Dominant Lethal Mutagenic Study with Three Polychlorinated Biphenyls in Albino Mice

G. L. Kennedy[1], M. L. Keplinger, Otis E. Fancher,

E. P. Wheeler[2] and J. C. Calandra

Industrial BIO-TEST Laboratories, Inc.

Northbrook, Illinois 60062

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[1] To whom proofs are to be sent

[2] Monsanto Company, St. Louis, Missouri

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INDEX TERMS

Aroclors

Aroclor 1242

Aroclor 1254

Aroclor 1260

Methyl methanesulfonate

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ABSTRACT

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Males were mated with untreated females 1, 2, 3, 4, 5, and 6 weeks following treatment. There was evidence of a dominant lethal effect among MMS-treated animals for matings after 1 and 2 weeks, but no evidence for dominant lethals following treatment with the Aroclors.

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A study was conducted to determine the mutagenic activity of 3 polychlorinated biphenyls (Aroclors 1242, 1254 and 1260) in albino mice. Treatment of male mice consisted of single intraperitoneal injections of test materials at levels of 500 and 100 mg/kg, administered as solutions in corn oil. Positive control males received 100 mg/kg of methyl methanesulfonate (MMS) in corn oil and control animals were treated with corn oil alone.

Males were mated with untreated females 1, 2, 3, 4, 5, and 6 weeks following treatment. There was evidence of a dominant lethal effect among MMS-treated animals for matings after 1 and 2 weeks, but no evidence for dominant lethals following treatment with the Aroclors.

The most readily detectable mutation in the mouse is the dominant lethal (Bateman, 1966), probably due to duplication of one chromosome segment and deficiency of another (Bateman, 1958), which leads to death in

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utero arising from affected gametes.

Dominant lethal mutations in sperm do not affect their capacity for fertilization but lead to death of the fertilized egg either before implantation (pre-implantation loss) or soon thereafter in the blastocyst stage with formation of a deciduoma, consisting entirely of maternal tissue stimulated into growth by an egg which shortly dies (Bateman, 1960). Deaths which occur at a later stage, after formation of placenta and foetal membranes, are generally considered to be unrelated to mutagenic activity (Bateman, 1958a).

When the mutation rate is high, because of an excessively high dose of mutagen, pre-implantation losses may be so high as to simulate failure of fertilization. Since the most sensitive test for mutagenicity is the proportion of all implants which are deciduomata, dose levels which permit a high proportion of implantations should be employed.

Mutagenicity can also be measured by comparing the mean number of viable embryos in a test group to that of the control group. Calculation of a mutation rate using this criterion assumes that pre-implantation losses among non-affected test animals would be analogous to those of control animals.

In the present study corpora lutea were not counted but were calculated assuming a mean of 13.5 per animal for untreated female mice (Kennedy, 1969). Epstein et al. (1970) have also omitted counting corpora lutea, achieving an indirect estimate of pre-implantation losses by comparing the numbers of total implants of test animals to those of controls. This method

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would be unreliable for a material which affects fertilization rate (Partington and Bateman, 1964), but has the advantage of including both pre-implantation losses and late deaths in calculation of the mutagenic rate.

The duration of the testicular phase of spermatogenesis in mice is 34.5 ± 1 day (Oakberg, 1956) and an additional 7.5 days are required for passage of the spermatozoa through the epididymis and Vas deferens (Sirlin and Edwards, 1957). With methyl methanesulfonate, used as the positive control material in the present studies, the maximal incidence of dominant lethal mutations has been shown to occur in mice in the second half of the first and the first half of the second week following intraperitoneal injection (Partington and Bateman, 1964). With high doses of MMS, the period of sensitivity may be somewhat extended in both directions. With most mutagens, dominant lethals are most readily induced in post-meiotic stages, end of pachytene or the beginning of dipotene (Moutschen, 1969), which are sampled during the first 3 weeks after treatment.

METHODS

The Charles River strain albino mice (Charles River Breeding Laboratories, North Wilmington, Mass.) were received at the age of 60-70 days. The male animals were divided into 8 groups of 12 animals each, a control group, a positive control group and 2 treatment groups for each of the 3 Aroclors.

A high dose level of 1000 mg/kg was chosen for each of the Aroclors when it was determined in pilot studies that this level was well tolerated.

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A lower dose level of 500 mg/kg was employed and the positive control animals were treated with 100 mg/kg of methyl methanesulfonate (MMS). The Aroclors were administered as 10 percent solutions in corn oil. The MMS was given as a 1 percent solution in corn oil and control males received corn oil alone in volumes equivalent to those administered to the high treatment level animals.

Each male, immediately after a single intraperitoneal treatment with the appropriate material, was placed in a cage with 3 untreated virgin females. At the end of 1 week, the females were removed from the cage and were 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 spermatocytes to mature spermatozoa.

The females were sacrificed by carbon dioxide asphyxiation approximately 1 week after removal from the breeding cage, at which time most females that had mated were at mid-pregnancy. 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 categorized as early deaths (deciduomata) and late deaths. Late deaths refer to embryos which develop to a relatively advanced stage prior to death as evidenced by the presence of placental and fetal membranes or remnants thereof.

The numbers of corpora lutea were calculated and pre-implantation losses were estimated using 2 methods.

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Mutation rates were calculated based on the proportion of implantations which were deciduomata and upon the mean number of viable embryos per test animal as compared to controls.

RESULTS

A. Mortality and Reactions

Mortality data for treated males are summarized in Table 1. No unusual reactions were noted and no female mice died during the study.

B. Mating Performance

Mating indices are shown in Table 2. The mating index for MMS treated animals was low for the first week post-treatment and those for animals treated with Aroclor 1254 at a dose level of 1000 mg/kg were low for weeks 4, 5, and 6. All other mating indices were considered to be normal.

C. Sacrifice Data

Data pertaining to the numbers of corpora lutea, implantation sites, resorption sites (early and late differentiated) and embryos are presented in Table 3. Animals treated with MMS showed a marked effect (increase in early resorptions and decrease in viable embryos at weeks 1 and 2). No significant effects on implantations, resorptions, and numbers of viable embryos resulted from treatment of male mice with any of the Aroclors at either dosage level.

D. Mutagenic Data

Pre-implantation Loss (in percent) is defined as:

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A. $\frac{\text{No. of Corpora Lutea - No. of Implantation Sites}}{\text{No. of Corpora Lutea}} \times 100$

or, as suggested by Epstein et al. (1970), as:

B. $\frac{\text{No. of Implantation Sites(control) - No. of Implantation Sites (test)}}{\text{No. of Implantation Sites (control)}} \times 100$

Method B indicates pre-implantation losses in excess of those of the simultaneous controls.

The pre-implantation losses calculated in accordance with the two procedures are shown in Table 4. It is evident from these data that MMS increases pre-implantation losses during weeks 1 and 2 following treatment. There is no clear, dose related pattern of effect for the Aroclors. The sporadic high values for pre-implantation losses calculated using Method A for Aroclors 1254 and 1260 are not confined by application of Method B. It is concluded, therefore, that the Aroclors do not adversely affect implantation.

Mutation rates were calculated by comparing the numbers of early resorptions (deciduomata) to the total number of implantations using the formula,

$$\text{Mutation Rate} = \frac{\text{No. of Early Resorptions/Female}}{\text{No. of Implantation Sites/Female}} \times 100$$

and by comparing the mean number of viable embryos in each test group to that of the control group using the formula,

$$\text{Mutation Rate} = 100 - \frac{\text{Viable Embryos/Test Female}}{\text{Viable Embryos/Control Female}} \times 100$$

The latter method includes consideration of pre-implantation losses and

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late deaths, and both contempory and cumulative control data (Kennedy, 1969) were employed in the calculations. Data regarding mutation rates are summarized in Table 5.

These data indicate that MMS exerts a dominant lethal effect during weeks 1 and 2 following treatment. There is no evidence that Aroclors 1242, 1254 and 1260 initiate dominant lethal mutations when administered intraperitoneally to male albino mice at dose levels of 500 and 100 mg/kg.

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TABLE I

Mortality Data for Treated Albino Mice

Test Material	Dose Level mg/kg	Incidence of Deaths	Time of Death (post-treatment)
Corn Oil	a	0	—
MMS[b]	100	1	Day 1
	100	1	During week 3
Aroclor 1242[c]	500	1	Day 1,
	1000	2	Day 1
Aroclor 1254[c]	1000	3	Day 2
Aroclor 1260[c]	500	1	During week 3

a Approximately 0.9 ml/kg
b As a 1% (W/V) solution in corn oil
c As a 10% (W/V) solution in corn oil

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TABLE 2

Mating Indices of Albino Mice Mated with Treated Males

Test Material	Dosage Level (mg/kg)	Mating Index[a] for Week No.					
		1	2	3	4	5	6
Corn Oil (control)	b	69.4	77.8	86.1	80.6	77.8	80.6
MMS[c]	100	42.4	57.6	69.7	86.7	76.7	80.0
Aroclor 1242[d]	500	66.7	81.8	90.9	84.8	100	81.8
	1000	63.3	86.7	90.0	76.7	73.3	83.3
Aroclor 1254[d]	500	72.2	66.7	66.7	77.8	86.1	83.3
	1000	66.7	59.2	63.0	48.1	51.8	44.4
Aroclor 1260[d]	500	86.1	86.1	72.2	84.8	87.9	97.0
	1000	83.3	83.3	69.4	86.1	75.0	77.8

a Mating Index = $\frac{\text{No. of animals pregnant}}{\text{No. of females mated}} \times 100$

b Approximately 0.9 ml/kg

c As a 1% (W/V) solution in corn oil

d As a 10% (W/V) solution in corn oil

Mis could be rounded off to nearest whole no.

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TABLE 3

Sacrifice Data for Female Albino Mice Mated with Males Treated with Aroclors 1242, 1254, and 1260

Treatment	Week No.	Pregnant Animals Examined	Calc'd Corpora Lutea	Implantation Sites	Resorption Sites		Viable Embryos
					Early	Late	
Corn Oil	1	25	338	293(11.7)	5(0.2)	1	287(11.5)
(Control)	2	28	378	355(12.7)	9(0.3)	0	346(12.4)
	3	31[a]	418	342(11.0)	14(0.4)	5	323(10.4)
	4	29	392	357(12.3)	15(0.5)	5	337(11.6)
	5	28	378	341(12.2)	13(0.5)	3	324(11.6)
	6	29	392	349(12.0)	12(0.4)	3	334(11.5)
Methyl	1	14	189	134(9.6)	41(2.9)	2	91(6.5)
Methanesulfonate	2	19	256	178(9.4)	47(2.5)	0	131(6.9)
100 mg/kg	3	23	310	294(12.8)	13(0.6)	3	278(12.1)
	4	26	351	334(12.8)	16(0.6)	4	314(12.1)
	5	23	310	291(12.6)	14(0.6)	5	272(11.8)
	6	24	324	292(12.2)	17(0.7)	2	273(11.4)
Aroclor 1242	1	22	297	268(12.2)	4(0.2)	0	264(12.0)
500 mg/kg	2	27	364	334(12.4)	7(0.2)	1	326(12.1)
	3	30[a]	405	375(12.5)	12(0.4)	1	362(12.1)
	4	28	378	340(12.1)	12(0.4)	3	325(11.6)
	5	33	446	424(12.8)	17(0.5)	3	404(12.2)
	6	27	364	337(12.5)	10(0.4)	5	322(11.9)
Aroclor 1242	1	19	256	235(12.4)	5(0.3)	1	229(12.0)
1000 mg/kg	2	26	351	324(12.5)	5(0.2)	1	318(12.2)
	3	27[a]	365	343(12.7)	8(0.3)	2	333(12.3)
	4	23	310	292(12.7)	8(0.3)	2	282(12.3)
	5	22	297	268(12.2)	5(0.2)	0	263(12.0)
	6	25	338	308(12.3)	14(0.6)	2	292(11.7)

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TABLE 3 continued

Sacrifice Data for Female Albino Mice Mated with Males Treated with Aroclors 1242, 1254, and 1260

Treatment	Week No.	Pregnant Animals Examined	Calc'd Corpora Lutea	Implantation Sites	Resorption Sites		Viable Embryos
					Early	Late	
Aroclor 1254	1	26	351	279(10.7)	3(0.1)	3	273(10.5)
500 mg/kg	2	24	324	281(11.7)	5(0.2)	1	275(11.4)
	3	24	324	315(13.1)	11(0.4)	0	304(12.7)
	4	28	378	345(12.3)	19(0.7)	2	324(11.6)
	5	31	418	403(13.0)	18(0.6)	4	381(12.3)
	6	30	405	354(11.8)	17(0.6)	4	333(11.1)
Aroclor 1254	1	18	243	214(11.9)	9(0.5)	2	203(11.3)
1000 mg/kg	2	16	216	197(12.3	3(0.2)	3	191(11.9)
	3	17	230	220(12.9)	3(0.2)	0	217(12.8)
	4	13	176	150(11.5)	5(0.4)	1	144(11.1)
	5	14	189	185(13.2)	9(0.6)	1	175(12.5)
	6	12	162	138(11.5)	4(0.3)	2	132(11.0)
Aroclor 1260	1	31	418	339(10.9)	13(0.4)	1	325(10.5)
500 mg/kg	2	31	418	384(12.4)	7(0.2)	2	374(12.1)
	3	26a	351	314(12.1)	7(0.2)	2	305(11.7)
	4	28	378	335(12.0)	11(0.4)	5	319(11.4)
	5	29	392	381(13.1)	14(0.5)	1	366(12.6)
	6	32	432	398(12.4)	14(0.4)	4	380(11.9)
Aroclor1260	1	30	405	342(11.4)	13(0.4)	1	328(10.9)
1000 mg/kg	2	30	405	371(12.4)	15(0.5)	2	354(11.8)
	3	25	338	312(12.5)	15(0.6)	4	293(11.7)
	4	31	418	376(12.1)	16(0,5)	3	357(11.5)
	5	27	364	330(12.2)	18(0.7)	1	311(11.5)
	6	28	378	349(12.5)	13(0.5)	1	335(12.0)

Note: Numbers in parentheses are means per female

a Includes one pseudo-pregnant female (corpora lutea only)

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TABLE 4

Pre-Implantation Losses among Female Albino Mice Mated with Males

Treated with Aroclors 1242, 1254 and 1260

Treatment	Week No.	Pre-Implantation Losses Method A	Method B
Corn Oil (Control)	1	13.3	—
	2	6.1	—
	3	18.0	—
	4	8.9	—
	5	9.8	—
	6	11.0	—
Methyl methanesulfonate 100 mg/kg (positive control)	1	29.1	18.0
	2	30.5	26.0
	3	5.2	-16.4
	4	4.8	- 4.1
	5	6.1	- 3.3
	6	9.9	- 1.7
Aroclor 1242	1	9.8	- 4.3
	2	8.2	2.4
	3	8.1	-13.6
	4	10.0	1.6
	5	4.9	- 4.9
	6	7.4	- 4.2
Aroclor 1242 1000 mg/kg	1	8.2	2.6
	2	7.7	1.6
	3	7.3	-15.5
	4	5.8	- 3.3
	5	9.8	0
	6	8.9	- 2.5

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TABLE 4 continued

Pre-Implantation Losses among Female Albino Mice Mated with Males

Treated with Aroclors 1242, 1254 and 1260

Treatment	Week No.	Pre-Implantation Losses	
		Method A	Method B
Aroclor 1254	1	20.5	9.3
500 mg/kg	2	13.3	7.9
	3	2.8	-19.1
	4	8.7	0
	5	3.6	- 6.6
	6	12.6	1.7
Aroclor 1254	1	11.9	- 1.7
1000 mg/kg	2	8.8	3.1
	3	4.3	1.8
	4	14.8	6.5
	5	2.1	- 1.6
	6	14.8	2.5
Aroclor 1260	1	18.9	6.8
500 mg/kg	2	8.1	2.4
	3	10.0	-10.0
	4	11.4	2.4
	5	2.8	- 7.4
	6	7.9	- 3.3
Aroclor 1260	1	15.6	2.6
1000 mg/kg	2	8.4	2.4
	3	7.7	-13.6
	4	10.0	1.6
	5	9.3	0
	6	7.7	- 4.2

[a] Pre-implantation Loss = $\frac{\text{No. of Corpora Lutea - No. of Implantation Sites}}{\text{No. of Corpora Lutea}} \times 100$

[b] Pre-implantation Loss = $\frac{\text{No. of Implantation Sites (control) - No. of Implantation Sites (test)}}{\text{No. of Implantation Sites (control)}} \times 100$

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TABLE 5

Mutation Rates Following Treatment of Male Albino Mice with

Aroclors 1242, 1254 and 1260

Treatment	Week No.	Mutation Rates		
		Method A[a]	Method B[b]	
			1[c]	2[d]
Corn Oil (control)	1	1.7	—	- 0.9
	2	2.5	—	-10.7
	3	4.1	—	10.4
	4	4.2	—	- 0.9
	5	3.8	—	0.9
	6	3.4	—	1.7
Methyl methanesulfonate 100 mg/kg (positive control)	1	30.6	43.5	43.0
	2	26.4	44.4	38.4
	3	4.4	-16.3	- 4.3
	4	4.8	- 4.3	- 5.2
	5	4.8	- 1.7	- 0.8
	6	5.8	0.9	2.6
Aroclor 1242 500 mg/kg	1	1.5	- 4.3	- 5.3
	2	2.1	2.4	- 8.0
	3	3.2	-16.3	- 4.3
	4	3.5	0.0	- 0.9
	5	4.0	- 5 2	- 4.2
	6	3.0	- 3.5	- 1.7
Aroclor 1242 1000 mg/kg	1	2.1	- 4.3	- 5.3
	2	1.5	1.6	- 8.9
	3	2.3	-18.3	- 6.0
	4	2.7	- 6.0	- 7.0
	5	1.9	- 3.4	- 2.6
	6	4.5	- 1.7	0.0

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TABLE 5 continued

Mutation Rates Following Treatment of Male Albino Mice with Aroclors 1242, 1254 and 1260

Treatment	Week No.	Mutation Rates		
		Method A[a]	Method B[b]	
			1[c]	2[d]
Aroclor 1254 500 mg/kg	1	1.1	8.7	7.9
	2	1.8	8.1	-1.8
	3	3.5	-22.1	-9.5
	4	5.5	0.0	-0.9
	5	4.5	- 6.0	-5.1
	6	4.8	3.5	5.1
Aroclor 1254 1000 mg/kg	1	3.7	1.7	0.9
	2	1.5	4.0	-6.2
	3	1.4	-23.1	-10.3
	4	3.3	4.3	3.5
	5	4.9	- 7.8	-6.8
	6	2.9	4.4	6.0
Aroclor 1260 500 mg/kg	1	3.8	8.7	7.9
	2	1.8	2.4	- 8.0
	3	2.2	-12.5	- 0.9
	4	3.3	1.7	0.9
	5	3.7	- 8.6	-7.7
	6	3.5	-3.5	-1.7
Aroclor 1260 1000 mg/kg	1	3.8	5.2	4.4
	2	4.0	4.8	-5.4
	3	4.8	-11.5	-0.9
	4	4.2	0.9	0.0
	5	5.4	0.9	1.7
	6	3.7	-4.3	-2.5

a Mutation Rate = $\frac{\text{No. of Early Resorptions/Female}}{\text{No. of Implantation Sites/Female}}$ X 100

b Mutation Rate = 100 - $\frac{\text{Viable Embryos/Test Female}}{\text{Viable Embryos/Control Female}}$ X 100

c Using contemporary controls

d Using cumulative controls

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FOOTNOTES

1 Aroclors 1242, 1254 and 1260, Monsanto Company, St. Louis, Missouri

2 Monsanto Company, St. Louis, Missouri

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