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Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

MONSANTO COMPANY

MUTAGENIC STUDY WITH AROCLOR 1260 IN ALBINO MICE

JANUARY 13, 1972

IBT NO. E623

0365755.01

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

January 13, 1972

Mr. Elmer P. Wheeler
Manager, Environmental Health
Monsanto Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166

Dear Mr. Wheeler:

Re: IBT No. E623 - Mutagenic Study with
Aroclor 1260 in Albino Mice

We are submitting herewith our laboratory report dated January 13, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/kjl

0365755.02

REPORT TO

MONSANTO COMPANY

MUTAGENIC STUDY WITH
AROCLOR 1260
IN ALBINO MICE

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I. Introduction

A mutation is a change in the character of a gene such that morphologic, physiologic, and/or biochemical alterations are produced. If this change in gene character occurs in the germinal cell, the alteration can be transmitted to succeeding generations. Changes of this nature can be artificially induced (irradiation, chemical exposure, etc.) or they may be spontaneous. A dominant lethal mutation, occurring in the male germinal cell, may lead to the inability of the affected cell to fertilize an egg or, once having fertilized, to the failure of development beyond the blastocyst stage (implantation). Male mice, treated with the test compound, are mated with untreated females. The numbers of pre-implantation losses and early resorptions in female mice, dissected at mid-gestation, are used to calculate the mutation rate based on the induction of dominant lethal mutations.

II. Summary

A dominant lethal mutagenic study was conducted using albino mice. Males were treated with a single intraperitoneal injection of Aroclor 1260 at levels of either 500 (T-I) or 1,000 (T-II) mg/kg of body weight. Another group of males received methyl methanesulfonate (MMS) at 100 mg/kg and was used as a positive control.

Mating indices for Aroclor 1260 treated animals were unaffected. Two positive control males and 1 T-I male died during the course of the study.

Data for treated animals regarding implantation sites, resorption sites, and embryos did not differ from the control data. Mutation rates were not unusual for the Aroclor treated animals.

MMS treated animals showed a positive response for the first 2 weeks following treatment.

Therefore, treating male mice with Aroclor 1260 via an intraperitoneal injection of 500 or 1,000 mg/kg did not cause a dominant lethal response.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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January 13, 1972

III. Procedure

A. Outline of Experiment

The test material was Aroclor 1260. Methyl methanesulfonate was used as a positive control. Charles River strain albino mice were received at this laboratory at 60 to 70 days of age for use in the study. The organization of groups is presented in Table I.

TABLE I

TEST MATERIAL: Aroclor 1260

Mutagenic Study - Albino Mice

Organization of Groups

Group	Dose Level* (mg/kg)	Number of Males Treated
C	-	12
PC	100	12
T-I	500	12
T-II	1,000	12

* Aroclor 1260 was administered as a 10 percent solution in corn oil. Control males received the vehicle in amounts equivalent to those received by the T-II males. Positive control males received methyl methanesulfonate in a 1 percent corn oil solution.

B. Dosage Levels

The treatment levels were selected by intraperitoneal administration of single graded doses of the test material to male mice. The maximum tolerated dose was determined and dose levels employed in the main study were based on these findings. The test material was administered intraperitoneally in a corn oil solution to male mice. Control animals received the vehicle in volumes equivalent to those giver the high test group and positive control males received methyl methanesulfonate.

C. Mating Schedule

Each group consisted of 12 male mice, each of which was placed in a cage with 3 untreated virgin females immediately after dose administration. At the end of 1 week, the females were removed from the cage and replaced by another group of 3 females. This procedure continued for 6 consecutive weeks, a period of time required for maturation of the male mouse germ cells from the spermatocyte to the mature spermatozoon. Experiments at this laboratory have shown that the reference mutagenic compound* affects germinal cells in the meiotic and post-meiotic stages prior to or during the spermatid stage. Recovery from the effects of MMS is generally seen 3 weeks post-treatment. Following the sixth week of mating, all males were sacrificed.

D. Female Sacrifice

The females were sacrificed approximately 1 week after removal from the breeding cage, at which time most females that had

* The reference mutagen is methyl methanesulfonate (MMS).

mated were at mid-pregnancy. All animals were sacrificed by carbon dioxide asphyxiation. The numbers of implantation sites, resorption sites, and embryos were recorded. Females were judged to be pregnant if corpora lutea were present in the ovaries.

Resorption sites were divided into 2 groups, early deaths (deciduomata) and late deaths. Late deaths refer to embryos which develop to a relatively advanced stage prior to death, so that the placenta and fetal membranes or remnants thereof are visible. The frequency of late deaths is apparently unaffected by mutagens and, therefore, would appear to be non-genetic in this test system. Deciduomata occur at the sites of implanted blastocysts which fail to develop following implantation. The mutagenicity of the chemical can be measured by the proportions of all implantations which are deciduomata.

Mutagenicity can also be measured by comparing the mean number of viable embryos in the test group to the number obtained in the control group. Calculation of the mutation rate using this criterion assumes that pre-implantation losses among non-affected test animals will be essentially the same as losses observed among control animals.

IV. Results

A. Treatment Levels

Doses were administered to groups of 4 male mice each to determine the levels for the main study. Exposures of 30, 100, 300, or 1,000 mg of Aroclor 1260 per kg of body weight failed to elicit any abnormal reactions and no deaths were recorded during the 14-day observation period following treatment.

B. Mortality and Reactions

Two positive control males (weeks 1 and 3) and 1 T-I male (week 3) died during the study. No other deaths occurred.

C. Mating Performance

Mating indices, defined as the number of animals pregnant divided by the number of females mated times 100, and the number of surviving males for each post-treatment week are shown in Table II. All mating indices for the Aroclor 1260 treated animals seen in the experiment are considered to be normal for the mouse strain employed. Positive control animals had a low mating index for the first week post-treatment.

TABLE II

TEST MATERIAL: Aroclor 1260

Mutagenic Study - Albino Mice

Mating Performance

Group	Test Week Number	Number of Surviving Males	Number of Animals Pregnant / Number of Females Mated	Mating Index (Percent)
C	1	12	25/36	69.4
	2	12	28/36	77.8
	3	12	31/36	86.1
	4	12	29/36	80.6
	5	12	28/36	77.8
	6	12	29/36	80.6
PC	1	11	14/33	42.4
	2	11	19/33	57.6
	3	11	23/33	69.7
	4	10	26/30	86.7
	5	10	23/30	76.7
	6	10	24/30	80.0

TABLE II continued

TEST MATERIAL: Aroclor 1260

Mutagenic Study - Albino Mice

Mating Performance

Group	Test Week Number	Number of Surviving Males	Number of Animals Pregnant / Number of Females Mated	Mating Index (Percent)
T-I	1	12	31/36	86.1
	2	12	31/36	86.1
	3	12	26/36	72.2
	4	11	28/33	84.8
	5	11	29/33	87.9
	6	11	32/33	97.0
T-II	1	12	30/36	83.3
	2	12	30/36	83.3
	3	12	25/36	69.4
	4	12	31/36	86.1
	5	12	27/36	75.0
	6	12	28/36	77.8

D. Sacrifice Data

Data pertaining to the numbers of corpora lutea, implantation sites, resorption sites (early and late differentiated), and embryos are presented in Table III. Laboratory data have shown that the untreated female mouse has a mean of 13.5 corpora lutea. Therefore, the number of pregnant animals is multiplied by 13.5 to obtain the total number of corpora lutea.

The corpora lutea for females in control and T-I (at week 3) that did not have implantations are included in the results. These data show no significant differences between test and control animals with regard to the numbers of implantations, resorptions, and embryos. Effects were noted for the first 2 weeks following treatment of the positive control animals.

TABLE III

TEST MATERIAL: Aroclor 1260

Mutagenic Study - Albino Mice

Summary of Sacrifice Data

Group	Test Week Number	Pregnant Animals Examined	Calculated Corpora Lutea	Implantation Sites	Resorption Sites Early	Resorption Sites Late	Embryos
C	1	25	338	293 (11.7)	5 (0.2)	1 (0.1)	287 (11.5)
	2	28	378	355 (12.7)	9 (0.3)	0 (0.0)	346 (12.4)
	3	31*	417	342 (11.0)	14 (0.4)	5 (0.2)	323 (10.4)
	4	29	392	357 (12.3)	15 (0.5)	5 (0.2)	337 (11.6)
	5	28	378	341 (12.2)	13 (0.5)	3 (0.1)	325 (11.6)
	6	29	392	349 (12.0)	12 (0.4)	3 (0.1)	334 (11.5)
PC	1	14	189	134 (9.6)	41 (2.9)	2 (0.1)	91 (6.5)
	2	19	256	178 (9.4)	47 (2.5)	0 (0.0)	131 (6.9)
	3	23	310	294 (12.8)	13 (0.6)	3 (0.1)	278 (12.1)
	4	26	351	334 (12.8)	16 (0.6)	4 (0.2)	314 (12.1)
	5	23	310	291 (12.6)	14 (0.6)	5 (0.2)	272 (11.8)
	6	24	324	292 (12.2)	17 (0.7)	2 (0.1)	273 (11.4)

Note: Numbers in parentheses are the means per female.

* Includes 1 pseudo-pregnant female (corpora lutea only).

TABLE III continued

TEST MATERIAL: Aroclor 1260

Mutagenic Study - Albino Mice

Summary of Sacrifice Data

Group	Test Week Number	Pregnant Animals Examined	Calculated Corpora Lutea	Implantation Sites	Resorption Sites Early	Resorption Sites Late	Embryos
T-I	1	31	418	339 (10.9)	13 (0.4)	1 (0.1)	325 (10.5)
	2	31	418	384 (12.4)	7 (0.2)	2 (0.1)	375 (12.1)
	3	26*	349	314 (12.1)	7 (0.3)	2 (0.1)	305 (11.7)
	4	28	378	335 (12.0)	11 (0.4)	5 (0.2)	319 (11.4)
	5	29	392	381 (13.1)	14 (0.5)	1 (0.1)	366 (12.6)
	6	32	432	398 (12.4)	14 (0.4)	4 (0.1)	380 (11.9)
T-II	1	30	405	342 (11.4)	13 (0.4)	1 (0.1)	328 (10.9)
	2	30	405	371 (12.4)	15 (0.5)	2 (0.1)	354 (11.8)
	3	25	338	312 (12.5)	15 (0.6)	4 (0.2)	293 (11.7)
	4	31	418	376 (12.1)	16 (0.5)	3 (0.1)	357 (11.5)
	5	27	364	330 (12.2)	18 (0.7)	1 (0.1)	311 (11.5)
	6	28	378	349 (12.5)	13 (0.5)	1 (0.1)	335 (12.0)

Note: Numbers in parentheses are the means per female.

* Includes 1 pseudo-pregnant female (corpora lutea only).

E. Mutagenic Data

A summary of results for the mutagenic study is presented in Table IV.

Pre-Implantation Loss is defined as:

$$\frac{\text{Number of Corpora Lutea - Number of Implantation Sites}}{\text{Number of Corpora Lutea}} \times 100$$

The mutation rate may be calculated by comparing the number of early resorptions (deciduomata) to the total number of implantations for each group.

$$\frac{\text{Number of Early Resorption Sites}}{\text{Number of Implantation Sites}} \times 100 \text{ (A in table)}$$

Another way of expressing the mutation rate, which takes into account pre-implantation losses, is by comparing the mean number of normal embryos in each test group to the mean number of embryos in the control group.

$$100 - \left(\frac{\text{Embryos Test Group/Female}}{\text{Embryos Control Group/Female}} \times 100 \right) \quad \text{(B in table)}$$

Values presented were obtained by comparing each group to the contemporary control group (a) and to cumulative control data (b). Values with a minus (-) sign indicate that the mean number of embryos for that group and week was greater than that of controls. The deviation from zero should not exceed 25 percent in the positive direction.

The mutation rates and pre-implantation losses among treated animals compared favorably to those of controls.

The mutation rates for the positive control animals indicated a response for the first 2 weeks post-treatment.

TABLE IV

TEST MATERIAL: Aroclor 1260

Mutagenic Study - Albino Mice

Summary of Mutagenic Data

Group	Test Week Number	Pre-Implantation Loss (Percent)	Pre-Implantation Loss and Mutation Rates: Mutation Rates A	Mutation Rates B: a	Mutation Rates B: b
C	1	13.3	1.7	-	- 0.9
	2	6.1	2.5	-	-10.7
	3	78.0	4.1	-	10.4
	4	8.9	4.2	-	- 0.9
	5	9.8	3.8	-	0.9
	6	11.0	3.4	-	1.7
PC	1	29.1	30.6	43.5	43.0
	2	30.5	26.4	44.4	38.4
	3	5.2	4.4	-16.3	- 4.3
	4	4.8	4.8	- 4.3	- 5.2
	5	6.1	4.8	- 1.7	- 0.8
	6	9.9	5.8	0.9	2.6

TABLE IV continued

TEST MATERIAL: Aroclor 1260

Mutagenic Study - Albino Mice

Summary of Mutagenic Data

Group	Test Week Number	Pre-Implantation Loss and Mutation Rates: Pre-Implantation Loss (Percent)	A	Mutation Rates B: a	b
T-I	1	18.9	3.8	8.7	7.9
	2	8.1	1.8	2.4	-8.0
	3	10.0	2.2	-12.5	-0.9
	4	11.4	3.3	1.7	0.9
	5	2.8	3.7	- 8.6	-7.7
	6	7.9	3.5	- 3.5	-1.7
T-II	1	15.6	3.8	5.2	4.4
	2	8.4	4.0	4.8	-5.4
	3	7.7	4.8	-11.5	-0.9
	4	10.0	4.2	0.9	0.0
	5	9.3	5.4	0.9	1.7
	6	7.7	3.7	- 4.3	-2.5

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