harmless. The application of pesticide combination is an unavoidable trend in the future practice of plant protection in this country. Their potential adverse effects to the health and genetic heritage of human being are consequently an important concern. Among them, genotoxicity is of special significant

1. Paper No. 298 of the Journal Series of the Institute of Zoology, Academia Sinica.

2. Please address correspondence to Dr. T. C. Wang, Institute of Zoology, Academia Sinica, Taipei 11529, the Republic of China.

because of the irreversible nature of its process and the long latent period required for the expression of its abnormalities. In this paper, we reported the genotoxicities of 12 pesticide combinations which recently have been applied for registration to commercialize in this country. The assay systems we used were sister chromatid exchanges (SCEs) and chromosome aberration inductions in Chinese hamster ovary (CHO) cells, which are sensitive and reliable short-term tests for this purpose (Williams *et al.*, 1980; Wolff, 1981; Takehisa, 1982; Brusick, 1984; Latt *et al.*, 1981).

MATERIALS AND 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 karyotypic 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% CO₂ in air. The growth medium was McCoy's 5A medium (Gibco) supplemented with 15% fetal bovine serum (FBS, Gibco), 2 mM glutamine (Gibco), 0.22% sodium bicarbonate (E. Merck, FRG) and antibiotics including penicillin (100 units/ml, Gibco) and streptomycin (100 µg/ml, Gibco).

Preparations of Pesticide Combinations

Pesticides used for combinations in the assays were all technical grades supplied by Taiwan Agricultural Chemical and Toxicant Substance Research Institute. They are listed in Table 1 for their common names, chemical names and purities. Stock solutions for each individual pesticide were prepared in dimethyl sulfoxide (DMSO, E. Merck, FRD) unless there were better alternatives available. In this experiment, polyoxin, Blasticidin-S and Bordeaux Mixture are highly water-soluble.

No organic solvents were needed for the preparations of their stock solutions. Rabcide, on the other hand, was found more satisfactory to dissolve in ethanol instead. Pesticide combinations then were made by mixing one with another according to the ratios indicated in the informations submitted by manufacturers on their applications for registration. From the maximum concentrations, each pesticide combination was diluted in the same solvent to a series of ten dose levels covering a range of five orders of magnitude. The concentration of the solvent was kept less than 1% in the culture for each treatment.

Quantitation of SCE Inductions

3 × 10⁵ cells were plated in a 60-mm petri dish and allowed to grow overnight. The cells were then treated with serum-free medium containing pesticide combination prepared as described previously or control chemical for two hours. At the end of the treatment, the medium was discarded and the cell cultures were washed twice with phosphate buffered saline (PBS, E. Merck, FRG). The petri dishes were refilled with 5 ml media containing BrdUrd (final concentration, 10 µM, Sigma) and the cell cultures were incubated in dark 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 and incubated for the final 2 hours. Metaphase cells were harvested by shake-off and chromosome preparation was done by air-dried techniques. The sister chromatid differential stain for chromosomes was prepared by a modified fluorescence plus Giemsa technique (Jan *et al.*, 1982). Thirty cells with chromosome numbers of 21 ± 2 were randomly sampled for each treatment from blind-coded slides to score the SCEs using an Olympus Vanox-S photomicroscope. At the same time, totally 300

TABLE 1
Pesticides used for assays in this report

Common names	Trade names ^a	Purity	Chemical names
MIPC	Etofolan (I)	97%	2-isopropyl-phenyl-N-methylcarbamate
carbofuran	Furadan (I)	95%	2, 3-Dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate
XMC	Macbal (I)	95%	3, 5-Xylyl methylcarbamate
cypermethrin	Ripcord (I)	90%	Alpha-cyano-3-phenoxybenzyl-2, 2-dimethyl-3-(2, 2-dichlorovinyl) cyclopropane carboxylate
butachlor	Machete (H)	90%	2-Chloro-2', 6'-diethyl-N-(butoxymethyl) acetanilide
chlomethoxynil	X-52 (H)	90%	2, 4-Dichloro-3'-methoxy-4'-nitrodiphenylether
pytazoxyfen	SL-49 (H)	97.5%	2-[4-(2, 4-dichlorobenzoyl)-1, 3-dimethyl pyrazol-5-yloxy]acetophenone
fthalide	Rabicide (F)	98%	4, 5, 6, 7-Tetrachlorophthalide
edifenphos	Hinosan (F)	88%	O-ethyl diphenyl phosphorodithiolate
Blasticidin-S	Bla-S (F)	96.7%	Blasticidin-S benzylaminobenzen sulfonate
Kasugamycin	Kasumin (F)	50%	D-3-0-(2-amino-4-[(1-carboxyimino-methyl) amino-2, 3, 4, 6-tetra-deoxy-alpha-D-arabinohexopyranosyl]-D-chiro-inositol)
thiophanatemethyl	Topsin M (F)	92%	Dimethyl[(1, 2-phenylene) bis-(imino-carbonothioyl) bis (carbamate)
cooper oxychloride	Cu-56 (F)	90%	Basic cupric chloride
carbendazim	Bavistin (F)	95%	2-(Methoxycarbonylamino)-benzimidazole
captafol	Difolatan (F)	95%	N-[(1, 1, 2, 2-Tetra-chloroethyl)thio] 4-cyclohexene-1, 2-dicarboximide
Polyoxin	Polyoxin (F)	95%	1-[5'- <i>n</i> -(5'-O-carbamoyl-2''-amino-2''-deoxy-L-xylo-nyl)-5'-amino-5'-deoxy-beta-D-allofulano-syluzonic acid]-5-hydroxymethyl-uracil
probanol	Oryzemat (F)	95%	3-Allyoxy-1, 2-benzixothiazole-1, 1-dioxide
isoprothiislam	Fuji-one (F)	95%	Diisopropyl-1, 3-dithiolam-2-ylidenemalonate
Bordeaux Mixture	Shimmel (F)	100%	Mixture of hydrated lime and cooper sulfate
curzate	Curzate (F)	95%	2-cyano-N-((ethylamine)thio)-carbonyl)-2-(methoxyimino) acetamide

a. F, H and I represent fungicide, herbicide and insecticide, respectively.

metaphase cells were randomly sampled for each treatment in order to examine the cell replication kinetics according to the method proposed by Schneider *et al.* (1981).

Quantitation of Chromosome Aberration Inductions

The method used for inducing chromosome aberrations in CHO cells was basically similar to that of the SCE assay except that BrdUrd was not added to the cultures and the period of post-treatment incubation was 18 instead of 24 hours. Metaphase cells were harvested and stained in 3% Giemsa solution. One hundred cells with chromosome numbers of 21 ± 2 were randomly sampled for each dose from blind-coded slides. Chromosome aberrations were scored using an Olympus Vanox-S photomicroscope. Identification of the types of chromosome aberration followed those described by Dean and Danford (1984). A further classification of chromosome aberrations into simple, complex and total types was made according to Galloway *et al.* (1985) in order to make the statistical analysis possible. However, according to Galloway *et al.* (1985), the final evaluation for each trial was based on the strongest evidence among the three for induction of chromosome aberration. In our experiment, it was the total type that showed the strongest evidence in every trial we assayed. We therefore presented only the % of aberrant cells of total type in the results.

For those combinations which showed positive in the inductions, assays using individual parent compounds were furtherly performed.

Data Analysis

The data of SCE and chromosome aberration were analyzed according to Galloway *et al.* (1985) and Margolin *et al.* (1986). Trend analysis was used for the examinations of the dose response of the inductions. Together with the results of trend analysis,

numbers of doses that elevated 20% over controls in SCE assay and numbers of individual doses elevated over controls at $p < 0.01$ level in chromosome aberration assay (Margolin *et al.*, 1983) were used to categorize the summary judgement of the inductions into positive (+), questionable positive (?+), questionable weak positive (?w) and negative (-). The result of positive but lack of dose response (+b) which also has been included by Galloway *et al.* (1985) was not observed in our experiment.

RESULTS AND DISCUSSION

MIPC and carbofuran [Fig. 1-(A), Table 2]

Both SCE and chromosome aberration inductions were questionable positive (?+). The chromosome aberration greatly increased as the dose reached to the highest level scored. Major types of chromosome aberration were breaks, fragments and exchanges. MIPC was found negative in bacterial reversion assay system either with (Jeang and Li, 1980) or without (Jeang and Li, 1978; Moriya *et al.* 1983) the metabolic activation. Carbofuran has been reported positive in the same system (Moriya *et al.*, 1983). Negative results of carbofuran, however, was observed in other reports using microorganism assaying systems (Jeang and Li, 1978; 1980; Gentile *et al.*, 1982; Klopman *et al.*, 1985; Sandhu *et al.*, 1985) and in higher organisms such as *Zea mays* (Gentile *et al.*, 1982) and *Drosophila* (Sandhu *et al.*, 1985). *In vitro* cytogenetic data were probably unavailable for the comparison.

XMC and cypermethrin [Fig. 1-(B), Table 2]

The inductions of SCE and chromosome aberration were questionable positive (?+). The chromosome aberration induced in the highest dose level scored was not as significant as those pesticide combinations described previously. XMC has been reported non-mutagenic in bacterial reversion assay (Moriya *et al.*, 1983). Cypermethrin also has been

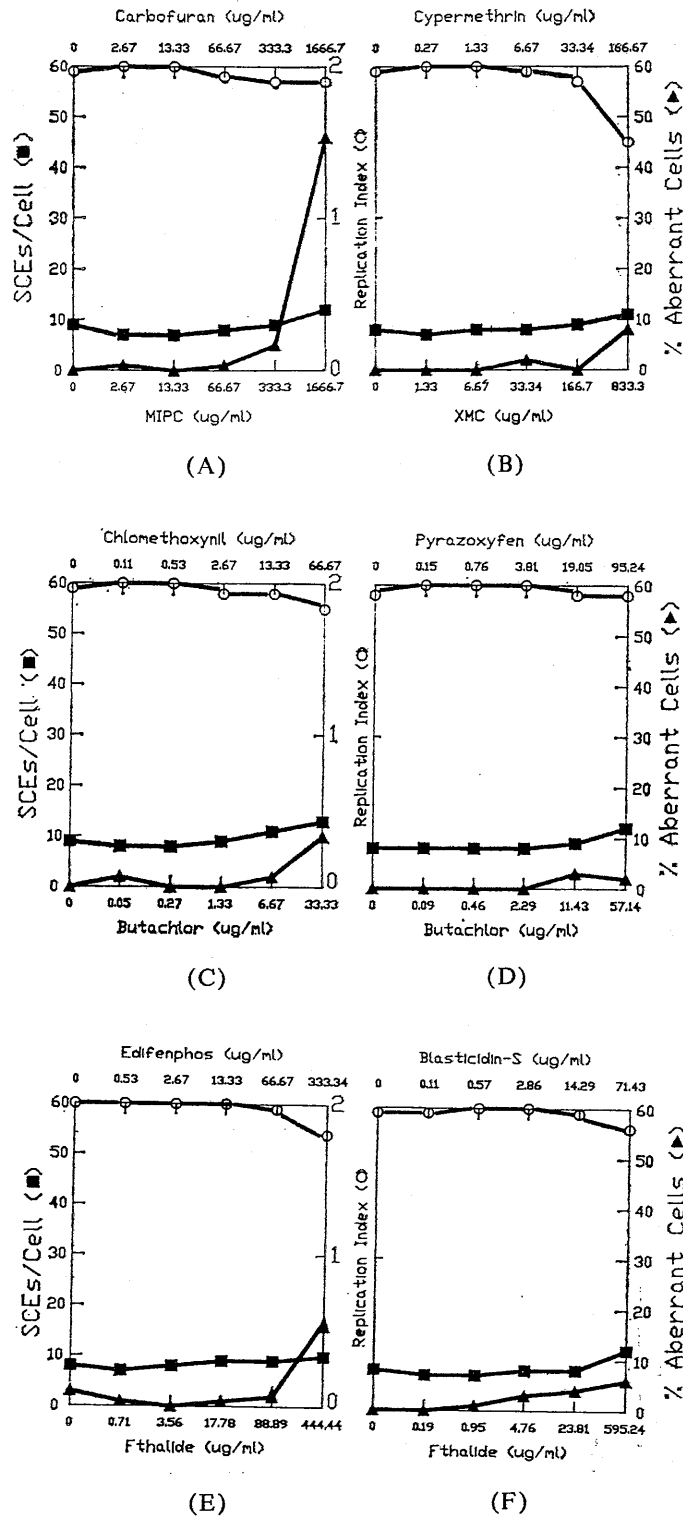


Fig. 1. (A) to (F) Sister chromatid exchanges, chromosome aberration and replication index induced in Chinese hamster ovary cells by 12 pesticide combinations.

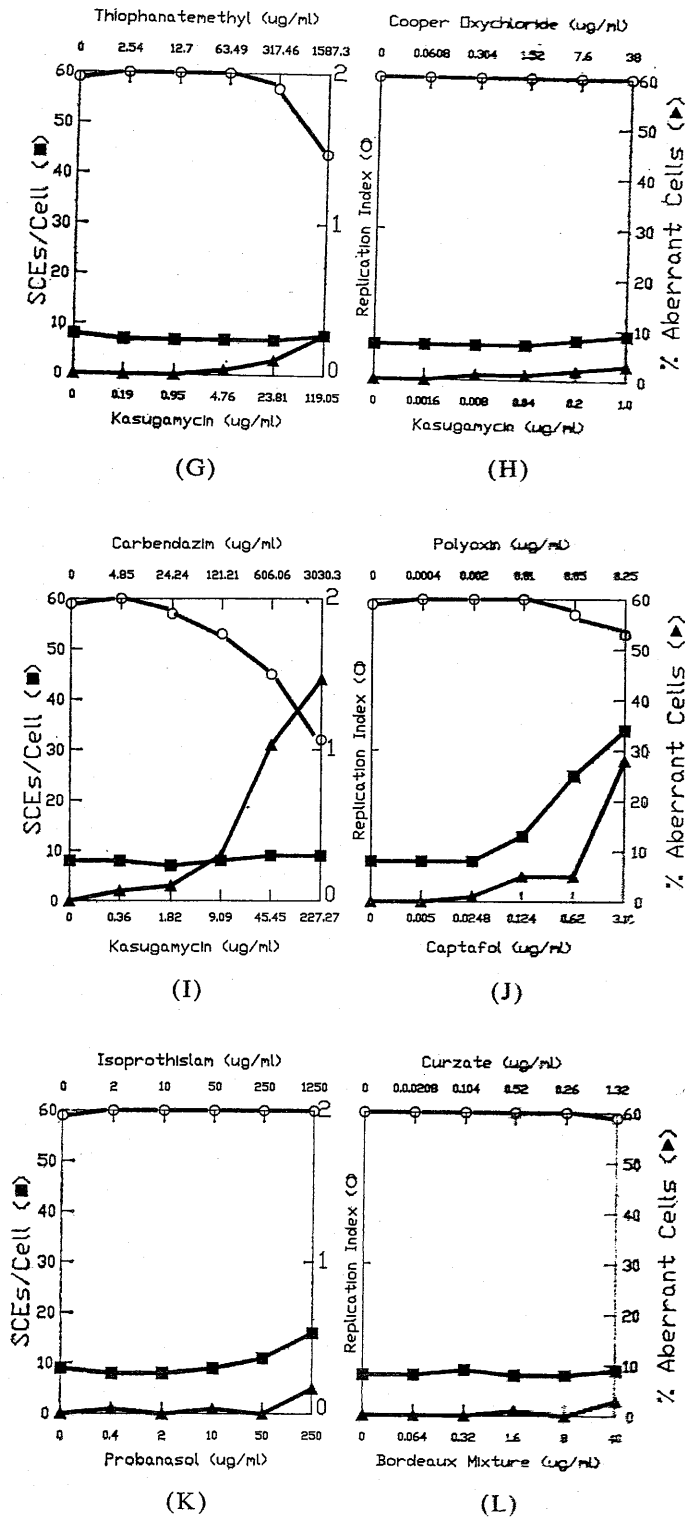


Fig. 1. (G) to (L) Sister chromatid exchanges, chromosome aberration and replication index induced in Chinese hamster ovary cells by 12 pesticide combinations.

TABLE 2

Numbers of treatment with significant induction, trend probability, and summary judgement in the SCE and chromosome aberration induced by pesticide combinations^a

Pesticide combinations or individual pesticides	SCE inductions			Chromosome aberration inductions		
	Numbers of treatment with significant induction	Trend probability	Summary judgement	Numbers of treatment with significant induction	Trend probability	Summary judgement
MIPC/carbofuran	1	<0.005	?+	1	<0.001	?+
XMC/cypermethrin	1	<0.005	?+	1	<0.001	?+
Butachlor/chlomethoxylinil	2	<0.005	+	1	<0.001	?+
Butachlor	1	<0.005	?+			
Chlomethoxylinil	0	0.43	—			
Butachlor/pyrazoxyfen	1	<0.005	?+	0	0.006	—
Fthalide/edifenphos	1	<0.005	?+	1	0.002	?w
Fthalide/Blasticidin-S	1	<0.005	?+	1	<0.001	?+
Kasugamycin/thiophanatemethyl	0	0.397	—	1	<0.001	?+
Kasugamycin/cooper oxychloride	1	<0.005	?+	0	0.009	—
Kasugamycin/carbendazim	0	0.018	—	3	<0.001	+
Kasugamycin				0	0.01	—
Carbendazim				2	<0.001	+
Captafol/polyoxin	3	<0.005	+	1	<0.001	?+
Captafol	4	<0.005	+	2	<0.001	+
Polyoxin	0	0.07	—	0	0.07	—
Probanasol/isoprothiislam	2	<0.005	+	0	<0.001	?w
Probanasol	2	<0.005	+			
Isoprothiislam	1	<0.005	?+			
Bordeaux mixture/curzate	0	0.206	—	0	0.009	—

a. Numbers of treatment with significant induction, trend probability and summary judgement followed methods described by Margolin *et al.* (1982), Galloway *et al.* (1985) and Margolin *et al.* (1986). The symbols '+', '?+', '?w' and '—', respectively indicate the positive, questionable positive, questionable weak positive and negative results of the induction.

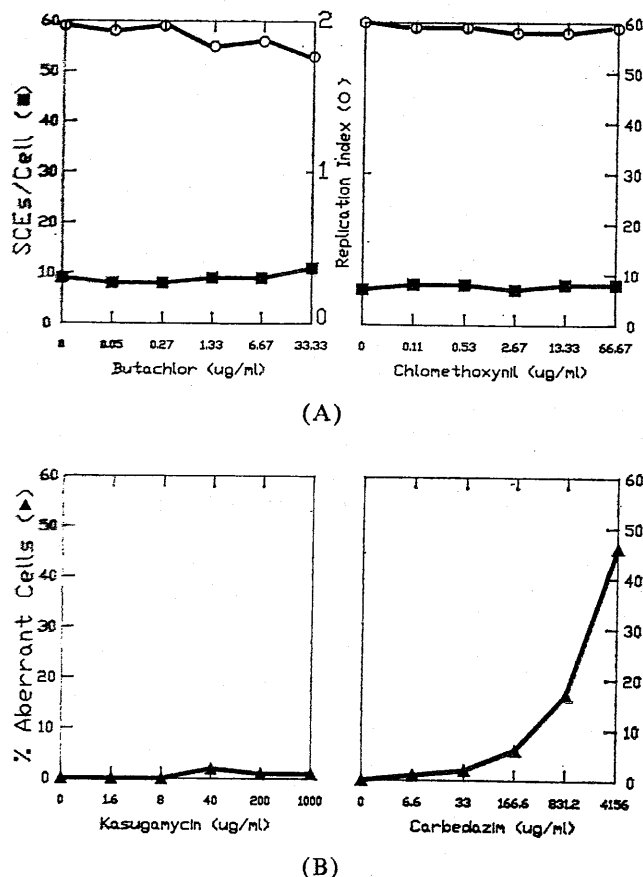


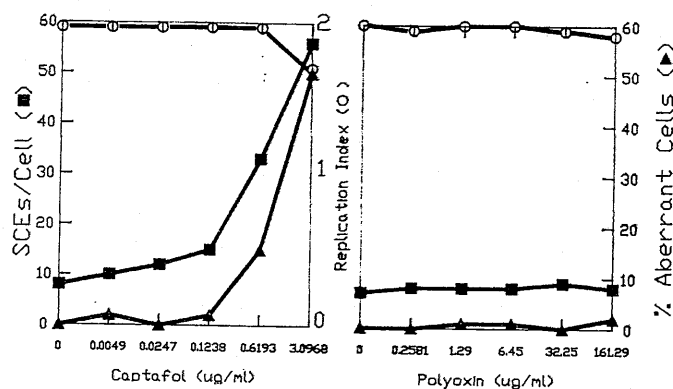
Fig. 2. (A) to (B) Sister chromatid exchanges and/or chromosome aberrations induced in Chinese hamster ovary cells by individual parent pesticides which show positive in corresponding assays when tested combinedly.

found negative in assay systems with several different microorganisms (Klopman *et al.*, 1985; Moriya *et al.*, (1983). It was reported to act as a weak mutagen in *Drosophila*, increasing significantly the frequency of gene mutation but is negative for chromosome damage assay (Batiste-Alentorn *et al.*, 1986). Induction of micronuclei in mouse bone marrow indicated that cypermethrin is mutagenic potential evidenced by a positive response of the assay (Amer and Aboul-era, 1985).

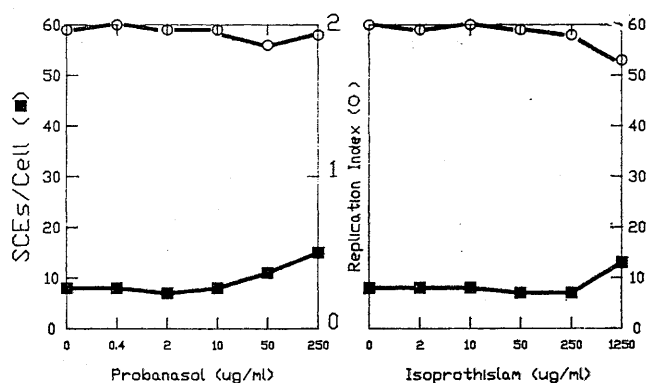
Butachlor and chlomethoxynil [Fig. 1-(C), Table 2]

The induction of SCEs was positive (+)

and that of chromosome aberration was questionable positive (?+). Assays with those two pesticides individually indicated that neither of them were positive in SCE induction [Fig. 2-(A), Table 2]. Butachlor was questionable positive (?+) and chlomethoxynil was negative (-). In a previous report (Moriya *et al.*, 1983), butachlor and chlomethoxynil were both positive in bacterial reversion assay. Butachlor, however, was found negative in several assays using *Bacillus subtilis* in another report (Shirasu *et al.*, 1976). Butachlor is an herbicide similar to alachlor in structures. They differs only in having a butoxy or methoxy group, respectively, on the *N*-methyl moiety. According to EPA's



(C)



(D)

Fig. 2. (C) to (D) Sister chromatid exchanges and/or chromosome aberrations induced in Chinese hamster ovary cells by individual parent pesticides which show positive in corresponding assays when tested combinedly.

report, alachlor is a potential human carcinogen inducing lung tumor in mice and stomach, thyroid and nasal turbinate tumors in rats (Anonymous, 1985b). Similar attentions, however, have not been because butachlor as well. This is probably because butachlor is applied mainly to rice fields which are common in certain Asian and South American areas and is not marketed in the United States. In view of the fact that butachlor has been the number one pesticide in quantity used in this country for past five years, more informations are necessary for the further assessment on the human risk of pesticides containing this herbicide as one of

their active ingredients.

Butachlor and pyrazoxyfen [Fig. 1-(D), Table 2]

The induction of SCEs was questionable positive (?+) while that of chromosome aberration was negative (-). There was probably no previous work has been done on the cytogenetic effects of pyrazoxyfen in mammalian cells *in vitro*.

Fthalide and edifenphos [Fig. 1-(E), Table 2]

The inductions of SCE and chromosome aberration were questionable positive (?+) and questionable weak positive (?w), respectively. The chromosome aberrations observed

were mainly breaks and fragments. The dose response of chromosome aberration induction was not significant at $p < 0.001$ level. No significant cell cycle delay was induced by this combination. The investigation provides information regarding the positive cytogenetic effects of edifenphos has been reported on the bone marrow cells of mice *in vivo* (Bhunya and Behera, 1984). There was probably no work has been done on the cytogenetic effects of fthalide on mammalian system *in vitro*. Non-mutagenic effects in bacterial testing system have been reported for fthalide (Moriya *et al.*, 1983) and for edifenphos (Moriya *et al.*, 1983; Shirasu *et al.*, 1976).

Fthalide and Blastocidin-S [Fig. 1-(F), Table 2]

Both the SCE and chromosome aberration inductions were questionable positive (?+) with significant dose response and induced significant result only in one dose level. Chromosome aberrations observed were mainly breaks and exchanges. There was no significant cell cycle delay. Negative result has been reported for Blastocidin-S in bacterial reversion assay systems (Moriya *et al.*, 1983). There is probably no previous report regarding the *in vitro* cytogenetic effects of Blastocidin-S on mammalian cells.

Kasugamycin and thiophanatemethyl [Fig. 1-(G), Table 2]

SCE induction was negative (-) but the chromosome aberration assay was questionable positive (?+). In a previous report, they were both negative in bacterial reversion assays (Moriya *et al.*, 1983).

Kasugamycin and cooper oxychloride [Fig. 1-(H), Table 2]

The SCE induction was questionable positive (?+) while that of chromosome aberration was negative. Previous data on the genotoxicity of cooper oxychloride are unavailable for the comparison.

Kasugamycin and carbedazim [Fig. 1-(I), Table 2]

The SCE induction was negative (-) while that of chromosome aberration was positive (+). There were three dose levels scored induced significant % of aberrant cells in the assay of chromosome aberration. Various types of chromosome aberration were induced by this combination including breaks, fragments, deletions, double minutes, exchanges, ring chromosomes and dicentric chromosomes. Cell cycles were significantly delayed in the highest two dose levels scored. Further investigation of chromosome aberration using those two fungicide individually indicated that carbendazim was clastogenic to CHO cells [Fig. 2-(B), Table 2]. Exchange type of aberration was extraordinarily numerous in the results. In previous papers, carbendazim has been reported negative in bacterial reversion assay either with or without the metabolic activation (Jeang and Li, 1978; 1980). However, due to the fact that in our experiment, the carbendazim was strongly clastogenic, we suggest that the application of carbendazim or any pesticide combination containing carbendazim should be a significant concern of human health.

Captafol and polyoxin [Fig. 1-(J), Table 2]

The SCE induction was positive (+) but the chromosome aberration induction was questionable positive (?+). According to our further investigation, captafol is responsible for the positive result of the assay (Fig. 2-(C), Table 2). The level of SCEs induced by captafol alone was higher than those by the combination with polyoxin. The induction of chromosome aberration also became positive when captafol was assayed individually. The polyoxin alone, on the other hand, was negative in both assays. This is consistent with what have been observed in several previous reports. Polyoxin was found negative in bacterial reversion assay (Shirasu *et al.*, 1976; Moriya *et al.*, 1983). Positive result of mutagenicity test

for captafol has been reported frequently (Shirasu *et al.*, 1976; Carere *et al.*, 1978; Moriya *et al.*, 1978; Sandhu *et al.*, 1985). In fact, a special review made by EPA has indicated that captafol is oncogenic in rats and mice (Anonymous, 1985a). Captan and folpet, two other fungicides of the same type of isoindole derivatives, also have been reported mutagenic in several assays (Shirasu *et al.*, 1976; Ahmed *et al.*, 1977; Ficsor *et al.*, 1977; Moriya *et al.*, 1978; Moriya *et al.*, 1983; Klopman *et al.*, 1985; Sandhu *et al.*, 1985). According to EPA's report (Anonymous, 1985c), captan poses a risk of cancer to human through dietary exposure. The use of pesticides containing isoindole derivatives as one of the active ingredients is thus a great concern for human health.

Probanasol and isoprothiisam [Fig. 1-(K), Table 2]

The induction of SCEs was positive (+) while that of chromosome aberration was questionable weak positive (?w). SCEs induced by probanasol alone was positive (+) while that by isoprothiisam alone was questionable positive (?+) [Fig. 2-(D), Table 2]. Both of them were found negative in microbial assays with (Jeang and Li., 1980) or without (Jeang and Li., 1978; Moriya *et al.*, 1983) the metabolic activation.

Bordeaux Mixture and curzate [Fig. 1-(L), Table 2]

Both SCE and chromosome aberration inductions were negative (-). Previous reports on the cytogenetic effects of those two fungicides in mammalian cells *in vitro* were unavailable for the authors.

Acknowledgements: Authors would like to extend their appreciations to Dr. T. Y. Ku of the Council of Agriculture and Drs. G. C. Li and S. C. Wang of the Taiwan Agricultural Chemical and Toxicant Research Institute for the supply of pesticides. This research was supported by the grant from the Council

of Agriculture, Executive Yuan, the Republic of China.

REFERENCES

- AHMED, F. E., HART, R. W. and LEWIS, N. J. 1977. Pesticide induced DNA damage and its repair in cultured human cells. *Mutat. Res.* **42**: 161-174.
- AMER, S. M. and ABOUL-ERA E. I. 1985. Cytogenetic effects of pesticides III. Induction of micronuclei in mouse bone marrow by the insecticides cypermethrin and rotenone. *Mutat. Res.* **155**: 135-142.
- ANONYMOUS. 1985a. Captafol; Special Review of Certain Pesticide Products. *Federal Register* **50**: 1103-1107.
- ANONYMOUS. 1985b. Alachlor; Special Review of Certain Pesticide Products. *Federal Register* **50**: 1115-1119.
- ANONYMOUS. 1985c. Intent to cancel registration of pesticide products containing captan; availability of position document 2/3. *Federal Register* **50**: 25884-25889.
- BATISTE-ALENTORN, M., XAMENA, N., VELAZQUEZ, A., CREUS, A. and MARCOR, R. 1986. Mutagenicity testing of the pyrethroid insecticide cypermethrin in *Drosophila*. *Mutagenesis* **5**: 343-346.
- BRUSICK, D. 1984. Cytogenetic assays: aberrations and SCE techniques. in *Carcinogenesis and Mutagenesis Testing* (Douglas, J. F. ed.), p. 265-278. Humana Press, Clifton, New Jersey.
- BHUNYA, S. P. and BEHERA, J. 1984. Clastogenicity of a fungicide, ediphenphos (Hinosan) in the bone marrow cells of mice *in vivo*. *Cytologia* **49**: 833-839.
- CARERE, A., ORTALI, V. A., CARDAMONE, G., TOROACCA, A. M. and RASCHETTI, R. 1978. Microbiological mutagenicity studies of pesticides *in vitro*. *Mutat. Res.* **57**: 277: 277-286.
- DEAN, B. J. and DANFORD, N. 1984. Assays for the detection of chemically-induced chromosome damage in cultured mammalian cells. in *Mutagenicity Testing—A Practical Approach* (Venitt, S. and Parry, J. M. eds.), pp. 187-232. IRL Press, Oxford.
- FICSOR, G., BORDAS, S., WADE, S. M., MUTHIANI, E., WERTZ, G. F. and ZIMMER, D. M. 1977. Mammalian host- and fluid-mediated mutagenicity assays of captan and streptozotocin in *Salmonella typhimurium*. *Mutat. Res.* **48**: 1-16.

- GALLOWAY, S. M., BLOOM, A. D., RESNICK, M., MARGOLIN, B. H., NAKAMURA, F., ARCHER, P. and ZEIGER, E. 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.
- GENTILE, J. M., GENTILE, G. J., BULTMAN, J., SECHRIEST, R., WAGNER, E. D. and PLEWA, M. J. 1982. An evaluation of the genotoxic properties of insecticides following plant and animal activation. *Mutat. Res.* 101: 19-29.
- JAN, K. Y., WANG-WUU, S. and WEN, W. 1982. A simplified fluorescence plus giemsa method for consistent differential staining of sister chromatids. *Stain Tech.* 57: 45-46.
- JEANG, C. L. and LI, G. C. 1978. Screening of pesticides for mutagenicity in the microbial systems. *Natl. Sci. Council Monthly* 6: 780-788.
- JEANG, C. L. and LI, G. C. 1980. Screening of pesticides for mutagenicity in the microbial system II. with mammalian microsomal activation. *Natl. Sci. Council Monthly* 8: 551-559.
- KLOPMAN, G., CONTRERAS, R., ROSENKRANZ, H. S. and WATERS, M. D. 1985. Structure-genotoxic activity relationships of pesticides: Comparisons of the results from several short-term assays. *Mutat. Res.* 147: 343-356.
- LATT, S. A., ALLEN, J., BLOOM, S. E., CARRANO, A., FALKE, E., KRAM, D., SCHENIDER, E., SCHRECK, R., TICE, R., WHITEFIELD, B. and WOLFF, S. 1981. Sister-chromatid exchanges: A report of the gene-tox program. *Mutat. Res.* 87: 17-62.
- LEE, T. C., HUANG, R. Y. and JAN, K. Y. 1985. Sodium arsenite enhances the cytotoxicity, clastogenicity and 6-thioguanine-resistant mutagenicity of ultraviolet light in Chinese hamster ovary cells. *Mutat. Res.* 148: 83-89.
- MARGOLIN, B. H., COLLINGS, B. J. and MASON, J. M. 1983. Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* 5: 705-716.
- MARGOLIN, B. H., RESNICK, M. A., LIMPO, J. Y., ARCHER, P., GALLOWAY, S. M., BLOOM, A. D. and ZEIGER, E. 1986. Statistical analysis for *in vitro* cytogenetic assays using Chinese hamster ovary cells. *Environ. Mutagen.* 8: 183-204.
- MORIYA, M., KATO, K. and SHIRASU, Y. 1978. Effects of cysteine and a liver metabolic activation system on the activities of mutagenic pesticides. *Mutat. Res.* 57: 259-263.
- MORIYA, M., OHTA, T., WATANABE, K., MIYAZAWA, T., KATO, K. and SHIRASU, Y. 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutat. Res.* 116: 185-216.
- SANDHU, S. S., WATERS, M. D., SIMON, V. F., MORTELMANS, K. E., MITCHELL, A. D., JORGENSEN, T., JONES, D. C. L., VALENCIA, R. and STACK, F. 1985. Evaluation of the genotoxic potential of certain pesticides used in Pakistan. in *Basic and Applied Mutagenesis* (Muhammed, A. and von Borstel, R. C. eds.), p. 185-219. Plenum Publ. Co., New York.
- SCHENIDER, E. L., NAKANISHI, Y., LEWIS, J. and STERNBERG, H. 1981. Simultaneous examination of sister chromatid exchanges and cell replication kinetics in tumor and normal cells *in vivo*. *Cancer Res.* 41: 4973-4975.
- SHIRASU, Y. M., MORIYA, K., KATO, A., FURUHASHI and T. KADA. 1976. Mutagenicity screening of pesticides in the microbial system, *Mut. Res.* 40: 19-30.
- TAKEHISA, S. 1982. Induction of sister chromatid exchanges by chemical agents. in *Sister Chromatid Exchange* (S. Wolff ed.), p. 87-148. John Wiley & Sons, Inc., NY.
- WATERS, M. D., S. NESNOW, V. F. SIMMON, A. D. MITCHELL, T. A. JORGESON and R. VALENCIA. 1980. Pesticides: mutagenic and carcinogenic potential. in *The Pesticide Chemist and Modern Toxicology* (S. K. Bandal, G. J. Marco, L. Golberg and M. L. Leng eds.), p. 89-114. Am. Chem. Soc. Washington, D. C.
- WILLIAMS, G. M., J. H. WEISBURGER and D. BRUSICK. 1980. The role of genetic toxicology in a scheme of systematic carcinogen testing. in *The Pesticide Chemist and Modern Toxicology* (S. K. Bandal, G. J. Marco, L. Golberg and M. L. Leng eds.), p. 89-114. Am. Chem. Soc. Washington, D. C.
- WOLFF, S. 1981. The sister chromatid exchange test. in *Short-Term Tests for Chemical Carcinogens* (H. F. Stich and R. H. C. San eds.), p. 236-242. Springer-Verlag, NY.

混合農藥對中國倉鼠卵巢細胞之遺傳毒性

王清澄 吳彰玲 林儒宏 唐中耀 林淑瑜

十二種在臺灣申請登記之混合農藥，經以姐妹子染色體與染色體變異探討其遺傳毒性所得主要結果如下：

- (一) Kasugamycin 與 Carbendazim 混合使用可造成顯著之染色體變異，主因來自 Carbendazim 之遺傳毒性。
- (二) Captafol 與 Polyoxin 混合使用可造成顯著之姐妹染色分體交換，主因來自 Captafol 之遺傳毒性。
- (三) Butachlor 與 Chlomethoxynil 混合使用可造成顯著之姐妹染色分體交換，主因可能來自 Butachlor 之遺傳毒性。
- (四) Probanasol 與 Isoprothiislam 混合使用可造成顯著之姐妹染色分體交換，主因來自 Probanasol 之遺傳毒性。
- (五) 其它混合使用之組合雖然無法由本研究中之結果證明具有致變性，然多數組合之遺傳毒性均有隨劑量上昇之趨勢是值得注意的現象。

