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DATA EVALUATION RECORD

HARMONY

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Brock, W. J. Two-generation, four-litter reproduction study in rats with INM-6316. (Unpublished report No. 432-85 by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for Agricultural Products Dept., E.I. du Pont de Nemours and Co., Wilmington, DE; dated December 3, 1985.) Accession No. 263758.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
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Signature: I. Cecil Felkner

Date: 3-16-87

1. CHEMICAL: Harmony; INM-6316; DPX-M6316; 2-thiophenecarboxylic acid, 3[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]-sulfonyl]-, methyl ester.

2. TEST MATERIAL: INM-6316, batch Nos. 20 and 25, were reportedly 95.6 and 98.0% pure, respectively.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Brock, W. J. Two-generation, four-litter reproduction study in rats with INM-6316. (Unpublished report No. 432-85 by Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE, for Agricultural Products Dept., E.I. du Pont de Nemours and Co., Wilmington, DE; dated December 3, 1985.) Accession No. 263758.

5. REVIEWED BY:

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Date: 3/17/87

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7. CONCLUSIONS:

A. The LOELs for parental, reproductive, or developmental toxicity of INM-6316 in rats could not be established since no toxic effects were demonstrated at any of the dose levels tested (i.e., 25, 500, and 2500 ppm); therefore, the NOEL for this study was 2500 ppm, the highest dose level tested.

B. This study is classified Core Supplementary.

8. RECOMMENDATIONS:

If further work is conducted, we recommend that:

1. The reproductive/developmental toxicity of the test material be tested at higher dose levels, levels that would produce parental or other toxicity.

2. More animals/sex/group be assigned to the study to provide at least 20 pregnant females per group.

3. More complete postmortem procedures for parental animals be implemented (see Section 14.C.3 of this review).

4. Individual and summarized data be presented for food consumption, gestation lengths, precoital intervals, and the numbers of breeding pairs with evidence of copulation.

9. BACKGROUND: In a 2-week study, male rats received 10 doses of INM-6316 at 2200 mg/kg/day. No gross or pathological changes were associated with the compound. In a 90-day dietary/one-generation reproduction study, no gross or histological changes were noted that were considered compound related; however, mean body weights, weight gains, and food efficiency were reduced for both sexes at 2500 and 7500 ppm. No compound-related reproductive effects were observed. The NOEL was 100 ppm.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: INM-6316 batch Nos. 20 and 25 were reported to be 95.6 and 98.0% pure, respectively. The test material was mixed weekly with the basal diet (Certified Purina Lab Chow #5002) at concentrations of 0 (control), 25, 500, and 2500 ppm. For the first 48 days of the study, the test diets

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[1] Only items appropriate to this DER are included.

were prepared by first crushing the compound with mortar and pestle and then mixing with basal diet. However, these procedures were altered since visual inspection of test diets (from a different study) containing the same compound revealed that mixing may not have been uniform. Subsequent diets were prepared by first mixing the compound with Mazola corn oil. The diet preparations, including the control diet, contained 1% (w/w) corn oil and were refrigerated until use.

Fresh diet samples were collected on days -1, 252, 323, and 444 for both homogeneity and stability assays. In addition, fresh samples were collected on day 95 for stability assays and on days 48, 223, and 266 for homogeneity assays.

2. Animals and Experimental Design: Male and female Crl:CD(SD)BR rats were obtained at 22 days of age from Charles River Breeding Laboratories, Inc., Kingston, NY. Animals of the same sex were housed three per cage, temporarily identified with colored tail marks, and acclimated for 13 days. During the acclimation period, the rats were weighed at approximately 3-day intervals and observed for "eating habits" and disease or injury. Basal diet and tapwater were provided ad libitum.

   After acclimation, 20 males and 20 females were randomly assigned (using weight stratification) to each of the four groups and designated F_0 parental animals. All F_0 rats were housed one per cage, toe clipped, and, except for controls, ear punched for identification. All animals were fed their respective diets throughout the study.

   After approximately 90 days of receiving their test diets, F_0 females were paired for 15 days with males from the same group to produce F_{1a} litters. Approximately 1 week after the last F_{1a} litter was weaned, F_0 females were bred with different males to produce F_{1b} litters.

   Twenty male and 20 female F_{1b} weanlings from each group were selected to be F_1 parental animals. One weanling of each sex was selected from each litter; if necessary, additional pups were taken from randomly selected litters to yield a total of 20 weanlings/sex/group. Each selected pup was reportedly representative of the pups in that group on the bases of general health and body size. The F_1 rats were maintained and bred using the same procedures as described for their parents to produce F_{2a} and F_{2b} litters. Sibling pairings were avoided.

3. Observations and Measurements: Animals were observed at least twice daily for mortality and abnormal behavior or appearance. Each rat was individually handled and examined at least once weekly throughout the F_0 and F_1 premating periods. Body weights and food consumption were also determined weekly prior to mating. In addition, dams were weighed when their litters were weaned.

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During the 15-day breeding periods, females were examined daily for copulatory plugs to detect evidence of mating. Six days after the breeding period, females were examined twice daily for the birth of pups. The numbers of live and dead pups were determined as soon as possible after all pups were delivered and again at 1, 4, 12, and 21 days postpartum. On day 4, litters were reduced to 10 pups (5 per sex, when possible). The study author reported that the pups remaining with the dam were, by gross appearance, representative of the health status of all pups in the litter.

Pups from the same litter were weighed collectively at approximately 24 hours and 4 days (both before and after litter size reduction) postpartum. On day 21, pups were weighed individually and their sexes were determined.

Ten male and 10 female F_{2b} weanlings in each group were randomly selected and necropsied; selected organs were weighed. Selected tissues and gross lesions from controls and high-dose weanlings were examined microscopically. Gross lesions from low- and mid-dose weanlings were also examined histologically; other collected tissues were preserved.

Parental rats that died or were euthanized were grossly necropsied. No further pathological examinations of parental animals were reported.

Statistical Methods: Body weight and organ weight data were evaluated using one-way analysis of variance. Significant differences in body weights between groups were identified with the least significant difference test. For organ weight data, Dunnett's test was used to identify group differences, and Bartlett's test was used to evaluate the homogeneity of variance. The Mann-Whitney U, Kruskal-Wallis, and Jonckheere's tests were used to analyze the reproductive and litter data. Pup survival rates were calculated on a per litter basis.

12. REPORTED RESULTS:

Test Material Analyses: Chemical analyses of freshly frozen diets yielded mean concentrations ranging from 84-102% of nominal values. Homogeneity data for diets used through day 48 of the study were not presented; however, the study author reported that the results indicated uniform mixing of the compound in the diet. Subsequent diets, prepared with corn oil, yielded comparable concentrations from different levels within the mixer.

Stability assays indicated decreases in compound concentration in low-dose diets at room temperature. Results from low-dose diets stored for 10 days were 51-100% of the values from fresh frozen diets (Table 1).

TABLE 1. Results of Stability Assays of INM-6316 in Rat Diets[a]

Date Prepared	Nominal Concentration (ppm)	% Recovery at Storage Conditions			
		Room Temperature			Refrigerated
		Fresh	24 hrs.	10 days	10 days
10-23-83	25	100	96	84	96
	500	106	108	104	104
	2500	114	110	108	112
1-27-84	25	84	--[b]	84	81
	500	102	92	88	--[b]
	2500	102	102	102	100
3-20-84	25	76	58	39	71
	500	94	81	72	85
	2500	97	97	96	101
7-16-84	25	91	76	75	86
	500	96	82	100	110
	2500	92	93	82	105
9-11-84	25	93	92	73	93
	500	90	94	86	92
	2500	94	96	96	94
1-10-85	25	104	92	84	100
	500	101	96	87	97
	2500	98	97	90	83

[a]These data were obtained from CBI Appendix B.

[b]Not available.

Parental Data: One mid-dose F_0 female died on day 23 of the study. In the F_1 generation, 1/20, 1/20, 2/20, and 1/19 males died or were euthanized in the control and low-, mid-, and high-dose groups, respectively. (An additional high-dose rat was missexed at weaning.) The study author reported that necropsy findings did not indicate that the cause of death or moribundity was compound related; details of necropsy findings were not presented.

Clinical observations noted in the F_0 generation were comparable for all groups. In the F_1 generation, the study author noted increased group incidences of clinical observations in all dosed groups when compared to controls; however, the findings noted are common in rats and were not associated with compound administration.

Body weight (Table 2) and food consumption (Table 3) data were generally comparable for all groups in both sexes of both generations.

Reproductive and Developmental Data: No data regarding the numbers of females mating, precoital intervals, or gestation lengths were presented. The percentages of cohabited females delivering litters were comparable for all groups at all litter intervals (Table 4). Except for an F_{1a} control litter and an F_{1b} low-dose litter, all litters had at least one live pup at birth. Litter sizes, pup survival (Table 5), and pup weights (Table 6) were comparable for all groups throughout lactation at all litter intervals.

No gross or microscopic findings at postmortem examinations of F_{2b} weanlings were noted as compound related. Low- and high-dose male weanlings had significantly reduced relative kidney weights; lung weights were significantly less than controls for the low- and mid-dose males.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author concluded that there were no compound-related effects on body weight, food consumption, clinical observations, pathology, reproduction, lactation performance, or pup weights. The NOEL was cited as 2500 ppm, the highest dose level tested.

B. A quality assurance statement was signed, but not dated.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material Analyses: Results of chemical analyses presented in the study report indicate that the test diets were accurately prepared and uniformly mixed. Stability assays, however, suggest that the low-dose diets may not have been stable for 10 days at room temperature. It is unclear why the mid- and high-dose diets were apparently stable whereas the concentration of the test material in the low-dose diets decreased. However, in the absence of toxicological findings at the high-dose level, we assess that the questionable assay results at the low-dose level did not negatively affect the integrity of this study.

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TABLE 2. Mean Parental Body Weights (g) of Rats Fed INM-6316

	Dose Level (ppm)	Week 0	1	4	7	10	13
F_0 Males	0	139	197	343	420	487	520
	25	139	198	353	438	508	548*
	500	140	197	349	429	493	524
	2500	141	196	342	421	486	520
F_0 Females	0	112	141	198	229	249	266
	25	113	142	204	237	259	281
	500	111	139	199	233	253	268
	2500	112	139	193	221	241	258
F_1 Males	0	163	218	374	460	522	563
	25	167	224	381	475	544	590
	500	156	212	385	488*	552	599
	2500	162	217	367	443	502	542
F_1 Females	0	134	162	225	260	283	307
	25	135	161	225	262	285	309
	500	128	159	224	263	287	312
	2500	133	157	216	250	270	290

*Significantly different from control value ($p < 0.05$).

TABLE 3. Mean Parental Food Consumption (g/rat/day) of Rats Fed INM-6316

	Dose Level (ppm)	Week						Premating Period
		1	2	4	7	10	13	
F_0 Males	0	21.7	24.7	25.5	29.4	27.9	27.3	26.7
	25	22.7	24.3	26.6	28.1	27.6	28.8	27.3
	500	22.0	24.2	26.0	28.0	26.4	25.8	26.2
	2500	22.2	24.0	26.1	27.9	27.4	26.8	26.4
F_0 Females	0	18.3	21.4	20.6	23.0	20.1	20.5	21.2
	25	19.6	19.9	20.5	24.0	21.1	22.1	21.6
	500	21.5	19.8	20.7	21.2	19.8	21.2	20.6
	2500	18.3	19.4	20.4	20.5	19.1	20.1	20.3
F_1 Males	0	24.2	26.7	27.5	28.8	28.0	26.9	27.2
	25	25.3	27.6	28.0	29.8	30.4	28.3	28.3
	500	27.9	29.3	30.2	30.5	30.7	29.6	29.7
	2500	24.5	27.0	28.3	29.4	30.9	29.7	28.5
F_1 Females	0	20.4	21.2	21.7	20.4	22.1	22.0	20.8
	25	20.6	21.3	20.1	20.6	21.7	21.0	20.7
	500	20.6	21.0	20.6	20.4	22.3	21.4	20.6
	2500	20.3	20.8	19.8	19.7	20.2	19.2	19.6

TABLE 4. Summary of Reproductive Data of Rats Fed INM-6316

	Dose Level (ppm)	No. Cohabited	Litters Delivered No.	Litters Delivered %
F_0 Females				
F_{1a} Interval	0	20	18	90
	25	20	17	85
	500	19	15	79
	2500	20	16	80
F_{1b} Interval	0	20	15	75
	25	20	15	75
	500	19	14	74
	2500	20	15	75
F_1 Females				
F_{2a} Interval	0	20	14	70
	25	19	12	63
	500	19	15	79
	2500	19	19	100
F_{2b} Interval	0	19	12	63
	25	19	14	74
	500	19	13	68
	2500	19	16	84

TABLE 5. Mean Litter Data of Rats Fed INM-6316

	Dose Level (ppm)	No. Live Pups at				% Survival on Days	
		Birth	24 Hrs.	Day 4	Day 21	0-4	4-21
F_{1a} Litters	0	10.8	10.8	10.8	8.7	100.0	100
	25	12.9	12.8	12.8	9.7	98.6	100
	500	10.8	10.8	10.7	8.8	98.0	100
	2500	12.3	12.2	12.2	9.6	99.5	100
F_{1b} Litters	0	12.3	12.8	12.8	9.1	100.0	100
	25	12.7	12.3	12.3	8.7	99.5	99
	500	11.1	11.1	11.1	8.6	100.0	99
	2500	13.3	13.1	13.1	9.8	98.8	99
F_{2a} Litters	0	11.4	11.1	11.1	8.6	99.5	93
	25	10.8	10.7	10.7	8.8	99.4	100
	500	11.7	11.4	11.3	9.1	95.8	100
	2500	13.6	13.4	13.3	9.8	97.7	99
F_{2b} Litters	0	13.0	13.0	13.0	9.2	100.0	100
	25	11.4	11.3	11.2	9.0	98.9	100
	500	13.2	13.2	13.2	9.5	100.0	99
	2500	14.1	14.1	13.9	10.0	98.1	100

TABLE 6. Mean Pup Weights (g) Per Litter of Rats Fed INM-6316

	Dose Level (ppm)	24 Hours	Day 4	Day 21	
				Male	Female
F_{1a} Litters	0	7.1	10.6	44	44
	25	7.2	10.7	45	44
	500	7.5	11.3	47	46
	2500	6.8	10.2	44	43
F_{1b} Litters	0	6.8	10.2	53	51
	25	6.7	10.2	50	47
	500	7.1	10.8	54	51
	2500	6.6	9.6	49	47
F_{2a} Litters	0	7.1	10.3	52	50
	25	7.0	10.6	51	50
	500	7.3	10.9	54	53
	2500	6.7	9.9	51	50
F_{2b} Litters	0	7.0	10.5	54	52
	25	7.4	11.4	55	53
	500	7.1	10.8	53	51
	2500	6.7	10.0	53	50

Parental Data: Mortality occurred with low frequency in all groups and was therefore not considered to be compound related.

We do not regard the incidences of clinical observations in either generation to be compound related. We also do not consider the body weight or food consumption data in this study to indicate compound toxicity at any of the dose levels tested.

Reproductive and Developmental Data: We assess that the percentages of cohabited females delivering did not reflect a compound effect. In addition, litter sizes, pup survival, and pup weights were comparable for all groups throughout lactation and did not suggest compound toxicity.

No compound effects were evident from the gross and microscopic findings of F_{2b} weanlings. The significant organ weight changes noted in F_{2b} males occurred in nondose-related patterns and were not associated with any histological changes; we therefore, do not consider these data to be compound related.

B. There were no major differences between the study author and the reviewers in interpreting the study results. We concur with the author that there were no compound-related effects evident in this study at any of the dose levels tested.

C. We note the following deficiencies in this study:

1. Parental toxicity was not demonstrated at even the highest dose level tested.

2. Only 20 animals/sex were assigned to each group in each generation. The number of litters/group available for examination ranged from 12 to 19 due to parental deaths and to mated females that were not pregnant. The 1982 EPA Guidelines, Subdivision F, recommend that at least 20 pregnant females be present at or near term.

3. Insufficient postmortem procedures were performed on parental animals. The EPA Guidelines recommend that all parental animals be grossly necropsied and that reproductive tissues and gross lesions be preserved. In addition, histological examinations should be conducted for all control and high-dose animals. In this study, only animals euthanized or found dead were reportedly necropsied. No tissues were apparently preserved or histologically examined for any parental animal.

4. No individual or summarized mating data were reported. This precluded our assessment of the percentages of breeding pairs that mated or of the lengths of precoital intervals. Similarly, no individual or summarized data on gestation length were presented.

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5. Individual food consumption data were not reported, precluding our validation of the summarized data.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 18-27; Appendix B, Protocol, CBI pp. 81-110.

APPENDIX A

Materials and Methods

Harmony Reviews

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Pages 16 through 56 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

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