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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

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Agency Agent: Fred P. Meyer Date: [illegible]

Director
National Fisheries Research Center
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Signature

GOOD LABORATORY PRACTICE STATEMENT

The submitter of this study was neither the sponsor of this study nor conducted it, and does not know whether it has been conducted in accordance with 40 CFR Part 160.

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16. ABSTRACT

The objectives of this project were to evaluate the mutagenicity of various compound mostly pesticides, using microbial and mammalian cell *in vitro* techniques, as well a *in vivo* techniques in Drosophila and mice, and to further develop and refine these p cedures for application as test batteries.

Seventy-nine compounds were evaluated for mutagenicity in one or more of 11 test sys *S. typhimurium* plate incorporation assay; *E. coli* WP-2 reverse mutation assay; *S. ce siae* D3 mitotic recombination assay; *S. cerevisiae* D7 assays; *E. Coli*, *B. subtilis*, *S. typhimurium* relative toxicity assays; siter-chromatid exchange in Chinese hamster ary cells assay; L5178Y mouse lymphoma cell forward mutation assay; unscheduled DNA s thesis assay; mouse micronucleus assay; *Drosophila* sex-linked recessive lethal assay; mouse dominant lethal assay.

The data from the evaluation of 41 pesticides and 10 industrial chemicals are present in this report. Qualitative interpretations of these data and of data obtained under previous contract, including those for an additional 28 pesticides, are summarized.

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In Vivo Assays

Mouse Micronucleus Test

Background

The micronucleus test is a rapid, in vivo cytogenetic screen. It is based on the observation that cells with chromosome breaks and/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 (40).

The cell population tested consists of erythroblasts undergoing their final chromosome replication and mitosis before expulsion of the nucleus. Bone marrow or peripheral blood smears made at intervals after treatment yield a population of young, enucleated erythrocytes that are known to have undergone mitosis during the time of treatment. These young erythrocytes are readily identifiable; they stain differently from mature cells and they are easily scored for the presence of micronuclei because they have no nucleus.

Because it indicates in vivo chromosome damage and/or spindle apparatus impairment, the micronucleus test is an important part of the battery of tests used to determine the mutagenic potential of chemicals. Correlated with the results of gene mutation assays and DNA-damage tests, the results of the micronucleus test contribute to the comprehensive evaluation of chemicals.

Method

The method of Schmid (40) is used for administration of test compounds and preparation of bone-marrow smears. Two sampling times (48 and 72 hours), in addition to the 30-hour time recommended by Schmid, are evaluated to test adequately compounds that may be slowly metabolized or absorbed.

Water-soluble compounds are administered in Hanks balanced salt solution (HBSS) or water. For compounds not soluble in water, solution in DMSO is attempted. Compounds not soluble in either water or DMSO are suspended in corn oil. Compounds are administered (5 ml of vehicle per kilogram of body weight) in three dilutions: 80%, 40%, and 20% of the LD50. The high-dose-level dilution is prepared using the appropriate amount of compound, and serial dilutions are made to prepare the 40% and 20% levels. Vehicle, positive, and negative (blank) control groups are included with each test. Trimethylphosphate (TMP) is the positive control agent. When more than one compound is tested simultaneously, common controls are used.

If toxicity data are available for a given compound, no preliminary procedures are necessary. If no guidelines are available, a preliminary range-finding test is done to determine appropriate dose levels. This range-finding test can be an informal procedure that allows selection of a toxic

dose level that permits the survival of enough animals for the three-day test period.

For the test itself, male Swiss-Webster mice weighing 20 to 30 g are housed four to a cage, with food and water ad libitum, and acclimated for one week. Animals are identified by cage number and ear punch or tail mark. Animals rooms are maintained at a temperature of 72 ± 2°F and on a 12-hour light cycle. The compound to be tested, dissolved in an appropriate vehicle, is administered by gavage or intraperitoneal injection to 30 animals at each dose level tested. Doses are expressed as mg/kg body weight. Vehicle-control animals are given the solvent in the same volume as that received by the treated animals of the same weight, the actual amount (5 or 10 ml/kg) to be determined during the dose range-finding study. Ten positive-control animals receive THP (1250 mg/kg), administered intraperitoneally in 5 ml/kg of DMSO (the intraperitoneal route is the most effective route for THP-induced effects). The test compound is weighed and dissolved in the vehicle immediately before dosing. Fresh solutions are made on each of the two days of dosing. Animals are weighed and treated at 0 and 24 hours. Sacrifice with CO_2 and smear preparation are done at 30, 48, and 72 hours after the first dose of test compound.

The eight animals to be sacrificed at each time are selected randomly (using a random-number table) from the animals in each treatment group. To ensure survival of at least eight animals at each dose-time point, each compound-treated group contains more animals than needed for scoring. Extra animals surviving past 72 hours are discarded. Two blood smears are made for each animal by mixing a drop of cardiac blood with a drop of FCS. Three bone marrow smears are made from each animal according to the method of Schmid (40). Slides are stained with buffered Giemsa stain, air-dried, and coverslipped. All slides are coded before they are scored so that the technician is unaware of the identity of the slides being scored. Five hundred polychromatophilic erythrocytes are evaluated for each animal. The number of micronucleated cells is tallied. The ratio of polychromatophilic to mature red cells is calculated from the number of mature cells encountered while scoring 200 polychromatophilic cells.

When scoring is completed, the slides are decoded and the results are compared to a decision table made according to the method of Mackey and MacGregor (41).

Data Manipulation and Presentation

The decision of positive or negative results for any one dose level is dependent on the total number of micronucleated cells observed in the group; therefore, the only data manipulation is the summation of micronucleated cells. The ratio of polychromatic (PCE) to normochromatic (RBC) cells is calculated for each animal. The data of interest—the number of micronucleated cells and the PCE to RBC ratio for each animal, the total number of micronucleated cells in each group, and the mean ratio for each group—are presented in tabular form in the final report.

The method of Mackey and MacGregor (41) is used to determine whether a dose group is positive or negative in the micronucleus test. This method is

based on the negative binomial distribution and uses a decision table constructed using the following parameters:

Parameter	Definition	Value in This Study
$\bar{x}_1$	The mean number of micronucleated cells per 500 polychromatophilic erythrocytes (PCE) scored in historical control animals.	0.9
$\bar{x}_2$	The true mean number of micronucleated cells per 1000 PCE needed to declare a compound mutagenic.	3.0
k	The negative binomial constant, estimated from historical control data using a FORTRAN program supplied by Bruce Mackey.	6.9
α	The chance of declaring mutagenic a group whose true mean equals $\bar{x}_1$ (false positive). The α error decreases as the true mean for a group increases.	(from table)
β	The chance of declaring nonmutagenic a group whose true mean equals $\bar{x}_2$ (false negative). The actual β error decreases as the true mean for any particular group decreases.	(from table)

Using these values in the formulae of Mackey and MacGregor, the following decision table has been calculated:

No. of Animals	Negative Decision β <					No. Decision α & β > 0.10	Positive Decision α <				
	0.001	0.005	0.01	0.05	0.10		0.10	0.05	0.01	0.005	0.001
5	< 1	2,3	—	4,5	6	7-10	11	12,13	—	14,15	16
6	< 3	4	5	--	7	8-12	13	14	15	16	18
7	< 4	6	7	8	9	10-14	--	15	17	18	20
8	< 6	7,8	--	10	11	12-15	16	17,18	19	20	21

To use the table, the row for the number of animals in the group is read across until the value for the total number of micronucleated cells found in the group is located. The decision and level of significance of the decision are indicated at the top of the column in which the total is located.

The data generated are considered unacceptable if the value for the positive or the negative control group does not fall within its range in the decision table. If either control group is unacceptable, slides are recoded and evaluated by another observer. If the results remain outside the acceptable range for the group, the study is repeated.

Criteria for Interpretation

Positive. A compound is judged mutagenic if at least two dose-time groups fall within the positive range.

Negative. A compound is judged nonmutagenic if the highest dose given is the maximum tolerated dose and all dose-time groups fall within the negative range. "Maximum tolerated dose" is assumed to have been achieved if there is significant weight loss in the treated groups or if some animals die before scheduled sacrifice.

Inconclusive. Results are considered inconclusive if all dose groups fall in the "no decision" category or if extreme bone marrow depression (mean PCE/RBC ratio < 0.1-0.15) occurs in all groups in a negative test.

Drosophila Sex-Linked Recessive Lethal Assay

Background

The sex-linked recessive lethal (SLRL) test using Drosophila melanogaster can detect lethal point mutations and small deletions on the X-chromosome, which constitutes approximately 20% of the Drosophila genome. It is one of the most sensitive and reliable short-term mutagenesis assays, and it is the most rapid and economical whole-animal test system for measuring mutation frequencies. The ability of the SLRL test to detect mutagenic effects of pesticides is well documented (42-44).

SRI uses a modification of the yellow-Bar test described by Würgler et al. (45) for SLRL determination. In this system, yellow-Bar males that carry BAR (B) and yellow (y) on the X-chromosome and two minute secondary translocations of the X-chromosome, bearing the wild-type allele of y (y+), one on each arm of the Y-chromosome, are exposed to the test chemical. The exposed males are crossed singly to females carrying the Inscy X-chromosome in homozygous condition. This doubly inverted X is marked with y and scute (sc). The F_1 female progeny, each of which represents one treated X-chromosome, are mated to their F_1 brothers. Each F_1 female is then placed in a separate vial for egg-laying. Lethality is scored by the absence of the treated male phenotype in the F_2 generation.

The yellow-Bar test has the following advantages: (a) XXY females can be excluded from the F_1 mating population quickly and easily, (b) reciprocal translocations can be detected using the same stocks, and (c) sex chromosome loss and nondisjunction can be detected. Two strains of Drosophila are used.

Yellow-Bar stock: C(1)DX,y f/y b/y^+ Y y^+. The females of this stock have an attached X-chromosome marked with the recessive mutations y and forked (f). The X-chromosome possessed by the male carries y and the dominant

TABLE 1. SEVENTY-NINE CHEMICALS EVALUATED (concluded)

Common Name	Alternate Name	Manufacturer	Identifying Number	Purity (%)	Supplier
Pentachlorophenol	Dowicide PC-7	Dow	PCP6168	88	EPA
Permethrin	NRDC-143	ICI-America	EK-61223	90.4% (38.3% cis/52.3% trans)	EPA
Phorate	Thimet	American Cyanamid	MO-85	85	Battelle
Polytrim	Metiram	FMC	CT606-29-8	Tech	EPA
Propanil	Stam Tech	Rohm and Haas	6-2402	88	EPA
Resmethrin	SBP-1382	S. B. Penick	3521-LTV-3	90	EPA
Rotenone	Protox	Fairfield Amer. Corp.	TNB-77-2-262	41.59	EPA/Battelle
Siduron	Tupersan	DuPont	I-70713-C	96.7	Battelle
Simazine	Primatol	Ciba-Geigy	FL-740846	97.7	EPA/Battelle
	Primatol	Ciba-Geigy	FL-740846	97.7	Battelle
Sumithrin	Penitrothion			94.4	EPA
2,4,5-T	Dow 2,4,5-T Acid	Dow		98.7	EPA
Trialate	Far-go	Monsanto	TTB-08017	46.3	Grand Forks Seed Co.
	Far-go	Monsanto		Tech	EPA
Trichlorfon	Dylox, Dipterex	Chemagro	5-00-7003	Tech	EPA/Battelle
	Dylox, Dipterex	Chemagro	5-00-7003	Tech	Battelle
Trifluralin	Treflan	Eli Lilly	X-26290	97.7	Battelle
Sulfallate[a]	Vegadex	Monsanto		Tech	EPA
Zineb	Dithane Z-78	FMC	337	Tech	EPA
INDUSTRIAL CHEMICALS					
Acetonitrile		Aldrich	TC112987	99	EPA
Chlorobenzene	Chlorobenzene d_5	Aldrich	102087 PC	99	EPA
Coal Tar - Flaked Pitch		Koppers			Koppers
Coal Tar - Mtumastic A	Mtumastic #300-M, Component A	Koppers	178C1		Koppers
Coal Tar - Mtumastic B	Mtumastic #300-M, Component B	Koppers	162C		Koppers
Coal Tar - Mtumastic A + B					
Ethanol	Ethyl Alcohol	Aldrich		99.5	EPA
Methanol	Methyl Alcohol	Aldrich		99.5	EPA
Toluene	Toluene Monograde	Mallinckrodt	809232	99.9	Mallinckrodt
Vinyl Fluoride		Linde		99.9	Linde

[a]Alternate name used in this report.

Rotenone

Micronucleus Test (Tables 293, 294). The dosing and sacrifice schedules are shown in Table 293. The incidences of micronuclei in PCEs and the PCE-to-RBC ratios are given in Table 294. Rotenone was negative at all dose levels and sampling times, with a β error of < 0.005 in all groups. The DMSO vehicle control group was negative at all times, and the TMP positive control group was positive ($\alpha < 0.001$). The mean PCE-to-RBC ratios were within normal limits (0.50-1.50) for all groups except the low-dose, 96-hour rotenone group, which was elevated because of an aberrant value for one animal. Rotenone meets all of the criteria for a nonmutagenic decision in the micronucleus test.

Table 293

DOSING AND SACRIFICE SCHEDULE

Compound	No. of Animals	Dose		Mean Body Wt Change (g)	Number Sacrificed			No. of Fatalities
		0 Hr	24 Hr		48 Hr	72 Hr	96 Hr	
DMSO								
Vehicle control	24*	5 ml/kg	5 ml/kg	-1.2	8	7	8	1
TMP								
Positive control	8*	1 g/kg	1 g/kg	-2.4	8	-	-	0
Rotenone								
Low dose	24	0.56 mg/kg	0.56 mg/kg	-1.3	7	8	8	1
Mid dose	24	1.13 mg/kg	1.13 mg/kg	-1.0	8	8	8	0†
High dose	24	2.25 mg/kg	2.25 mg/kg	-1.0	8	8	7	1

* The control groups are the same as those reported with trichlorophon, which was treated at the same time.

† One animal sacrificed at 72 hr was cyanotic and moribund.

Table 294

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*

(500 PCE scored per animal)

Group	48 Hours			72 Hours			96 Hours		
	Animal No.	PCE with MN	PCE RBC	Animal No.	PCE with MN	PCE RBC	Animal No.	PCE with MN	PCE RBC
DMSO 2 x 5 ml/kg Vehicle Control	101♂	1	1.30	103♂	1	1.98	102♂	0	0.70
	101r	0	1.49	103r	1	1.83	102r	1	1.00
	101ℓ	2	1.09	103ℓ	0	1.61	102ℓ	0	1.09
	101b	0	0.88	103b	2	0.59	102b	0	0.81
	104♂	1	0.81	106r	1	1.28	105♂	1	0.98
	104r	1	0.75	106ℓ	1	1.47	105r	1	1.56
	104ℓ	1	0.92	106b	1	1.23	105ℓ	1	1.24
	104b	0	1.47				105b	0	1.67
Total		6†			7§			4†	
Mean			1.09			1.43			1.13
Rotenone 2 x 0.56 mg/kg	112♂	0	1.44	111♂	0	0.93	110♂	0	1.45
	112r	1	1.79	111r	0	1.08	110r	1	1.62
	112ℓ	0	0.73	111ℓ	0	1.26	110ℓ	1	1.74
	115♂	0	1.17	111b	0	1.45	110b	0	0.93
	115r	0	1.18	114♂	0	1.61	113♂	0	2.94
	115ℓ	0	1.18	114r	0	1.76	113r	1	1.77
	115b	0	1.09	114ℓ	0	0.92	113ℓ	0	1.28
				114b	1	0.73	113b	0	1.44
Total		1†			1†			3†	
Mean			1.23			1.14			1.65
Rotenone 2 x 1.13 mg/kg	117♂	0	1.14	116♂	1	0.98	118♂	0	1.26
	117r	0	1.09	116r	0	1.26	118r	0	0.87
	117ℓ	0	1.09	116ℓ	1	1.30	118ℓ	1	1.58
	117b	1	0.66	116b	1	1.27	118b	0	0.74
	119♂	0	0.92	120♂	0	0.10	121♂	1	0.84
	119r	0	1.80	120r	0	0.81	121r	1	2.27
	119ℓ	1	0.99	120ℓ	0	2.51	121ℓ	1	1.13
	119b	0	1.37	120b	1	0.60	121b	2	0.90
Total		2†			4†			6†	
Mean			1.13			1.10			1.20

* PCE = Polychromatophilic erythrocytes, RBC = mature erythrocytes, MN = micronucleus.
† $P \leq 0.001$.
§ $P \leq 0.01$.

Table 294 (Concluded)

Group	48 Hours			72 Hours			96 Hours		
	Animal No.	PCE with MN	PCE RBC	Animal No.	PCE with MN	PCE RBC	Animal No.	PCE with MN	PCE RBC
Rotenone 2 x 2.25 mg/kg	125♂	0	1.40	122♂	0	1.35	124♂	0	1.00
	125r	1	1.28	122r	0	1.63	124	1	0.64
	125ℓ	1	1.90	122ℓ	0	1.26	124b	1	1.59
	125b	0	1.18	122b	1	1.08	126♂	0	1.64
	127♂	0	1.40	123♂	0	0.91	126r	2	1.59
	127r	2	1.34	123r	2	1.41	126ℓ	1	1.20
	127ℓ	1	1.30	123ℓ	0	1.11	126b	0	0.83
	127b	2	1.16	123b	0	0.78			
Total		7†			3†			5†	
Mean			1.37			1.19			1.21
DMP 2 x 1 g/kg Positive Control	107♂	4	0.92						
	107r	18	0.62						
	107ℓ	2	0.92						
	107b	5	1.51						
	108♂	3	0.71						
	108r	4	1.32						
	108ℓ	0	1.01						
	108b	2	0.90						
Total		38**							
Mean			0.99						

† $\leq$ 0.001.
†† $\leq$ 0.005.
** $\leq$ 0.001.