UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

009619

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

JUL 2 2 1992

MEMORANDUM

SUBJECT: EPA Id# 057801: Diazinon: Review of an in vivo sister chromatid exchange study in mouse bone marrow.

TOX CHEM No.: 342
PC No.: 057801
TOX PROJECT No.: 2-1005
Submission No.: S409008

FROM: John Doherty 7/9/92
Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: Larry Schnaubelt/Robert Richards
Product Manager #72
Special Review and Reregistration Division
(H7508W)

THROUGH: Marion Copley, DVM, Section Head
Section IV, Toxicology Branch I
Health Effects Division (H7509C) 7/13/92

I. CONCLUSION

The sister chromatid exchange study in mouse bone marrow following oral administration was reviewed and determined to be ACCEPTABLE for males. No evidence of induction of sister chromatid exchange in males was evident at dose levels up to and including 100 mg/kg the highest dose levels tested.

The study was determined to be UNACCEPTABLE for females. An additional study testing higher doses in females will have to be provided by the registrant.

II. ACTION REQUESTED

The Ciba-Geigy Company has submitted an in vivo sister chromatid exchange study using mouse in order to satisfy the Agency's requirement for additional mutagenicity testing. The

study was reviewed and a copy of the DER is attached.

III. Study Reviewed.

Study Identification and Classification	TB-I comments
84-4. Sister Chromatid exchange in vivo in mouse bone marrow cells. Hazleton Labs Study # 1226-0-458, October 10, 1990 MRID # 416877-01 Classification: ACCEPTABLE for males; UNACCEPTABLE for females.	No evidence of increased sister chromatid exchange in mouse bone marrow cells. Target cell toxicity and symptoms in males but not females. Dose levels tested 0, 10, 50 and 100 mg/kg in PEG by gavage. ICR strain mouse.

Reviewed by: John Doherty 7/9/92
Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Irving Mauer,Ph.D., Geneticist
Section IV, Toxicology Branch I (H7509C)

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07-09-92

DATA EVALUATION RECORD

MRID No.: 416877-01
Tox Chemical No.: 342
PC No.: 057801

STUDY TYPE: 84-4. Sister Chromatid Exchange - Mouse Bone Marrow

TEST MATERIAL: Diazinon MG8

STUDY NUMBER(S): 12226-0-458 (Lab #), TX-90-0093 (Sponsor's #)

SPONSOR: Ciba Geigy

TESTING FACILITY: Hazleton Laboratories America, Inc.

TITLE OF REPORT: In vivo Sister Chromatid Exchange Assay

AUTHOR(S): Hemalatha Murli, PhD.

REPORT ISSUED: October 10, 1990

STUDY DATES: May 29 to July 16, 1990 (in life phase)

CONCLUSIONS: No evidence of increased sister chromatid exchanges in mouse bone marrow cells.

DOSE LEVELS TESTED: 0, 10, 50 and 100 mg/kg by gavage in peg 400. ICR strain mouse. Positive control: cyclophosphamide.

CLASSIFICATION: ACCEPTABLE for males, UNACCEPTABLE for females (dose levels tested too low).

QUALITY ASSURANCE STATEMENT: Provided

GOOD LABORATORY PRACTICE STATEMENT: Provided

Review

TEST MATERIAL: Diazinon MG8. Identification No.: FL-880045 ARS-10061, Batch Code 790701-M?5755 stated as being 88.0% purity. No analytical data were presented in the report, but data to confirm the test material concentration in the dosing solution were reportedly generated and in a separate report. Polyethylene Glycol 400 was used as the vehicle.

TEST SYSTEM: ICR strain mice, obtained from Harlan Sprague Dawley, Inc. Frederick, MD. The mice were reported to be 8 weeks old at start of dosing.

PRELIMINARY EXPERIMENTS: The first "dose limit test" indicated that mice (3/sex) dosed by gavage with 1000 and 1500 mg/kg died starting about five hours after dosing. Based on these data, dose levels of 80, 260, 440, 620 and 800 mg/kg (3/sex) were selected for the pilot study for the evaluation of toxicity and inhibition of cellular proliferation. These dose levels proved to be too toxic and a second study using 100, 233, 367 and 500 mg/kg was initiated (3 mice/sex). These mice were subcutaneously implemented with a 50 mg BrdURD pellet approximately one hour prior to dosing. The mice were scheduled for sacrifice 24 hours after dosing with the diazinon. Two hours prior to scheduled sacrifice, they were dosed with 2.0 mg/kg colchicine. Females tolerated the treatment better than males since none dosed with 100 and 233 mg/kg died. One 100 mg/kg and two 233 mg/kg males died. Significant cell cycle delays were evident in the males but not females dosed with 233 mg/kg/day.

On the basis of this study, the dose levels of 10, 50 and 100 mg/kg were selected for the definitive study.

DEFINITIVE STUDY:

A. Experimental

Five groups of male (wt range 28.9 to 38 gms) and females (wt range 21.3 to 26.4 gms) mice were dosed as either 0, 10, 50 or 100 mg/kg of diazinon technical (by gavage), the fifth group was dosed with 10 mg/kg cyclophosphamide (Sigma, intraperitoneally as a saline solution). Approximately one hour (0.87 to 1.47 hours) prior to dosing with diazinon, the mice were implanted with 50 mg BrdURD and were sacrificed 24 hours after dosing with diazinon. Prior to sacrifice, the mice were dosed with 2 mg/kg of colchicine.

Following sacrifice by CO_2 inhalation, the marrow from both tibia was flushed from the bone with Hank's balanced salt solution. The marrow was centrifuged and the "button" collected

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and resuspended in saline, fixed in methanol: acetic acid and refrigerated overnight. Before slide preparation the "button" was washed twice with fixative. The cells in suspension were dropped onto glass slides and dried. The slides were stained with fluorescence plus Giesma (method described). The slides were evaluated or scored by an individual unaware of the treatment group assignment. Twenty-five cells (when available) were analyzed for sister chromatid exchange and 100 cells per animal were analyzed for cell cycle kinetics.

B. Results.

1. Reactions to treatment. One male (dose with 50 mg/kg) was found dead after about 20 hours following treatment. Several males in the high (100 mg/kg) dose group were reported appearing languid with squinted eyes, with a slight colored crust around their eyes. No reactions were reported in females.

2. Cell cycle. Based on percent of cells in metaphase 1, 2 or 3 (M1, M2, M3) there was an indication that treatment of males with 100 mg/kg resulted in an induction of a prolongation of the cell cycle. No effect was evident in other groups either male or female or with the positive control.

3. Sister Chromatid Exchange. Table 3 (xeroxed from the study report) illustrates the results of this study. No effect of diazinon treatment was indicated in either sex. The positive control produced the expected positive result.

CONCLUSION: This study is ACCEPTABLE for males, UNACCEPTABLE for females. The dose levels used for females should have produced target cell reactions such as cell cycle delay if not overt toxicity and the study is thus considered to be tested at too low a dose level for females.

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TABLE 2
CELL CYCLE KINETICS DATA - SCE ASSAY

SPONSOR: Ciba-Geigy TEST ARTICLE: Diazinon MG8

ASSAY NO.: 12226

	FEMALES					MALES				
DOSE	ANIMAL #	ZM1	ZM2	ZM3	AGT*	ANIMAL #	ZM1	ZM2	ZM3	AGT*
VEHICLE CONTROL	8559	13	42	45	10.78	8560	27	69	4	14.12
	8568	8	62	30	11.26	8570	12	70	18	12.14
	8618	8	49	43	10.64	8584	23	53	24	12.44
	8619	18	56	26	12.02	8591	14	61	25	11.85
	8633	45	52	3	15.82	8624	48	41	11	15.34
GROUP MEAN					12.10					13.18
POSITIVE CONTROL	8565	35	45	20	13.51	8581	40	50	10	14.71
	8585	3	59	38	10.64	8582	19	49	32	11.74
	8587	10	48	42	10.78	8586	17	63	20	12.32
	8627	15	45	40	11.11	8614	20	50	30	11.90
	8632	47	44	9	15.43	8629	22	70	8	13.44
GROUP MEAN					12.29					12.82
TEST ARTICLE 10 mg/kg	8563	10	56	35	11.01	8561	20	72	8	13.30
	8571	6	51	43	10.55	8566	53	38	9	16.03
	8572	14	56	30	11.57	8597	24	48	28	12.25
	8604	7	49	44	10.55	8612	14	69	17	12.32
	8608	6	40	54	10.08	8626	47	48	5	15.82
GROUP MEAN					10.75					13.94
50 mg/kg	8575	40	60	0	15.63	8579	36	47	17	13.81
	8592	12	58	30	11.47	8583	4	72	24	11.36
	8605	3	50	47	10.25	8599	13	64	23	11.90
	8607	19	57	24	12.20	8610	28	50	22	12.89
	8630	12	47	41	10.92	8628	30	60	10	13.89
GROUP MEAN					12.09					12.77
100 mg/kg	8595	15	78	7	13.02	8558	100	0	0	25.00
	8602	5	64	31	11.06	8576	94	6	0	23.58
	8615	45	54	1	16.03	8590	34	57	9	14.29
	8616	18	59	23	12.20	8593	19	48	33	11.68
	8631	32	35	33	12.44	8623	70	20	10	17.86
GROUP MEAN					12.95					18.48**

Hours of Brdurd exposure = 25.0.

* Average Generation Time = $\frac{\text{Hours of Brdurd Exposure (25.0)}}{ZM_1*(0.01) + ZM_2*(0.02) + M_3*(0.03)}$

**Significantly greater than the vehicle control $p<0.05$.

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TABLE 3

SISTER CHROMATID EXCHANGE DATA

SPONSOR: Ciba Geigy TEST ARTICLE:Diazinon MG8

ASSAY NO.: 12226

	FEMALES				MALES			
DOSE	ANIMAL #	MEAN	±	S.E.	ANIMAL #	MEAN	±	S.E.
VEHICLE CONTROL	8559	3.44	±	0.45	8560	3.20	±	0.33
	8568	4.84	±	0.72	8570	4.08	±	0.36
	8618	6.64	±	0.70	8584	2.92	±	0.24
	8619	5.20	±	0.53	8591	3.28	±	0.38
	8633	3.40	±	0.45	8624	2.64	±	0.29
GROUP MEAN S.E.		4.70	±	0.60		3.22	±	0.24
POSITIVE CONTROL	8565	17.24	±	1.11	8581	7.64	±	0.89
	8585	12.20	±	1.62	8582	10.12	±	0.81
	8587	21.28	±	1.57	8586	12.08	±	0.84
	8627	12.68	±	1.00	8614	11.72	±	0.93
	8632	20.28	±	1.41	8629	20.20	±	1.27
GROUP MEAN S.E.		16.74*	±	1.88		12.35*	±	2.11
TEST ARTICLE 10 mg/kg	8563	4.84	±	0.36	8561	2.60	±	0.39
	8571	5.40	±	0.50	8566	3.28	±	0.49
	8572	4.20	±	0.41	8597	2.48	±	0.21
	8604	4.76	±	0.55	8612	4.16	±	0.42
	8608	5.80	±	0.85	8626	3.08	±	0.21
GROUP MEAN S.E.		5.00	±	0.28		3.12	±	0.30
50 mg/kg	8575	3.68	±	0.47	8579	4.04	±	0.31
	8592	4.88	±	0.51	8583	4.44	±	0.39
	8605	4.60	±	0.43	8599	3.28	±	0.23
	8607	3.68	±	0.52	8610	3.76	±	0.41
	8630	5.84	±	0.81	8628	2.92	±	0.25
GROUP MEAN S.E.		4.54	±	0.41		3.69	±	0.27
100 mg/kg	8595	4.00	±	0.50	8558	3.00	±	0.58**
	8602	6.08	±	0.94	8576	2.71	±	0.40**
	8615	6.36	±	0.90	8590	2.52	±	0.22
	8616	5.32	±	0.62	8593	4.08	±	0.31
	8631	2.44	±	0.34	8623	3.16	±	0.24
GROUP MEAN S.E.		4.84	±	0.73		3.25	±	0.45

25 cells, if available, were analyzed for SCE from each animal.

* Significantly greater than the vehicle control, $p<0.05$.

**Only 3 (#8558) and 14 (#8576) M2 cells were available for analysis and these data were not used for calculation of mean and S.E..

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84-2 Mutagenicity Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

General Requirements

1. ✓ Technical form of the active ingredient tested.
2. ✓ Negative, solvent and/or vehicle control(s) for the test system.
3. ✓ Positive control(s) for the test system.
4. ✓ Fully identified test system, species, strain, source etc.
5. ✓ Fully described method for maintaining test system.
6. ✓ Fully described method for preparing test environment and administering test compound.
7. ✓ Fully described metabolic activation system, if required.
8. ✓ Determination of maximum and range of concentrations/doses used under test conditions.
9.* ✓ Criteria for determination of a positive effect.

Test Specific Requirements

Salmonella reverse mutation assay

1. ___ Minimum of four strains, TA98, TA100, TA1535 and TA1536. (alternatives need rationale)
2. ___ Strain specific positive controls.
3. ___ Highest concentration limited by toxicity, solubility or 5000 ug/plate.
4.* ___ At least 5 different concentrations of test material at adequate intervals.
5.* ___ A single positive response confirmed by testing over a narrow range of concentrations.
6.* ___ At least three plates experimental point.

Gene mutation in somatic cells in culture

1. ___ Highest concentration limited by toxicity (10-20% relative survival), solubility or 5000 ug/ml.
2.* ___ At least 4 different concentrations of test material to yield a concentration related toxic effect.
3. ___ Determination of the number of cell cultures used.

In vitro mammalian cytogenetics

1. ___ Highest concentration limited by toxicity (e.g. reduced mitotic activity; alteration of cell cycle; cytotoxicity), solubility or 5000 ug/ml.
2.* ___ Multiple concentrtions used to define the response.
3.* ___ At least two independent cultures for each experimental point.
4. ___ Determination of culture harvest time.

In vivo mammalian cytogenetics - bone marrow

1. ✓ At least 5 male and 5 female animals per experimental group.
2. ✓ Highest dose limited by toxicity or 5000 mg/kg.
3. ___ Determination of sampling times.

Aberrations; a) one treatment - 3 times in range of 6-48 hours after treatment adequately spaced with central sample at 24 hour (may be altered based on cell cycle time). b) repeated treatments - samples taken 6 and 24 hours after last treatment (may be

Criteria marked with a * are supplemental and may not be required for every study.

1 Problem with females less sensitive than males.

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