GENETIC BIOASSAYS OF SELECTED PESTICIDES, WITH EMPHASIS ON COMPOUNDS USED IN EGYPT

Michael D. Waters,[1] Shahbeg S. Sandhu,[1]
Vincent F. Simmon,[2] Kristien E. Mortelmans,[3]
Ann D. Mitchell,[3] Ted A. Jorgenson,[3] David C.L. Jones,[3]
Ruby Valencia,[4] A.H. El-Sebae,[5] and S.A. Soliman[5]*

[1]Genetic Toxicology Division
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

[2]Genex Laboratories
Rockville, MD 20852

[3]SRI International
Menlo Park, CA 94025

[4]Zoology Department, University of Wisconsin
Madison, WI 53706

5 Laboratory of Environmental Chemistry and Toxicology
Department of Pesticide Chemistry
Faculty of Agriculture
Alexandria University
Alexandria, Egypt

INTRODUCTION

In Egypt, a semi-arid country whose agriculture is concentrated in a 2.5-million-acre strip along the Nile River, pesticides must be used to limit the loss of agricultural production to an acceptable level. Indeed, the urgency of maximizing agricultural productivity worldwide has stimulated efforts to develop new, more effective pesticide chemicals, many of which have been used or tested in Egypt. Because of concern over the possible genotoxic (i.e., DNA-mediated toxic) properties of some of these agents, the U.S. Environmental Protection Agency (EPA) established, initially under its Substitute Chemicals Program, a research project to evaluate the mutagenicity and related biological properties of selected pesticides. Of these chemicals, 21 are or have been used in Egyptian agriculture.

* To whom correspondence should be addressed.

The purpose of this paper is to summarize the qualitative results of the EPA study of pesticide chemicals, with particular attention to the methods used and to the results for chemicals that are or have been used extensively in Egypt.

PESTICIDES

Battelle Memorial Laboratories, Columbus, Ohio, obtained the pesticides from the manufacturers and provided samples to SRI International, Menlo Park, California, and WARF Institute, Inc., Madison, Wisconsin, for the studies reported here. A few of the chemicals were obtained from the manufacturers by the EPA Office of Pesticide Programs, Washington, D.C. Each pesticide was a technical-grade product or the equivalent. Product information is available from the first author. Monocrotophos was tested as a formulated product, Azodrin 5 (Shell). Mancozeb was tested as two different products: Dithane M-45 (Rohm and Haas) and Manzate 200 (du Pont). Maneb also was tested as two different products: Dithane M-22 (Rohm and Haas) and Manzate D (du Pont).

BIOASSAYS

WARF Institute, Inc., performed 29 of the Drosophila sex-linked recessive lethal tests. All other bioassays were performed by SRI International, under contract to EPA. Table 1 lists the 14 bioassays used in this study, organized into three classes of genetic damage and subdivided according to the phylogenetic level of organization of the test organism (prokaryote or eukaryote). The latter distinction is important because of the substantial differences in cell structure, metabolism, and genetic replicative processes between the two groups. Each bioassay is denoted by the three-character code used in the EPA GENE-TOX Program (6, 19), with two exceptions: "YE3" and "SAR" represent enhanced mitotic recombination in S. cerevisiae D3 and differential toxicity in S. typhimurium (described below). These two code words are not employed in the GENE-TOX data file.

The bioassays are classified as "initial" or "confirmatory," indicating the sequence in which they were performed. In the initial stage of this study, most of the pesticides were evaluated in a battery of six bioassays (SAL, WPU, REP, REW, YE3, and UDH). Many of the compounds that exhibited some genotoxic effect in the initial tests, or were considered on the basis of other information to be potentially genotoxic, were later tested more extensively in confirmatory bioassay systems. In general, a result had to be observed repeatedly for a chemical to be judged genetically active or inactive in a given assay. Mixed or ambiguous results are identified as such.

Table 1. Genotoxicity Bioassays Employed

Code	Organism	Property Examined	Reference
Point/Gene Mutations in Prokaryotes			
SAL*	S. typhimurium (5 strains)	Reverse mutation	(1)
WPU*	E. coli WP2 uvrA	Reverse mutation	(2)
Point/Gene Mutations in Eukaryotes			
YER	S. cerevisiae D7	Reverse mutation	(24)
L5T	Mouse lymphoma L5178Y cells	Forward mutation	(4)
SRL	D. melanogaster	Recessive lethality	(22)
Primary DNA Damage in Prokaryotes			
REP*	E. coli polA	Differential toxicity	(17)
REW*	B. subtilis rec	Differential toxicity	(11)
SAR	S. typhimurium uvrB, rec	Differential toxicity	(1)
Primary DNA Damage in Eukaryotes			
YE3*	S. cerevisiae D3	Enhanced mitotic recombination	(3)
YEH	S. cerevisiae D7	Gene conversion and crossing-over	(23)
UDH*	Human lung fibroblasts WI-38	Unscheduled DNA synthesis	(15)
Chromosomal Effects			
SCC	Chinese hamster ovary cells	Sister-chromatid exchange	(13, 18)
MNM	Mouse bone marrow and cardiac blood	Chromosome breakage (micronuclei)	(14)
DLM	Mouse	Dominant lethality	(16)

*A bioassay from the initial battery of screening tests.

The following mutation bioassays were carried out in both the presence and absence of an Aroclor-1254-induced metabolic activation system: SAL, WPU, YER, L5T, YE3, YEH, UDH, and SCC. Liver homogenates for the metabolic activation systems were prepared from adult male Sprague-Dawley rats weighing 250 to 300 g, obtained from Simonsen Laboratories, Gilroy, California.

POINT/GENE MUTATIONS IN PROKARYOTES

Salmonella typhimurium Plate-Incorporation Assay (SAL)

The S. typhimurium strains used in this test, TA1535, TA1537, TA1538, TA98, and TA100, are histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown in minimal medium with a trace of histidine, only cells that revert to histidine independence (his^+) can form colonies. The spontaneous mutation frequency is relatively constant for each strain. When a mutagen is added to the agar medium, the reversion frequency increases, usually in proportion to the dose of mutagen added. The tester strains were obtained from Dr. Bruce Ames, of the University of California at Berkeley, and the test procedures were those of Ames et al. (1). All the indicator strains have a mutation (rfa^-) that results in a defective lipopolysaccharide coat; they also have a deletion that includes genes involved in the synthesis of biotin (bio^-) and in the repair of ultraviolet-induced DNA damage ($uvrB^-$). The $uvrB^-$ mutation results in decreased repair of some types of chemically or physically damaged DNA, thereby enhancing the strain's sensitivity to some mutagens. Strain TA100 is derived from TA1535 by the introduction of the resistance transfer factor pKM101, which also increases sensitivity to some mutagens. Similarly, strain TA98 is derived from TA1538 by the addition of plasmid pKM101.

The chemicals were usually tested at a minimum of six concentrations; the highest nontoxic concentration tested was 10 mg/plate, unless specific solubility restricted the maximum concentration. For toxic chemicals, the concentrations tested were reduced appropriately; attempts were made to test each chemical at a minimum of four nontoxic concentrations.

In these assays, mutagenicity was defined as a dose-related increase in the number of histidine-independent colonies per plate. A positive result in this assay was considered to be a dose-related increase in the number of revertants in at least one strain for at least three concentration levels. The result for a compound was considered negative when at least two independent experiments failed to yield a dose-related increase in the number of revertants.

If neither of these criteria was met, the results were classified as inconclusive.

Escherichia coli Reverse-Mutation Assay (WPU)

E. coli strain WP2 is a tryptophan auxotroph (trp⁻) by virtue of a base-pair substitution in the tryptophan operon. It also is deficient in the repair of some physically or chemically induced DNA damage (uvrA⁻), which makes the strain more sensitive to certain mutagens. The procedure used for this bioassay was that described by Bridges (2). Concurrent positive (known mutagen) and negative (solvent) controls were included in each assay. The positive-control chemicals were 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) without metabolic activation and 2-aminoanthracene with metabolic activation. Each test chemical was assayed at a minimum of six concentrations, and the highest nontoxic concentration tested was 10 mg/plate (or lower for more toxic chemicals). The criteria for mutagenicity were the same as for the S. typhimurium assay.

POINT/GENE MUTATIONS IN EUKARYOTES

Saccharomyces cerevisiae D7 Reverse-Mutation Assay (YER)

The yeast S. cerevisiae strain D7 is homozygous at the ilvi92/ilvi92 gene locus and requires isoleucine for growth. The isoleucine requirement caused by the homozygous condition can be alleviated by true reverse mutation and by allele-nonspecific suppressor mutation. When the yeast is plated on medium that lacks isoleucine (selective medium), only such mutants are capable of growing. Thus, the frequency of reverse mutation can be measured by counting the revertant colonies appearing on agar plates that lack isoleucine. The S. cerevisiae D7 strain used was originally obtained from Dr. F.K. Zimmermann (Fachbereich Biologie Technische Hochschule, Darmstadt, West Germany), who described the procedures for use of this strain (24). Solvent was used as the negative control, and 1,2,3,4-diepoxybutane as the positive control. Five concentrations of the test chemical were assayed both with and without metabolic activation. For toxic compounds, the concentration range was reduced until about 50% final toxicity was obtained. The test substance was judged mutagenic if a dose-response relationship in revertant-colony formation was observed repeatedly. In other respects, results were interpreted as for the S. typhimurium reverse-mutation assay.

Mouse Lymphoma Forward-Mutation Assay (L5T)

Most mammalian cells produce thymidine triphosphate by two metabolic pathways: a major pathway that includes the enzyme thymidine synthetase and a second, "salvage" pathway that uses the enzyme thymidine kinase to process exogenous thymidine or thymidine derived from DNA degradation. The thymidine analogue trifluorothymidine (TFT) is lethal to cells that have the salvage pathway, because TFT is incorporated into the cells and irreversibly binds to and inhibits an enzyme required for DNA synthesis. Thus, cells possessing thymidine kinase will die, while thymidine-kinase-deficient ($\underline{TK}^{-/-}$) cells survive. In the L5178 mouse lymphoma cell mutagenesis assay (4), cells heterozygous for thymidine kinase ($\underline{TK}^{+/-}$) were incubated with the test chemical and then cloned in the presence of TFT; surviving colonies indicated mutation of cells to $\underline{TK}^{-/-}$. The results were considered positive if the mutation frequency in response to at least one test concentration was at least twice the background mutation frequency and the mutation frequency increased with increasing concentration. Results were considered negative if none of the test concentrations produced a mutation frequency more than double the background rate and the mutation frequency was not dose-related. Results were considered inconclusive if the compound showed limited or no cytotoxicity at the highest concentrations tested or the results could not be reproduced in repeated experiments.

Drosophila melanogaster Sex-Linked Recessive Lethal Assay (SRL)

The sex-linked recessive lethal test using the fruit fly D. melanogaster can detect lethal point mutations and small deletions on the X chromosomes, which constitute about 20% of the Drosophila genome. The sex-linked recessive lethal test used by WARF, Inc., was described by Waters et al. (21). The sex-linked recessive lethal test used by SRI International was a modification of the yellow-bar test described by Würgler et al. (22). In this system, males that carry the genes bar (B) and yellow (y) on the X chromosome plus two minute secondary translocations of the X chromosome, bearing the wild-type allele (y^{+}), one on each arm of the Y chromosome, were exposed to the test chemical. The exposed males were crossed to females carrying the In sc y X chromosome in homozygous condition; this doubly inverted X is marked with y and scute (sc). The male and female progeny of this mating (F_1 generation) were mated with each other, and the resulting progeny (F_2 generation) were examined. Absence of the treated-male phenotype (bar-eyed) in the F_2 generation was considered evidence of recessive lethal mutation.

Cultures of the F_2 generation were examined under a dissecting microscope approximately two weeks after the broods were initiated. If at least two bar-eyed males were present in a culture, the result was considered negative. If there were at least 20 progeny and no bar-eyed males in a culture, it was retested to confirm the presence of a lethal mutation, as were cultures with fewer than 20 progeny and a low ratio of bar-eyed males to bar-eyed females. In the retest, three F_2 females of the yellow-bar phenotype were mated with their F_2 male siblings, and their progeny (the F_3 generation) were examined. If no bar-eyed males occurred among at least 20 offspring, the result for that culture was considered positive. If some females of an F_2 culture produced bar-eyed males and others did not, then their gonads were mosaic for a sex-linked recessive lethal mutation, and the result for this group was also scored as positive. If none of the F_3 cultures contained at least 20 progeny, this test was eliminated from final calculations. After all the tests were scored, the results were statistically analyzed to determine an overall mutation frequency. An increase in the background mutation rate of at least 0.2% at the 95% confidence level was considered a positive response. Compounds that were tested with a progeny sample large enough to allow mutation to be detected at the 95% confidence level but did not elicit a 0.2% increase in mutation rate over the background rate were classified as nonmutagenic in this assay. Compounds for which the progeny sample was too small to permit detection of a 0.2% increase in mutation rate at the 95% confidence level were considered inconclusively tested.

PRIMARY DNA DAMAGE IN PROKARYOTES

E. coli polA Differential Toxicity Assay (REP)

E. coli strain p3478 is a derivative of strain W3110 that is deficient in DNA polymerase I (polA$^-$) (5). This enzyme is involved in resynthesizing DNA segments that have been damaged and excised. Bacterial strains deficient in DNA polymerase I are thus especially sensitive to DNA damage and grow less readily in the presence of DNA-damaging agents than does the parent strain. Thus, test compounds that are more toxic to p3478 than to W3100 may be assumed to react with DNA.

The two E. coli strains were obtained from Dr. H. Rosenkranz (Case Western Reserve University, Cleveland, Ohio), who described the test procedure (17). To determine a test compound's relative toxicity to the two bacterial strains, disks of filter paper inoculated with the test substance were placed on the surfaces of two agar plates, each containing nutrient broth and one of the bacterial strains. The plates were incubated for a day, and the

zone of inhibition of bacterial growth was measured. Comparison of the diameters of the zones of growth inhibition for the polA and normal strains indicated whether the test compound caused DNA damage. The positive control in this assay was methyl methanesulfonate. The negative controls were ampicillin, kanamycin, and chloramphenicol, which all induce equal zones of inhibition, because their toxic effects are not due to DNA damage. A positive response was indicated by a larger zone of inhibition for the repair-deficient strain than for the normal strain; a negative response was indicated by equal zones of growth on both plates. A result was considered inconclusive if growth was not inhibited on either of the test plates. Compounds were tested at at least two concentrations, and final testing consisted of two repetitions of the experiments, performed on different days.

Bacillus subtilis recA Differential Toxicity Assay (REW)

B. subtilis strain M45 is a recombination-deficient ($recA^-$) derivative of strain H17; both strains were obtained from Dr. T. Kada (National Institute of Genetics, Mishima, Japan). Recombination is required for repair of damaged DNA. Except for the difference in test strains, the method of testing (11) and criteria for interpretation were the same as for the REP assay.

S. typhimurium SL4525(rec^+)/SL4700(rec^-) Differential Toxicity Assay (SAR)

S. typhimurium strain SL4700 is a recombination-deficient derivative of strain SL4525; both strains were obtained from Dr. Bruce Stocker (Stanford University, Stanford, California). These two strains also have an rfa mutation that leads to a defective lipopolysaccharide coat, which makes them more permeable to large molecules and thus more suitable for testing possible mutagens. A second pair of S. typhimurium strains, TA1978 and TA1538, also contain the rfa mutation; they were obtained from Dr. Bruce Ames. Strain TA1538 lacks the uvrB gene, which is involved in the repair of DNA damage caused by exposure to ultraviolet light. The methods for testing and evaluating the ability of pesticides to induce DNA damage in these pairs of bacterial strains (1) were similar to those described for the REP assay.

PRIMARY DNA DAMAGE IN EUKARYOTES

Saccharomyces cerevisiae D3 Assay (YE3)

The yeast S. cerevisiae strain D3 is a diploid microorganism heterozygous for a mutation leading to a defective enzyme in the

adenine-metabolizing pathway. When grown on medium containing adenine, cells homozygous for this mutation produce a red pigment. These homozygous mutants can be generated from the heterozygotes by mitotic recombination. The frequency of this recombinational event is increased by incubating the cells in the presence of recombinogenic agents. The recombinogenic activity of a compound or its metabolites was determined from the number of red-pigmented colonies appearing on test plates, as described by Brusick and Mayer (3). Five concentrations of the test chemical were tested both with and without metabolic activation. For toxic compounds, the concentration range was lowered until maximum toxicity was approximately 50%. A positive response in this assay was indicated by dose-related increases of more than threefold in the numbers of mitotic recombinants per milliliter and per 10^5 surviving cells. A negative response was indicated by no recombinogenic activity in any of the assays. When recombinogenic activity was not dose-related, the test was considered inconclusive.

Saccharomyces cerevisiae D7 Gene Conversion and Mitotic-Crossing-Over Assays (YEH)

S. cerevisiae strain D7 is heterozygous for a mutation leading to a defective enzyme in the adenine-metabolizing pathway. This heteroallelic condition, ade2-40/ade2-119, gives rise to white colonies. Homozygous mutants can be generated by mitotic crossing over. When grown on medium containing adenine, cells homozygous for this mutation produce pink and red twin-sectored colonies of the genotypes ade2-40/ade2-40 (deep red) and ade2-119/ade2-119 (pink). Thus, the frequency of mitotic crossing over at this locus is determined by counting the numbers of twin-sectored colonies appearing on the plates. The methods used for testing with this strain were described by Zimmermann (23).

A variety of genetic events, such as point mutation, gene conversion, chromosomal deletion, and aneuploidy, give rise to additional mutant phenotypes: colonies may be pink, red, white and pink, or red and white. These types of colonies appear more frequently than the twin-sectored colonies. No attempt was made to distinguish these phenotypic aberrants or their causes--they were grouped as total aberrants.

S. cerevisiae D7 is also heterozygous for a mutation leading to a specific growth requirement. Cells of the genotype trp5-12/trp5-27 require exogenous tryptophan for growth. Transfer of the intact region of one mutant allele to replace the defective nucleotide sequence in the other mutant allele (gene crossover) will restore a true wild-type genotype that allows growth in the absence of tryptophan. Thus, the frequency of mitotic gene

conversion can be determined from the number of colonies appearing on medium that lacks tryptophan.

The numbers of mitotic recombinants and of total aberrants per 10^5 survivors and the number of gene convertants per 10^6 survivors were calculated. Mitotic crossing over and mitotic gene conversion were indicated by dose-related increases in the numbers of aberrant or convertant colonies. If no dose-related increases in aberration or conversion were observed, the result was considered negative; results were considered inconclusive when responses were not unequivocally positive or negative.

Human Lung Fibroblast Unscheduled DNA Synthesis Assay (UDH)

Unscheduled DNA synthesis is the incorporation of nucleotides into the DNA of cells during repair of damage induced by physical or chemical agents. In this test, the incorporation of tritiated thymidine, [^{3}H]-dT, into human lung fibroblast cells (strain WI-38) is monitored by liquid scintillation counting. The methods used were a variation of those described by Simmon (15). The cells are grown in synchronous culture, and the assay was conducted only when the cells were not in the synthetic (S) phase, when normal DNA synthesis would have overwhelmed any measurement of DNA repair synthesis. Cells were incubated with the test chemical and [^{3}H]-dT, with or without a metabolic activation system, for a few hours; then their DNA was extracted, and the [^{3}H]-dT content measured by liquid scintillation counting. Positive controls were 4-nitroquinoline-N-oxide, which induces unscheduled DNA synthesis in the absence of metabolic activation, and dimethylnitrosamine, which induces unscheduled DNA synthesis only with metabolic activation. The negative control was DMSO in culture medium. An appropriate concentration range was selected in preliminary studies. Five concentrations of test compound were used for each assay, with six replicate samples for each concentration, to facilitate statistical analysis of the results. A test result was considered positive if there was a significant concentration-related increase in thymidine incorporation in test cells compared with the negative controls. A result was considered negative if thymidine incorporation did not increase significantly and inconclusive if neither of the above criteria was met.

CHROMOSOMAL EFFECTS

Sister-Chromatid Exchange Assay (SCC)

The induction of DNA lesions by chemical mutagens leads to exchanges between two chromatids of a chromosome (SCE), which may

be related to a recombinational or postreplicative repair of DNA damage. SCEs are observed in cells that have been grown in the presence of bromodeoxyuridine (BrdU) for two rounds of replication. Because DNA replication is semiconservative, such chromosomes possess one chromatid that is half BrdU-substituted and one that is fully substituted. These chromatids are differentially stained by the fluorescence-plus-Giemsa technique; hence, exposure to a chromosome-damaging material results in an increased frequency of SCEs, revealed by a "harlequin" pattern of dark and light stained chromatid segments.

The Chinese hamster ovary (CHO) cells used in this assay were obtained from the American Type Culture Collection (ATCC-CCL-61). Procedures for maintaining these cells and for demonstrating SCEs were described by Perry and Evans (13) and by Stetka and Wolff (18). The positive controls were ethyl methanesulfonate without metabolic activation and dimethyl-nitrosamine with metabolic activation. Because test chemicals may affect the duration of the cell cycle and treated cells must divide twice to be evaluated for SCE induction, a series of five dilutions of the compound and positive and negative controls were tested with and without metabolic activation. For each test, two cytogeneticists analyzed duplicate coded samples for at least three concentrations of the test compound, as well as the controls. Each observer analyzed 25 cells per sample for the total number of SCEs per cell and for the number of chromosomes per cell. A test compound was considered to produce positive results if both cytogeneticists agreed either (1) that it induced SCEs at at least twice the baseline frequency or (2) that at least three test concentrations caused a progressive increase in SCE frequency, and at least one value was significantly greater than the background frequency ($p < 0.001$). A result was considered negative if both cytogeneticists agreed that the above criteria were not met and inconclusive if only one observer judged the criteria to have been met.

Mouse Micronucleus Assay (MNM)

The micronucleus test is a rapid, *in vivo* assay based on the observation that cells with chromosome breaks or exchanges often have disturbances in the distribution of chromatin during cell division. After division, the daughter cells contain this displaced chromatin as distinct micronuclei in the cytoplasm. The cell population examined consisted of erythrocytes taken from bone marrow or peripheral blood smears of mice. Because erythrocytes normally do not contain DNA, the presence of micronuclei in these cells was considered evidence of chromosome breakage.

Test compounds were administered according to the method of Schmid (14). The test compound was dissolved in an appropriate solvent and administered by oral gavage or intraperitoneal injection to a group of male Swiss-Webster mice. Trimethylphosphate (TMP) was the positive control, and solvent was the negative control. Eight mice selected randomly from each treatment group were sacrificed, and cardiac blood and bone marrow were extracted from each animal and smeared on slides. The slides were stained with Giemsa stain, and the number of micronucleated cells per 500 polychromatophilic cells was recorded for each slide. The results were analyzed by the statistical methods of Mackey and MacGregor (12).

Mouse Dominant Lethal Assay (DLM)

A final test for chromosomal effects of chemicals was the mouse dominant lethal assay, in which male mice were fed the test compound and mated with fertile females. Pregnant females were sacrificed and sectioned at midterm, and the numbers of corpora lutea and dead and live fetuses were counted. Comparison of these mice with those impregnated by untreated males allowed judgement of whether genotoxic effects of the test chemical affected the sperm. The methods for this test were those of Simmon (16).

Adult ICR/SIM mice from a closed, random-bred colony were used to determine the acute toxicity and maximum tolerated dose, as well as for the dominant lethal assay. The mice were supplied by Simonsen Laboratories, Gilroy, California. The males were 3- to 4-month-old proven breeders, and the females were 10- to 12-week-old virgin stock.

Each pesticide to be tested was dissolved or suspended in corn oil. The compound-oil concentrate then was added at a concentration of 3% to a finely ground commercial diet of known composition. Untreated control animals were given a diet containing 3% corn oil. Positive controls were administered a single intraperitoneal injection of 0.2 mg/kg of triethylenemelamine (TEM) two hours before the first mating. Treated animals were given diets containing the maximum tolerated dose (or 5 g/kg, whichever was lower), one-half, and one-quarter of the maximum dose. For this work, the maximum tolerated dose was defined as the dietary level that may produce up to a 20% weight loss, mild but transient clinical signs, no inhibition of breeding performance, and no mortality.

Each control and experimental test group contained 20 adult male mice. The treatment and control diets were administered for seven weeks. At the end of the treatment period, each male was

allowed to breed with two virgin females over a period of seven days. Females were replaced weekly for eight weeks.

Females were sacrificed at midterm of pregnancy. A complete autopsy was performed to determine if infection was present; such a condition can induce preimplantation loss and early fetal deaths. At sacrifice, each female was scored for early fetal deaths, late fetal deaths, and living fetuses (which together provided a total implant score). The index of dead implants per total implants was analyzed statistically by the t-test on angular-transformed data (6). A result was judged positive only if the increase in mortality of implants was statistically significant.

RESULTS AND DISCUSSION

The qualitative test results obtained in each system for each chemical are presented in Tables 2, 3, and 4 for insecticides, herbicides, and fungicides, respectively. Results are indicated as "+" for positive responses, "-" for negative responses, and "?" for uninterpretable results. In vitro tests that gave positive responses only with metabolic activation are denoted by asterisks. Of the 65 chemicals, 35 gave positive results in one or more test systems: 19 caused point/gene mutations, 29 caused DNA damage, and 9 caused chromosomal effects. Eleven of the chemicals produced a positive response in only one test system; however, testing is not complete.

Detection and Confirmation of Genetic Activity

Six tests were performed with 51 or more of the pesticides:

S. typhimurium plate-incorporation assay (SAL)
E. coli reverse-mutation assay (WPU)
E. coli polA differential toxicity assay (REP)
B. subtilis recA differential toxicity assay (REW)
S. cerevisiae D3 assay (YE3)
human lung fibroblast unscheduled DNA synthesis assay (UDH)

Five of the test systems were used as confirmatory tests for pesticides giving positive results in the initial test battery:

S. cerevisiae D7 reverse-mutation assay (YER)
mouse lymphoma forward-mutation assay (L5T)
S. cerevisiae D7 gene conversion and mitotic-crossing-over assays (YEH)
sister-chromatid exchange assay (SCC)
mouse micronucleus assay (MNM)

Table 2. Genotoxicity Bioassay Results for Insecticides[a]

Compound	Point/Gene Mutation					DNA Damage						Chromosomal Effects		
	Prokaryote		Eukaryote			Prokaryote			Eukaryote					
	SAL	WPU	YER	L5T	SRL	REP	REW	SAR	YE3	YEH	UDH	SCC	MNM	DLM
Acephate	+	-	+*	+	-	?	?	-	+	+	+	+	-	
Allethrin	+*	-				?	-		-		-			
Aspon	-	-			-	?	?		-		-			
Azinphos-methyl	-	-	-	+*	-	?	?	-	+	-	-	-	-	-
Carbofuran	-	-			-	?	?		-		-			
Chlordimeform	-	-				?	-		-		-			
Chlorpyrifos	-	-			-	+	+	+	-		-			
Chrysanthemic acid	-	-				-	+		-		-			
Crotoxyphos	-	-	-	+	-	?	?	-	+	-	-	-	-	
Cypermethrin	-	-				?	?		-		-			
Demeton	+	+	+	+	-	-	+	-	+	+	+	+	-	
Diazinon	-	-				?	?		-		-			
m-Dichlorobenzene	-	-				+	-		+					
o-Dichlorobenzene	-	-				+	-		-					
p-Dichlorobenzene	-	-				-	-		-					
Disulfoton	-	-	-	+	-	?	?	-	-	-	+	+*	-	
Endrin	-	-				-	-		-		-			
Ethion	-	-				-	-		-		-			

(continued)

[a] "?" indicates that results were inconclusive or equivocal; "*" indicates that results were positive only with metabolic activation.

Table 2 (continued)

Compound	Point/Gene Mutation					DNA Damage						Chromosomal Effects		
	Prokaryote		Eukaryote			Prokaryote			Eukaryote					
	SAL	WPU	YER	L5T	SRL	REP	REW	SAR	YE3	YEH	UDH	SCC	MNM	DLM
Ethyl chrysanthemate	-	-				-	-		+		-			
Fensulfothion	-	-				?	?		-		-			
Fenthion	-	-			-	?	?		-		-			
Fonofos	-	-				?	?		-		-			
Formetanate	-	-				-	-		-					
Malathion	-	-			-	-	-		-		-			-
Methomyl	-	-			-	-	-		-		-			
Methoxychlor	-	-			-	?	?		-		-			
Methyl parathion	-	-	-	+	-	?	?	-	+	-	-	+*		-
Monocrotophos	+	-	+	+	-	?	?	+	+	+	+*	+	-	-
Parathion	-	-			-	-	-		-		-			-
Permethrin	-	-				?	?		-		-			
Phorate	-	-			-	-	-		-		-			-
Resmethrin	-	-				?	?		-		-			
Rotenone													-	
Sumithrin	-	-				?	?		-		-			
Trichlorfon	+	+	+	+	-	?	?	+	+	+	+	+	-	

[a] "?" indicates that results were inconclusive or equivocal; "*" indicates that results were positive only with metabolic activation.

Table 3. Genotoxicity Bioassay Results for Herbicides[a]

Compound	Point/Gene Mutation					DNA Damage						Chromosomal Effects		
	Prokaryote		Eukaryote			Prokaryote			Eukaryote					
	SAL	WPU	YER	L5T	SRL	REP	REW	SAR	YE3	YEH	UDH	SCC	MNM	DLM
Bromacil	-	-	-	+*	+	-	-*	-	-	-	-	-	-	-
Cacodylic acid	-	-	+	+	-	?	?	-	+	+	-	-	+	
Diallate	+*	-	-	+	+				+*	-				
Dicamba	-	-			-	+	+	-	-		-			
Dinoseb	-	-			-	+	+	+	-		-			
DSMA	-	-				-	-		-		-			
Monuron	-	-	-	+	-	-	-		-	-	-	+*	+	
MSMA	-	-			-	-	-		-		-			
Pentachlorophenol	-	-				-	+		+					
Propanil	-	-				-	+	+	-		-			
Siduron	-	-			-	?	?		-		-			
Simazine	-	-	-	+*	+	?	?	-	-	-	-	-	-	
Sulfallate	+*	-				-	-		-					
Triallate	+*	-	-	+	-				+*	-				
Trifluralin	-	-			-	-	-		-		-			
2,4-D	-	-				-	+	-	-		-			
2,4-DB	-	-				+	-	-	-		-			
2,4,5-T	-	-				-	+		-					

[a] "?" indicates that results were inconclusive or equivocal; "*" indicates that results were positive only with metabolic activation.

Table 4. Genotoxicity Bioassay Results for Fungicides[a]

Compound	Point/Gene Mutation					DNA Damage						Chromosomal Effects		
	Prokaryote		Eukaryote			Prokaryote			Eukaryote					
	SAL	WPU	YER	L5T	SRL	REP	REW	SAR	YE3	YEH	UDH	SCC	MNM	DLM
Benomyl	1	2		+3			4			5		+	+6	
Biphenyl	-	-				?	?		-		-			
Captan	+	+		+	+	+	+	+	+		-			-
Dichloran	-	-				-	-		-					
Folpet	+	+		+	+	+	+	+	+		-			-
Mancozeb	-	-				-	-		+		+[b]			
Maneb	-	-				-	-		+		+[c]			
PCNB	-	-			-	?	?		-		-			-
Polyram	-	-				-	-		-		-			
sec-Butylamine	-	-				+	-		-					
sec-Butylamine H_3PO_4	-	-				-	-		-					
Zineb	-	-				-	-		+		-			

[a] "?" indicates that results were inconclusive or equivocal.

[b] Dithane M-45: negative; Manzate 200: positive without metabolic activation, negative with metabolic activation.

[c] Dithane M-22: negative; Manzate D: positive without metabolic activation, negative with metabolic activation.

Of the 35 pesticides that gave at least one positive result, only a few were detected by any one test in the initial battery.

Among the six tests used initially, YE3 detected the largest number of genetically active chemicals, and WPU the smallest. Of the chemicals that gave positive results in one or more tests in the initial test battery, the prokaryotic systems REW, SAL, REP, and WPU detected 11/32 (34%), 10/34 (29%), 9/32 (28%), and 4/34 (12%), respectively. One tester strain in the SAL assay, TA100, detected all 10 of the chemicals that gave positive results in this system. The eukaryotic systems in the initial test battery, YE3 and UDH, detected 18/34 (53%) and 7/31 (23%) chemicals, respectively. Correlation with initial test results was highest for the L5T assay (17/17, or 100%) and lowest for the MNM test (3/13, or 23%). When the test results for the functional classes of pesticides were considered independently, it was seen that of the initial tests, YE3 detected the largest numbers of the genetically active insecticides and fungicides, 8/14 (57%) and 5/6 (83%), respectively.

Of the 18 pesticides that gave positive results in YE3, 12 subsequently gave positive results in L5T (the other 6 have not yet been tested in L5T). Hence, it appears that L5T confirms the genetic activity of many of the chemicals detected in YE3. Of 10 pesticides that gave positive results in SAL, 8 also gave positive results both in YE3 and in L5T (the other 2 pesticides have not yet been tested in L5T). Thus, L5T also appears to confirm the genetic activity detected by SAL for many pesticides. On the other hand, SAL appears to miss some genetically active chemicals: of 10 pesticides giving negative results in SAL, all gave positive results in YE3, and 4 gave positive results in L5T (the remaining 6 have not been tested in L5T). Furthermore, of 34 pesticides that gave positive results in the initial test battery, 24 gave negative results in SAL.

Clearly, no single test of the initial battery was sufficiently sensitive to detect all genetically active pesticides. The results underscore the need for a battery of screening tests to detect the various kinds of genetic activity displayed by pesticide chemicals.

The genotoxicity test results for 15 pesticides are compared with results of carcinogenicity studies in Table 5. Four herbicides (diallate, monuron, sulfallate, and trifluralin) and one fungicide (captan) evaluated in the present study cause tumors in rodents (7, 10). One of these compounds, trifluralin, was shown to be contaminated with dipropyl nitrosamine in the National Cancer Institute carcinogenesis bioassay (21). Three insecticides

Table 5. Comparison of Genotoxicity and Carcinogenicity Test Results

Pesticide[a]	Genotoxicity Results	Carcinogenicity Results[b] Mice (m/f)	Rats (m/f)	Reference
Carcinogenic				
Captan (F)	+ : SAL, WPU, L5T, SRL, REP, REW, SAR, YE3 - : UDH, DLM	+/±	-/-	(7)
Diallate (H)	+ : SAL, L5T, SRL, YE3 - : WPU, YER, YEH	+/+	±/±	(8)
Monuron (H)	+ : L5T, SCC, MNM - : 9 tests	+	±	(10)
Sulfallate[c] (H)	+ : SAL - : 4 tests	+/+	+/+	(7)
Trifluralin (H)	- : 7 tests	-/+	-/-	(7)
Possibly Carcinogenic				
Azinphos-methyl (I)	+ : L5T, YE3 - : 10 tests	-/-	±/±	(7)
Fenthion (I)	- : 5 tests	±/-	-/-	(7)
Parathion (I)	- : 8 tests	-/-	±/±	(7)
Not Carcinogenic (under assay conditions)				
Diazinon (I)	- : 4 tests	-/-	-/-	(7)
Endrin (I)	- : 6 tests	-/-	-/-	(7)

(continued)

Table 5 (continued)

Pesticide[a]	Genotoxicity Results	Carcinogenicity Results[b] Mice (m/f)	Rats (m/f)	Reference
Malathion (I)	- : 8 tests	-/-	-/-	(7)
Methoxychlor (I)	- : 5 tests	-/-	-/-	(7)
Methyl parathion (I)	+ : L5T, YE3, SCC - : 8 tests	-/-	-/-	(7)
PCNB (F)	- : 6 tests	-/-	-/-	(7)
2,4-D and esters (H)	+ : REW, REP - : 5 tests	-	-	(10)

[a] F = fungicide; H = herbicide; I = insecticide.
[b] + = positive response; - = negative response; ± = equivocal response.
[c] Mutagenicity testing of sulfallate is incomplete.

(azinphos-methyl, fenthion, and parathion) show equivocal evidence of carcinogenicity (7). Overall, stronger relationships between genetic bioassays and carcinogenicity are apparent for the eukaryotic tests (L5T, YE3, SRL, and, to a lesser extent, SCC, MNM, and UDH). The noncarcinogens generally gave negative results in the genotoxicity assays. The genotoxicity test results obtained in this study are discussed in more detail elsewhere (20).

Pesticides Used In Egypt

Table 6 lists the pesticides that have been or are now used in tonnage quantities in Egypt. Those tested in the present study are underlined. Other insecticides tested that are used in significant amounts (or have been tested for large-scale use) in Egypt include acephate, carbofuran, chlordimeform, demeton, diazinon, disulfoton, endrin, ethion, fenthion, fonofos, malathion, methoxychlor, methyl parathion, parathion, and phorate. The genetic toxicology of the most important pesticides used in Egypt can be summarized from the findings of the present study.

Table 6. Major Pesticides Used in Egypt[a]

Insecticides	Herbicides	Fungicides
Azinphos-methyl	Ametryn	Benomyl
Carbaryl	Atrazine	Captafol
Chlorpyriphos	Bensulide	Captan
Cypermethrin	Cacodylic acid	Choranil
DDT	Dalapon	Dichlone
Decamethrin	Dicamba	Dinocap
Endrin	Dinoseb	Ediphenphos
Fenvalerate	Diuron	Ethylmercury chloride
Leptophos	Fluometuron	Ferbam
Lindane	Hexazinone	Folpet
Mephosfolan	Monuron	IBP
Methomyl	Paraquat	Mancozeb
Monocrotophos	Propanil	Maneb
Phosfolan	Simazine	Thiram
Profenofos	Trifluralin	Triazbutil
Toxaphene	2,4-D	Zineb
Triazophos	2,4,5-T	
Trichlorfon		

[a]Underlined compounds were tested in the present study.

Insecticides

Azinphos-methyl. This compound was tested in all 14 bioassays. In the initial battery, it gave positive results only in the S. cerevisiae D3 assay for DNA damage (YE3). In the E. coli polA and B. subtilis recA differential toxicity assays (REP and REW), the results were inconclusive. The confirmatory assays gave negative results, with one exception: azinphos-methyl caused gene mutations in mouse lymphoma cells (L5T) when a metabolic activation system was present.

Chlorpyrifos. In the initial battery of assays, chlorpyrifos did not produce point mutations in prokaryotic systems (SAL and WPU). In the DNA-damage assays, it gave positive results in the prokaryotic systems (REP and REW), but negative results in the eukaryotic systems (YE3 and UDH). In confirmatory assays, it did not cause sex-linked recessive lethal mutations in Drosophila (SRL), but it gave positive results in the S. typhimurium differential toxicity assay (SAR).

Cypermethrin. This insecticide was tested only in the initial battery of assays. It gave negative results in all of these except the two DNA-damage assays in prokaryotic systems (REP and REW), for which the results were inconclusive.

Methomyl. The results for this compound were negative in all six assays of the initial battery. Methomyl was also tested in the Drosophila sex-linked recessive lethal test (SRL), with negative results.

Monocrotophos. Monocrotophos was tested in all 14 bioassays. It produced point/gene mutations in S. typhimurium (SAL), S. cerevisiae D7 (YER), and mouse lymphoma cells (15T), but not in E. coli WP2 (WPU) or the Drosophila sex-linked recessive lethal test (SRL). Results were inconclusive in the E. coli polA and B. subtilis recA differential toxicity assays (REP and REW), but positive in the other assays for DNA damage. In the unscheduled DNA synthesis assay (UDH), monocrotophos gave positive results only with metabolic activation. It induced sister-chromatid exchange (SCC), but gave negative results in the other two assays for chromosomal effects.

Trichlorfon. This insecticide was tested in all of the assays except the mouse dominant lethal assay (DLM). It caused point/gene mutations in both prokaryotic test systems (SAL and WPU), in S. cerevisiae D7 (YER), and in mouse lymphoma cells (L5T), but gave negative results in the Drosophila sex-linked recessive lethal test (SRL). DNA damage was induced in all three eukaryotic test systems and in the S. typhimurium differential toxicity assay (SAR), but results were inconclusive in the E. coli polA and B. subtilis recA differential toxicity assays (REP and REW). Trichlorfon also induced sister-chromatid exchange, but gave negative results in the mouse micronucleus assay.

Herbicides

Cacodylic acid. This compound was tested in all of the bioassays except the mouse dominant lethal assay (DLM). Cacodylic acid produced point/gene mutations in S. cerevisiae D7 (YER) and mouse lymphoma cells (L5T), but not in the Drosophila sex-linked recessive lethal test (SRL) or in either prokaryotic test system. DNA-damage results were positive in S. cerevisiae D3 (YE3) and S. cerevisiae D7 (YEH), inconclusive in E. coli polA (REP) and B. subtilis recA (REW), and negative in the S. typhimurium differential toxicity assay (SAR) and the unscheduled DNA synthesis assay (UDH). Cacodylic acid gave positive results in the mouse micronucleus assay (MNM), but negative results in the sister-chromatid exchange assay (SCC).

Dicamba. In the initial battery of assays, dicamba did not cause point mutations in the prokaryotic systems. It caused DNA damage in both prokaryotic test systems (REP and REW), but not in the two eukaryotic systems. Two confirmatory assays, the Drosophila sex-linked recessive lethal test (SRL) and the S. typhimurium differential toxicity assay (SAR), both yielded negative results.

Dinoseb. In the initial battery, dinoseb produced positive results only in the two assays for DNA damage in prokaryotic systems (REP and REW). A third such assay, for differential toxicity in S. typhimurium (SAR), also gave positive results. Negative results were obtained in the Drosophila sex-linked recessive lethal test.

Monuron. The results for this compound were negative in all six assays of the initial battery. However, monuron gave positive results in three of the confirmatory assays: the mouse lymphoma forward-mutation assay (L5T), the mouse micronucleus assay (MNM), and the sister-chromatid exchange assay (SCC). Sister-chromatid exchanges were produced only with metabolic activation. Monuron was not tested in the S. typhimurium differential toxicity assay (SAR) or in the mouse dominant lethal assay (DLM).

Propanil. In the initial battery of assays, propanil gave positive results only in the B. subtilis recA differential toxicity assay (REW). The one confirmatory test, the S. typhimurium differential toxicity assay (SAR), also gave positive results.

Simazine. This herbicide was tested in all of the assays except the mouse dominant lethal assay (DLM). Simazine caused gene mutations in mouse lymphoma cells (L5T) with metabolic activation and in the Drosophila sex-linked recessive lethal test (SRL). The results were inconclusive for the E. coli polA and B. subtilis recA differential toxicity assays (REP and REW) and negative for all of the other assays.

Trifluralin. Trifluralin gave negative results in all of the assays of the initial battery and also in the Drosophila sex-linked recessive lethal test (SRL).

2,4-D. In the initial battery, 2,4-D gave positive results only in the B. subtilis recA differential toxicity assay (REW). Results were negative for the other five initial assays and also for the S. typhimurium differential toxicity assay (SAR).

2,4,5-T. This herbicide has been tested in five of the six assays of the initial battery. It gave positive results only in the B. subtilis recA differential toxicity assay (REW); it was not tested in the unscheduled DNA synthesis assay (UDH).

Fungicides

Benomyl. Benomyl was tested in only three assays, none of which is part of the initial battery. It produced gene mutations in mouse lymphoma cells (L5T), and it caused sister-chromatid exchanges (SCC) and micronucleus formation (MNM).

Captan. In the initial battery, captan gave positive results in all of the assays except that for unscheduled DNA synthesis (UDH). It also caused gene mutations in mouse lymphoma cells (L5T) and sex-linked recessive lethal mutations in Drosophila (SRL). The results for captan were positive in the S. typhimurium differential toxicity assay (SAR) and negative in the mouse dominant lethal assay (DLM).

Folpet. Folpet was tested in the same ten assays as was captan and gave the same qualitative results in each case.

Mancozeb. Mancozeb was tested in the initial battery. It gave negative results in the four prokaryotic test systems and positive results in the two eukaryotic assays for DNA damage. In the unscheduled DNA synthesis assay (UDH), the results differed for the two formulations tested: for Dithane M-45, the results were negative; for Manzate 200, they were negative with metabolic activation, but positive without activation.

Maneb. In the initial battery, Maneb gave the same qualitative results as did Mancozeb. In the unscheduled DNA synthesis assay (UDH), the results were negative for Dithane M-22; for Manzate D, they were negative with metabolic activation, but positive without.

Zineb. In the initial battery of assays, zineb gave positive results only in the S. cerevisiae D3 assay for DNA damage (YE3). It was not tested in any of the confirmatory assays.

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