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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

008091

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: MOLINATE - RAT DEVELOPMENTAL TOXICITY STUDY
RESPONSE TO COMPREHENSIVE DATA CALL-IN

TO: E. DOBBINS
PRODUCT MANAGER (50)
GENERIC CHEMICAL SUPPORT BRANCH
SPECIAL REVIEW AND REREGISTRATION DIVISION (H7508C)

FROM: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 8/22/90
TOXICOLOGY BRANCH II SECTION II
HEALTH EFFECTS DIVISION (H7509C)

THRU: K. CLARK SWENTZEL K. Clark Swentzel 8/28/90
SECTION II HEAD, TOXICOLOGY BRANCH II
HEALTH EFFECTS DIVISION (H7509C)
AND

MARCIA VAN GEMERT, PH.D. Marcia Gemert 8/28/90
CHIEF, TOXICOLOGY BRANCH/HFAS/HED (H7509C)

REGISTRANT: ICI AMERICAS INC.
CHEMICAL: MOLINATE;
PROJECT: 0-1256
CASWELL NO.: 444
RECORD NO.: 264034
IDENTIFYING NO.: 2435
MRID NO.: 414734-01
ACTION REQUESTED: PLEASE REVIEW "A TERATOLOGY STUDY IN CD RATS WITH R-4572 TECHNICAL".

COMMENT: THE REGISTRANT HAS SUBMITTED A RAT DEVELOPMENTAL TOXICITY (TERATOLOGY) STUDY IN RESPONSE TO THE MOLINATE COMPREHENSIVE DATA CALL-IN; CROSS REFERENCE TO PHASE 2, CASE 2435.

THE STUDY HAS BEEN REVIEWED AND THE RESULTS ARE SUMMARIZED BELOW. THE DER IS ATTACHED.

MATERNAL REPRODUCTIVE PARAMETERS WERE NOT AFFECTED BY TREATMENT OF PREGNANT RATS WITH MOLINATE AT DOSE LEVELS OF 2.2, 35, AND 140 MG/KG FROM DAY 6 TO DAY 16 OF GESTATION.

THE NUEL FOR MATERNAL TOXICITY CAN BE SET AT 35 MG/KG; THE LEL AT 140 MG/KG, BASED ON DECREASED BODY WEIGHT, BODY-WEIGHT GAIN, AND FOOD CONSUMPTION, AND INCREASED SALIVATION AND RBC CHOLINESTERASE INHIBITION. THE DEVELOPMENTAL NUEL CAN BE SET AT 35 MG/KG; THE LEL AT 140 MG/KG,

BASED ON AN INCREASE IN POST-IMPLANTATION LOSS, LOWER FETAL BODY WEIGHT, AND INCREASED INCIDENCES OF RUNTS, EXTERNAL, SOFT TISSUE, AND SKELETAL VARIANTS. DEVELOPMENTAL TOXICITY OCCURRED ONLY AT A MATERNALLY-TOXIC DOSE LEVEL OF MOLINATE.

THIS STUDY IS CLASSIFIED AS CORE MINIMUM. THIS STUDY SATISFIES THE GUIDELINE (83-3) REQUIREMENTS FOR A DEVELOPMENTAL TOXICITY STUDY.

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REVIEWED BY: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 8/28/90
TOX. BRANCH II, SECTION II, HED (H7509C)
SECONDARY REVIEWER: K. CLARK SWENTZEL K Clark Swentzel 8/28/90
SECTION II HEAD, TOX. BRANCH, HED (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: DEVELOPMENTAL TOXICITY - RAT TOX. CHEM. NO.: 444

MRID NO.: 414734-01

TEST MATERIAL: R-4572 TECHNICAL

SYNONYMS: MOLINATE

STUDY NUMBER: T-13266

SPONSOR: ICI AMERICAS INC. WILMINGTON, DELAWARE

TESTING FACILITY: CIBA-GEIGY ENVIRONMENTAL HEALTH CENTER - FARMINGTON, CT

TITLE OF REPORT: A TERATOLOGY STUDY IN CD RATS WITH R-4572 TECHNICAL

AUTHOR: J.L. MINOR

REPORT ISSUED: MARCH 30, 1990

QUALITY ASSURANCE: A QUALITY ASSURANCE STATEMENT WAS PROVIDED.

CONCLUSION: THE ADMINISTRATION OF MOLINATE TO PREGNANT RATS AT DOSE LEVELS OF 2.2, 35, AND 140 MG/KG ON DAYS 6 THROUGH 16 OF GESTATION PRODUCED MATERNAL TOXICITY, EMBRYOTOXICITY, AND FETOTOXICITY AT THE HIGH-DOSE LEVEL. THE NOEL FOR MATERNAL TOXICITY CAN BE SET AT 35 MG/KG/DAY, THE LEL AT 140 MG/KG/DAY, BASED ON DECREASED BODY WEIGHT, BODY-WEIGHT GAIN, AND FOOD CONSUMPTION, AND INCREASED SALIVATION AND RBC CHOLINESTERASE INHIBITION. THE NOEL FOR FETOTOXICITY CAN BE SET AT 35 MG/KG/DAY, THE LEL AT 140 MG/KG/DAY, BASED ON AN INCREASE IN POST-IMPLANTATION LOSS, LOWER FETAL BODY WEIGHT, AND INCREASED INCIDENCES OF RUNTS, EXTERNAL, SOFT TISSUE, AND SKELETAL VARIANTS. DEVELOPMENTAL TOXICITY OCCURRED ONLY AT A MATERNALLY-TOXIC DOSE LEVEL OF MOLINATE.

CLASSIFICATION: CORE MINIMUM. THIS STUDY SATISFIES THE GUIDELINE (83-3) REQUIREMENTS FOR A DEVELOPMENTAL TOXICITY STUDY.

A. MATERIALS:

1. TEST COMPOUND: R-4572 TECHNICAL; DESCRIPTION: NONE PROVIDED; BATCH #: WRC 4921-8-22*; PURITY: 97.6%; SOURCE: ICI AMERICAS WESTERN RESEARCH CENTER, RICHMOND, CA.

   *NOT LISTED AS A BATCH #; EHC # 0866-30

2. TEST ANIMALS: SPECIES: RAT; STRAIN: CRL:CD®(SD) BRVAF/PLUS™; AGE: FEMALES - APPROXIMATELY 10 WEEKS OLD AT START OF MATING (72-81 DAYS OLD ON DAY 0 OF GESTATION; MALES-PROVEN BREEDERS); WEIGHT: MALES: NOT PROVIDED, FEMALES: 255 GRAMS ON DAY 0; SOURCE: CHARLES RIVER BREEDING LABORATORIES, KINGSTON, NY.

3. STATISTICS: INCIDENCE DATA (CLINICAL OBSERVATIONS) - ANALYZED BY THE FISHER EXACT PROBABILITY TEST (SIEGEL, 1956), WITH BONFERRONI'S CORRECTION FOR MULTIPLE COMPARISONS TO A SINGLE CONTROL LEVEL; ENUMERATIVE DATA FOR EACH LITTER (% INCIDENCE OF FETAL VARIATIONS AND MALFORMATIONS, # CORPORA LUTEA PER DAM) - ANALYZED BY THE NONPARAMETRIC MANN-WHITNEY U TWO-SAMPLE RANK TEST (GOLDSTEIN, 1967); QUANTITATIVE OR CONTINUOUS DATA (BODY WEIGHT AND FOOD CONSUMPTION) - ANALYZED BY ONE-WAY ANALYSIS OF VARIANCE (STEEL AND TORRIE, 1960) AND THE DUNNETT'S T-TEST (DUNNETT, 1964).

B. STUDY DESIGN:

1. MATING: ONE OR TWO FEMALES WERE COHABITED OVERNIGHT WITH EACH PROVEN MALE BREEDER. VAGINAL SMEARS WERE EXAMINED THE NEXT MORNING TO DETERMINE THE STAGE OF THE ESTROUS CYCLE AND THE PRESENCE OF SPERM OR A COPULATORY PLUG. THE DAY EVIDENCE OF MATING WAS FOUND WAS DESIGNATED DAY 0 OF GESTATION. ASSIGNMENT OF MATED, SPERM-POSITIVE FEMALES TO THE STUDY GROUPS WAS DONE IN A MANNER TO ELIMINATE ANY BIASES ORIGINATING FROM THE DAY ON WHICH THEY WERE MATED, THE MALE TO WHICH THEY WERE MATED, AND THEIR BODY WEIGHT ON DAY 0 OF GESTATION; NO OTHER DETAILS WERE PROVIDED.

   DOSING: THE TEST MATERIAL WAS GIVEN AS A SOLUTION IN CORN OIL AND WAS ADMINISTERED ORALLY (GAVAGE) AT A CONSTANT VOLUME OF 5 ML/KG FROM DAY 6 THROUGH DAY 15 OF GESTATION. DOSING WAS BASED ON GESTATIONAL DAY 6 BODY WEIGHT. THE DOSE LEVELS WERE 0, 2.2, 35, AND 140 MG/KG/DAY, AND EACH GROUP CONSISTED OF 26 ANIMALS. ALTHOUGH NOT STATED IN THE METHODS SECTION OF THE REPORT, IT IS ASSUMED THAT THE CONTROLS RECEIVED CORN OIL. THE DOSING SOLUTIONS WERE ANALYZED FOR CONCENTRATION, AND A SAMPLE OF THE TEST MATERIAL WAS RETAINED BY THE SPONSOR. DOSING SOLUTIONS WERE PREPARED AT THE BEGINNING OF THE STUDY BY A WEIGHT/VOLUME METHOD AND STORED IN THE REFRIGERATOR. THE INTENDED CONCENTRATIONS WERE 0, 0.44, 7.0, AND 28 MG/ML.

   THE ANIMALS HAD ACCESS TO PURINA RODENT CHOW (#5002) AND TAP WATER AD LIBITUM.

RESULTS

ANALYSES OF THE DOSING SOLUTIONS INDICATED THAT THE MEAN MEASURED CONCENTRATIONS OF TEST MATERIAL WERE WITHIN 10% OF THE INTENDED CONCENTRATIONS FOR ALL PREPARATIONS. THE SOLUTIONS WERE FOUND TO BE ADEQUATELY HOMOGENEOUS. SINCE R-4572 TECHNICAL (LOT NO. EHC-0469-29) IN CORN OIL AT A CONCENTRATION OF 4 MG/ML HAD BEEN SHOWN PREVIOUSLY TO BE UNCHANGED AFTER BEING STORED AT ROOM TEMPERATURE FOR 6 WEEKS, IT WAS STATED THAT THE DOSING SOLUTIONS USED IN THIS STUDY WERE STABLE FOR THE PERIOD OF THE STUDY.

2. CLINICAL OBSERVATIONS

ALL ANIMALS WERE OBSERVED DAILY FOR MORIBUNDITY AND MORTALITY, APPEARANCE, BEHAVIOR, AND SIGNS OF TOXICITY. BODY WEIGHTS WERE RECORDED ON DAYS 0, 6, 7, 9, 12, 16, AND 21 OF GESTATION AND FOOD CONSUMPTION WAS MEASURED FOR GESTATION DAY INTERVALS 0-6, 6-9, 9-12, 12-16, AND 16-21. ERYTHROCYTE CHOLINESTERASE ACTIVITY WAS DETERMINED ON BLOOD SAMPLES DRAWN AT SACRIFICE. THE FEMALES WERE SACRIFICED ON DAY 21 OF GESTATION.

3. NECROPSY

MATERNAL

EACH DAM WAS WEIGHED AND SACRIFICED FOLLOWING THE COLLECTION OF BLOOD FROM THE ABDOMINAL AORTA. COMPLETE NECROPSIES WERE PERFORMED ON ALL MATED FEMALE RATS, WHICH INCLUDED A MACROSCOPIC EXAMINATION OF THE ORAL CAVITY AND ALL ORGANS OF THE THORACIC AND ABDOMINAL CAVITIES. THE OVARIES AND UTERUS WITH CERVIX WERE REMOVED IMMEDIATELY, TRIMMED, WEIGHED INTACT, AND EXAMINED AS DESCRIBED BELOW.

OVARIES WERE EXAMINED AND THE CORPORA LUTEA COUNTED. THE UTERUS WAS OPENED AND EXAMINED FOR THE NUMBER AND DISTRIBUTION OF FETUSES AND RESORPTIONS. RESORPTION SITES WERE NOTED AS EARLY, IF ONLY A PLACENTA WAS PRESENT; AS MID, IF BOTH A PLACENTA AND EMBRYONIC TISSUES WITHOUT RECOGNIZABLE FEATURES WERE PRESENT; OR LATE, IF THE CONCEPTUSES SHOWED EITHER EXTERNAL DEGENERATIVE CHANGES OR AN ARRESTED STATE OF DEVELOPMENT. PLACENTAS AND ASSOCIATED FLUIDS WERE INSPECTED VISUALLY. UPON REMOVAL FROM THE UTERUS, FULLY DEVELOPED FETUSES WERE CONSIDERED DEAD IF REFLEXES WERE ABSENT WHEN THE NECK WAS PRESSED. ALL FETUSES WERE WEIGHED, SEXED, AND EXAMINED FOR EXTERNAL VARIATIONS AND MALFORMATIONS. FETUSES THAT WEIGHED LESS THAN THREE-FOURTHS OF THE LITTER MEAN WERE DESIGNATED RUNTS. LIVE FETUSES WERE SACRIFICED.

FETAL

ONE-HALF OF THE FETUSES WERE PROCESSED AND EXAMINED FOR SOFT TISSUE VARIATIONS AND MALFORMATIONS OF THE HEAD AND TRUNK [MODIFICATION OF THE WILSON (1965) SERIAL CROSS-SECTION AND STAPLES (1974) TECHNIQUES]. THE REMAINING FETUSES WERE PROCESSED AND EXAMINED FOR SKELETAL VARIATIONS AND MALFORMATIONS (ALIZARIN RED, MODIFIED FROM CRAREY, 1962).

RESULTS

1. MATERNAL TOXICITY

A. CLINICAL OBSERVATIONS AND SURVIVAL

THERE WAS ONE DEATH (SACRIFICE ON DAY 15 AFTER NINE DOSES) AT THE HIGH-DOSE LEVEL. ANTEMORTEM SIGNS INCLUDED CHROMODACRYORRHEA EVIDENT ON THE FIRST DAY OF DOSING, FOLLOWED BY DEHYDRATION AND BODY-WEIGHT LOSS WITHOUT RECOVERY. FOOD CONSUMPTION WAS LOW BEFORE DOSING BEGAN. AT NECROPSY, THE ADRENALS WERE MODERATELY ENLARGED AND THE STOMACH SHOWED DISCOLORATIONS IN THE MUCOSA. THE OVARIES HAD 14 CORPORA LUTEA AND THE UTERUS HAD 3 IMPLANTS, WHICH WERE BEING RESORBED.

ONLY THE HIGH-DOSE DISPLAYED SALIVATION, WHICH WAS OBSERVED IN 9 DAMS. DEHYDRATION OCCURRED IN ONE CONTROL, ONE MID-DOSE, AND 5 HIGH-DOSE DAMS. RBC CHOLINESTERASE ACTIVITY WAS SIGNIFICANTLY DECREASED (56% OF CONTROL VALUE; $^{**}P<0.01$) IN THE HIGH DOSE AT STUDY TERMINATION.

	C	L	M	H
RBC CHOLINESTERASE (IU-PACKED RBC)	1667	1618	1478	940**

$^{**}P<0.01$

THE AUTHORS ALSO PRESENTED DATA ON THE RBC CHOLINESTERASE ACTIVITY IN THOSE DAMS THAT DISPLAYED SALIVATION. THESE ANIMALS DISPLAYED LOWER VALUES THAN THE HIGH-DOSE GROUP AS A WHOLE (747 IU-PACKED RBC).

B. BODY WEIGHT AND BODY-WEIGHT GAIN

BODY WEIGHT WAS DECREASED AT THE HIGH-DOSE LEVEL FROM DAY 12 ONWARD COMPARED TO CONTROL VALUE. CORRECTED (BODY WEIGHT ON DAY 21 MINUS GRAVID REPRODUCTION TRACT WEIGHT) BODY WEIGHT WAS ALSO SIGNIFICANTLY DECREASED AT THIS DOSE LEVEL (89% OF CONTROL).

	BODY WEIGHT (GRAMS)			
	CONTROL	LOW	MID	HIGH
DAY 0	255	253	255	254
6	285	283	289	285
12	306	310	308	288** (94)°
16	334	338	334	301** (90)
21	398	401	397	323** (81)
CORRECTED	316	314	315	280** (89)

** $P<0.01$; ° (% OF CONTROL VALUE)

BODY-WEIGHT GAIN (GRAMS)

	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected† BW Gain	Corrected†† BW Gain
Days	0-6	6-16	16-21	0-21	6-21	0-21
Group						
Control	30	50	76	143	32	61
Low	31	53	79	148	29	61
Mid	34	44	74	142	25	59
High	32	16**	31**	67**	-7.2**	25**

† CORRECTED BODY WEIGHT GAIN = BODY-WEIGHT GAIN FOR DAYS 6-21 MINUS GRAVID UTERUS WEIGHT

†† CORRECTED BODY WEIGHT GAIN = BODYWEIGHT GAIN FOR ENTIRE GESTATION PERIOD MINUS GRAVID UTERUS WEIGHT

** $p<0.01$

C. Food Consumption: THE HIGH DOSE FEMALES DISPLAYED LOWER FOOD CONSUMPTION FOLLOWING TEST MATERIAL ADMINISTRATION, WHICH CONTINUED THROUGHOUT GESTATION, COMPARED WITH THE OTHER GROUPS. DATA WERE PROVIDED ON AN INTERVAL BASIS ONLY AND ONLY AS GRAMS/DAY. TWO INTERVALS OF INTEREST, WHICH WERE NOT REPORTED, WERE FOR DAYS 6-16 AND DAYS 0-21.

FOOD CONSUMPTION (GM/DAY)

Group	Days 0-6	Days 6-9	Days 9-12	Days 12-16	Days 16-21
Control	23	21	22	23	29
Low	22	21	22	23	27
Mid	23	20	22	23	25**
High	22	16**	20*	19**	20**

*$p<0.05$; **$p<0.01$

D. Gross Pathology: THERE WERE NO FINDINGS THAT COULD BE RELATED TO TEST MATERIAL ADMINISTRATION.

E. Histology: THERE WAS A SLIGHT INCREASE IN THE INCIDENCE OF PALE LIVERS IN THE HIGH-DOSE GROUP COMPARED TO CONTROLS, BUT THE AUTHOR INDICATED THAT THIS FINDING CAN BE SEEN IN ANIMALS WHICH ARE EXSANGUINATED THOROUGHLY AND THAT THE BIOLOGICAL SIGNIFICANCE OF THE FINDING IS EQUIVOCAL. THE REGISTRANT INDICATED THAT LIVER EFFECTS HAVE BEEN OBSERVED IN OTHER STUDIES IN RODENTS AND, THEREFORE, HE CONSIDERED THE OBSERVATION AS POSSIBLY TREATMENT RELATED.

F. REPRODUCTIVE PERFORMANCE:

REPRODUCTIVE DATA AT CEASAREAN SECTION

DOSE GROUP	CONTROL	LOW	MID	HIGH
# ANIMALS ASSIGNED	26	26	26	26
# ANIMALS MATED	23	23	24	24
PREGNANCY RATE (%)	88	88	92	92
MATERNAL WASTAGE				
# DIED	0	0	0	1
# ABORTED	0	0	0	0
# PREMATURE DELIVERY	0	0	0	0
# NOT PREGNANT	3	3	2	2
# OF CORPORA LUTEA	381	378	402†	402†
CORPORA LUTEA/DAM	16.6	16.4	16.8	17.5
TOTAL # OF IMPLANTATIONS	317	345	345	347
IMPLANTATIONS/DAM	13.8	15.0	14.3	15.1
TOTAL # OF LIVE FETUSES	299	332	327	175
LIVE FETUSES/DAM	13.0	14.4	13.6	7.6** (8.3)
TOTAL # RESORPTIONS	15	13	18	175
EARLY (MEAN)	10	9	14	135
MID (MEAN)	5	2	4	24
LATE (MEAN)	0	2	0	16
# LITTERS WITH RESORPTIONS (%)	10(43)	9(39)	14(58)	23(100)**
TOTAL # DEAD FETUSES	3	0	0	0
PREIMPLANTATION LOSS*	17.9	8.3	13.1†	11.6†
SEX RATIO (% FEMALE)	49.5	53.9	45.4	48.6

* PREIMPLANTATION LOSS = $\frac{\text{CORPORA LUTEA} - \text{IMPLANTS}}{\text{CORPORA LUTEA}}$

** $p<0.01$

† CORPORA LUTEA WERE MIS-COUNTED FOR SEVERAL DAMS

() # CALCULATED BY LLT

2. DEVELOPMENTAL TOXICITY

A. FETAL WEIGHT AND VIABILITY: FETAL WEIGHT (ALL FETUSES) WAS DEPRESSED AT THE HIGH DOSE (82% OF CONTROL VALUE); THE SAME RESULT WAS SEEN WHEN THE WEIGHT WAS CALCULATED BY SEX.

FETAL WEIGHT	CONTROL	LOW	MID	HIGH
MALE FETUSES°	5.6			4.6
FEMALE FETUSES°	5.2			4.2
ALL FETUSES	5.4	5.4	5.2	4.4**

** $p<0.01$
° # CALCULATED BY LLT

B. EXTERNAL AND VISCERAL EXAMINATIONS: WITH REGARD TO EXTERNAL ANOMALIES, THE MEAN LITTER % OF NORMAL FETUSES WAS DECREASED AT THE HIGH DOSE, WHICH IS OUTSIDE THE HISTORICAL CONTROL INCIDENCE. THERE WAS A DOSE-RELATED INCREASE IN THE NUMBER OF RUNTS OBSERVED AND A CORRESPONDING INCREASE IN THE NUMBER OF LITTERS WITH RUNTS. POINTED SNOUT WAS OBSERVED IN ONE LOW-DOSE AND ONE HIGH-DOSE FETUS, BUT THIS ANOMALY WAS NOT REPORTED IN THE HISTORICAL CONTROL.

	EXTERNAL ANOMALIES			
OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	299(23)	332(23)	328(24)	175(21)
NORMAL				
FETUSES WITH OBSERVATION	298	328	321	163
LITTERS WITH OBSERVATION	23	23	24	21
MEAN LITTER %	99.7	98.7	98.4	93.6*
SNOUT POINTED				
FETUSES WITH OBSERVATION	0	1	0	3
LITTERS WITH OBSERVATION	0	1	0	3
MEAN LITTER %	0	0.43	0	1.57
RUNT				
FETUSES WITH OBSERVATION	0	2	4	9
LITTERS WITH OBSERVATION	0	2	4	9
MEAN LITTER %	0	0.71	1.13	4.38*

SEVERAL EXTERNAL ANOMALIES (ARRESTED DEVELOPMENT, CRANIUM DOMED, HEMATOMA IN HIND LIMB, TAIL) WERE NOTED ONLY AT THE HIGH DOSE, BUT EACH IN ONLY 1 FETUS. HOWEVER, 2 OF THESE ANOMALIES (ARRESTED DEVELOPMENT AND CRANIUM DOMED) WERE NOT REPORTED IN THE HISTORICAL CONTROLS. THE ANIMAL WITH ARRESTED DEVELOPMENT WAS ALSO A RUNT (FEMALE); FEMALE WITH DOMED CRANIUM ALSO DISPLAYED A POINTED SNOUT. OVERALL, THERE WAS A STATISTICALLY SIGNIFICANT INCREASE IN THE MEAN LITTER % OF VARIANTS AT THE HIGH-DOSE LEVEL, BUT NOT IN THE INCIDENCE OF EXTERNAL MALFORMATIONS.

OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	299(23)	332(23)	328(24)	175(21)
TOTAL EXTERNAL VARIANTS				
FETUSES WITH OBSERVATION	1	3	5	12
LITTERS WITH OBSERVATION	1	3	5	10
MEAN LITTER %	0.31	0.96	1.41	6.36*
TOTAL EXTERNAL MALFORMATIONS				
FETUSES WITH OBSERVATION	0	1	3	1
LITTERS WITH OBSERVATION	0	1	3	1
MEAN LITTER %	0	0.31	0.81	0.53

WITH REGARD TO SOFT TISSUE OBSERVATIONS OF THE HEAD (WILSON), THE MEAN LITTER % NORMAL WAS DECREASED AT THE HIGH-DOSE LEVEL COMPARED TO CONCURRENT CONTROL AND HISTORICAL CONTROL VALUES.

SOFT TISSUE EXAMINATION (WILSON)

OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	153(23)	166(23)	160(24)	88(19)
NORMAL				
FETUSES WITH OBSERVATION	152	164	159	63
LITTERS WITH OBSERVATION	23	23	24	17
MEAN LITTER %	99.46	98.84	99.31	71.05**

SOFT TISSUE ANOMALIES OF THE HEAD THAT WERE SLIGHTLY INCREASED AT THE HIGH DOSE ARE LISTED BELOW.

OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	153(23)	166(23)	160(24)	88(19)
FOURTH VENTRICLE DILATED				
FETUSES WITH OBSERVATION	0	0	0	3
LITTERS WITH OBSERVATION	0	0	0	2
MEAN LITTER %	0	0	0	2.51

THE HISTORICAL CONTROL DATA LIST ONE OF 5 STUDIES IN WHICH THIS ANOMALY (MODERATE) WAS NOTED (2 LITTERS/2 FETUSES; 1.3 MEAN LITTER %).

LATERAL VENTRICLE(S) DILATED				
FETUSES WITH OBSERVATION	0	1	0	7
LITTERS WITH OBSERVATION	0	1	0	4
MEAN LITTER %	0	0.54	0	9.65
LATERAL VENTRICLE(S) DILATED MODERATE				
FETUSES WITH OBSERVATION	0	0	0	18
LITTERS WITH OBSERVATION	0	0	0	8
MEAN LITTER %	0	0	0	19.3*

THE HISTORICAL CONTROL DATA LIST ONE OF 5 STUDIES IN WHICH LATERAL VENTRICLE(S) DILATED WAS NOTED (1 LITTER/1 FETUS; 0.52 MEAN LITTER %).

SOFT TISSUE VARIATIONS IN THE TRUNK (STAPLES) WERE SIGNIFICANTLY DECREASED IN THE HIGH DOSE WITH A CONCOMITANT STATISTICALLY SIGNIFICANT INCREASE IN THE MEAN LITTER % OF FETUSES CONSIDERED NORMAL.

SOFT TISSUE EXAMINATION (STAPLES)

OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	153(23)	166(23)	160(24)	88(19)
NORMAL				
FETUSES WITH OBSERVATION	120	125	134	84
LITTERS WITH OBSERVATION	21	23	24	19
MEAN LITTER %	75.36	75.77	82.57	94.74**

SOFT TISSUE ANOMALIES OF THE TRUNK THAT WERE INCREASED AT THE HIGH-DOSE LEVELS ARE LISTED BELOW.

OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	153(23)	166(23)	160(24)	88(19)
SMALL HEART				
FETUSES WITH OBSERVATION	0	2	3	2
LITTERS WITH OBSERVATION	0	2	3	2
MEAN LITTER %	0	1.0	1.71	3.07

FOUR OF THE FIVE STUDIES FROM WHICH HISTORICAL CONTROL DATA WERE PRESENTED DISPLAYED ZERO INCIDENCE OF SMALL HEART. ONE STUDY HAD AN INCIDENCE IN 2 LITTERS AND 3 FETUSES, WITH A MEAN LITTER % OF 1.70.

THERE WERE DECREASES IN ALL URINARY TRACT VARIATIONS, BUT THE INCIDENCE OF URETERS CURVING REPEATEDLY WAS STATISTICALLY SIGNIFICANTLY DECREASED. THE AUTHOR CONSIDERED THIS PATTERN OF ALTERED INCIDENCE TO BE CONSISTENT WITH THE REDUCTION IN WEIGHT/SIZE OF THE FETUSES IN THE HIGH-DOSE GROUP.

OBSERVATION	CONTROL	LOW	MID	HIGH
URETER CURVING REPEATEDLY (CONVOLUTED) BOTH				
FETUSES WITH OBSERVATION	13	20	9	0
LITTERS WITH OBSERVATION	10	9	7	0
MEAN LITTER %	12.6	11.8	6.6	0*
URETER CURVING REPEATEDLY (CONVOLUTED) LEFT				
FETUSES WITH OBSERVATION	13	8	3	2
LITTERS WITH OBSERVATION	11	6	3	2
MEAN LITTER %	6.9	4.5	1.8*	2.2

THERE WAS A STATISTICALLY SIGNIFICANT INCREASE IN TOTAL SOFT TISSUE VARIANTS OF THE HEAD (WILSON) AND A STATISTICALLY SIGNIFICANT DECREASE IN TOTAL SOFT TISSUE VARIANTS OF THE TRUNK (STAPLES) AT THE HIGH-DOSE LEVEL BUT NO INCREASE IN THE INCIDENCE OF MALFORMATIONS IN EITHER THE HEAD OR TRUNK.

OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	153(23)	166(23)	160(24)	88(19)
WILSON				
TOTAL VISCERAL VARIATIONS				
FETUSES WITH OBSERVATION	0	1	0	25
LITTERS WITH OBSERVATION	0	1	0	11
MEAN LITTER %	0	0.54	0	28.95**
TOTAL VISCERAL MALFORMATIONS				
FETUSES WITH OBSERVATION	1	2	1	0
LITTERS WITH OBSERVATION	1	2	1	0
MEAN LITTER %	0.54	1.16	0.69	0
STAPLES				
TOTAL VISCERAL VARIATIONS				
FETUSES WITH OBSERVATION	33	40	25	4
LITTERS WITH OBSERVATION	16	15	14	4
MEAN LITTER %	24.6	23.5	16.8	5.3**
TOTAL VISCERAL MALFORMATIONS				
FETUSES WITH OBSERVATION	2	2		0
LITTERS WITH OBSERVATION	2	2	1	0
MEAN LITTER %	1.16	1.35	0.60	0

c. SKELETAL EXAMINATION: WITH REGARD TO SKELETAL EFFECTS, THE MEAN LITTER % NORMAL WAS DECREASED AT THE HIGH-DOSE LEVEL COMPARED TO CONCURRENT CONTROLS. THE INCIDENCE OF SEVERAL ANOMALIES OF THE RIBS, STERNEBRAE, AND THORACIC VERTEBRAE THAT DISPLAYED AN INCREASE IN THE HIGH-DOSE GROUP COMPARED TO CONCURRENT AND HISTORICAL CONTROL IS PRESENTED BELOW.

SKELETAL EXAMINATIONS

OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	146(23)	166(23)	166(24)	87(21)
NORMAL				
FETUSES WITH OBSERVATION	83	83	93	22
LITTERS WITH OBSERVATION	22	23	23	11
MEAN LITTER %	56.06	49.40	57.02	27.70**
RIBS RUDIMENTARY BOTH				
FETUSES WITH OBSERVATION	0	0	3	7
LITTERS WITH OBSERVATION	0	0	2	6
MEAN LITTER %	0	0	1.6	7.98

TWO OF THE FIVE HISTORICAL CONTROL STUDIES DISPLAYED THIS ANOMALY (2 FETUSES/2 LITTERS; MEAN LITTER % 4.89 AND 1 FETUS/1 LITTER; MEAN LITTER % 0.65)

RIBS RUDIMENTARY RIGHT				
FETUSES WITH OBSERVATION	1	0	4	4
LITTERS WITH OBSERVATION	1	0	3	4
MEAN LITTER %	0.9	0	2.6	3.86

ONE STUDY DISPLAYED 3 LITTERS/4 FETUSES WITH THIS ANOMALY; MEAN LITTER % WAS 2.59.

STERNEBRA INCOMPLETELY OSSIFIED 5TH				
FETUSES WITH OBSERVATION	29	44	32	39
LITTERS WITH OBSERVATION	12	16	15	16
MEAN LITTER %	16.7	27.1	18.2	46.96**

THE INCIDENCE OF THIS ANOMALY IN THE HISTORICAL CONTROL DATA (STUDIES A-E) WAS VARIABLE, AS SHOWN BELOW.

STERNEBRA INCOMPLETELY OSSIFIED 5TH	A	B	C	D	E
FETUSES WITH OBSERVATION	39	50	58	61	1
LITTERS WITH OBSERVATION	18	19	17	21	2
MEAN LITTER %	23.2	29.7	33.0	45.2	7

SKELETAL EXAMINATIONS

OBSERVATION	CONTROL	LOW	MID	HIGH
STERNEBRA IRREGULARLY SHAPED				
FETUSES WITH OBSERVATION	0	2	3	4
LITTERS WITH OBSERVATION	0	2	2	4
MEAN LITTER %	0	1.27	1.88	4.01

THIS ANOMALY WAS NOT LISTED IN THE HISTORICAL CONTROL TABLES.

OBSERVATION	CONTROL	LOW	MID	HIGH
STERNEBRA(E) INCOMPLETELY OSSIFIED OTHER				
FETUSES WITH OBSERVATION	1	3	3	6
LITTERS WITH OBSERVATION	1	3	3	5
MEAN LITTER %	0.48	2.2	1.58	6.35

THE INCIDENCE OF THIS ANOMALY IN THE HISTORICAL CONTROLS IS SHOWN BELOW.

	A	B	C	D	E
FETUSES WITH OBSERVATION	1	1	3	1	0
LITTERS WITH OBSERVATION	1	1	2	1	0
MEAN LITTER %	0.51	0.54	1.56	0.54	0

OBSERVATION	CONTROL	LOW	MID	HIGH
STERNEBRA MALALIGNED				
FETUSES WITH OBSERVATION	1	5	3	10
LITTERS WITH OBSERVATION	1	5	3	7
MEAN LITTER %	0.72	3.46	1.58	14.68

THE INCIDENCE OF THIS ANOMALY IN THE HISTORICAL CONTROLS IS LISTED BELOW.

	A	B	C	D	E
FETUSES WITH OBSERVATION	6	5	3	8	6
LITTERS WITH OBSERVATION	6	4	2	5	6
MEAN LITTER %	3.22	2.67	1.56	4.37	3.51

OBSERVATION	CONTROL	LOW	MID	HIGH
STERNEBRA(E) SPLIT				
FETUSES WITH OBSERVATION	0	0	2	3
LITTERS WITH OBSERVATION	0	0	2	3
MEAN LITTER %	0	0	1.12	6.43

THIS ANOMALY WAS NOT LISTED IN THE HISTORICAL CONTROL DATA.

OBSERVATION	CONTROL	LOW	MID	HIGH
THORACIC VERTEBRA(E) CENTRA IRREGULARLY SHAPED				
FETUSES WITH OBSERVATION	0	0	1	2
LITTERS WITH OBSERVATION	0	0	1	3
MEAN LITTER %	0	0	0.60	6.35

FROM THE HISTORICAL CONTROL DATA, ONLY ONE OF THE FIVE STUDIES DISPLAYED THORACIC VERTEBRA(E) CENTRA MALFORMED (1 FETUS/1 LITTER; MEAN LITTER % 0.62).

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OBSERVATION	CONTROL	LOW	MID	HIGH
THORACIC VERTEBRA(E) CENTRA SPLIT				
FETUSES WITH OBSERVATION	2	0	1	6
LITTERS WITH OBSERVATION	2	0	1	5
MEAN LITTER %	1.09	0	0.83	6.91

THIS ANOMALY WAS NOT LISTED IN THE HISTORICAL CONTROL TABLES.

THERE WAS A STATISTICALLY SIGNIFICANT INCREASE IN THE TOTAL VARIANTS OF THE SKELETON AT THE HIGH-DOSE LEVEL, BUT NO STATISTICALLY SIGNIFICANT DIFFERENCE IN MALFORMATIONS, ALTHOUGH ONLY THE TREATED ANIMALS DISPLAYED SKELETAL MALFORMATIONS.

	SKELETAL EXAMINATIONS			
OBSERVATION	CONTROL	LOW	MID	HIGH
# PUPS (LITTERS) EXAMINED	146(23)	166(23)	166(24)	87(21)
TOTAL SKELETAL VARIANTS				
FETUSES WITH OBSERVATION	63	82	71	65
LITTERS WITH OBSERVATION	20	22	23	19
MEAN LITTER %	43.94	49.98	41.69	72.30**
TOTAL SKELETAL MALFORMATIONS				
FETAL INCIDENCE (%)	0	2	2	2
LITTER INCIDENCE (%)	0	2	2	2
MEAN LITTER %	0	1.24	1.29	1.43

* $p<0.05$; ** $p<0.01$ - SIGNIFICANTLY DIFFERENT FROM CONTROL, TWO-TAILED

DISCUSSION

THERE WAS NO EFFECT ON SURVIVAL OF THE DAMS, AND THERE WERE COMPARABLE NUMBERS OF CORPORA LUTEA AND IMPLANTATION SITES AMONG THE GROUPS. MOLINATE WAS MATERNALLY TOXIC AT THE HIGH-DOSE LEVEL (140 MG/KG/DAY), AS EVIDENCED BY THE DECREASED BODY WEIGHT, BODY-WEIGHT GAIN, AND FOOD CONSUMPTION, THE INCREASED SALIVATION, AND RBC CHOLINESTERASE INHIBITION.

FETOTOXICITY WAS DEMONSTRATED AT THE SAME DOSE LEVEL, AS EVIDENCED BY AN INCREASE IN POST-IMPLANTATION LOSS, LOWER FETAL BODY WEIGHT, AN INCREASED INCIDENCE OF RUNTS, AND AN INCREASED INCIDENCE OF EXTERNAL, SOFT TISSUE (HEAD), AND SKELETAL VARIANTS.

CONCLUSION

THE NOEL FOR MATERNAL TOXICITY CAN BE SET AT 35 MG/KG/DAY, THE LEL AT 140 MG/KG/DAY, BASED ON DECREASED BODY WEIGHT, BODY-WEIGHT GAIN, AND FOOD CONSUMPTION, AND INCREASED SALIVATION AND RBC CHOLINESTERASE INHIBITION. THE NOEL FOR FETOTOXICITY CAN BE SET AT 35 MG/KG/DAY, THE LEL AT 140 MG/KG/DAY, BASED ON AN INCREASE IN POST-IMPLANTATION LOSS, LOWER FETAL BODY WEIGHT, AND INCREASED INCIDENCES OF RUNTS, EXTERNAL, SOFT TISSUE, AND SKELETAL VARIANTS. DEVELOPMENTAL TOXICITY OCCURRED ONLY AT A MATERNALLY-TOXIC DOSE OF MOLINATE.

DISCREPANCIES

THERE WERE SEVERAL DISCREPANCIES NOTED BETWEEN THE SUMMARY TABLES AND THE INDIVIDUAL DATA. THESE INCLUDE:

1) PAGE 33, TABLE 8 - # FETUSES/DAM IN THE HIGH DOSE IS LISTED AS 7.6; USING THE DATA IN APPENDIX G, PAGES 121-123, THE # IS 8.3.
2) PAGE 33, TABLE 8 - THE SEX RATIO OF THE CONTROL GROUP IS LISTED AS 49.2 AND THE HIGH-DOSE GROUP AS 48.0; USING THE DATA IN APPENDIX G, THE RATIOS ARE 49.5 AND 48.6, RESPECTIVELY.
3) PAGES 35-38, TABLE 9 - # OF FETUSES IN THE MID-DOSE GROUP IS LISTED AS 327; ADDING THE # OF FETUSES LISTED AS EXAMINED IN TABLES 10 AND 11 RESULTS IN 326 FETUSES.
4) PAGE 119, DAM # 95 IS LISTED AS HAVING 7 MALE AND 8 FEMALE FETUSES; APPENDIX I SHOWS 6 MALE AND 9 FEMALE.
5) PAGE 121, DAM # 132 IS LISTED AS HAVING 5 MALE AND 8 FEMALE FETUSES; APPENDIX I SHOWS 6 MALE AND 7 FEMALE.