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HAZLETON LABORATORIES

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Document Title
INITIAL SUBMISSION: 13-WEEK SUBCHRONIC FEEDING STUDY IN RATS WITH DECABROMODIPHENYL OXIDE

Chemical Category
DECABROMODIPHENYL OXIDE

FYI-0794-001093

HAZLETON
LABORATORIES AMERICA, INC.

SPONSOR: Tracor-Jitco, Inc.
Subcontract No. 78-52-106002

DATE: July 27, 1979

MATERIAL: Decabromodiphenyl Oxide (C44287)

Contains No CBI

SUBJECT: Final Report
13-Week Sub-Chronic Feeding Study - Rat

SUMMARY

The test material Decabromodiphenyl Oxide (C44287) was administered in the diet of five groups of 10 male and 10 female rats for 13 weeks at levels of 0.31, 0.62, 1.25, 2.5, and 5.0%. A sixth group of rats served as control and received the basal diet only.

Througnout the study, there was no evidence of a compound effect with regard to survival, physical appearance and behavior, body weight gain, food consumption, and gross and microscopic pathology.

Based on these results, the following dose levels are recommended for the 104-week chronic study in rats on Decabromodiphenyl Oxide:

Males - 2.5 and 5.0%

Females - 2.5 and 5.0%

FYI-94-001093
INIT 07/26/94

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INTRODUCTION

The purpose of this study was to evaluate and characterize the effects of Decabromodiphenyl Oxide (C55287) in the rat after 13 weeks of dietary administration and to determine the maximum test dose that could be utilized for a subsequent chronic (104 weeks) feeding study. The study was initiated on February 27, 1979 and terminated on June 1, 1979.

TEST MATERIAL

1. **Identification**

   Decabromodiphenyl Oxide (C55287), Lot No. 08287-2 Batch No. 1 and Lot No. D12478 Batch No. 2.

2. **Description**

   Lot No. 08287-2 and D12478; white crystalline powder

3. **Receipt Date**

   Lot No. 08287-2 was received on September 21, 1978 from Midwest Research Institute and was used from week 1 to week 6.
   Lot No. D12478 was received on December 18, 1978 from Midwest Research Institute and was used from week 7 to week 13.

4. **Purity**

   Considered 100 % active for purpose of dose calculation. Thin layer chromotography indicated the major spot only according to the Midwest Research report of September 5, 1978.

5. **Handling**

   Both lot numbers were contained in fiberboard drums lined with a polyethylene bag. The chemical was stored in a light-resistant container in the freezer for the duration of the study.

6. **Test Diet Formulation**

   The basal diet used was Purina Rodent Laboratory Chow 5001 (see attached analysis/ingredients). Each dose level was prepared in 10 kg batches once every two weeks. The chemical DBDPO was mixed directly into the basal diet.

The desired amount of compound was weighed out and brought to 5 kg with the control diet and premixed in the Hobart mixing bowl for approximately five minutes. The mixture was then placed in a PK V-blender adding 5 kg control diet (not less than 10 kg capacity) and mixed for 12 minutes (approximately 1 min/kg). After blending the test diet, the mixture was placed in an airtight color-coded container and stored at room temperature. Fresh diet was mixed every two weeks and 6 kg placed in the freezer. Prior to use, the feed was brought to room temperature. The feed was mixed for the rat and mouse study at the same time.

7. Experimental Animals

Sixty male and sixty female Fischer 344 rats were used in the study. The animals were 30 days of age when received on January 30, 1979 from Charles River Breeding Laboratories, Portage, Michigan. The rats were quarantined in the test room for three weeks and were placed on test at approximately 9 weeks of age.

Shortly after arrival, two animals of each sex were sacrificed and examined grossly for any unusual lesions and endo and ecto parasites. During quarantine, observations for mortality were made twice daily. Body weights and clinical observations were recorded weekly.

Weight range at initiation: Males 177 to 271 grams
Females 130 to 173 grams

8. Selection for Groups

Assignment to the treatment groups was performed using a computer that generated random numbers assigned to the available animals, ranked the numbers, and assigned the random numbers and corresponding animals to the treatment groups.

9. Animal Maintenance

Rats and mice were housed together in a temperature and humidity-controlled room. The temperature was maintained at 70°F ± 1°F and the relative humidity at 45% ± 5% during the study. The animals were housed five per cage in polycarbonate cages (Hazleton Systems, Inc.) suspended on stainless steel drawer-type racks (Hazleton Systems, Inc., Aberdeen, Md.) supplied

with filter sheets. All cages were furnished with heat-treated hardwood chip bedding (Sani-Chips®, P.J. Murphy, Moonachie, N.J.). Cages and bedding were changed twice a week. Racks were sanitized every two weeks. Diet and county-supplied water (automatic waterers) were provide ad libitum.

10. Experimental Design

The treatment groups, feeding levels, and duration of the study are shown in Table 1.

11. Observations and Records

All animals were observed twice daily (8:30 a.m. to 9:00 a.m. and at 3:00 p.m. to 3:30 p.m.) for mortality.

Individual body weights, cage food consumption, appearance and behavior were recorded weekly using the automatic data recording system. DBDPO was fed in the diet for a period of 13 weeks.

12. Termination

After 13 weeks, all surviving rats were exsanguinated following I.P. injections of sodium pentobarbital (Diabutal-Diamond Laboratories, Inc., Des Moines, Iowa). Sera from five male and five female control rats and four male and four female control mice were submitted to Microbiological Associates, Bethesda, Maryland for viral screen tests.

13. Postmortem Procedures

Necropsies were performed and representative tissues were preserved on all rats that died during the study and those sacrified after 13 weeks. Necropsy procedures followed were those specified and described in the Bioassay Operations Protocol (78-52-106002).

14. Tissues Preserved

The following tissues were preserved in 10% neutral buffered formalin:

Gross Lesions
Skin
Mandibular Lymph Node
Mammary Gland
Salivary Gland
Thigh Muscle
Sciatic Nerve
Sternebrae, Vertebrae or Femur including Marrow
Costochondral Junction, Rib
Thymus
Larynx
Trachea
Lungs and Bronchi
Heart
Thyroid
Parathyroids
Esophagus
Stomach
Duodenum
Jejunum
Tissue Masses or Suspect Tumors and Regional Lymph Nodes
Ileum
Colon
Cecum
Rectum
Mesenteric Lymph Nodes
Liver
Pancreas
Spleen
Kidneys
Adrenals
Urinary Bladder
Seminal Vesicles
Prostate
testes
Ovaries
Uterus
Nasal Cavity
Brain
Pituitary
Spinal Cord
Eyes

15. Microscopic Pathology

The following tissues from the control and high-dose rats were processed histologically and examined microscopically:

- Gross Lesions and Tissue Masses (and Regional Lymph Nodes, if possible)
- Blood Smear (as required by the pathologist)
- Mandibular or Mesenteric Lymph Node
- Salivary Gland
- Sternebrae, Femur, or Vertebrae including Marrow
- Thyroid
- Parathyroids
- Small Intestine (one section)
- Colon
- Liver
- Gall Bladder (mouse only)
- Prostate
- Testes
- ~~Ovaries~~
- Lungs and Mainstem Bronchi
- Heart
- Esophagus
- Stomach
- Uterus
- Brain (three sections, including Frontal Cortex and Basal Ganglia, Parietal Cortex and Thalmus, and Cerebellum and Pons)
- Thymus
- Trachea
- Pancreas
- Spleen
- Kidneys
- Adrenals
- Urinary Bladder
- Pituitary
- Spinal Cord (if neurologic signs are present)
- Eyes (if grossly abnormal)
- Mammary Gland

RESULTS

1. Appearance and Behavior

Throughout the study there was not evidence of compound effect with regard to physical appearance and behavior among the rats at any treatment level. Incidental signs commonly seen among Fischer 344 rats were noted sporadically in a few animals and were observed at a comparable rate between the control and treated groups. These signs included reddened eyelids and a few small areas of alopecia. One high-dose male developed a small firm nodule and one showed a small opaque left eye.

2. Body Weight, Food Consumption and Survival Data

Group mean body weights, group mean food consumptions, and survival data are presented in appended Table No. 2 and 2a. Graphs of mean body weights for each week on appended figure 1 and 1a. Calculated differential weight gain relative to controls in appended Table 2b and 2c. Initial and terminal individual body weight on Tables 2b and 2c.

Throughout the study there was no distinct indication of compound effect upon body weight gain and food consumption. Group mean body weight gains for the treated male rats were generally comparable to those of the control male rats. There was a slight trend to suppression of mean body weight gains in treated female rats compared to control female rats.

3. Observation of Necropsy

Necropsy findings in the animals sacrificed at termination did not reveal any consistent tissue or organ changes which could be attributed to the administration of the test material. Findings are presented in appended Table No. 3 and 3a.

4. Histopathology

A detailed incidence of microscopic findings and tissues examined for individual animals is presented in appended Tables No. 4 and 4a. No compound-related histomorphologic alterations were observed in any of the rats.

One male high-dose animal developed a small nodule in the axillary area. Histopathologically, it is a mammary gland fibroadenoma. It is not considered a compound-related alteration, but a natural development rarely observed in rats this age.

Other histopathology findings included chronic murine pneumonia in all animals, hemorrhage of the mandibular and cervical lymph node, heart focal or multifocal chronic inflammation. These lesions occurred in male and female animals in both treated and control groups.

All other tissues examined were within normal histologic limits and comparable in appearance between control and high-dose treated groups.

TABLE NO. 1

EXPERIMENTAL DESIGN
Group and Dose Level

Group No.	No. of Animals Male	Female	Dose Level %	Weeks
R2-2D1	10	10	0	13
R2-2A2	10	10	0.31	13
R2-2B3	10	10	0.62	13
R2-2C4	10	10	1.25	13
R2-2D5	10	10	2.5	13
R2-2E6	10	10	5.0	13

Subchronic Test - 90-Day Rat, Males

Chemical - Decabromophenyloxide C55287

TABLE 2

976-614

	Group 1 Control			Group 2 0.31%			Group 3 0.63%			Group 4 1.25%			Group 5 2.5%			Group 6 5.0%		
Week No.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Su
0	214		10/10	216		10/10	229		10/10	214		10/10	211		10/10	224		10/1
1	236	127		237	131		238	125		236	122		232	129		239	143	
2	255	119		259	115		267	130*		256	113		254	118		270	122	
3	275	109		281	129		287	128*		276	127		277	132		280	149	
4	290	116		297	116		306	116*		290	110		292	110		298	118	
5	302	128		310	127		314	133		299	121		305	129		310	135	
6	310	136		316	132		322	139		308	121		314	117		314	141	
7	314	103		324	124		328	121		314	124		324	141		324	138	
8	329	120		339	142		341	132		327	113		335	127		336	145	
9	340	126		353	134		350	125		335	124		344	127		346	131	
10	350	126		359	131		359	118		343	139		354	126		353	129	
11	350	122		356	126		362	124		344	120		359	129		355	131*	
12	352	115		364	121		368	124		353	117		361	111		365	131	
13	369	122		377	125		380	120		362	116		374	124		372	150	

*Food consumption avr for 5 animals

**Body Weight and food consumption in grams

Sub-Chronic Test - 90-Day Rat, Females

Chemical - Decabromopehyloxide C55287

TABLE 2a

976-614

	Group 1 Control			Group 2 0.31%			Group 3 0.63%			Group 4 1.25%			Group 5 2.5%			Group 6 5.0%		
Week No.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv.	Body Wt**	Food Cons.	Surv
0	149		10/10	150		10/10	146		10/10	150		10/10	150		10/10	151		10/1
1	157	100		157	97		152	105		158	92		159	116		157	106	
2	165	106		164	74*		159	72		166	79		164	74		169	73	
3	173	107		174	107		169	116		174	114		173	117		175	115	
4	181	111		180	93		175	103		181	108		181	101		182	101	
5	185	95		188	100		170	96		187	106		185	94		186	99	
6	187	92		190	97		181	99		188	107		187	83		187	90	
7	188	119		192	85		184	88		190	100		191	112		192	95	
8	198	92		200	80		189	92		197	91		194	115		196	113	
9	203	87		203	80		192	119		208	85		196	91		198	89	
10	206	93		208	74		197	88		203	84		201	84		201	102	
11	203	79		204	76		196	78		203	83		199	118		196	79	
12	207	77		209	79		199	88		209	80*		204	85		202	87	
13	211	82		213	74		203	87		211	77		208	90		205	68	

*Food consumption AVR for 5 animals

**Body weight and food consumption in grams

TABLE 2b
INDIVIDUAL BODY WEIGHT IN GRAMS - MALE RATS

976-614
Decabromodiphenyl Oxide

At Start

Group No.	201		2A2		2B3		2C4		2D5		2E6	
	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight
	01	177.7	21	205.6	41	228.0	61	213.1	81	223.5	101	244.4
	02	208.4	22	219.9	42	238.5	62	166.4	82	233.8	102	242.1
	03	201.0	23	209.1	43	224.1	63	233.0	83	227.6	103	204.3
	04	200.9	24	184.9	44	223.3	64	217.1	84	186.3	104	223.4
	05	204.4	25	224.9	45	231.7	65	216.4	85	210.6	105	202.7
	06	271.0	26	217.4	46	268.6	66	179.7	86	231.7	106	219.0
	07	212.8	27	223.3	47	211.1	67	234.0	87	186.6	107	215.5
	08	214.0	28	219.5	48	227.3	68	228.8	88	203.6	108	246.7
	09	243.7	29	226.0	49	223.3	69	226.8	89	196.7	109	235.7
	10	213.1	30	226	50	210.0	70	226.9	90	209.0	110	206.8
	Avg. BWT	214.7		215.7		228.6		215.2		211.5		224.1

At Termination

Group No.	201		2A2		2B3		2C4		2D5		2E6	
	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight
	01	382.6	21	375.8	41	357.2	61	372.4	81	383.0	101	400.4
	02	350.8	22	352.5	42	390.8	62	310.7	82	389.9	102	406.3
	03	356.5	23	356.2	43	345.2	63	389.4	83	423.7	103	341.6
	04	347.3	24	390.3	44	390.7	64	373.3	84	309.3	104	386.9
	05	373.0	25	379.9	45	422.7	65	348.2	85	390.9	105	359.6
	06	384.2	26	383.5	46	395.6	66	353.2	86	389.5	106	366.3
	07	376.6	27	285.1	47	348.4	67	372.3	87	349.4	107	373.0
	08	361.0	28	399.2	48	405.5	68	327.9	88	359.3	108	377.7
	09	405.5	29	368.4	49	368.3	69	385.3	89	391.5	109	410.2
	10	350.5	30	381.7	50	377.5	70	384.8	90	350.3	110	209.1
	Avg BWT.	368.8		377.3		380.6		361.7		373.7		372.0
	Wt GAIN	154.1		161.6		152.0		147.0		162.0		147.9
	%			+4.87		-1.36		-4.61		+5.26		-4.03

TABLE 2c
INDIVIDUAL BODY WEIGHT IN GRAMS - FEMALE RATS

976-614
Decabromodiphenyl Oxide

At Start

Group No.	2D1		2A2		2B3		2C4		2D5		2E6	
	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight
	11	173.5	31	153.6	51	148.9	71	133.4	91	151.1	111	152.4
	12	151.2	32	152.6	52	142.0	72	145.1	92	151.9	112	155.8
	13	153.0	33	151.4	53	159.7	73	162.9	93	145.7	113	158.0
	14	153.7	34	161.9	54	140.6	74	152.9	94	145.9	114	148.6
	15	151.1	35	146.6	55	137.9	75	155.1	95	138.9	115	147.9
	16	139.4	36	162.5	56	158.9	76	148.3	96	145.4	116	141.8
	17	149.2	37	129.6	57	141.1	77	144.8	97	165.6	117	158.7
	18	141.0	38	150.9	58	139.7	78	152.7	98	155.9	118	166.2
	19	141.4	39	141.3	59	149.9	79	147.7	99	150.3	119	137.8
	20	136.9	40	154.7	60	145.3	80	157.5	100	146.2	120	143.1
	Avg BWT	149.0		150.5		146.4		150.0		149.7		151.0

At Termination

Group No.	2D1		2A2		2B3		2C4		2D5		2E6	
	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight	Animal No.	Body Weight
	11	238.9	31	209.7	51	203.2	71	192.7	91	198.9	111	191.1
	12	215.5	32	217.0	52	190.1	72	196.7	92	206.9	112	219.2
	13	218.3	33	203.7	53	232.9	73	215.8	93	203.0	113	206.5
	14	205.2	34	214.4	54	183.5	74	227.2	94	210.8	114	213.4
	15	223.3	35	217.5	55	200.0	75	213.2	95	196.4	115	195.0
	16	191.4	36	222.8	56	211.4	76	209.5	96	205.0	116	200.9
	17	217.0	37	189.3	57	199.5	77	223.6	97	224.7	117	218.5
	18	206.2	38	219.5	58	206.0	78	214.2	98	219.0	118	211.3
	19	207.5	39	217.7	59	189.7	79	204.4	99	208.7	119	189.8
	20	191.7	40	219.8	60	211.9	80	218.0	100	203.2	120	195.2
	Avg BWT	211.4		213.1		202.8		211.5		207.7		204.9
	Wt GAIN	62.4		62.6		56.4		61.5		58.0		53.9
	%			+0.32		-9.61		-1.44		-7.05		-13.62

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TABLE 3

Clinical Signs, Gross Observations and Pathology Male Rats

Dose%	Mortality	Weight Gain In Grams	Clinical Signs	Gross Observations	Pathology
Control	0/10		Animals appeared normal	Mandibular lymph nodes enlarged 1/10	Mandibular lymph nodes Hemorrhage 5/10
				Dark (red or purple) 3/10	
				Thymus - red foci 2/10	Thymic lymph node Hemorrhage 1/10
				Thymic lymph node Enlarged 1/10	Heart Focal chronic inflammation 2/10
				Cervical lymph node Red 1/10	Multifocal chronic inflammation 3/10
				U. Bladder Pale soft plug material 1/10	Lung Chronic murine inflammation 10/10
				Kidney Blotchy surface 1/10	Cervical lymph node Hemorrhage 2/10
				Lung Pale to dark areas 3/10	Seminal Vesicles Focal acute seminal vesiculitis 1/10
				Liver Enlarged rounded margins and dark red areas 1/10	Liver Congestion 1/10
5.0%	0/10			Lung Small firm gray nodule 3/10	Lung Chronic murine pneumonia 10/10

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TABLE 3-continued

Clinical Signs, Gross Observations and Pathology — Male Rats

Dose %	Mortality	Weight Gain In Grams	Clinical Signs	Gross Observations	Pathology
			1/10 tissue mass left axilla	mottled 1/10 dark foci 3/10	Cecum Congestion 1/10
			(first noted at week 8)	Cecum: walls reddened 2/10	Hemorrhage 2/10 Edema 1/10
			1/10 left eye cloudy and small	Mandibular lymph node Red 1.10	Mandibular lymph node Hemorrhage 1/10
				Slightly enlarged 1/10 Thymus: red foci 1/10	Mammary Gland Fibroadenoma 1/10
				Subcutaneous mass 1/10	Heart 1/10 Multifocal chronic inflammation
					Eye Unilateral retinal atrophy
0.31	0/10		10/10 Appeared Normal	Lung Dark red areas 3/10	
				Mandibular lymph Nodes Dark red 2/10	
				Cecum red Area 1/10 (injection site)	
				No gross lesion 6/10	
0.63	0/10		10/10 Appeared Normal	Lung: mottled 6/10	
				Spleen: Surface appeared rough 1/10	

TABLE 3 - continued

976-614
Clinical Signs, Gross Observations and Pathology Male Rats

Dose %	Mortality	Weight Gain in Grams	Clinical Signs	Gross Observations	Pathology
				Mandibular lymph node dark 3/10 Enlarged 4/10	
				Epididimal fat pad has a yellow tissue. 1/10	
				Duodenum : Sirucosal surface granular 1/10	
				Cecum: Red injection site 1/10	
				Peribronchial lymph node: Enlarged 1/10	
				No gross lesion 1/10	
1.25	0/10		10/10 Appeared normal	Mandibular lymph node: Dark 2/10	
				Enlarged 4/10 Lung: red areas 3/10	
				white foci 1/10 Thymus: red foci 2/10	
				Thymic lymph nodes red 1/10 no gross lesions	

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TABLE 3

Clinical Signs, Gross Observations and Pathology

Male Rats

Dose %	Mortality	Weight Gain in Grams	Clinical Signs	Gross Observations	Pathology
2.5	0/10		1/10 stain on right front leg 1/10 ears red	Thymus: Red foci 1/10	
				Lung: red areas 3/10 white foci 1/10	
				Mandibular lymph nodes Enlarged 1/10	
				U. Bladder: Contains small white material 3/10	
				Cecum: Red area injection site 1/10	

TABLE 3 a

976-614
Clinical Signs, Gross Observations and Pathology Female Rats

Dose	Mortality	Weight Gain in Grams	Clinical Signs	Gross Observations	Pathology
Control	0/10		1/10 Alopecia on right front paw	Mandibular lymph node enlarged 2/10	Lung Chronic murine pneumonia 1C
				dark 6/10	Mandibular lymph node Hemorrhage 5/10
				Pituitary slightly enlarged 1/10	Cervical lymph node
				Ovary Watery cyst 1/10	Hemorrhage 3/10
				Thymic lymph node red 1/10	Heart 1/10 Multifocal chronic inflamma
				Liver Diaphragmatic hernia 1/10	
				Cecum Red area 1/10	
				Front paw Alopecia 1/10	
				No gross observations 3/10	

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TABLE 3 a - continued

Clinical Signs, Gross Observations and Pathology

Female Rats

Dose	Mortality	Weight Gain in Grams	Clinical Signs	Gross Observations	Pathology
5.0%	0/10		1/10 wheezing	Thymus Red foci 1/10	Lung Chronic murine pneumonia 10/10
				Cecum Red area 3/10	
				Uterus Distended with clear fluid 1/10	
				No gross observation 6/10	

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TABLE 3 a

Clinical Sigsn, Gross Observations and Pathology

Female Rats

Dose %	Mortality	Weight Gain in Grams	Clinical Signs	Gross Observations	Pathology
0.31	0/10			no gross lesion 2/10	
			1/10 left eye cloudy and small	Mandibular lymph nodes Appear dark 2/10	
				Cecum: Red Injection site 2/10	
				Uterine horns Distended with clear fluid	
				Renal lymph node Slightly enlarged 1/10	
				Liver: small section protuding through diaphragm	

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TABLE 3 a

Clinical Signs, Gross Observations and Pathology

Female Rats

Dose %	Mortality	Weight Gain in Grams	Clinical Signs	Gross Observations	Pathology
				Left Ovary: Watery Cyst 1/10	
				left eye cirudy and small 1/10	
				no gross lesion 3/10	
0.62%	0/10		3/10 Areas of alopecia	Lung: gray foci all lobes 3/10	
				dark area 2/10	
				uterus: distended with clear fluid 1/10	
				Cecum: red area injection site 1/10	
				no gross lesion 4/10	
1.25	0/10		1/10 left eye lacrimated	Cecum: red area injection site 4/10	
			1/10 alopecia on head	Uterus: Distended with clear fluid 1/10	
				Liver: Small section protuding through diaphragm 1/10	
				U. Bladder: contains white material 1/10	
				Lung: Red foci 1/10	
				No gross lesion 5/10	
2.5	0/10		10/10 appeared normal	Lung: red areas 1/10	
				pale areas 1/10	

TABLE 3 a

976-614
Clinical Signs, Gross Observations and Pathology Female Rats

Dose %	Mortality	Weight Gain in Grams	Clinical Signs	Gross Observations	Pathology
				Cecum: Red area injection site 1/10	
				Uterus: Distended with fluid 2/10	
				Thymus: Red foci 1/10	
				Peribronchial lymph nodes Enlarged 1/10	
				No gross lesion 5/10	

KEY TO DETAILED HISTOPATHOLOGY INCIDENCE TABLE

TYPE OF FINDING

0 = Tissue Absent

X = Tissue Examined and Not Remarkable

A = Autolysis

P = Finding Present

GRADING OR DEGREE OF FINDING

1 = Minimal

2 = Slight

3 = Moderate

4 = Moderately Severe

5 = Severe

TABLE 4

RATS - MALES

INDIVIDUAL PATHOLOGY FINDINGS

ORGANS	R2-201M Control										R2-2E6M 5.0%									
	01	02	03	04	05	06	07	08	09	10	101	102	103	104	105	106	107	108	109	110
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PITUITARY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ADRENAL	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HEART:																				
Focal chronic inflammation	P	X			X	X	X	P		X	X	X	X	X	X	X	X		X	X
Multifocal chronic inflammation			P	P					P									P		
LUNG:																				
Chronic murine pneumonia	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
SPLEEN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LIVER																				
Congestion	X	X	X	X	X	X	X	X	X	P	X	X	X	X	X	X	X	X	X	X
KIDNEY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
STOMACH	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SMALL INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CECUM:																				
~~Congestion~~																				
Hemorrhage														P						
Edema														P	P					
															P					
LARGE INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PANCREAS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TESTES	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PROSTATE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SEMINAL VESCILES:																				
Focal acute seminal vesiculitis					P															
SALIVARY GLAND	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

RATS - MALES

INDIVIDUAL PATHOLOGY FINDINGS

ORGANS	R2-201M Control										R2-2E6M 5%									
	01	02	03	04	05	06	07	08	09	10	101	102	103	104	105	106	107	108	109	110
MESENTERIC LYMPH NODE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MANDIBULAR LYMPH NODE: Hemorrhage	P	P	P	P	P	X	X	X	X	X	X	X	X	X	X	X	P	X	X	X
THYMIC LYMPH NODE: Hemorrhage	P																			
CERVICAL LYMPH NODE: Hemorrhage	X	X	X		P	P	X	X	X	X	X	X	X	X	X	X	X	X	X	X
URINARY BLADDER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EYE: Unilateral retinal atrophy																			P	
BONE MARROW	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MAMMARY GLAND: Fibroadenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	P	X	X
PARATHYROID	X	X	X	X	X	0	X	X	X	0	X	X	X	X	X	X	0	0	X	X
THYMUS	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X
TRACHEA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ESOPHAGUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

TABLE 4a

RATS - FEMALES

INDIVIDUAL PATHOLOGY FINDINGS

ORGANS	R2-201F Control										R2-2E6F 5.0%									
	11	12	13	14	15	16	17	18	19	20	111	112	113	114	115	116	117	118	119	120
BRAIN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PITUITARY	X	X	X	X	X	X	O	X	X	X	X	X	X	X	X	X	X	X	X	O
THYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	O	X
ADRENAL	X	X	X	X	X	X	X	X	O	X	X	X	X	X	X	X	X	X	X	X
HEART	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Multifocal chronic inflammation			P																	
LUNG																				
Chronic murine pneumonia	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
SPLEEN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LIVER	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
KIDNEY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
STOMACH	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SMALL INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CECUM																				
Hemorrhage																		P	X	
LARGE INTESTINE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PANCREAS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
OVARY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
UTERUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SALIVARY GLAND	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MESENTERIC LYMPH NODE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
MANDIBULAR LYMPH NODE	X					X		X	X	X	X	X	X	X	X	X	X	X	X	X
Hemorrhage		P	P	P	P		P													

TABLE 4a

RATS - FEMALES

INDIVIDUAL PATHOLOGY FINDINGS

ORGANS	R2-201F Control										R2-2E6F 5.0%									
	11	12	13	14	15	16	17	18	19	20	11	12	13	14	15	16	17	18	19	20
CERVICAL LYMPH NODE: Hemorrhage	X		X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
URINARY BLADDER		P			P		P													
BONE MARROW	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X
MAMMARY GLAND	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PARATHYROID	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X
THYMUS	X	X	X	X	0	X	0	0	X	X	X	X	0	X	X	0	X	X	0	X
TRACHEA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ESOPHAGUS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X																		
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

HAZLETON
LABORATORIES AMERICA, INC.

Decabromodiphenyl Oxide Requirements

Dose Level	No. of Animals male	No. of Animals female	kg diet mix/week	comp/1 kg gm	comp/1 week gm	kg comp/103 wk
rat						
5.0%	50	50	25	50	1250	129
2.5%	50	50	25	25	625	64.53
					Total	193.53
mice						
5.0%	50	50	5	50	250	25.75
2.5%	50	50	5	25	125	12.87
					Total	38.62
					Rats & Mice total comp.	232.15 kg

ompound received fter Prechronic Studies	15.558 kg 5.480
ompound to be ordered	226.67 kg

Submitted by Henry A. Rutter Jr.
HENRY A. RUTTER, Jr., Ph.D.
Principal Investigator

Microbiological Associates
8221 RIVER ROAD • BETHESDA, MARYLAND 20816
TELEPHONE: (301) 654-3400

DIAGNOSITC SERVICES TEST REPORT

Our Code A 4326
Contract N01-CM-67069 SM

TO: Henry Rutter
Hazelton Labs
9200 Leesburg Pike
Vienna, VA

FROM: Michael J. Collins, Jr., Ph.D. (MC)
DATE: June 11, 1979
TEST: Murine virus antibody determination
SPECIMEN: 10 rat and 9 mouse sera
RECEIVED: June 1, 1979

Sample #	Hemagglutination Inhibition								Complement Fixation				
	PVM	Reo3	GDVII	Poly	MVM	KRV	H-1	Ectro	Send	M.Ad	MHV	LCM	RCV
71616-71620	-					-	-		≥80				-
71626-71630	-					-	-		≥80				-
976-614001	-	-	-	-	-			-	-	-	10	-	
976-614002	-	-	-	-	-			-	-	-	AC	-	
976-614003	-	-	-	-	-			-	-	-	AC	-	
976-614004	-	-	-	-	-			-	-	-	10	-	
976-614008	-	-	-	-	-			-	20	AC	AC	-	
976-614012	-	-	-	-	-			-	-	-	-	-	
976-614014	-	-	-	-	-			-	-	-	-	-	
976-614019	-	-	-	-	-			-	-	-	-	-	
976-614020	-	320	-	-	-			-	-	-	-	-	
Significant Titer	20	20	20	20	20	20	20	20	10	10	10	10	10

AC = Anticomplementary serum

LABORATORIES AMERICA, INC.
8203 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

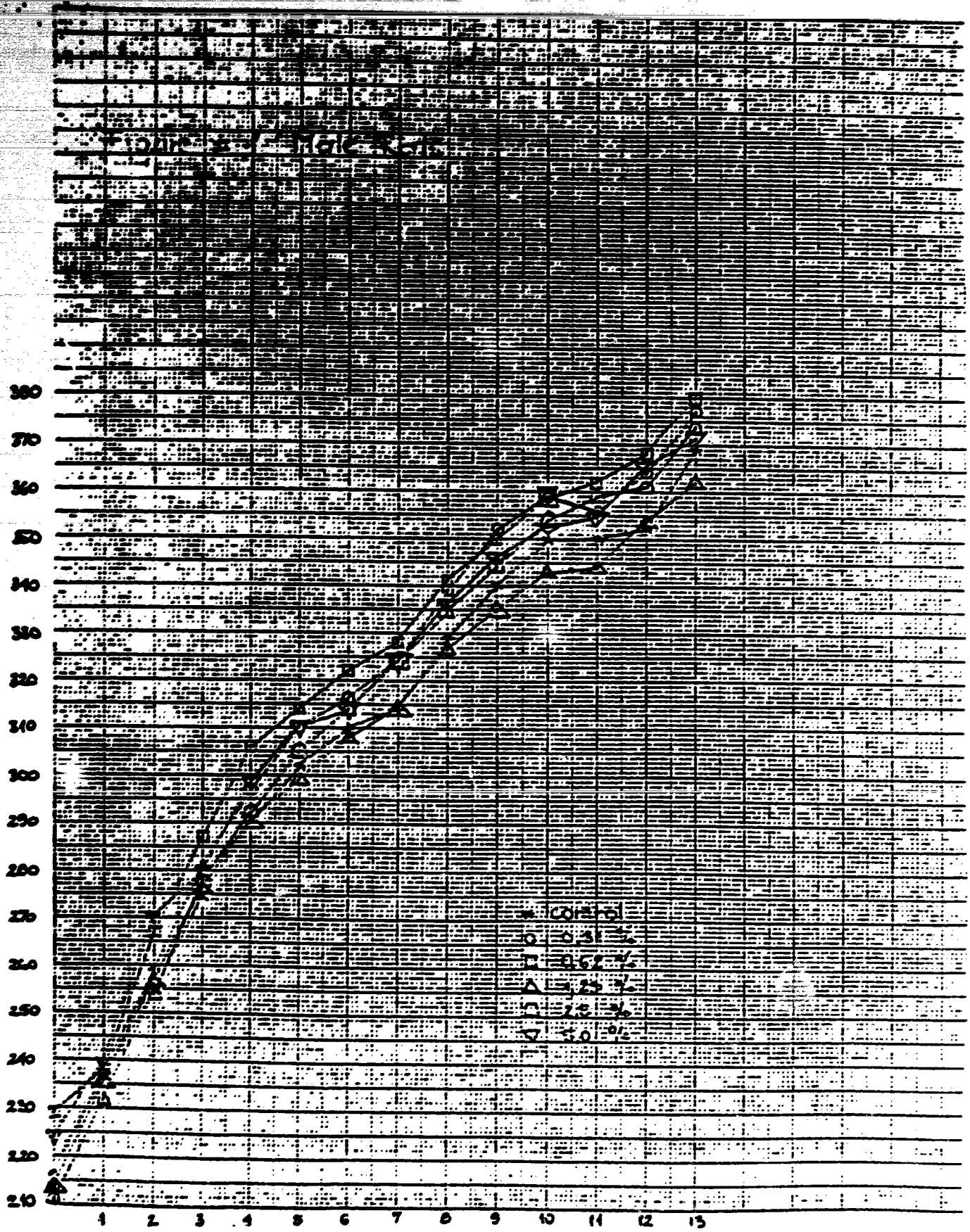
Rodent Laboratory Chow®
5001

Guaranteed Analysis/ Ingredients

Crude protein, min.	23.0%
Crude fat, min.	4.5%
Crude fiber, max.	6.0%
Ash, max.	8.0%
Added minerals, max.	2.5%

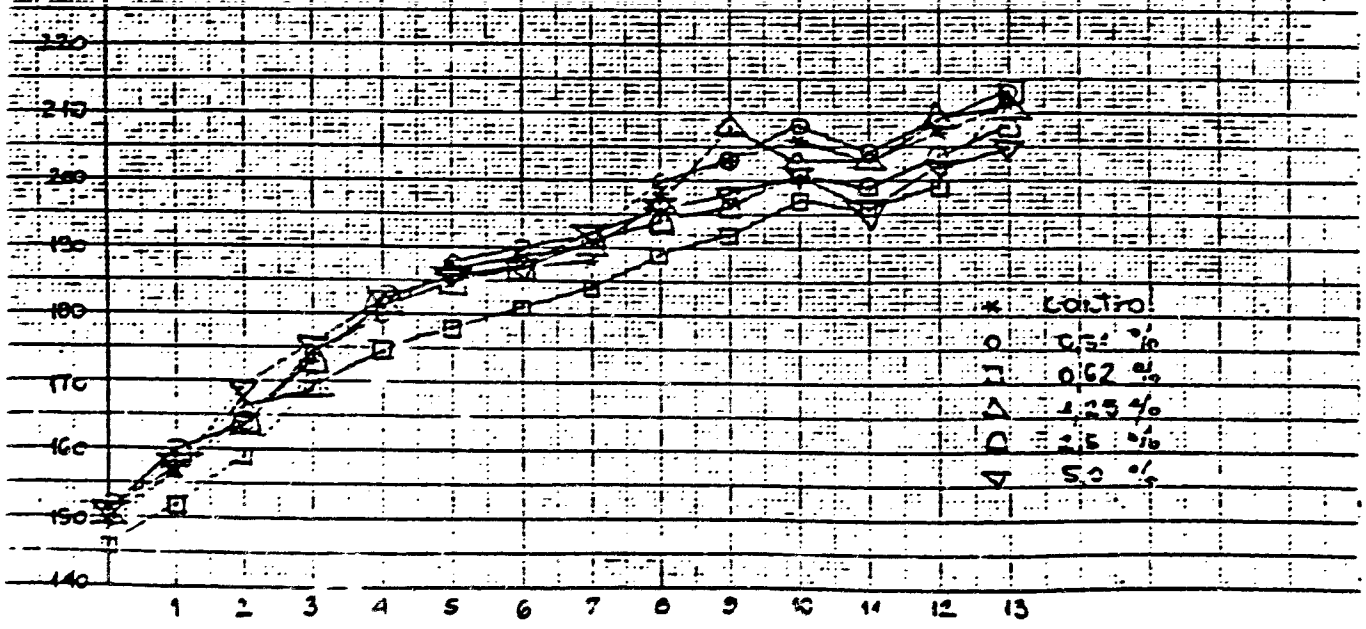
Ground extruded corn, soybean meal, ground oat groats, dried beet pulp, wheat germ meal, fish meal, brewers' dried yeast, dehydrated alfalfa meal, cane molasses, dried milk products, meat and bone meal, wheat middlings, animal fat preserved with BHA, calcium carbonate, dicalcium phosphate, salt, animal liver meal, calcium iodate, vitamin B_{12} supplement, methionine hydroxy analogue calcium, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, thiamin, niacin, pyridoxine hydrochloride, ferrous sulfate, vitamin A supplement, D activated animal sterol, vitamin E supplement, iron oxide, manganous oxide, cobalt carbonate, copper oxide, zinc oxide.

PHONE (703) 893-5400, TELEX 899436 (HAZLABS VINA), CABLE HAZLABS WASH DC


380
370
360
350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
1
2
3
4
5
6
7
8
9
10
11
12
13
control
0,31 %
0,62 %
1,25 %
2,5 %
5,0 %

0 0 , 3 2

Figure # 1a Tensile Rots



ANALYSIS OF DOSAGE MIXTURE

Subcontractor Name Project 976-605 Date 3/3/79

Chemical Name Decabromodiphenyl oxide

Vehicle (or Feed) Feed

1. Methods:

   a. Method of sample selection: Arbitrary by analyst

   b. Detailed description of extraction or other workup procedure and analytical method(s) used:
   See addendum attached

   c. Storage temperature of mixture before analysis: -20°C

2. Results: (continued)

Sample	Data Mixed	Data Analyzed	Theor. Conc.	Concentration Found #1	#2	Average	Corrected
Group 1 (Control)	2/6/79	2/22/79	0.00%	0.00%	0.00%	0.00%	0.00%
Group 2 (Top left)	2/6/79	2/22/79	0.31%	0.31%	0.29%	0.30%	0.285 (7)%
Group #2 (Top right)	2/6/79	2/22/79	0.31%	0.28%	0.31%	0.30%	0.285 %
Group #2 (Bottom)	2/6/79	2/22/79	0.31%	0.33%	0.33%	0.33%	0.314 %
Ref.			(continued on attached)				

3. Conclusions:
Group 3 and Group 4 were not within the ± 10% limit, and will be remixed and reanalyzed.

4. Actions taken as a result:
Another mixture of group 3 was prepared for reanalyses, and is included as a separate report. The results from Group 4 will follow at a [illegible] date.

[illegible]
Signature of Responsible Chemist

HLA Notebook No.
2/79 (rev.)

ANALYSIS OF DOSAGE MIXTURE

Subcontractor Name Project 976-605 Date 3/3/79

Chemical Name Decabromodiphenyl oxide C#

Vehicle (or Feed) Feed

1. Methods:

   a. Method of sample selection: same

   b. Detailed description of extraction or other workup procedure and analytical method(s) used:

   c. Storage temperature of mixture before analysis: -20°C

2. Results: (continued)

Sample	Data Mixed	Data Analyzed	Theor. Conc.	Concentration Found #1	#2	Average	Corrected
Group #3	2/6/79	2/22/79	0.63%	0.57%	0.51%	0.540%	0.544%
Group #4	2/6/79	2/22/79	1.25%	1.40%	1.34%	1.370%	1.413%
Group #5	2/6/79	2/22/79	2.50%	2.68%	2.65%	2.665%	2.759%
Group #6 (Top 4 of 6)	2/6/79	2/22/79	5.00%	5.45%	5.40%	5.425%	5.298%
Ref.							

3. Conclusions:

4. Actions taken as a result:

[signature]
Signature of Responsible Chemist

HLA Notebook No.
2/79 (rev.)

ANALYSIS OF DOSAGE MIXTURE

Subcontractor Name Project 97[illegible]-665 Date 3/[illegible]/79

Chemical Name Dextromethorphenyl oxide C# _____

Vehicle (or Feed) Feed

1. Methods:

   a. Method of sample selection: Same

   b. Detailed description of extraction or other workup procedure and analytical method(s) used:

   c. Storage temperature of mixture before analysis: -20°C

2. Results: (continued)

Sample	Data Mixed	Data Analyzed	Theor. Conc.	Concentration Found #1	#2	Average	Corrected
Group 6 (Top right)	2/6/79	2/22/79	5.00%	5.30%	5.45%	5.375%	5.249%
Group 6 (Bottom)	2/6/79	2/22/79	5.00%	5.40%	5.25%	5.325%	5.200%
Ref.							

3. Conclusions:

4. Actions taken as a result:

[signature]
Signature of Responsible Chemist

HLA Notebook No.
2/79 (rev.)

ANALYSIS OF DOSAGE MIXTURE

Subcontractor Name Project 976-605 Date 3/1/79

Chemical Name Dec bromodiphenyl oxide C#

Vehicle (or Feed) Feed

1. Methods:

   a. Method of sample selection: Same

   b. Detailed description of extraction or other workup procedure and analytical method(s) used:

   c. Storage temperature of mixture before analysis: -20°C

2. Results:

Ref.	Sample	Data Mixed	Data Analyzed	Theor. Conc. (Ng)	Concentration Found #1 (Ng)	#2 (Ng)	#3 (Ng)	Average (Ng)	Corrected (% Recovery)
/Control 0%	1	3/1/79	3/1/79	0.00	0.00	0.00	0.00	0.00	100%
0.31%	2	3/1/79	3/1/79	1616.67	1800	1700	1600	1700	105 %
0.63%	3	3/1/79	3/1/79	3150	3125	3125	—	3125	99.2 %
1.25%	4	3/1/79	3/1/79	6318.3	6100	6000	6200	6100	96.6 %
5.00%	5	3/1/79	3/1/79	25,150	26,750	25,500	25,000	25,750	102.4 %

3. Conclusions:
All the recoveries were ± 5% and were used to correct all the feed samples.

4. Actions taken as a result:

[signature]
Signature of Responsible Chemist

HLA Notebook No.
2/79 (rev.)

ANALYSIS OF DOSAGE MIXTURE

Subcontractor Name Dosage 976-605 Date 3/7/79

Chemical Name Dicarbamodiphenyl oxide C# 55297

Vehicle (or Feed) Feed LH 191028 LOT-012477 Baten-02

1. Methods:

   a. Method of sample selection: Arbitrary by analyst

   b. Detailed description of extraction or other workup procedure and analytical method(s) used:
   same as reported on 3/2/79

   c. Storage temperature of mixture before analysis: -20°C

2. Results: 90 Day subchronic toxicity ... rats
   weeks on study – Day 0 and week 2

Sample	Date Mixed	Date Analyzed	Theor. Conc.	Concentration Found #1	#2	Average	Corrected *
sup 6-(1)	2/27/79	3/7/79	1.25%	1.16	1.16	1.16	1.25%
sup 4-(2)	2/27/79	3/7/79	1.25%	1.16	1.16	1.16	1.25%
3							
4							
5							
Tubing Ref. 1.25%	3/7/79	3/7/79	1.25%	1.16	1.16	1.16%	93% recovery

3. Conclusions: * - Corrected for 93% recovery from fortification.
   The remix group 4 samples were right on target.

4. Actions taken as a result:

[signature]
Signature of Responsible Chemist

HLA Notebook No.
2/79 (rev.)

March 2, 1979

Project 976-605

1. Methods

(b) Detailed description of extraction or other workup procedure and analytical method used:

A 5.00 gm sample in a 50 ml test tube was extracted with 40 ml of tetrahydrofuran for 10 minutes on a horizontal shaker. The sample was centrifuged at 2500 rpm for 15 minutes, and the supernatant transferred to a 125 ml erlenmeyer flask. The feed residue was reextracted again with 40 ml of tetrahydrofuran. The combined extracts were filtered through Whatman #1 filter paper into a 100 ml vilumetric flask. The solutions were brought up to volume with tetrahydrofuran. Dilutions from 1:2 to 1:10 were made in order to inject 10 µl aliquots on the high pressure liquid chromotograph under the following conditions:

Instrument:	Waters - Model 6000A high pressure liquid chromotograph with absorbance detector model 440 and WISP sample processor model 710A equipped with a Houston Instrument recorder.
Column:	µ Bondapak C18 300 mm x ~~25~~ 3.9 mm
Mobile phase:	10% water: 90% acetonitrite
Flow rate:	1.0 ml/min.
Detector:	Ultraviolet 254 nm
Chart speed:	0.5in/min
Attentuation:	0.1
Retention time:	19.5 min for major peak

All feed samples are processed in duplicate, including control feed. Samples were quantitated against a standard curve prepared by injecting known amounts of decabromodiphenyl oxide. A series of spiked samples were processed simultaneously with the feed samples to adjust for average recovery losses.

ANALYSIS OF DOSAGE MIXTURE

Subcontractor Name Project 976-605 Date [illegible]

Chemical Name Decabromodiphenyl oxide C#

Vehicle (or Feed) Feed

1. Methods:

   a. Method of sample selection: Relating to analyst

   b. Detailed description of extraction or other workup procedure and analytical method(s) used:

   c. Storage temperature of mixture before analysis: -20°C

2. Results:

Sample	Date Mixed	Date Analyzed	Theor. Conc.	Concentration Found #1	#2	Average	Corrected
Group 3 - 1	2/27/79	3/1/79	0.63%	0.668%	0.700%	0.684%	0.689(5)%
Group 3 - 2	2/27/79	3/1/79	0.63%	0.680%	0.600%	0.640%	0.645 %
3							
4							
5							
Ref.	3/1/79	3/1/79	0.63%	0.625%	0.625%	0.625%	0.630 (99.2% recovery)

3. Conclusions: The repeated samples were within the ± 10% limit, therefore the mixtures were correctly prepared.

4. Actions taken as a result:
None

[illegible]
Signature of Responsible Chemist

HLA Notebook No.
2/79 (rev.)

AMTEK CORP.

CERTIFICATE OF AUTHENTICITY

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Data produced 7 8 97 Marcia Rubolino

(Month) (Day) (Year) Camera Operator

Place Syracuse New York

(City) (State)