EPA: 68-02-4225
DYNAMAC No. 244A
March 16, 1987

DATA EVALUATION RECORD

HARMONY

Teratogenicity Study in Rabbits

STUDY IDENTIFICATION: Solomon, H. M., Alvarez, L., and Staples, R. E. INM-6316. Developmental toxicity study in rabbits dosed by gavage on days 7-19 of gestation. (Unpublished study No. MR-7108-001 by Haskell Laboratory of E.I. Dupont de Nemours and Co., Inc., Newark, DE; dated April 10, 1985.) Accession No. 263758.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 3-16-87

1. CHEMICAL: Harmony; INM-6316; 2-thiophenecarboxylic acid, 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-, methyl ester (INM-6316-26, N.B. 9283-180).

2. TEST MATERIAL: INM-6316, batch No. 26, was described as an off-white solid with a molecular weight of 387.4 and a purity of 95.4%.

3. STUDY/ACTION TYPE: Teratogenicity study in rabbits.

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5. REVIEWED BY:

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Teratogenicity and Reproductive Effects
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Date: 3-16-87

Marcia Van Gemert, Ph.D.
EPA Reviewer and Section Head

Signature: M Van Gemert
Date: 3/17/87

WJB

7. CONCLUSIONS:

A. The NOEL and LOEL for maternal toxicity are approximately 158 and 511 mg/kg/day (these are the reported doses received by animals in the 200- and 650-mg/kg/day groups, respectively), based on mild reductions in maternal body weight gain at 511 mg/kg/day.

Although the fetotoxic potential of the test material could not be ruled out (based on slight, nonsignificant decreases in fetal body weights at 511 mg/kg/day and on mild, nonsignificant increases in fetal and litter incidences of subcutaneous hemorrhages in all groups exposed to the test material), the noted effects were of negligible biological importance. Therefore, the NOEL for fetotoxicity was greater than 511 mg/kg/day, the highest dose tested.

B. This study is classified Core Minimum.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

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

The test material (INM-6316) was suspended daily in 0.5% aqueous methyl cellulose at a concentration of 130 mg/mL. This stock suspension was used for the 650-mg/kg/day group and diluted to 40 and 6 mg/mL for the 200- and 30-mg/kg/day dose groups, respectively. Control animals received aqueous methyl cellulose. Test suspensions were sampled on four different dosing days and analyzed to determine the concentration and stability of the test material. The samples were frozen at -70°C immediately after preparation or after 4 hours at room temperature.

Female New Zealand White rabbits (nulliparous) were obtained from Hazleton-Dutchland Laboratories, Inc., Denver, PA, and quarantined for 14 days. These animals were housed in a room maintained at 68±4°F and 40-60% relative humidity; the room was artificially illuminated for 12 hours daily.

Twenty females were randomly assigned to each of four study groups. These does were artificially inseminated with semen from one of two males of proven fertility; the day of insemination was designated gestation day (GD) 0. Ovulation was induced by injection of chorionic gonadotropin within 3 hours after insemination. Water was available ad libitum. Certified Purina Rabbit Chow was

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[1]Only items appropriate to this DER have been included.

reportedly available at 125 g/animal/day prior to insemination, and 150 g/animal/day thereafter.

Inseminated females were dosed by gavage on GD 7-19. Female body weights were obtained 1 day after arrival, before insemination, and on GD 0, 7-20, 24, and 29. Clinical signs were assessed 1 day after arrival, prior to insemination, each morning from GD 0-29, and each afternoon from GD 7-19. Food consumption was measured daily from GD 0-29. Females were killed on GD 29 by injection of T-61 solution and then necropsied. Livers and gravid uteri were weighed. The numbers of corpora lutea were counted under 2.5 X magnification. Uteri were opened and the numbers and positions of live and dead fetuses and resorptions were recorded. Uteri appearing to be nongravid were stained with ammonium sulfide to detect early resorptions.

Live fetuses were weighed and externally examined under 2.5 X magnification. These fetuses were injected with sodium pentabarbital, examined viscerally by methods described by Staples, and internally sexed. Fresh brains were examined by making a transverse section through the head at the frontoparietal suture. The eyes were examined to detect microphthalmia. Following these procedures, fetal carcasses were fixed, processed, and stained with alizarin red S for skeletal examination.

Dams were coded before being killed to preclude bias during maternal and fetal examinations and during data collection and classification.

Data were statistically analyzed using the litter as the experimental unit. The level of statistical significance used in data analyses was $p \leq 0.05$. Incidences of pregnancy, clinical signs, maternal deaths, and litters with total resorptions were analyzed using the Cochran-Armitage and Fisher's exact tests. Maternal body weight, weight change, liver weight, and food consumption were analyzed by an orthogonal polynomial test of dose ranks and by one-way ANOVA followed by Dunnett's test. Data on nidations, live and dead fetuses, resorptions, corpora lutea, male and female fetuses, fetal weight, percent resorptions, and incidence of fetal findings were analyzed by Jonckheere's and Mann-Whitney U tests.

Two-way ANOVA was used to detect differences among groups. When differences were significant, data were analyzed by Student's t-test. When ties exceeded 75% in reproductive and fetal parameters, Fisher's exact test was used to detect significant differences between groups, and Cochran-Armitage test was used to detect dose responses.

B. Protocol (and protocol amendments): See Appendix B.

12. REPORTED RESULTS:

Test Material Analyses: Results from chemical analyses indicate that the recovery concentrations of the test material in dosing suspensions ranged from 65-88% of nominal values (Table 1); the concentrations of fresh frozen samples were comparable to those of samples frozen after 4 hours at room temperature (Table 1). The mean values obtained from the samples indicate that the actual doses of the test material were 0.0±0.0, 21.9±1.89, 157.8±12.42, and 511.3±33.70 mg/kg for the nominal 0-, 30-, 200-, and 650-mg/kg/day groups, respectively.

Maternal Data: One female from the low-dose group died due to a gavage accident. No other deaths were reported. Females in the 200- and 650-mg/kg/day group had significant, dose-related increases in the incidence of orange-tinted urine; this effect was noted only during the treatment period. Incidences of other clinical signs were comparable for all groups.

Food consumption was comparable among all groups (Table 2). Although maternal body weight changes showed a significant dose-related decreasing trend on GD 7-9 and an increasing trend on GD 9-11, none of the dosed-group values were significantly different from controls (Table 3). The study authors noted, however, that the body weight loss for the 650-mg/kg/day group was significant at $p = 0.07$. The 650-mg/kg/day group also had nonsignificantly reduced weight gains for the treatment period (GD 7-20). No compound-related effects were noted at necropsy. Liver weights were comparable for all groups.

Developmental Data: No compound-related effects were reported on pregnancy rate, the incidences of abortions, and totally resorbed litters, or the numbers of corpora lutea, nidations, resorptions, and live fetuses per litter (Table 4). Fetal body weights were similar among all groups (Table 4).

The authors reported that no compound-related effects on malformations, variations, or developmental retardations occurred (Table 5).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that slight maternal toxicity was demonstrated at 650 mg/kg/day and that no fetal toxicity was evident at any dosage in this study. The NOEL for maternal toxicity was assessed at 200 mg/kg/day, and the NOEL for fetoxicity was greater than 650 mg/kg/day.

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

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material Analyses: Results from chemical analyses indicate that the test material was stable in the dosing suspensions for 4 hours at room temperature and that the concentrations of the test

TABLE 1. Results from Chemical Analyses of Dosing Suspensions

Preparation Date	Percent Recovery at Dosage (mg/kg/day) 30	200	650
11/26/84			
A	80	88	79
B	78	82	85
11/30/84			
A	65	75	86
B	65	71	72
12/5/84			
A	72	76	75
B	73	78	72
12/10/84			
A	75	78	75
B	75	81	78

A = Fresh frozen sample.
B = Sample frozen after 4 hours at room temperature.

TABLE 2. Effects of INM-6316 on Mean Maternal Food Consumption (g/rabbit/day)

Gestation Days	Dosage (mg/kg/day)			
	0	30	200	650
0-7	150.3	151.2	150.4	150.9
7-9	145.0	149.3	150.6	146.0
9-11	146.3	149.1	150.5	143.5
11-13	143.8	151.9	148.4	145.3
13-15	148.8	150.0	141.2	140.7
15-17	144.1	145.9	132.6	136.8
17-20	130.9	145.6	125.4	126.2
7-20	142.2	148.4	140.2	138.7
20-29	128.6	141.0	127.2	134.4

TABLE 3. Effects of INM-6316 on Mean Maternal Body Weight Changes (g/rabbit/day)

Gestation Days	Dosage (mg/kg/day)			
	0	30	200	650
0-7	242.2	203.8	197.6	245.7
7-9[a]	4.0	20.5	11.7	-36.0
9-11[a]	-1.5	-4.5	17.3	29.5
11-13	71.6	82.4	51.5	52.8
13-15	79.4	64.0	55.0	68.9
15-17	-11.1	11.3	-6.2	-2.7
17-20	9.2	-1.6	-24.5	-24.6
7-20	151.6	172.1	104.8	88.0
20-29	166.5	179.6	165.0	225.4

[a] Study authors reported a significant trend ($p \leq 0.05$).

TABLE 4. Effects of INM-6316 on Values for Reproductive and Developmental Parameters in Rabbits

	Dosage (mg/kg/day)			
	0	30	200	650
No. of Females				
Inseminated	20	20	20	20
Dead	0	1	0	0
Pregnant	15	17	17	15
Aborted	0	1	0	1
With totally resorbed litters	3	3	2	0
Mean per Litter				
Corpora lutea	10.3	9.2	9.1*	10.7
Nidations	5.8	5.2	4.7	6.7
Resorptions	0.5	0.8	0.1*	0.7
Live fetuses	5.4	4.4	4.6	6.0
Fetal Body Weight (g)	50.27	51.78	50.70	48.84

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

TABLE 5. Effects of INM-6316 on Malformations and Variations in Rabbit Fetuses

	Dosage (mg/kg/day)			
	0	30	200	650
No. Fetuses Examined	59	57	69	84
No. Litters Examined	11	13	15	14
Malformations[a]				
No. fetuses (mean %/litter)	4 (6.1)	6 (9.0)	5(13.3)	10(16.3)
No. litters (%)	4(36.4)	4(30.8)	4(26.7)	5(35.7)
Variations[a,b]				
No. fetuses (mean %/litter)	14(23.4)	12(20.8)	12(22.1)	19(22.7)
No. litters (%)	8(72.7)	8(61.5)	9(60.0)	11(78.6)
Developmental Retardation[a]				
No. fetuses (mean %/litter)	3 (5.6)	5(13.1)	11(16.8)	10(10.6)
No. litters (%)	3(27.3)	4(30.8)	7(46.7)	5(35.7)

[a]Includes external, visceral, cranial, and skeletal findings.
[b]Excluding variations due to developmental retardation.

material in the sample suspensions were stable (Table 1); however, the percentages of test material recovery ranged from 65-88 percent of the nominal values (Table 1). The actual dosage levels in this study were approximately 0, 22, 158, and 511 mg/kg/day, based on reported average concentrations.

Maternal Data: We assess that the only evidence of maternal toxicity was a slight (not significant) reduction in maternal body weight gain during the treatment period, followed by a slight increase during posttreatment in the 650-mg/kg/day group. The biological significance of orange-tinted urine among animals in the 200- and 650-mg/kg/day groups was not clear; however, we assess that the reported change in urine color was not toxicologically important.

Developmental Data: We assess that the data on pregnancy rates, abortions, numbers of corpora lutea, nidations, resorptions, and live fetuses did not indicate adverse compound-related effects at any dosage level.

The body weights of fetuses in the 650-mg/kg/day group were slightly (but not significantly) lower than those of controls. We assess that the difference was minimal and, therefore, not toxicologically important.

In general, the total incidences of malformations, variations, and developmental delays were comparable for all groups. Although certain malformations (including cleft palate, small lung, and hydrocephaly) were reported only for the groups dosed with the test material, their incidences were very low and not dose related. We regard these findings to be incidental.

The following is a summary of fetal and litter incidences of subcutaneous hemorrhages:

	Dosage (mg/kg/day)			
	0	30	200	650
Fetuses affected	1/59 (1.7%)	2/57 (3.5%)	4/69 (5.6%)	5/84 (5.9%)
Litters affected	1/11 (9.0%)	2/13 (15.4%)	3/15 (20.0%)	4/14 (28.6%)

The above data suggest a slight dose-related increase in fetal and litter incidences of subcutaneous hemorrhages; however, our analyses indicate that no significant differences exist in pairwise comparisons against controls using Fisher's exact test and no significant dose-related trends were detected using the Cochran-Armitage trend test. Also, in the absence of other vascular findings, these hemorrhages were considered to be of negligible biological importance.

B. The study authors reported that 150 g of food/animal/day were available; however, as shown in Table 2 of this review, maternal food consumption exceeded this value several times.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 7-11; Appendix B, Protocol and Protocol Amendments, CBI pp. 23-42.

APPENDIX A

Materials and Methods

Harmony Reviews

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APPENDIX B

Protocol and Protocol Amendments

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