REPORT TO

MONSANTO COMPANY

THREE-GENERATION REPRODUCTION STUDY WITH AROCLOR 1254 IN ALBINO RATS

NOVEMBER 1, 1971

IBT NO. B7297

I. Introduction

At the request of Monsanto Company, a reproduction study was initiated in order to ascertain potential toxicological effects of subacute oral administration of Aroclor 1254, Lot No. AK-38, upon the fertility and offspring of three consecutive generations of albino rats.

This report presents the data collected from the initiation of the investigation to the weaning of the second (F3b) litters obtained from the third (F2) parental generation.

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II. Summary

A three-generation reproduction study employing albino rats fed diets containing 1, 10 or 100 ppm Aroclor 1254 was conducted. The following results were obtained during the investigation.

A. Parental Animals

1. Body Weights

Body weights and body weight gains revealed no differences between test and control animals which could be attributed to the ingestion of Aroclor 1254.

2. Mortality and Reactions

Several animals succumbed during each of the three generations. None of these deaths could be attributed to the ingestion of Aroclor 1254.

3. Pathologic Studies

Gross pathologic examination revealed no differences between test and control rats.

The liver weights and liver to body weight or brain weight ratios were significantly elevated in males fed 100 ppm Aroclor 1254. The liver weights for females in the same group (T-III) were not elevated; however, liver to brain weight ratios were significantly higher than control values. Statistical evaluations of other organ weights, organ to body weight and organ to brain weight ratios disclosed several randomly occurring intergroup differences. The lack of any consistent dose or

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generation related response and the absence of any deleterious histopathologic change confirm that these differences were not related to the ingestion of Aroclor 1254.

4. Reproductive Performance

Reproductive indices of rats fed 1 or 10 ppm did not differ from those of the controls during any of the three generations. During the second generation (F2a and F2b litters), rats fed 100 ppm had lowered mating indices and a reduced incidence of pregnancy. The other indices were normal.

B. Progeny

1. Population Data

The number of live pups born per litter was not affected at any level of Aroclor 1254 tested. The number of pups weaned per litter was reduced in all three generations in the group fed 100 ppm (T-III). The average number weaned per litter was normal for the groups fed 1 or 10 ppm. No external abnormalities were present that could be attributed to treatment. No untoward behavioral reactions were observed among test or control progeny.

2. Survival

Pup survival was lower in litters from dams fed 100 ppm Aroclor 1254 than for any other group. Pup survival was not reduced when 1 or 10 ppm was fed.

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3. Body Weights

Body weights for all progeny were normal at weaning.

4. Pathologic Studies

At autopsy of the F3b weanlings no gross or microscopic tissue changes were present which could be associated with the ingestion of Aroclor 1254.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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III. Procedure

A. Experimental Animals

The animals employed were weanling Charles River albino rats*. A total of 96 weanling rats (32 males and 64 females) was selected to form three test groups and a control group of Fo generation animals. Parental animals were allowed to reach maturity, mate and produce two litters. Eight males and 16 females from the second litters of each dietary group (F1b and F2b) were selected at weaning as parental animals for the succeeding generations. The study terminated following the weaning of the F3b litters.

B. Organization of Groups

A structural outline of the experiment is given in Table I. A flow chart depicting the three generations is shown in Figure I.

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

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TABLE I

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Outline of Experiment

Fo Generation

Group	Dietary Level (ppm)	Number of Animals Male	Female
C	None	8	16
T-I	1	8	16
T-II	10	8	16
T-III	100	8	16

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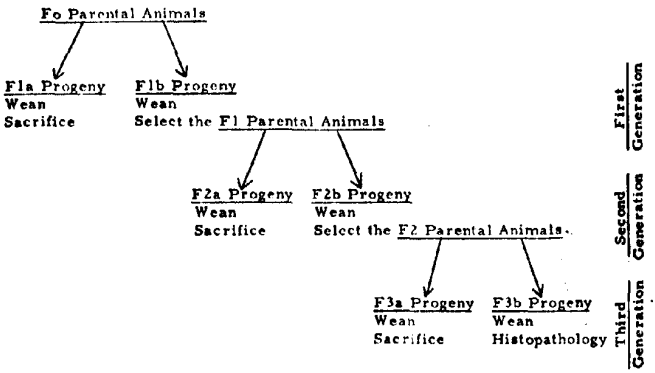
FIGURE 1

TEST MATERIAL: Aroclor 1254

Design of Experiment



Gross and histopathologic examinations were conducted upon parental animals of all three generations following the weaning of the second (b) litters.

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C. Diets and Feeding

Diets were prepared in the central diet room of this laboratory. The diet for any given test group was prepared by incorporating a calculated weight of Aroclor 1254 into a standard pulverized stock ration*, so that the final concentrations of test material in the diets were 1, 10 and 100 parts per million respectively. The animals in the control group were fed the basic ration without test material. Animals in all groups were maintained on their respective diets without interruption until their sacrifice, which followed the weaning of the second litters. Feeding was conducted on an ad libitum basis; fresh diets were prepared weekly.

D. Body Weights and Weight Gains

Initially, the body weight of each rat was determined and recorded at 21 days of age. All animals were then weighed weekly until mating trials commenced. No further body weight data were obtained until the sacrifice of each parental generation, at which time final body weights were recorded.

E. Mortality, Reactions and General Observations

Observations among animals of each generation for mortality and abnormal behavioral reactions were made daily. In addition, these animals were observed for fertility, length of gestation and lactation performance.

* Special Mix Mouse Chow, Ralston Purina Company, St. Louis, Mo.

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All pups were examined for physical abnormalities at birth and the numbers of viable and stillborn members of each litter were recorded. Records of survival at designated intervals during the lactation period were maintained and a final examination for physical abnormalities was made at the weaning of each litter. Litters of more than ten pups were reduced to that number by random sacrifice on the fifth day of the lactation period.

F. Mating Procedure

Mating trials were initiated when the parental animals were 100 days old. Females were caged in pairs and mated with a male from within the same treatment group. Daily examinations were conducted to determine if copulation had occurred. Males were rotated within their dietary group at ten day intervals until conception was confirmed or until each female had been paired with a maximum of three males.

The first litters (F1a, F2a, and F3a) obtained were weaned at 21 days post-partum. The parental females were then given a ten-day rest period and again mated, the above procedure being repeated in order to obtain second litters (F1b, F2b, and F3b). Females failing to become pregnant during the first mating trials were not rebred.

Eight males and 16 females from the second litters of each group were selected at weaning on the basis of health and body weight as parental animals for each succeeding generation.

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G. Gross and Microscopic Pathologic Studies

Whenever a parental animal succumbed during the test period, a gross autopsy was performed. In those instances when postmortem changes were not advanced, representative tissues were taken for microscopic study.

After the second litters were weaned, all surviving males and eight females from each group were sacrificed and subjected to gross pathologic examination. The weights of the liver, kidneys, spleen, gonads, heart, brain, adrenal glands and thyroid gland were recorded, along with the final body weight of each rat.

Statistical analyses were conducted upon the absolute organ weights of the sacrificed animals and upon the corresponding organ to body weight and organ to brain weight ratios. An Analysis of Variance was conducted first and significant effects disclosed by that treatment were further studied by "t"-tests.

Microscopic pathologic studies were conducted upon five males and five females from the control and T-III groups.

The following tissues and organs were examined:

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Tissues and Organs Examined Grossly	Tissues and Organs Examined Microscopically
Skin	Heart (right and left ventricles)
Heart	Trachea
Trachea	Lung
Lungs	Liver
Liver	Pancreas
Pancreas	Stomach (cardiac, fundic and pyloric regions)
Esophagus	Small Intestine (duodenum, jejunum and ileum)
Stomach	Caecum
Intestinal Tract	Colon
Spleen	Spleen
Lymph Nodes	Lymph Node (cervical and mesenteric)
Kidneys	Kidney
Urinary Bladder	Urinary Bladder
Testes	Testis
Ovaries	Ovary
Prostate	Prostate
Seminal Vesicles	Uterus
Uterus	Pituitary Gland
Vagina	Adrenal Gland
Pituitary Gland	Salivary Gland (submaxillary)
Adrenal Glands	Thyroid Gland
Salivary Glands	Parathyroid Gland
Thyroid Gland	Skeletal Muscle
Skeletal Muscle	Bone Marrow (sternum and femur)
Bone	Peripheral Nerve (sciatic)
Peripheral Nerve	Brain (cerebrum, cerebellum and pons)
Eyes	Seminal Vesicles
Brain	Esophagus
Thymus	Spinal Cord
Optic Nerve	
Aorta	

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IV. Results

A. Parental Animals

1. Body Weights

Body weight data are presented in Tables II and III. Included in the data on these tables are total weight gains over the first 77 days of the feeding period.

There were no differences between test and control animals which could be related to the ingestion of Aroclor 1254.

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TABLE II

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Body Weight and Weight Gain Data

Parental Male Rats

Group	Generation	Mean Body Weight (grams) Day Number: 0	7	14	21	28	35	42	49	56	63	70	77	Total Weight Gain (grams)	Final Body Weight at Sacrifice
C	Fo	-	95	142	202	253	309	354	393	426	456	480	492	397	709
	F1	47	77	112	152	192	234	271	316	349	382	416	438	391	636
	F2	49	77	108	140	164	200	234	265	295	319	345	354	305	592
T-I	Fo	-	95	136	192	240	293	354	375	404	432	453	468	373	681
(1 ppm)	F1	47	79	122	164	203	247	288	329	362	389	405	422	375	642
	F2	55	97	135	184	232	279	317	348	375	400	425	445	390	679
T-II	Fo	-	95	135	184	230	280	339	361	391	417	446	460	365	661
(10 ppm)	F1	47	78	124	171	202	240	278	314	347	373	396	412	365	650
	F2	56	88	118	157	193	206	264	297	325	350	373	389	333	579
T-III	Fo	-	95	135	186	231	285	353	376	417	449	479	496	401	678
(100 ppm)	F1	42	73	111	160	201	242	283	306	343	374	397	421	376	640
	F2	47	77	118	157	167	228	278	300	313	339	368	386	339	569

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TABLE III

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Body Weight and Weight Gain Data

Parental Female Rats

Group	Generation	Mean Body Weight (grams) Day Number: 0	7	14	21	28	35	42	49	56	63	70	77	Total Weight Gain (grams)	Final Body Weight at Sacrifice
C	Fo	-	95	142	202	253	309	354	393	426	456	480	492	397	709
	F1	47	77	112	152	192	234	271	316	349	382	416	438	391	636
	F2	49	77	108	140	164	200	234	265	295	319	345	354	305	592
-I	Fo	-	95	136	192	240	293	354	375	404	432	453	468	373	681
ppm)	F1	47	79	122	164	203	247	288	329	362	389	405	422	375	642
	F2	55	97	135	184	232	279	317	348	375	400	425	445	390	679
-II	Fo	-	95	135	184	230	280	339	361	391	417	446	460	365	661
0 ppm)	F1	47	78	124	171	202	240	278	314	347	373	396	412	365	650
	F2	56	88	118	157	193	206	264	297	325	350	373	389	333	579
-III	Fo	-	95	135	186	231	285	353	376	417	449	479	496	401	678
00 ppm)	F1	42	73	111	160	201	242	283	306	343	374	397	421	379	640
	F2	47	77	118	157	167	228	278	300	313	339	368	386	339	569

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2. Mortality

Mortality data are presented in Tables IV and V.

Gross autopsy performed on animals which succumbed during the test period failed to reveal any relationship between the ingestion of Aroclor 1254 and the death of the animal.

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TABLE IV

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mortality Data

Parental Generation Males

Group	Generation	First Mating			Second Mating		
		Pre-M	M	Post-M	M	Post-M	Total
C	Fo	0	0	0	0	0	0
	F1	0	0	0	0	0	0
	F2	0	0	0	0	1	1
T-I	Fo	0	0	0	0	0	0
(1 ppm)	F1	0	0	0	0	0	0
	F2	0	0	0	0	0	0
T-II	Fo	0	0	0	0	0	0
(10 ppm)	F1	0	0	0	0	0	0
	F2	0	0	0	0	0	0
T-III	Fo	0	0	0	0	0	0
(100 ppm)	F1	0	0	0	0	0	0
	F2	0	1	0	0	0	1

Pre-M = Pre-Mating Period
M = Mating Period
Post-M = Post-Mating Period

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TABLE V

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mortality Data

Parental Generation Females

Group	Generation	First Mating Pre-M	M	G	P	L	R	Second Mating M	G	P	L	Post-W	Total
C	Fo	0	0	0	1	0	0	0	0	0	0	0	1
	F1	0	0	0	0	0	0	0	0	0	0	0	0
	F2	0	0	0	0	0	1	0	0	0	0	0	1
T-I	Fo	0	0	0	0	0	0	0	0	0	0	1	1
(1 ppm)	F1	0	0	0	0	0	0	0	0	0	0	0	0
	F2	0	0	0	0	0	0	0	0	0	0	0	0
T-II	Fo	0	0	0	0	0	0	0	1	0	0	0	1
(10 ppm)	F1	0	0	0	0	0	0	0	0	0	1	1	2
	F2	0	0	0	0	0	0	0	0	1	1	0	2
T-III	Fo	0	0	0	0	0	0	0	0	0	0	0	0
(100 ppm)	F1	0	0	0	0	0	0	0	0	0	0	0	0
	F2	0	0	0	1	0	0	0	0	0	0	0	1

Pre-M = Pre-Mating Period
M = Mating Period
G = Gestation Period
P = Parturition
L = Lactation Period
R = Resting Period
Post-W = Post-Weaning Period

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3. Reactions

No untoward reactions were noted among the parental animals during the investigation.

4. Pathologic Findings

a. Gross Pathologic Findings

There were no gross pathologic findings which could be correlated with ingestion of the test material. Findings among test animals were comparable to those noted among control animals.

b. Organ Weights and Ratios

Mean organ weights, organ to body weight ratios and organ to brain weight ratios are assembled in Tables VI through XIII.

Liver weights and liver to body or brain weight ratios of males fed diets containing 100 ppm Aroclor 1254 were elevated. The absolute liver weights for females in the same group (T-III) were not elevated; however, liver to brain weight ratios were significantly higher than the control values for these females. Statistical evaluation of other organ weights, organ to body weight and organ to brain weight ratios disclosed several randomly occurring intergroup differences. The lack of any consistent dose or generation related response and the absence of any deleterious histopathologic change confirm that these differences were not related to the ingestion of Aroclor 1254.

Statistical evaluations of other organ weights, organ to body weight and organ to brain weight ratios disclosed several randomly

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occurring intergroup differences. The lack of any consistent dose or generation related response and the absence of any deleterious histopathologic change confirm that these differences were not related to the ingestion of Aroclor 1254.

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TABLE VI

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Organ Weights and Ratios

Parental Animals

Organ: Liver

Group	Generation	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
		Males	Females	Males	Females	Males	Females
C	Fo	28.0	16.3	3.96	4.26	12.9	8.68
	F1	24.7	15.2	3.90	4.41	11.9	7.92
	F2	23.6	12.2	3.97	3.87	11.6	6.97
T-I	Fo	24.2**	14.6	3.56*	4.00	11.5	7.79
(1 ppm)	F1	25.5	15.3	3.95	4.66	11.9	7.82
	F2	27.9	17.1**	4.16	4.73	12.5	8.48
T-II	Fo	23.6*	15.8	3.55*	4.16	11.8	8.34
(10 ppm)	F1	27.9	14.3	4.25	4.04	13.4	7.84
	F2	21.5	14.2	3.72	4.21	10.1	7.50
T-III	Fo	33.4**	17.3	4.95**	4.90*	15.9**	9.28
(100 ppm)	F1	32.8*	17.3	5.14**	4.85	17.5**	10.70**
	F2	32.8	17.4	5.79	5.20	15.7	9.11**

* Statistically significant difference at the 95 percent confidence level.
** Statistically significant difference at the 99 percent confidence level.

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TABLE VII

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Organ Weights and Ratios

Parental Animals

Organ: Kidneys

Group	Generation	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
		Males	Females	Males	Females	Males	Females
C	Fo	4.82	2.77	0.680	0.724	2.22	1.48
	F1	4.44	2.68	0.709	0.771	2.14	1.40
	F2	4.01	2.43	0.681	0.774	1.99	1.39
T-I	Fo	4.25*	2.82	0.625	0.776	2.00	1.50
(1 ppm)	F1	4.44	2.87*	0.693	0.878	2.08	1.45
	F2	4.54	2.98**	0.677	0.821	2.03	1.47
T-II	Fo	4.02**	2.79	0.614	0.734	2.00	1.47
(10 ppm)	F1	4.56	2.79	0.697	0.793	2.20	1.53
	F2	3.84	2.56	0.667	0.752	1.82	1.34
T-III	Fo	4.68	2.75	0.697	0.782	2.23	1.48
(100 ppm)	F1	4.08	2.61	0.638	0.735	2.14	1.62
	F2	3.90	2.51	0.700	0.754	1.87	1.31

*Statistically significant difference at the 95 percent confidence level.
**Statistically significant difference at the 99 percent confidence level.

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TABLE VIII

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Organ Weights and Ratios

Parental Animals

Organ: Spleen

Group	Generation	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
		Males	Females	Males	Females	Males	Females
C	Fo	0.83	0.63	0.12	0.16	0.38	0.33
	F1	0.74	0.61	0.12	0.18	0.36	0.32
	F2	0.96	0.63	0.16	0.20	0.48	0.36
T-I (1 ppm)	Fo	0.93	0.58	0.14	0.16	0.44	0.31
	F1	0.72	0.48	0.11	0.15	0.34	0.24
	F2	0.91	0.63	0.14	0.17*	0.41	0.31*
T-II (10 ppm)	Fo	0.78	0.54	0.12	0.14	0.39	0.29
	F1	0.87	0.50	0.13	0.14	0.42	0.28
	F2	0.76	0.73	0.13	0.21	0.36	0.38
T-III (100 ppm)	Fo	0.78	0.56	0.12	0.16	0.37	0.30
	F1	0.72	0.48	0.11	0.13	0.38	0.30
	F2	0.77	0.57	0.14	0.17*	0.37	0.30*

* Statistically significant difference at the 99 percent confidence level.

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TABLE IX

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Organ Weights and Ratios

Parental Animals

Organ: Gonads

Group	Generation	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
		Males	Females	Males	Females	Males	Females
C	Fo	3.62	0.117	0.510	0.0302	1.67	0.0627
	F1	3.57	0.099	0.576	0.0283	1.72	0.0524
	F2	3.18	0.132	0.530	0.0424	1.57	0.0757
T-I (1 ppm)	Fo	3.74	0.126	0.551	0.0344	1.76	0.0668
	F1	3.17*	0.092	0.492*	0.0280	1.48**	0.0469
	F2	3.78	0.127	0.563	0.0356	1.69	0.0636
T-II (10 ppm)	Fo	3.41	0.122	0.523	0.0322	1.69	0.0641
	F1	4.03**	0.108	0.624	0.0313	1.96**	0.0615
	F2	3.11	0.148	0.543	0.0439	1.47	0.0781
T-III (100 ppm)	Fo	3.52	0.111	0.525	0.0313	1.67	0.0587
	F1	3.68	0.105	0.577	0.0303	1.97	0.0665
	F2	3.52	0.135	0.623	0.0410	1.68	0.0705

* Statistically significant difference at the 95 percent confidence level.
** Statistically significant difference at the 99 percent confidence level.

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TABLE X

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Organ Weights and Ratios

Parental Animals

Organ: Heart

Group	Generation	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
		Males	Females	Males	Females	Males	Females
C	Fo	1.86	1.28	0.261	0.332	0.858	0.684
	F1	1.74	1.24	0.276	0.360	0.844	0.647
	F2	1.84	1.19	0.314	0.379	0.914	0.682
T-I	Fo	1.93	1.29	0.283	0.355	0.910	0.688
(1 ppm)	F1	1.71	1.24	0.266	0.376	0.804	0.630
	F2	2.01	1.43**	0.301	0.394	0.909	0.706
T-II	Fo	1.69	1.23	0.258	0.325	0.838	0.650
(10 ppm)	F1	1.81	1.10**	0.279	0.314*	0.881	0.608
	F2	1.66*	1.16	0.292	0.343	0.788	0.610
T-III	Fo	1.84	1.24	0.271	0.352	0.877	0.[illegible]
(100 ppm)	F1	1.87	1.41**	0.292	0.401	0.930	0.884
	F2	1.68*	1.23	0.298	0.369	0.805	0.[illegible]

*Statistically significant difference at the 95 percent confidence level.
**Statistically significant difference at the 99 percent confidence level.

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TABLE XI

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Organ Weights and Ratios

Parental Animals

Organ: Brain

Group	Generation	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)	
		Males	Females	Males	Females
C	Fo	2.17	1.90	0.307	0.509
	F1	2.07	1.93	0.334	0.566
	F2	2.01	1.74	0.345	0.557
T-I (1 ppm)	Fo	2.13	1.89	0.313	0.521
	F1	2.13	1.97	0.335	0.607
	F2	2.23*	2.03**	0.332	0.560
T-II (10 ppm)	Fo	2.02	1.90	0.310	0.502
	F1	2.05	1.82	0.317	0.525
	F2	2.10	1.90**	0.370	0.564
T-III (100 ppm)	Fo	2.11	1.86	0.316	0.530
	F1	1.89*	1.62**	0.296*	0.461
	F2	2.09	1.91**	0.370	0.581

*Statistically significant difference at the 95 percent confidence level.
**Statistically significant difference at the 99 percent confidence level.

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TABLE XII

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Organ Weights and Ratios

Parental Animals

Organ: Adrenal Glands

Group	Generation	Organ Weight (g)		Organ/Body Weight Ratio (g/100 g)		Organ/Brain Weight Ratio (g/g)	
		Males	Females	Males	Females	Males	Females
C	Fo	0.072	0.095	0.010	0.025	0.033	0.050
	F1	0.066	0.091	0.010	0.026	0.032	0.047
	F2	0.071	0.083	0.012	0.025	0.035	0.049
T-I	Fo	0.077	0.092	0.011	0.025	0.036	0.049
(1 ppm)	F1	0.077*	0.092	0.012*	0.028	0.036	0.046
	F2	0.084	0.012*	0.012	0.032	0.037	0.058
T-II	Fo	0.059	0.087	0.008	0.022	0.029	0.046
(10 ppm)	F1	0.056	0.075	0.009*	0.021	0.027	0.042
	F2	0.064	0.073	0.011	0.021	0.030*	0.038
T-III	Fo	0.070	0.082	0.010	0.023	0.033	0.044
(100 ppm)	F1	0.069	0.083	0.012	0.023	0.037*	0.051
	F2	0.058	0.074	0.010	0.022	0.027**	0.038

* Statistically significant difference at the 95 percent confidence level.
** Statistically significant difference at the 99 percent confidence level.

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TABLE XIII

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Mean Organ Weights and Ratios

Parental Animals

Organ: Thyroid Gland

Group	Generation	Organ Weight (g) Males	Organ Weight (g) Females	Organ/Body Weight Ratio (g/100 g) Males	Organ/Body Weight Ratio (g/100 g) Females	Organ/Brain Weight Ratio (g/g) Males	Organ/Brain Weight Ratio (g/g) Females
C	Fo	0.035	0.028	0.0050	0.0075	0.016	0.015
	F1	0.025	0.025	0.0040	0.0074	0.012	0.013
	F2	0.033	0.028	0.0056	0.0089	0.016	0.016
T-I (1 ppm)	Fo	0.031	0.025	0.0045	0.0069	0.014	0.013
	F1	0.042**	0.034**	0.0066**	0.0104**	0.020**	0.018**
	F2	0.034	0.031	0.0051	0.0088	0.015	0.015
T-II (10 ppm)	Fo	0.034	0.029	0.0052	0.0076	0.017	0.015
	F1	0.035**	0.023	0.0054*	0.0066	0.017**	0.013
	F2	0.040	0.024	0.0069	0.0073	0.019	0.012
T-III (100 ppm)	Fo	0.047*	0.023*	0.0069*	0.0067	0.022	0.013
	F1	0.044**	0.044**	0.0068**	0.0123**	0.023**	0.027**
	F2	0.035	0.021	0.0062	0.0066	0.016	0.011

* Statistically significant difference at the 95 percent confidence level.
** Statistically significant difference at the 99 percent confidence level.

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e. Histopathologic Findings

Histopathologic findings among sacrificed animals are summarized in Tables XIV through XVI. Table XVII summarizes the histopathologic examination of the urinary bladder conducted on all animals sacrificed from the final generation.

Three animals with epithelial hyperplasia of the urinary bladder were present in the T-III group of the F2 generation. All three of these rats were from the same litter. One control rat had similar urinary bladder hyperplasia.

The changes found in the other organs examined for each of the three generations did not differ from those present in the control group.

TOXSTUDIES1453

TABLE XIV

TEST MATERIAL: Arocior 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Control Group - Fo Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Trachea	5 Males	Focal inflammation	3	0.5
		Tracheitis	1	2.0
	5 Females	Focal inflammation	1	1.0
Lung	5 Males	Focal inflammation	2	1.0
		Hyperemia	5	2.0
	5 Females	Focal inflammation	2	0.5
		Hyperemia	5	1.0
Pancreas	5 Males	Prominent islands of Langerhans	1	1.0
Kidney	5 Males	Focal inflammation	1	0.5
		Hyperemia	1	0.5
	5 Females	Hyperemia	2	1.0
Adrenal glands	5 Males	Hyperemia	3	1.0

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TABLE XIV continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Test Group III - Fo Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Heart	5 Males	Focal inflammation	1	1.0
Trachea	5 Males	Focal inflammation	2	2.0
		Tracheitis	1	2.0
	5 Females	Tracheitis	3	2.0
Lung	5 Males	Focal inflammation	4	1.0
		Hyperemia	2	1.0
	5 Females	Focal inflammation	1	0.5
		Hyperemia	5	3.0

TOXSTUDIES1455

TABLE XIV continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Test Group III - Fo Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Liver	5 Males	Focal vacuolization	2	1.0
		Hyperemia	1	0.5
Kidney	5 Males	Nephritis	1	1.0
	5 Females	Focal inflammation	1	1.0
Thyroid	5 Males	Thyroiditis	3	1.0
Thymus	5 Females	Hyperemia	1	2.0

All other tissues and organs examined were normal histologically.

Grading System
0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

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TABLE XV

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Control Group - F1 Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Trachea	5 Males	Tracheitis	3	2.0
		Focal inflammation	1	1.0
	5 Females	Tracheitis	3	2.0
Lung	5 Males	Focal inflammation	5	2.0
	5 Females	Focal inflammation	4	1.0
		Focal inflammation with abscess	1	5.0
		Hyperemia	1	2.0
Liver	5 Females	Hyperemia	1	0.5
Kidney	5 Males	Nephrosis	2	0.5
		Nephritis	1	0.5
	5 Females	Nephrosis	1	0.5
Prostate	5 Males	Focal inflammation	1	0.5

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TABLE XV continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Test Group III - F1 Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Trachea	5 Males	Focal inflammation	3	0.5
Lung	5 Males	Hyperemia	3	1.0
		Focal inflammation	4	1.0
	5 Females	Hyperemia	5	3.0
		Focal inflammation	2	1.0
Liver	5 Females	Hyperemia	1	2.0
Kidney	5 Males	Nephritis	1	0.5
	5 Females	Nephritis	1	0.5

All other tissues and organs examined were normal histologically.

Grading System
0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TOXSTUDIES1458

TABLE XVI

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Control Group - F2 Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Trachea	5 Males	Focal inflammation	1	0.5
		Inflammation	2	3.0
	5 Females	Focal inflammation	1	0.5
		Inflammation	1	1.0
Lung	5 Males	Focal inflammation	4	1.0
		Inflammation with abscess	1	3.0
		Hyperemia	2	1.0
	5 Females	Focal inflammation	1	2.0
		Inflammation with abscess	1	4.0
		Hyperemia	3	2.0

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TOXSTUDIES1459

TABLE XVI continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Control Group - F2 Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Colon	5 Males	Focal inflammation	2	0.5
Liver	5 Females	Hyperemia	1	0.5
Kidney	5 Males	Focal inflammation	1	0.5
	5 Females	Nephrocalcinosis	1	0.5

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TABLE XVI continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Test Group III - F2 Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Trachea	7 Males	Focal inflammation	1	2.0
		Inflammation	1	2.0
	8 Females	Focal inflammation	4	0.5
		Inflammation	1	1.0
Lung	7 Males	Focal inflammation	5	1.0
		Hyperemia	5	2.0
	8 Females	Focal inflammation	5	1.0
		Hyperemia	5	2.0
Liver	7 Males	Focal inflammation	2	2.0
	8 Females	Hyperemia	2	2.0
		Mononuclear cell infiltration	1	0.5
		Focal vacuolization	1	0.5

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TABLE XVI continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes

Test Group III - F2 Generation

Organ	Number of Animals Examined	Findings	Incidence	Average Grade
Colon	7 Males	Focal inflammation	2	1.0
	8 Females	Focal inflammation	1	0.5
Testis	7 Males	Atrophied	1	4.0
		Focal inflammation	1	2.0
Kidney	7 Males	Focal inflammation, pyelonephritis	1	4.0
	8 Females	Nephrocalcinosis	1	0.5

All other tissues and organs examined were normal histologically.

Grading System
0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

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TABLE XVII

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Histopathologic Changes of the Urinary Bladder

F2 Generation

Group	Sex	Number Examined	Findings*	Incidence	Average Grade
C	M	7	None	-	-
	F	10	Focal epithelial hyperplasia associated with focal cystitis (11)	1	1.0
T-I (1 ppm)	M	8	Proteinaceous plug (103)	1	-
	F	14	None	-	-
T-II (10 ppm)	M	8	Proteinaceous plug (123 and 124)	2	-
	F	13	None	-	-
T-III (100 ppm)	M	7	Diffuse epithelial hyperplasia associated with focal cystitis and mineralised calculi (152)	1	4.0
	F	10	Focal epithelial hyperplasia (167 and 168)	2	1.0

* Individual animal numbers are shown in parenthesis.

Grading System
1.0 = minimal or trace
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

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5. Reproductive Data

Reproductive data for parental animals are presented in Table XVIII.

The indices employed are defined below:

$$\text{Mating Index} = \frac{\text{Number of Copulations*}}{\text{Number of Estrus Cycles Required}} \times 100$$

$$\text{Fertility Index} = \frac{\text{Number of Pregnancies}}{\text{Number of Copulations}} \times 100$$

$$\text{Incidence of Pregnancy} = \frac{\text{Number of Pregnancies}}{\text{Number of Females Mated}} \times 100$$

$$\text{Incidence of Parturition} = \frac{\text{Number of Parturitions}}{\text{Number of Pregnancies}} \times 100$$

The addition of 1 or 10 ppm Aroclor 1254 to the diet did not affect the reproductive performance of rats during the three-generation study. During the second generation (F2a and F2b litters), rats fed 10 (T-II) or 100 ppm (T-III) had lowered mating indices. The other indices were normal. During the third generation (F3a and F3b litters) the reproductive performance of all treatment groups was equal to that of the contemporary control.

* Only one copulation counted per estrus cycle. Five days equal one estrus cycle.

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TABLE XVIII

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

General Data

Parental Animals

Reproductive Performance

Litter	Mating Index Fraction	Mating Index Percent	Incidence of Pregnancy Fraction	Incidence of Pregnancy Percent	Fertility Index Fraction	Fertility Index Percent	Incidence of Parturition Fraction	Incidence of Parturition Percent	Gestation Time (Days) Mean	Gestation Time (Days) Range
F1a	17/25	68.0	16/16	100.0	16/17	94.1	15/16	93.8	22	19-22
F1b	15/20	75.0	15/15	100.0	15/15	100.0	15/15	100.0	22	21-22
F2a	16/37	43.2	14/16	87.5	14/16	87.5	14/14	100.0	21	20-22
F2b	14/26	53.8	14/14	100.0	14/14	100.0	14/14	100.0	22	21-23
F3a	15/66	22.7	13/16	81.2	13/15	86.7	11/13	84.6	22	21-22
F3b	12/16	75.0	12/12	100.0	12/12	100.0	11/12	91.6	22	20-22
F1a	16/29	55.2	16/16	100.0	16/16	100.0	16/16	100.0	22	21-22
F1b	16/29	55.2	16/16	100.0	16/16	100.0	15/16	93.8	22	20-23
F2a	16/42	38.1	12/16	75.0	12/16	75.0	12/12	100.0	21	20-22
F2b	11/28	39.3	11/12	91.7	11/11	100.0	11/11	100.0	22	20-23
F3a	18/58	31.0	12/18	66.7	12/16	75.0	9/12	75.0	22	22-23
F3b	14/24	58.3	11/14	78.6	11/12	91.7	11/11	100.0	22	21-23

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TABLE XVIII continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

General Data

Parental Animals

Reproductive Performance

		Mating Index		Incidence of Pregnancy		Fertility Index		Incidence of Parturition		Gestation Time (Days)	
	Litter	Fraction	Percent	Fraction	Percent	Fraction	Percent	Fraction	Percent	Mean	Range
[illegible]	F1a	16/23	69.6	16/16	100.0	16/16	100.0	16/16	100.0	22	21-23
[illegible] ppm)	F1b	16/21	76.2	16/16	100.0	16/16	100.0	15/16	93.8	22	20-23
	F2a	14/63	22.2	11/16	68.8	11/14	78.6	10/11	90.9	22	21-23
	F2b	11/32	34.4	10/11	90.9	10/11	90.9	9/10	90.0	23	22-23
	F3a	16/54	29.6	14/16	87.5	14/16	87.5	12/14	85.7	22	21-22
	F3b	14/35	40.0	13/14	92.9	13/14	92.9	13/13	100.0	22	20-23
[illegible]-III	F1a	16/28	57.1	16/16	100.0	16/16	100.0	15/16	93.8	22	21-23
[illegible] ppm)	F1b	16/26	61.5	16/16	100.0	16/16	100.0	13/16	81.3	21	19-22
	F2a	14/73	18.2	13/16	81.2	13/14	92.9	13/13	100.0	22	22-24
	F2b	10/52	19.2	9/13	69.2	9/10	90.0	9/9	100.0	22	-
	F3a	11/36	30.6	11/11	100.0	11/11	100.0	10/11	90.9	22	22-23
	F3b	8/31	25.8	8/8	100.0	8/8	100.0	8/8	100.0	22	-

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B. Progeny

1. Population Data

Population data for all litters are presented in Tables XIX and XX. The number of live pups born per litter was not affected by feeding 1, 10 or 100 ppm Aroclor 1254. The number of pups weaned per litter was reduced in all three generations in the group fed 100 ppm (T-III). The average number of pups weaned per litter for the groups fed 1 or 10 ppm was not lowered.

All pups delivered appeared outwardly normal and displayed normal growth through the lactation period. At weaning, an examination of each pup for external abnormalities was conducted and all pups were judged to be free of gross external abnormalities.

TOXSTUDIES1467

TABLE XIX

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Population Data

Progeny

	Number of Pups Delivered		Number of Pups Stillborn		Number of Pups Cannibalized		Number of Viable Pups Born	
Litter	Total	Mean/Litter	Total	Mean/Litter	Total	Mean/Litter	Total	Mean/Litter
F1a	178	11.9	13	0.9	0	-	165	11.0
F1b	191	12.7	12	0.8	1	0.1	178	11.9
F2a	147	10.5	7	0.5	0	-	140	10.0
F2b	164	11.7	0	-	0	-	164	11.7
F3a	102	9.3	10	0.9	0	-	92	8.4
F3b	109	9.9	15	1.4	1	0.1	93	8.5
F1a	187	11.7	1	0.1	0	-	186	11.6
F1b	207	13.8	4	0.3	1	0.1	202	13.4
F2a	132	11.0	0	-	0	-	132	11.0
F2b	124	11.3	3	0.3	0	-	121	11.0
F3a	85	9.4	12	1.3	1	0.1	70	7.8
F3b	120	10.9	8	0.7	1	0.1	111	10.1

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TABLE XIX continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Population Data

Progeny

	Litter	Number of Pups Delivered Total	Number of Pups Delivered Mean/Litter	Number of Pups Stillborn Total	Number of Pups Stillborn Mean/Litter	Number of Pups Cannibalized Total	Number of Pups Cannibalized Mean/Litter	Number of Viable Pups Born Total	Number of Viable Pups Born Mean/Litter
	F1a	163	10.2	11	0.7	4	0.2	148	9.2
m)	F1b	192	12.8	14	0.9	5	0.3	173	11.5
	F2a	97	9.7	5	0.5	0	-	92	9.2
	F2b	88	9.8	1	0.1	0	-	87	9.7
	F3a	99	8.2	6	0.5	-	-	93	7.8
	F3b	118	9.1	15	1.2	-	-	105	8.1
	F1a	167	11.1	0	-	0	-	167	11.1
ppm)	F1b	163	12.5	8	0.6	3	0.2	152	11.7
	F2a	115	8.8	8	0.6	5	0.4	102	7.8
	F2b	97	10.8	3	0.3	12a	1.3	82	9.1
	F3a	95	9.5	14	1.4	4	0.4	77	7.7
	F3b	83	10.4	2	0.2	-	-	79	9.9

aSeven pups cannibalized from one litter.

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TABLE XX

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Population Data

Progeny

Litter	Number of Pups at Lactation Day 1		Number of Pups at Lactation Day 5		Number of Pups Retained at Lactation Day 5		Number of Weanlings			
	Total	Mean/Litter	Total	Mean/Litter	Total	Mean/Litter	Male	Female	Total	Mean/Litter
F1a	146	9.7	70	4.7	66	4.4	34	28	62	4.1
F1b	176	11.7	117	7.8	103	6.9	31	35	66	4.4
F2a	132	9.4	90	6.4	78	5.6	30	34	64	4.6
F2b	163	11.6	135	9.6	115	8.2	39	46	85	6.1
F3a	90	8.2	69	6.3	65	5.9	26	25	51	4.6
F3b	91	8.3	79	7.2	75	6.8	16	26	42	3.8
F1a	182	11.4	165	10.3	144	9.0	74	60	134	8.4
F1b	159	10.6	150	10.0	131	8.7	55	57	112	7.5
F2a	131	10.9	119	9.9	88	7.3	30	43	73	6.1
F2b	120	10.9	103	9.4	84	7.6	35	39	74	6.7
F3a	59	6.5	57	6.3	53	5.9	18	29	47	5.2
F3b	111	10.1	106	9.6	95	8.6	37	49	86	7.8

EIO-TEST

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TABLE XX continued

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Population Data

Progeny

	Litter	Number of Pups at Lactation Day 1 Total	Mean/Litter	Number of Pups at Lactation Day 5 Total	Mean/Litter	Number of Pups Retained at Lactation Day 5 Total	Mean/Litter	Number of Weanlings Male	Female	Total	Mean/Litter
	F1a	143	8.9	114	7.1	104	6.5	45	42	87	5.4
ppm)	F1b	159	10.6	150	10.0	131	8.7	55	57	112	7.5
	F2a	66	6.6	54	5.4	50	5.0	18	24	42	4.2
	F2b	66	7.3	40	5.0*	37	4.6	20	15	35	4.4
	F3a	92	7.7	89	7.4	82	6.8	31	35	66	5.5
	F3b	99	7.6	93	7.2	76	5.8	36	28	64	4.9
	F1a	158	10.5	98	6.5	92	6.1	38	26	64	4.3
ppm)	F1b	148	11.4	110	8.5	101	7.8	13	13	26	2.0
	F2a	90	6.9	66	5.1	59	4.5	11	26	37	2.8
	F2b	61	6.8	44	4.9	40	4.4	13	12	25	2.8
	F3a	54	5.4	14	1.4	14	1.4	6	3	9	0.9
	F3b	77	9.6	39	4.9	39	4.9	10	10	20	2.5

*...n per litter from day 5 of lactation through weaning reported on eight females due to death of one female and ...sacrifice of progeny.

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TOXSTUDIES1471

2. Survival Data

Survival data for progeny are presented in Table XXI.

The indices employed are defined below.

$$\text{Live Birth Index} = \frac{\text{Number of Viable Pups Born}}{\text{Total Number of Pups Born}} \times 100$$

$$\text{24-Hour Survival Index} = \frac{\text{Number of Pups Viable at Lactation Day 1}}{\text{Number of Viable Pups Born}} \times 100$$

$$\text{5-Day Survival Index} = \frac{\text{Number of Pups Viable at Lactation Day 5}}{\text{Number of Viable Pups Born}} \times 100$$

$$\text{Lactation Index} = \frac{\text{Number of Pups Viable at Lactation Day 21}}{\text{Number of Pups Viable at Lactation Day 5}} \times 100$$

Pup survival was lower in litters from dams fed 100 ppm Aroclor 1254 than for any other group. Pup survival was not reduced when 1 or 10 ppm was fed.

TOXSTUDIES1472

TABLE XXI

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Survival Indices

Progeny

Group	Litter	Live Birth Index	24-Hour Survival Index	5-Day Survival Index	Lactation Index
C	F1a	92.7	88.5	42.4	93.9
	F1b	93.2	98.9	65.7	64.1
	F2a	95.2	94.3	64.3	82.1
	F2b	100.0	99.3	82.3	73.9
	F3a	90.2	97.8	75.0	78.5
	F3b	85.3	97.8	84.9	56.0
T-I (1 ppm)	F1a	99.5	97.8	88.7	93.1
	F1b	97.6	78.7	91.6	78.8
	F2a	100.0	99.2	90.2	83.0
	F2b	97.6	99.1	85.1	88.1
	F3a	82.4	84.3	81.4	88.7
	F3b	92.5	100.0	95.9	90.5
T-II (10 ppm)	F1a	90.8	96.6	77.0	83.7
	F1b	90.1	91.9	86.7	85.5
	F2a	94.8	71.7	58.7	84.0
	F2b	98.9	75.8	50.0*	94.6
	F3a	93.9	98.9	95.7	80.5
	F3b	89.0	94.3	88.6	84.2
T-III (100 ppm)	F1a	100.0	94.6	58.7	69.6
	F1b	93.3	97.4	72.4	25.7
	F2a	88.7	88.2	64.7	62.7
	F2b	84.5	74.4	53.7	62.5
	F3a	81.1	70.1	18.2	64.3
	F3b	95.2	97.5	49.4	51.3

* Five-day survival index corrected to allow for sacrificed progeny due to the death of one female.

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3. Body Weight Data

Body weight data for weanlings are presented in Table XXII.

There were no significant differences between test and control weanling body weights.

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TABLE XXII

TEST MATERIAL: Aroclor 1254

Three-Generation Reproduction Study - Albino Rats

Progeny

Weanling Body Weight Data

Group	Litter	Mean Body Weight (grams) Male	Female
C	F1a	48	44
	F1b	47	43
	F2a	46	43
	F2b	41	39
	F3a	45	44
	F3b	42	42
T-I (1 ppm)	F1a	49	46
	F1b	45	45
	F2a	52	45
	F2b	48	47
	F3a	47	41
	F3b	50	48
T-II (10 ppm)	F1a	51	48
	F1b	51	47
	F2a	52	48
	F2b	45	49
	F3a	45	42
	F3b	48	45
T-III (100 ppm)	F1a	46	42
	F1b	43	40
	F2a	40	42
	F2b	47	46
	F3a	43	47
	F3b	40	32

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4. Pathologic Studies

At autopsy of the F3b weanlings no gross or microscopic tissue changes were present which could be associated with the ingestion of Aroclor 1254.

TOXSTUDIES1476