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DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity in Mice

TOX. CHEM. NO.: 573S

ACCESSION NUMBER: 263756

MRID NO.: ?

TEST MATERIAL: INM-6316

SYNONYMS: Harmony

STUDY NUMBER(S): HLR 685-85

SPONSOR: Dupont de Nemours

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine, EI duPont de Nemours and Co. Newark, Del.

TITLE OF REPORT: Long term feeding study in mice with INM-6316

AUTHOR(S): J.C. Summers

REPORT ISSUED: 6/26/85

CONCLUSIONS: At terminal sacrifice there was a significant drop in body weight at the 750 and 7500 ppm dose groups. Based on these effects, the NOEL = 25 ppm, and the LEL = 750 ppm.

No individual pathology sheets for each animal accompanied this submission, and these will need to be evaluated before a final conclusion can be reached concerning this study. There the classification is core-supplementary.

Special Review Criteria (40 CFR 154.7)

A. MATERIALS:

1. Test compound: INM-6316, Description - not given,
Batch #15,172-01, Purity 95.6%, contaminants: list in CBI appendix
#14,172-02, " 98.0%

2. Test animals: Species: Mice, Strain:Crl:CD-1(ICR)BR
, Age: weanlings
Weight: 18-25 gms, males Source: Charles River Breeding Labs
15-24 gms, females Kingston, N.Y.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 18 months male	female
1 Cont.	0	80	80
2 Low (LDT)	25	80	80
3 Mid (MDT)	750	80	80
4 High(HDT)	7500	80	80

2. Diet preparation

Diet was prepared weekly and stored at refrigerator temperature. Samples of treated food were analyzed for stability and homogeneity at days -1, 20, 209, 232, 364 and 546. Stability samples included freshly prepared test diet, fresh diet stored at room temperature for 24 hours and 10 days, and fresh diet stored refrigerated for 10 days. Homogeneity samples were collected at 3 levels (top, middle and bottom) of the mixing vessel.
Diet was originally made in a mixer, but after 20 days on test and visual inspection indicated that particles of test compound could be seen in the diet, the test material was suspended in corn oil.
Results - Analysis of diet:
The data indicate little variability in homogeneity and stability of the diet. The data indicated that the compound was not as stable in the diet at room temperature for 10 days. Variability was greatest in the low dose groups. However, the highest variability was 12% from frozen samples. These data are appended on page 2 of the appendix.

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3. Animals received food and water ad libitum.

4. Statistics - Statistical analyses of the data are appended on page 1.

5. Quality assurance statement was signed and numerous inspections were documented.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of abnormal behavior and appearance, moribundity and mortality.

Toxicity/Mortality (survival)
1. Toxicity: There did not appear to be any compound-related increases in clinical observations.
2. Mortality: There was no treatment-associated increase in mortality during the 18-month study. A table of the mortality data is on appended page 11. Survival curves are on appended pages 9 and 10.

2. Body weight

Animals were weighed weekly for the first six months, then every other week until study completion.

Results: Male and female body weights were not significantly different from controls, although there were sporadic increases and decreases seen in all treated groups relative to controls throughout the study. However, concerning body weight gains high dose females showed a decreased overall weight gain for the 0-547 days combined, as well as 182-365 days combined. ($p > 0.05$) Data for females are on appended page 3 and males are on appended page 4. Body weight curves are on appended pages 7 and 8. Females that were sacrificed at 187 ~~months~~ weeks for organ weights showed a significant drop at the mid and high dose. See appended page 12.

3. Food consumption and compound intake

Consumption was determined weekly and mean daily diet consumption was calculated. Group mean food efficiency and compound intake were calculated from the consumption and body weight gain data.

Food consumption/Food Efficiency/Compound

No compound-related changes were seen in food consumption or food efficiency. Compound intake data for both males and females are on appended pages 5 and 6.

4. Ophthalmological examinations- were not performed.

5. Blood was collected before treatment and at 3,6,9, 12, and 18 months for hematology and clinical analysis from 10 animals/group. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte counts were taken but not evaluated
	Blood Clotting Measurements (Thromboplastin time) (Clotting time) (Prothrombin time)		

* Required for subchronic and chronic studies

For the hematological findings, the statistical summary tables were written in a way that is extremely confusing. This is also true of table 7, where only p values are given. The (+) is not well defined. However, some groups have a (+) sign while others don't. The legend should clarity this problem. Also it would have been more readable it tables 1 and 2 were combined with tables 3,4,5 and 6. There did not appear to be any treatment related effects on hematological parameters.

b. Clinical Chemistry - was not evaluated.

6. Urinalysis - was not evaluated.

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7. Sacrifice and Pathology -
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed. Sacrifice was by chloroform anaesthesia and exanguination.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	.Aorta*	XX	.Brain*†
X	.Salivary glands*	XX	.Heart*	X	Periph. nerve*#
X	.Esophagus*	X	.Bone marrow*	X	Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*	X	.Pituitary*
X	.Duodenum*	XX	.Spleen*	X	Eyes (optic n.)*#
X	.Jejunum*	X	Thymus*		Glandular
X	.Ileum*		Urogenital		.Adrenals*
X	.Cecum*	XX	.Kidneys*†		Lacrimal gland#
X	.Colon*	X	.Urinary bladder*	X	Mammary gland*#
X	.Rectum*	XX	.Testes*†	X	.Parathyroids*††
XX	.Liver*†	X	Epididymides	X	.Thyroids*††
X	Gall bladder*#	X	Prostate		Other
X	.Pancreas*	X	Seminal vesicle	X	Bone*#
	Respiratory	X	Ovaries*†	X	Skeletal muscle*#
X	.Trachea*	X	.Uterus*	X	Skin*#
X	.Lung*	X	Vagina	X	All gross lesions and masses*
X	Nose°			X	Harderian gland
	Pharynx°				
	Larynx°				

All tissues were examined from control and high dose groups at termination of the study, and in all mice that died before the end of the study.

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

Liver (with gall bladder) kidneys, lungs and gross lesions were examined in low and mid dose groups. Bone marrow smears were prepared for all mice at final sacrifice, but were not evaluated.

a. Organ weight

There were no changes in absolute organ weights associated with compound administration. There was a statistically significant drop in body weight in the females. This drop is illustrated on appended page 12. As a result of the decreased body weight there was an increase in mean relative organ weight in the mid and high dose groups in brain-to-body weight ratios. However, this appears to be a phenomen only associated with the decreased body weight, and not a toxicological effect to the brain of the compound.

b. Gross pathology

There were no treatment-related gross lesions. Gross lesions evident were found in both control and experimental animals with similar frequency.

c. Microscopic pathology

There were no treatment-related changes in microscopic pathology evident from the summary tables. However, no individual pathology sheets accompanied the text submission, so final judgment concerning the pathological changes in the study await receipt of the individual pathology sheets.

D. DISCUSSION:

There was a decrease in terminal body weights evident in the female animals on test in the mid and high doses. There were enough animals (55 and 54 respectively) in each dose group to indicate that the effect was probably a toxicological consequence of treatment with the compound. Therefore, the NOEL will be set at 25 ppm, and the LOEL = 750 ppm.
Since individual pathology data were not submitted with this study, the gross and histopathological data cannot be adequately assessed. Therefore, the firm will need to be requested to submit the raw data for our analysis. In the meantime, the study will be considered core supplementary.

Appended p8.1

I. Statistical Analyses

Body weights, body weight gains, absolute and relative organ weights, and clinical laboratory measurements were analyzed by a one-way analysis of variance. When the test for differences among group means (F-test) was significant, pairwise comparisons were made between control and test groups. For body weights and weight gains, these comparisons were made with the least significant difference (LSD) test. The clinical laboratory measurements were compared by Kruskal-Wallis, Mann-Whitney U, and Dunnett's tests. Bartlett's test for homogeneity of variances was performed on organ weights and clinical laboratory measurements. Organ weights were examined for pairwise comparisons by both LSD and Dunnett's tests and by a test for linear trend. Tumor incidence and clinical signs were analyzed by Fisher's Exact test and the Cochran-Armitage test for trend. Clinical observations were analyzed by Fisher's Exact test. Tests for the comparison of means were considered significant at the $p < 0.05$ probability level.

Results and Discussion

A. Diet Analyses

The analytical methods and results of diet analyses are in Appendix B with the results summarized in Table 1.

After initiation of this study, visual inspection of the prepared diet for another study that utilized INM-6316 as the test material revealed that the compound may not have been uniformly mixing with the GCPLC, i.e. particles

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TABLE 1

SUMMARY OF DIETARY ANALYSES FOR INM-6316 DURING THE 18-MONTH FEEDING STUDY IN MICE

Nominal INM-6316 Dietary Concentration (ppm)	Measured INM-6316 Dietary Concentration (ppm)[a]				
Test Day[b]:	20	209	232	364	546
25	22+1 (88%)[c]	25+1 (100%)	27+2 (108%)	23+2 (92%)	25+1 (100%)
750	742+12 (99%)	757+25 (101%)	758+33 (101%)	703+30 (94%)	716+28 (95%)
7,500	7950+173 (106%)	7500+304 (100%)	7020+172 (94%)	6860+307 (91%)	7541+313 (101%)

[a] Mean (± SD) of all determinations (stability and/or homogeneity) for each dietary concentration at each sampling period. The results were not corrected for recovery which ranged from 84% to 112%.

[b] The results of diet samples collected at the initiation of this study (day -1) are not included in this table since the method of diet preparation was changed to include corn oil. The data presented in this table represents those diets prepared with corn oil.

[c] Number in parentheses represents the percent of nominal INM-6316 concentration.

Appendix B

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TABLE 5 (Continued)

MEAN BODY WEIGHT GAINS OF FEMALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 INM-6316

	MEAN BODY WEIGHT GAINS (g)			
GROUP:	II	IV	VI	VIII
CONCENTRATION(ppm):	0	25	750	7,500
DAYS ON TEST				
351-365	3.8	3.9	4.8	3.0
365-379	0.3	0.0	-0.1	0.6
379-393	0.3	0.3	0.3	0.3
393-407	-0.4	-0.3	0.1	0.0
407-421	0.3	0.6	0.2	0.1
421-435	0.9	0.8	0.4	0.4
435-449	-0.1	0.2	-0.2	0.1
449-463	0.4	0.0	0.4	0.3
463-477	0.4	0.4	0.0	0.3
477-491	-0.1	0.2	0.2	-0.2
491-505	0.8	0.6	0.8	0.8
505-519	-1.4	-0.8	-0.8	-0.7
519-533	0.7	-0.3*	0.4	0.4
533-547	0.7	0.7	0.3	0.2
0- 91	6.3	6.1	6.2	6.2
91-182	1.0	1.8	1.8	1.9
182-365	4.4	3.9	3.6	2.7*
365-547	2.7	2.5	1.9	1.9
0-547	14.9	14.5	13.6	12.9*

* Different from control at $p < 0.05$ level of significance.

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TABLE 4 (Continued)

MEAN BODY WEIGHT GAINS OF MALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 INM-6316

	MEAN BODY WEIGHT GAINS (g)			
GROUP:	I	III	V	VII
CONCENTRATION(ppm):	0	25	750	7,500
DAYS ON TEST				
351-365	5.9	6.1	5.6	4.6
365-379	0.5	0.7	0.4	0.3
379-393	0.1	-0.2	0.0	0.1
393-407	0.1	0.1	0.1	0.2
407-421	-0.2	-0.3	0.1	0.2
421-435	0.8	1.0	0.7	0.2*
435-449	0.3	0.0	0.2	0.3
449-463	0.1	-0.1	-0.2	0.0
463-477	-0.4	-0.1	0.0*	0.2*
477-491	-0.3	-0.8*	-0.1	-0.2
491-505	0.9	0.6	0.3*	0.4*
505-519	-1.7	-0.9*	-0.6*	-0.4*
519-533	-0.3	-0.6	-0.9	-0.8
533-547	0.5	0.5	0.7	0.6
0- 91	10.1	10.7	9.8	9.2
91-182	2.9	2.3	2.6	1.8
182-365	3.4	3.1	3.1	3.2
365-547	1.7	1.6	2.1	1.5
0-547	18.1	17.8	17.8	16.0

* Different from control at $p < 0.05$ level of significance.

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TABLE 10 (Continued)

MEAN DAILY INTAKE OF INM-6316 BY MALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 INM-6316

	MEAN DAILY INTAKE (mg INM-6316/kg BODY WT/day)			
GROUP:	I	III	V	VII
CONCENTRATION(ppm):	0	25	750	7,500
DAYS ON TEST				
351-365	0	3.6	101	835
365-379	0	2.4	104	814
379-393	0	2.6	83	828
393-407	0	2.7	84	894
407-421	0	2.6	82	817
421-435	0	2.7	80	844
435-449	0	2.7	78	830
449-463	0	2.8	83	851
463-477	0	2.7	81	835
477-491	0	2.8	84	858
491-505	0	2.7	78	839
505-519	0	2.6	76	769
519-533	0	2.4	76	814
533-547	0	2.7	80	811
0- 91	0	4.2	128	1307
91-182	0	3.4	104	1093
182-365	0	3.1	92	907
365-547	0	2.6	82	831
0-547	0	3.2	97	979

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Appended Page 6

TABLE 11 (Continued)

MEAN DAILY INTAKE OF INM-6316 BY FEMALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 INM-6316

MEAN DAILY INTAKE (mg INM-6316/kg BODY WT/day)

GROUP:	II	IV	VI	VIII
CONCENTRATION(ppm):	0	25	750	7,500
DAYS ON TEST				
351-365	0	4.2	126	1328
365-379	0	3.6	96	1130
379-393	0	3.5	101	1112
393-407	0	3.6	105	1152
407-421	0	3.6	105	1105
421-435	0	3.7	119	1238
435-449	0	3.5	107	1113
449-463	0	3.8	111	1147
463-477	0	3.5	109	1165
477-491	0	3.5	100	1169
491-505	0	3.4	93	1048
505-519	0	3.1	102	1012
519-533	0	3.2	98	959
533-547	0	3.3	100	995
0- 91	0	5.6	168	1641
91-182	0	4.9	147	1554
182-365	0	4.0	124	1235
365-547	0	3.5	104	1103
0-547	0	4.3	128	1312

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FIGURE 1
GROWTH CURVES OF MALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 ppm INM-6316

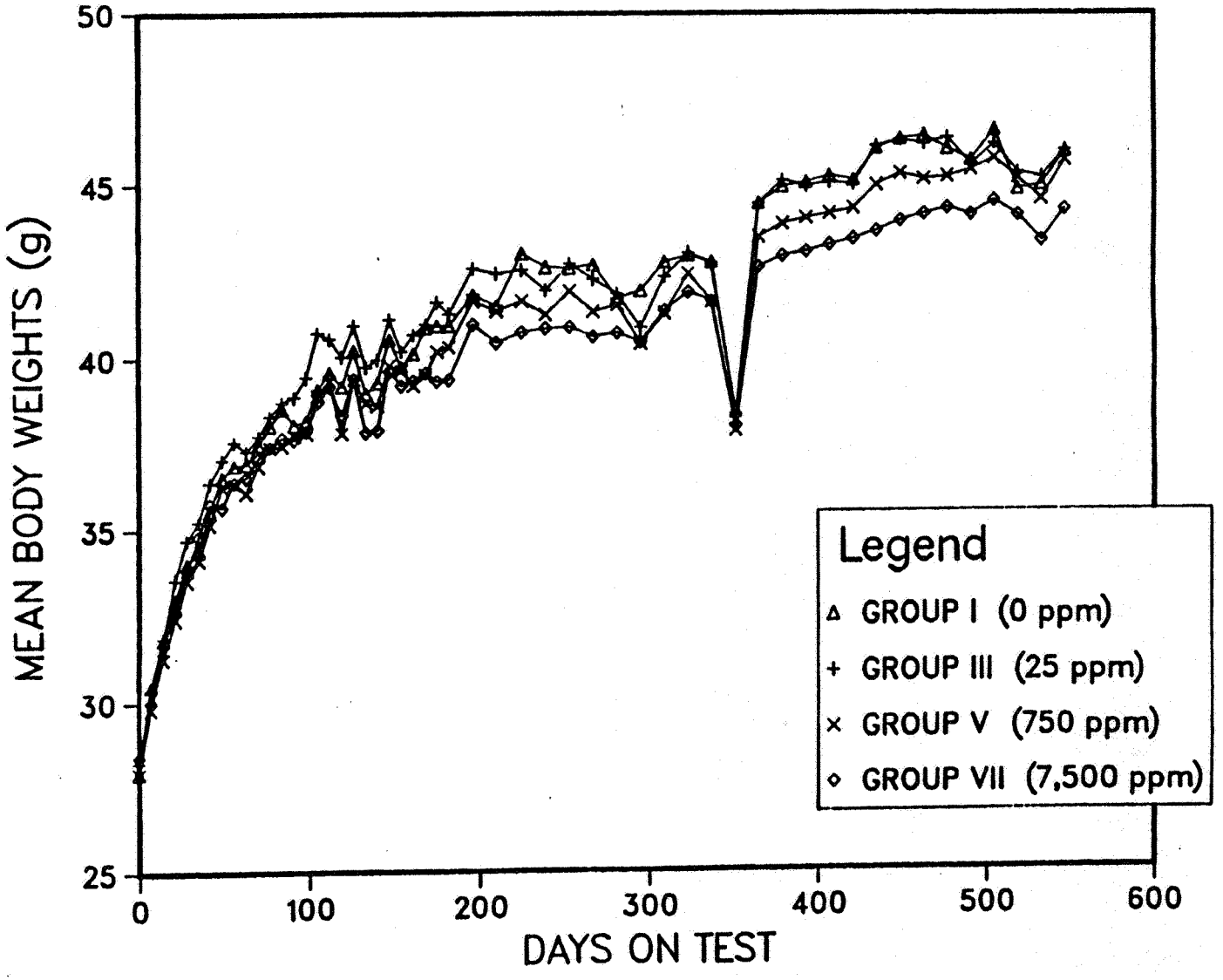

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FIGURE 2
GROWTH CURVES OF FEMALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 ppm INM-6316

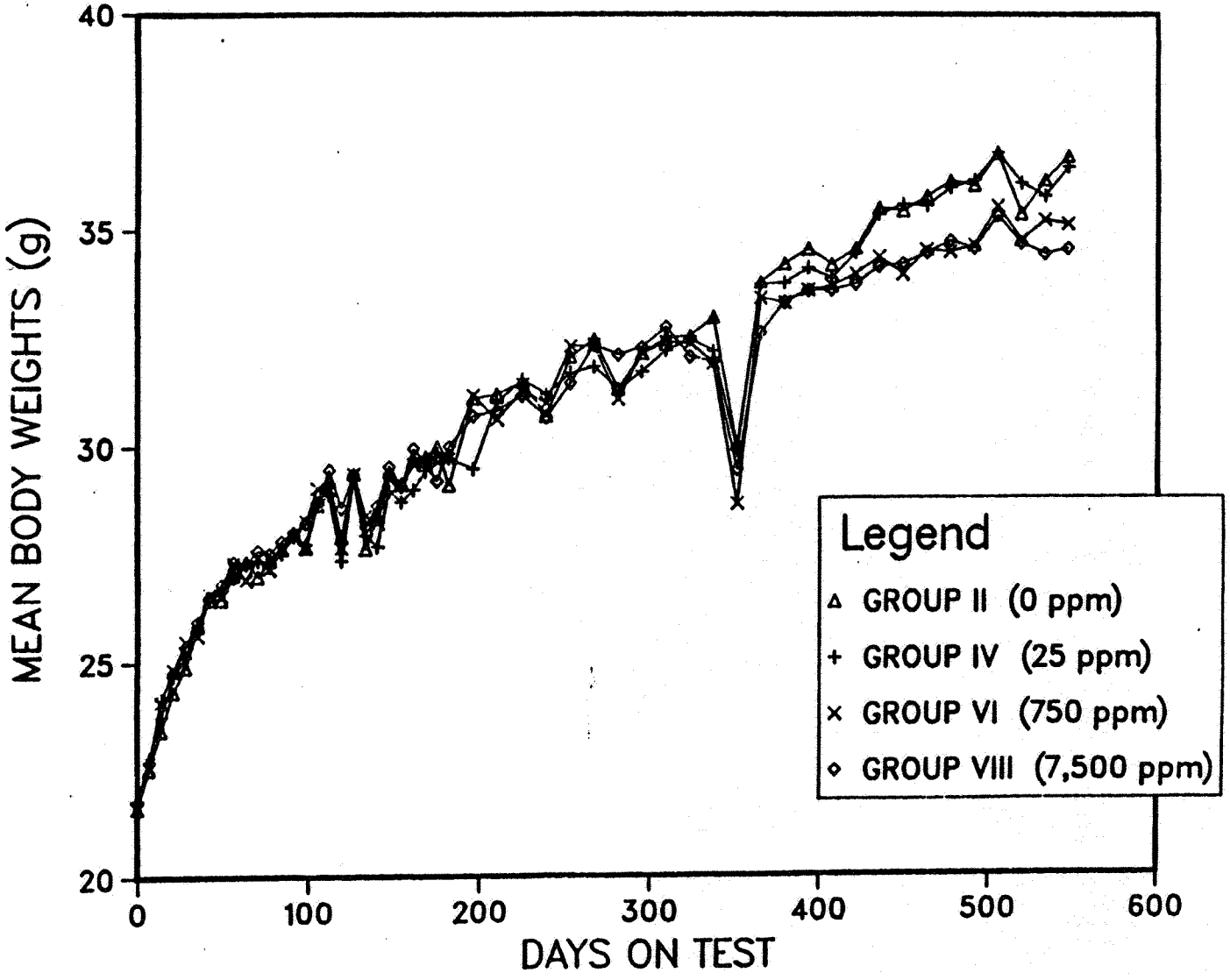

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FIGURE 3

SURVIVAL CURVES OF MALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 ppm INM-6316

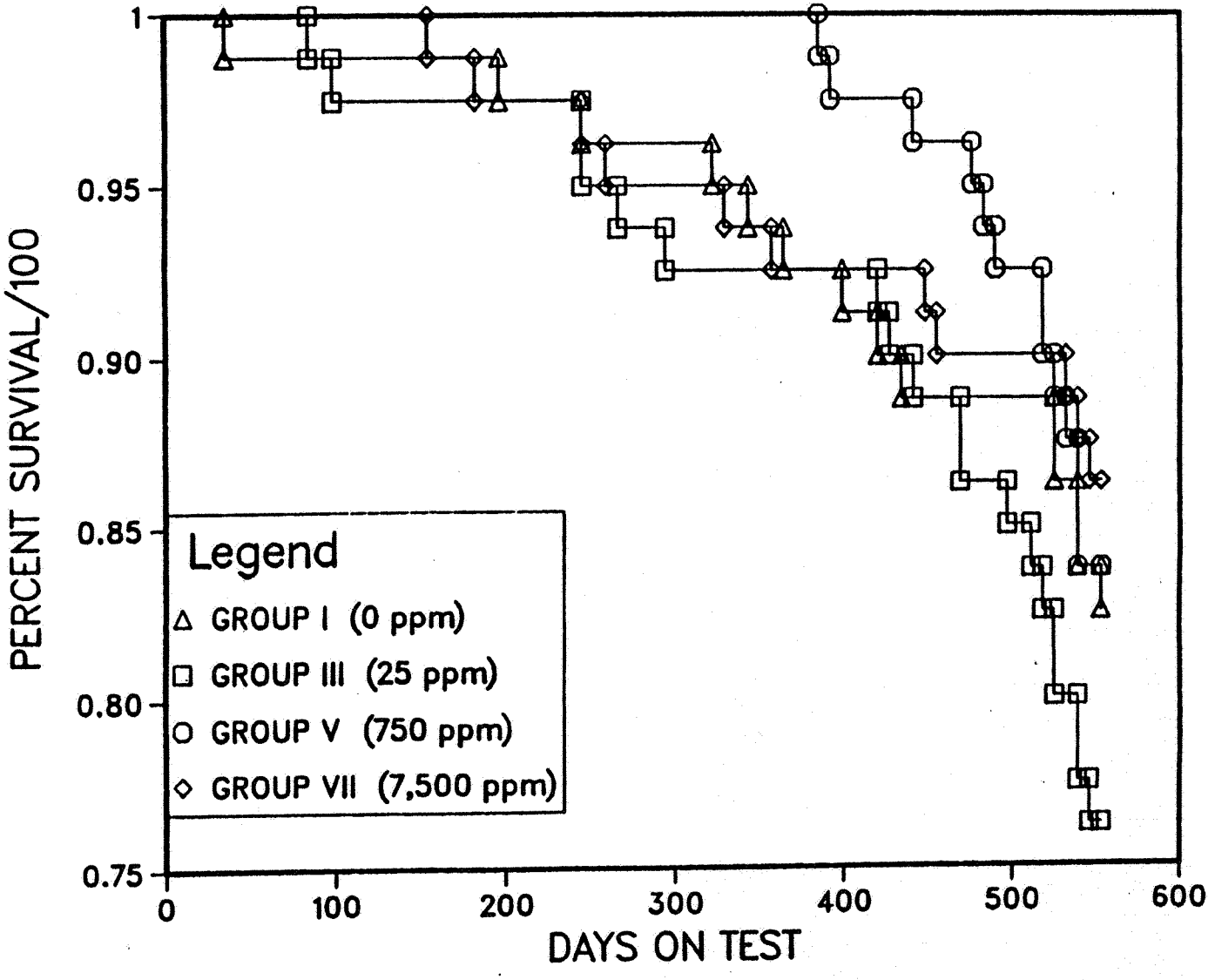

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FIGURE 4
SURVIVAL CURVES OF FEMALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 ppm INM-6316

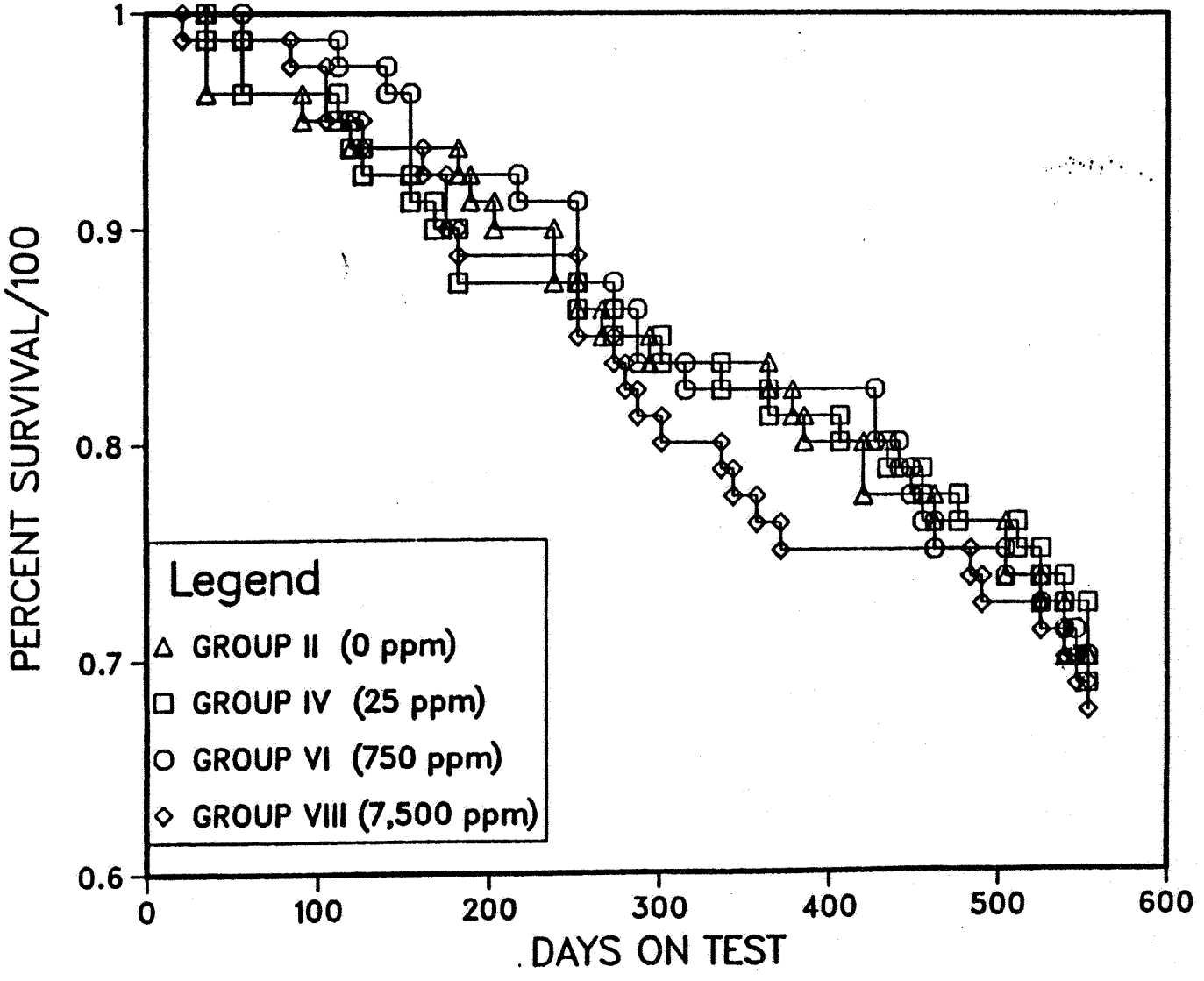

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TABLE 14

INCIDENCE OF MORTALITY AMONG MALE AND FEMALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7,500 ppm INM-6316[a]

DIETARY CONCENTRATION (ppm)	NUMBER OF DEATHS (% MORTALITY)[b]	
	MALES	FEMALES
0	15 (19%)[c]	25 (31%)[d]
25	22 (28%)[e]	26 (32%)[f]
750	13 (16%)[g]	24 (30%)[h]
7,500	12 (15%)[i]	26 (32%)[j]

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[a] This table does not include any scheduled deaths. All mice were found dead except where noted.

[b] % Mortality = (number of deaths per group/number of mice per group at study start) X 100%.

[c] Includes three mice sacrificed in extremis.

[d] Includes two mice sacrificed in extremis.

[e] Includes six mice sacrificed in extremis and one mouse accidentally killed.

[f] Includes four mice sacrificed in extremis.

[g] Includes three mice sacrificed in extremis.

[h] Includes six mice sacrificed in extremis. Percent mortality is based on 80 mice. Mouse #274 (Animal #32335) was sacrificed in extremis on day 16 and was replaced by mouse #281 (Animal #32549). Mouse #274 is not included in this mortality table.

[i] Includes two mice sacrificed in extremis.

[j] Includes two mice sacrificed in extremis and one mouse accidentally killed.

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TABLE IX

MEAN FINAL BODY AND ORGAN WEIGHTS (g) OF FEMALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7500 PPM INM-6316

AGRICULTURAL PRODUCTS DEPARTMENT

GROUP	CONC.	BODY WEIGHT		HEART		LIVER	
II	CONTROL N=55	38.4(	4.0)	0.189(	0.023)	1.943(	0.670)
IV	25 PPM N=54	38.3(	4.8)	0.187(	0.024)	1.857(	0.568)
VI	750 PPM N=56	36.7(	3.7)+	0.185(	0.033)	1.926(	1.171)
VIII	7500 PPM N=54	36.3(	4.3)#	0.189(	0.032)	1.866(	0.392)

GROUP	CONC.	SPLEEN		KIDNEYS*		BRAIN	
II	CONTROL	0.163(	0.139)	0.596(	0.093)	0.533(	0.032)
IV	25 PPM	0.188(	0.221)	0.631(	0.348)	0.534(	0.040)
VI	750 PPM	0.236(	0.606)	0.610(	0.239)	0.534(	0.033)
VIII	7500 PPM	0.161(	0.152)	0.607(	0.139)	0.535(	0.035)

STANDARD DEVIATION IN PARENTHESES
+ - SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY LSD
# - SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY LSD AND DUNNETT'S TEST
* - KIDNEYS WERE WEIGHED WITH ADRENALS ATTACHED

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TABLE XI

MEAN RELATIVE ORGAN WEIGHTS (%) OF FEMALE MICE FED FOR 18 MONTHS WITH DIETS THAT CONTAINED 0, 25, 750, OR 7500 PPM INM-6316

AGRICULTURAL PRODUCTS DEPARTMENT

GROUP	CONC.		HEART		LIVER		SPLEEN	
II	CONTROL	N=56	0.494(	0.060)	5.030(	1.328)	0.430(	0.367)
IV	25 PPM	N=54	0.495(	0.075)	4.864(	1.332)	0.495(	0.565)
VI	750 PPM	N=56	0.506(	0.090)	5.210(	2.641)	0.615(	1.402)
VIII	7500 PPM	N=54	0.525(	0.097)	5.144(	0.912)	0.439(	0.396)

GROUP	CONC.	KIDNEYS*		BRAIN	
II	CONTROL	1.553(	0.197)	1.401(	0.163)
IV	25 PPM	1.650(	0.862)	1.416(	0.188)
VI	750 PPM	1.671(	0.684)	1.467(	0.151)+
VIII	7500 PPM	1.679(	0.360)	1.490(	0.152)#

STANDARD DEVIATION IN PARENTHESES
+ - SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY LSD
# - SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY LSD AND DUNNETT'S TEST
* - KIDNEYS WERE WEIGHED WITH ADRENALS ATTACHED