#29

FOOD AND DRUG LABORATORIES, INC.

MAURICE AVENUE AT 58TH STREET, MASPETH, NEW YORK 11378

FINAL

April 16, 1973

Teratologic Evaluation of FDA 71-30

(Oil of clove)

in

Mice, Rats, ~~and~~ Hamsters and Rabbits

Final report-Teratologic Evaluation of FDA 71-30 (Oil of Clove) in Mice, Rats, Hamsters & Rabbits

4/16/73

M I C E

Food and Drug Research Laboratories
INCORPORATED

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date April 16, 1973

Laboratory No. 1573 c
Contract No. FDA 71-260

Sample: Clear yellow liquid

Marking: FDA 71-30 (Oil of clove)

Examination Requested: Teratologic evaluation of FDA 71-30 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 215 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

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Vice President

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 221 & 222; 227 through 230

Material: FDA 71-30

Date: March 16, 1973

Laboratory No.: 1573 c

Table 1

Fate Summary
(Mice)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
221	Sham	0.0	25	22	25	22
222	Aspirin*	150.0	24	23	24	23
227	FDA 71-30	2.2	25	22	25	22
228	FDA 71-30	10.0	23	23	23	23
229	FDA 71-30	46.0	25	24	25	24
230	FDA 71-30	215.0	24	22	24	22

* Positive Control: 150.0 mg/kg

** Administered as a corn oil solution (10 ml per kg of body weight)

1) Includes all dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 221 & 222; 227 through 230

Date: March 16, 1973

Material: FDA 71-30

Laboratory No. 1573 c

Table 2
Reproduction Data
(Mice)

Group:	221	222	227	228	229	230
Dose (mg/kg):	Sham	Aspirin**	2.2	10.0	46.0	215.0
Pregnancies						
Total No.	22	23	22	23	24	22
Died or Aborted (before Day 17)	0	0	0	0	0	0
To term (on Day 17)	22	23	22	23	24	22
Live Litters						
Total No.*	22	23	22	23	24	22
Implant Sites						
Total No.	264	274	241	277	268	246
Average/dam*	12.0	11.9	11.0	12.0	11.2	11.2
Resorptions						
Total No.*	14	20	10	3	11	3
Dams with 1 or more sites resorbed	8	12	8	3	9	3
Dams with all sites resorbed	--	--	--	--	--	--
Per cent partial resorptions	36.4	52.2	36.4	13.0	37.5	13.6
Per cent complete resorptions	--	--	--	--	--	--
Live Fetuses						
Total No.	249	253	230	272	255	240
Average/dam*	11.3	11.0	10.5	11.8	10.6	10.9
Sex ratio (M/F)	0.98	0.85	1.08	0.77	1.08	0.89
Dead Fetuses						
Total*	1	1	1	2	2	3
Dams with 1 or more dead	1	1	1	2	2	3
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	4.55	4.35	4.55	8.70	8.34	13.6
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	0.90	0.85	0.94	0.87	0.90	0.86

* Includes only those dams examined at term.
** Positive Control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230 Laboratory No. 1573 c

Table 3

Material FDA 71-30 Date March 16, 1973

Summary of Skeletal Findings*

(Mice)

Findings / Group No.:	221	222	227	228	229	230
Dose (mg/kg):	Sham	Aspirin**	2.2	10.0	46.0	215.0
Live Fetuses Examined (at term)	176/22	177/23	161/22	186/23	180/24	168/22
Sternebrae						
Incomplete oss.	91/18	73/20	37/14	74/19	61/20	67/17
Scrambled						
Bipartite	9/8	4/4	2/2	3/3	4/3	6/5
Fused						
Extra						
Missing	14/8	27/12	11/7	26/11	17/12	27/9
Other						
Ribs						
Incomplete oss.						
Fused/split						1/1
Wavy						
Less than 12						
More than 13	45/16	34/12	53/18	46/16	49/17	38/16
Other						
Vertebrae						
Incomplete oss.	5/4	6/2	4/2	6/4	3/2	2/2
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis						
Tail defects						
Other						
Skull						
Incomplete closure	4/3	3/2		1/1		
Missing						
Craniostosis						
Other						
Extremities						
Incomplete oss.	6/4	5/1	3/2	5/5	5/3	2/2
Missing						
Extra						
Miscellaneous						
Hyoid; missing	37/15	59/18	23/12	38/13	35/17	44/15
Hyoid; reduced	36/14	26/13	29/16	19/9	20/12	27/16

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230 Date March 16, 1973

Material FDA 71-30 Laboratory No. 1573 c

Table 3-a
Summary of Soft Tissue Abnormalities
(Mice)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
228	FDA 71-30	10.0	C 3032	1	Meningoencephalocele

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230

Date March 16, 1973

Species Mice

Laboratory No. 1573 c

Table 4

Average Body Weights*

Group	Material	Dose Level	Day 0	Day 6	Day 11	Day 15	Day 17**
		mg/kg	g				
221	Sham	0.0	31.2	35.4	38.0	46.2	51.9 (22)
222	Aspirin***	150.0	30.1	34.4	36.3	44.4	49.7 (23)
227	FDA 71-30	2.2	31.1	33.4	35.0	48.1	50.6 (22)
228	FDA 71-30	10.0	31.4	33.7	37.0	45.3	50.9 (23)
229	FDA 71-30	46.0	30.5	33.6	36.0	43.4	48.6 (24)
230	FDA 71-30	215.0	30.8	34.1	36.7	45.8	49.4 (22)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 150.0 mg/kg

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were gang-housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubaticn; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 221

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date March 16, 1973

Laboratory No. 1573

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 3666	P		11	10		5	6	1	1.00	
S 3667	P		10	10		5	5		0.87	
S 3668	P		13	11		7	4	2	0.94	
S 3669	P		11	11		6	5		0.98	
S 3670	P		11	11		3	8		0.97	
S 3671	P		11	11		5	6		0.83	
S 3672	P		10	10		6	4		1.18	
S 3673	P		13	11		4	7	2	0.92	
S 3674	NP		0						----	
S 3675	P		9	9		4	5		0.88	
S 3676	P		12	12		5	7		0.85	
S 3677	P		14	14		8	6		0.70	
S 3678	P		12	12		6	6		0.84	
S 3679	NP		0						----	
S 3680	P		13	12		4	8	1	0.85	
S 3681	P		10	7		2	5	3	0.96	
S 3682	P		14	13		5	8	1	0.89	
S 3683	P		12	12		10	2		0.99	
S 3684	P		15	12		8	4	3	0.93	
S 3685	NP		0						----	
S 3686	P		13	12		11	1	1	0.87	
S 3687	P		14	13	1	6	7		0.79	
S 3688	P		11	11		3	8		0.83	
S 3689	P		14	14		6	8		0.94	
S 3690	P		11	11		5	6		0.81	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 222

Material Aspirin

Dose 150.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date March 16, 1973

Laboratory No. 1573

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 3666	P		16	16		9	7		0.94	
A 3667	P		12	9		5	3	3	0.94	
A 3668	P		13	13		6	6		0.80	
A 3669	P		13	13		4	9		0.86	
A 3670	NP		0						----	
A 3671	P		11	11		6	5		0.81	
A 3672	P		7	7		2	5		0.60	
A 3673	P		10	10		6	4		0.81	
A 3674	P		13	11		6	5	2	0.77	
A 3675	P		11	10		5	5	1	0.72	
A 3676	P		10	8	1	2	6	1	0.91	
A 3677	P		9	8		5	3	1	0.80	
A 3678	P		15	15		7	8		0.73	
A 3679	P		10	8		3	5	2	0.86	
A 3680	P		14	13		5	8	1	0.72	
A 3681	P		14	14		6	8		0.70	
A 3682	P		13	13		6	7		0.87	
A 3683	P		14	13		4	9	1	0.89	
A 3684										Not Assigned
A 3685	P		9	9		4	5		0.87	
A 3686	P		12	11		3	8	1	0.95	
A 3687	P		12	12		3	9		0.89	
A 3688	P		13	10		5	5	3	0.97	
A 3689	P		12	10		8	2	2	0.98	
A 3690	P		11	9		5	4	2	1.20	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 227

Material FDA 71-30

Dose 2.2 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date March 16, 1973

Laboratory No. 1573 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 3001	NP		0						----	
C 3002	P		11	10		4	6	1	0.82	
C 3003	P		10	9		4	5	1	0.98	
C 3004	P		9	9		5	4		0.88	
C 3005	NP		0						----	
C 3006	P		9	9		4	5		0.83	
C 3007	P		11	11		5	6		1.13	
C 3008	P		13	12		6	6	1	1.04	
C 3009	P		11	11		4	7		0.98	
C 3010	P		14	14		9	5		0.91	
C 3011	P		12	11	1	5	6		0.91	
C 3012	P		13	13		8	5		0.86	
C 3013	P		12	12		8	4		0.99	
C 3014	P		7	6		4	2	1	0.92	
C 3015	P		6	4		1	3	2	1.15	
C 3016	P		16	15		4	11	1	0.92	
C 3017	P		13	12		5	7	1	0.90	
C 3018	P		11	11		4	7		0.69	
C 3019	P		13	11		7	4	2	0.91	
C 3020	P		11	11		9	2		1.07	
C 3021	P		8	8		4	4		1.06	
C 3022	P		10	10		8	2		0.92	
C 3023	P		10	10		5	5		0.94	
C 3024	P		11	11		6	5		0.86	
C 3025	NP		0						----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 228

Material FDA 71-30

Dose 10.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date March 16, 1973

Laboratory No. 1573 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 3031	P		13	13		7	6		0.82	
C 3032	P		13	13		3	10		0.86	
C 3033	P		12	12		5	7		1.12	
C 3034	P		12	12		5	7		0.85	
C 3035	P		13	12	1	5	7		0.73	
C 3036	P		13	13		6	7		0.85	
C 3037	P		8	7		3	4	1	0.99	
C 3038	P		15	15		6	9		1.08	
C 3039	P		12	12		3	9		0.83	
C 3040	P		14	14		6	8		0.98	
C 3041	P		12	12		6	6		0.72	
C 3042	P		10	9	1	7	2		0.78	
C 3043	P		12	12		6	6		0.92	
C 3044	P		9	9		5	4		0.84	
C 3045	P		12	12		5	7		0.92	
C 3046	P		11	10		4	7	1	1.20	
C 3047	P		13	13		3	10		0.80	
C 3048										Not Assigned
C 3049	P		11	11		3	8		0.66	
C 3050	P		12	12		5	7		0.86	
C 3051	P		11	10		5	5	1	0.92	
C 3052										Not Assigned
C 3053	P		15	15		11	4		0.81	
C 3054	P		13	13		6	7		0.73	
C 3055	P		11	11		3	8		0.78	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 229

Material FDA 71-30

Dose 46.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date March 16, 1973

Laboratory No. 1573 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 3061	P		14	14		7	7		0.88	
C 3062	P		14	13	1	7	6		0.84	
C 3063	P		12	12		5	7		0.92	
C 3064	P		9	8		6	2	1	1.15	
C 3065	P		11	11		6	5		0.92	
C 3066	P		12	12		5	7		0.70	
C 3067	P		13	12		9	3	1	0.80	
C 3068	P		9	8		2	6	1	0.82	
C 3069	P		11	11		5	6		1.00	
C 3070	P		13	11		6	7	2	0.83	
C 3071	P		12	10	1	4	6	1	0.96	
C 3072	P		4	4		1	3		1.03	
C 3073	P		11	11		10	1		0.81	
C 3074	NP		0						----	
C 3075	P		9	8		4	4	1	0.90	
C 3076	P		14	13		6	7	1	0.95	
C 3077	P		13	13		7	6		0.98	
C 3078	P		9	8		4	4	1	0.92	
C 3079	P		9	9		6	3		0.91	
C 3080	P		10	10		7	3		0.95	
C 3081	P		12	12		6	6		0.86	
C 3082	P		12	12		5	7		0.89	
C 3083	P		10	8		3	5	2	0.74	
C 3084	P		13	13		6	7		0.86	
C 3085	P		12	12		6	6		0.87	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 230

Material FDA 71-30

Dose 215.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date March 16, 1973

Laboratory No. 1573 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 3091	P		13	13		4	9		0.79	
C 3092	P		6	6		4	2		0.82	
C 3093	P		10	10		4	6		0.82	
C 3094	P		10	10		4	6		0.88	
C 3095	P		7	7		4	3		0.92	
C 3096	P		9	9		4	5		0.97	
C 3097	P		12	11	1	5	6		0.98	
C 3098	P		13	13		5	8		0.82	
C 3099	P		10	10		9	1		0.95	
C 3100	P		12	12		5	7		0.85	
C 3101	P		14	14		9	5		0.92	
C 3102	P		10	10		5	5		0.81	
C 3103	P		14	14		9	5		0.69	
C 3104	P		12	12		4	8		0.90	
C 3105	P		14	14		5	9		0.85	
C 3106	P		10	8	1	2	6	1	0.77	
C 3107	P		11	10		4	6	1	0.87	
C 3108	NP		0						----	
C 3109										Not Assigned
C 3110	P		10	10		3	7		0.76	
C 3111	P		13	12		6	6	1	0.91	
C 3112	NP		0						----	
C 3113	P		9	8	1	3	5		0.78	
C 3114	P		13	13		6	7		0.95	
C 3115	P		14	14		9	5		0.92	

* P = Pregnant; NP = Not Pregnant

R A T S

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date April 16, 1973

Laboratory No. 1574 c
Contract No. FDA 71-260

Sample: Clear Yellow liquid

Marking: FDA 71-30 (Oil of clove)

Examination Requested: Teratologic evaluation of FDA 71-30 in rats

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 280 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 221 & 222; 227 through 230

Material: FDA 71-30

Date: March 16, 1973

Laboratory No.: 1574 c

Table 1

Fate Summary
(Rats)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
221	Sham	0.0	25	21	25	21
222	Aspirin*	250.0	23	21	23	21
227	FDA 71-30	2.8	25	23	25	23
228	FDA 71-30	13.0	25	25	25	25
229	FDA 71-30	60.0	25	23	25	23
230	FDA 71-30	280.0	25	24	25	24

* Positive Control: 250.0 mg/kg
** Administered as a corn oil solution (See Appendix I)
1) Includes all dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 221 & 222; 227 through 230

Date: March 16, 1973

Material: FDA 71-30

Laboratory No. 1574 c

Table 2
Reproduction Data
(Rats)

Group:	221	222	227	228	229	230
Dose (mg/kg):	Sham	Aspirin**	2.8	13.0	60.0	280.0
Pregnancies						
Total No.	21	21	23	25	23	24
Died or Aborted (before Day 20)	0	0	0	0	0	0
To term (on Day 20)	21	21	23	25	23	24
Live Litters						
Total No.*	21	19	23	25	23	24
Implant Sites						
Total No.	253	254	257	294	275	257
Average/dam*	12.1	12.1	11.2	11.8	12.0	10.7
Resorptions						
Total No.*	2	61	2	2	4	2
Dams with 1 or more sites resorbed	2	9	2	2	1	1
Dams with all sites resorbed	--	2	--	--	--	--
Per cent partial resorptions	9.52	42.9	8.70	8.00	4.35	4.17
Per cent complete resorptions	--	9.52	--	--	--	--
Live Fetuses						
Total No.	251	191	255	290	270	255
Average/dam*	12.0	9.10	11.1	11.6	11.7	10.6
Sex ratio (M/F)	1.06	1.07	0.78	0.82	0.91	0.71
Dead Fetuses						
Total*	--	2	--	2	1	--
Dams with 1 or more dead	--	2	--	1	1	--
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	--	9.52	--	4.00	4.35	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	3.91	2.52	3.98	4.17	4.00	3.95

* Includes only those dams examined at term.
** Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230 Laboratory No. 1574 c

Table 3

Material FDA 71-30 Date March 16, 1973

Summary of Skeletal Findings*
(Rats)

Findings / Group No.:	221	222	227	228	229	230
Dose (mg/kg):	Sham	Aspirin**	2.8	13.0	60.0	280.0
Live Fetuses Examined (at term)	175/21	133/19	177/23	201/25	187/23	181/24
Sternebrae						
Incomplete oss.	49/15	108/19	63/19	53/16	51/17	57/20
Scrambled						
Bipartite		21/9	2/2	1/1	3/3	
Fused						
Extra			1/1			
Missing	4/2	108/19	9/6	4/3	5/5	4/4
Other						
Ribs						
Incomplete oss.		2/2				1/1
Fused/split		17/7		1/1		
Wavy	9/6	62/16	18/10	28/14	18/9	20/12
Less than 12						
More than 13	2/2	104/19		2/2	5/3	3/3
Other						
Vertebrae						
Incomplete oss.	8/7	127/19	15/8	6/5	15/5	14/9
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis		1/1				
Tail defects						
Other						
Skull						
Incomplete closure	23/12	84/18	26/12	38/16	45/11	54/17
Missing		5/4				
Craniostosis						
Other						
Extremities						
Incomplete oss.		7/5	1/1	2/2		1/1
Missing						
Extra						
Miscellaneous						
Hyoid; missing	27/11	78/18	40/15	17/9	42/12	25/12
Hyoid; reduced	25/13	7/6	8/6	29/13	20/11	34/17
Pelvic bone; incomplete		1/1				

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230 Date March 16, 1973

Material FDA 71-30 Laboratory No. 1574 c

Table 3-a

Summary of Soft Tissue Abnormalities

(Rats

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
222	Aspirin*	250.0	A 4669	1	Encephalomyelocele
			A 4670	1	Encephalomyelocele; umbilical hernia
				1	Encephalomyelocele
			A 4674	1	Encephalomyelocele
			A 4677	1	Exencephaly
			A 4678	1	Exencephaly; ateloglossia
			A 4685	1	Encephalomyelocele
			A 4690	1	Encephalomyelocele

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230

Date March 16, 1973

Table 4

Species Rats

Laboratory No. 1574 c

Average Body Weights*

Group	Material	Dose Level	Day 0	6	11	15	20**
		mg/kg	g				
221	Sham	0.0	220	240	258	274	341 (21)
222	Aspirin***	250.0	216	234	248	267	313 (21)
227	FDA 71-30	2.8	217	241	259	279	344 (23)
228	FDA 71-30	13.0	227	248	265	288	356 (25)
229	FDA 71-30	60.0	225	248	267	288	357 (23)
230	FDA 71-30	280.0	214	236	252	272	333 (24)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 250.0 mg/kg

Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubations. The controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose. The test material was prepared and doses calculated according to the following table:

Dosage (mg/kg)	Dose (ml/kg)	Concentration (mg/ml)
≦ 250	1	≦ 250
251 - 500	2	125 - 250
501 - 750	3	133 - 250
751 - 1000	4	187 - 250
1001 - 1250	5	200 - 250
1251 - 1500	6	208 - 250
1501 - 1600	6.4	235 - 250

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam

was examined in detail for anatomical normalcy.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 221

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date March 16, 1973

Laboratory No. 1574

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 4666	P		15	15		12	3		4.25	
S 4667	P		10	10		4	6		3.93	
S 4668	P		14	14		7	7		3.87	
S 4669	P		13	12		4	8	1	3.66	
S 4670	P		14	14		8	6		3.40	
S 4671	NP		0						----	
S 4672	P		14	14		6	8		3.88	
S 4673	P		10	10		3	7		4.10	
S 4674	P		11	11		1	10		3.91	
S 4675	P		12	12		8	4		3.81	
S 4676	P		7	7		4	3		4.11	
S 4677	P		13	13		7	6		3.74	
S 4678	P		11	11		3	8		3.91	
S 4679	P		8	8		5	3		4.03	
S 4680	NP		0						----	
S 4681	P		13	13		7	6		4.07	
S 4682	P		12	12		7	5		4.13	
S 4683	P		11	11		8	3		3.66	
S 4684	P		12	12		6	6		3.95	
S 4685	P		13	13		7	6		4.10	
S 4686	P		16	16		10	6		3.74	
S 4687	NP		0						----	
S 4688	P		13	13		9	4		3.88	
S 4689	P		11	10		3	7	1	3.97	
S 4690	NP		0						----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 222

Material Aspirin

Dose 250.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date March 16, 1973

Laboratory No. 1574

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 4666	P		12	12		7	5		2.79	
A 4667	P		10	10		7	3		3.04	
A 4668	P		13	5		3	2	8	2.22	
A 4669	P		12	12		2	10		2.15	
A 4670	P		13	13		7	5		2.20	
A 4671	P		11					11	----	
A 4672	P		16	15		8	7	1	2.77	
A 4673	P		12	12		6	6		2.52	
A 4674	P		11	11		6	5		2.68	
A 4675	P		11	11		6	5		3.07	
A 4676	NP		0						----	
A 4677	P		12	12		8	4		2.52	
A 4678	P		13	12	1	9	3		3.13	
A 4679	NP		0						----	
A 4680	P		12	11		5	6	1	1.98	
A 4681	P		11					11	----	
A 4682	P		12	12		5	7		2.55	
A 4683	P		10	10		4	6		2.66	
A 4684										Not Assigned
A 4685	P		11	4	1	3	1	6	2.19	
A 4686	P		12	4		2	2	8	2.67	
A 4687	P		12	12		7	5		2.45	
A 4688										Not Assigned
A 4689	P		13	11		3	8	2	2.33	
A 4690	P		15	2		0	2	13	1.99	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 228

Material FDA 71-30

Dose 13.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date March 16, 1973

Laboratory No. 1574 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 4031	P		10	10		7	3		4.10	
C 4032	P		8	8		3	5		3.92	
C 4033	P		12	12		5	7		3.56	
C 4034	P		15	15		7	8		3.76	
C 4035	P		11	11		8	3		3.79	
C 4036	P		13	13		8	5		3.55	
C 4037	P		13	13		5	8		4.35	
C 4038	P		14	14		7	7		4.15	
C 4039	P		13	13		6	7		3.85	
C 4040	P		9	9		2	7		3.85	
C 4041	P		11	10		7	3	1	3.96	
C 4042	P		13	13		3	10		5.62	
C 4043	P		10	10		6	4		4.38	
C 4044	P		15	15		9	6		4.28	
C 4045	P		12	12		7	5		3.91	
C 4046	P		11	11		1	10		4.11	
C 4047	P		13	13		2	11		6.27	
C 4048	P		14	14		6	8		4.00	
C 4049	P		14	14		6	8		4.07	
C 4050	P		12	12		5	7		3.84	
C 4051	P		9	9		4	5		4.32	
C 4052	P		14	14		7	7		3.84	
C 4053	P		9	8		4	4	1	3.97	
C 4054	P		11	11		3	8		4.06	
C 4055	P		8	6	2	3	3		4.74	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 229

Material FDA 71-30

Dose 60.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date March 16, 1973

Laboratory No. 1574 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 4061	P		13	13		7	6		3.92	
C 4062	NP		0						----	
C 4063	P		14	14		8	6		3.47	
C 4064	P		16	16		6	10		4.03	
C 4065	P		12	12		4	8		3.84	
C 4066	P		14	14		9	5		4.05	
C 4067	P		12	12		4	8		4.22	
C 4068	P		14	14		8	6		3.33	
C 4069	P		10	10		5	5		3.46	
C 4070	P		10	10		6	4		3.72	
C 4071	P		9	9		4	5		4.17	
C 4072	P		13	13		7	6		4.10	
C 4073	P		10	10		6	4		4.34	
C 4074	P		12	12		8	4		4.37	
C 4075	NP		0						----	
C 4076	P		13	13		6	7		4.04	
C 4077	P		9	9		6	3		4.05	
C 4078	P		10	10		4	6		4.09	
C 4079	P		15	15		8	7		3.91	
C 4080	P		9	9		4	5		4.39	
C 4081	P		13	13		5	8		4.37	
C 4082	P		10	9	1	4	5		4.16	
C 4083	P		12	8		4	4	4	3.91	
C 4084	P		9	9		1	8		3.97	
C 4085	P		16	16		5	11		4.00	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 230

Material FDA 71-30

Dose 280.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date March 16, 1973

Laboratory No. 1574 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 4091	P		12	12		5	7		3.48	
C 4092	P		11	11		2	9		3.83	
C 4093	P		11	11		5	6		3.71	
C 4094	P		11	11		4	7		3.61	
C 4095	P		11	11		5	6		4.01	
C 4096	P		11	11		5	6		4.33	
C 4097	P		10	10		6	4		3.62	
C 4098	P		8	8		6	2		3.94	
C 4099	P		11	11		5	6		3.92	
C 4100	NP		0						----	
C 4106	P		14	14		9	5		3.78	
C 4107	P		12	12		5	7		4.14	
C 4108	P		7	7		1	6		3.92	
C 4109	P		13	13		5	8		4.10	
C 4110	P		14	14		6	8		4.22	
C 4111	P		9	9		6	3		3.72	
C 4112	P		10	10		3	7		3.97	
C 4113	P		12	10		2	8	2	3.67	
C 4114	P		13	13		7	6		4.13	
C 4115	P		9	9		4	5		4.03	
C 4116	P		11	11		2	9		4.38	
C 4117	P		11	11		5	6		3.99	
C 4118	P		10	10		4	6		4.17	
C 4119	P		5	5		0	5		4.10	
C 4120	P		11	11		4	7		4.10	

* P = Pregnant; NP = Not Pregnant

HAMSTERS

Food and Drug Research Laboratories
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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date April 16, 1973

Laboratory No. 1575 c
Contract No. FDA 71-260

Sample: Clear Yellow liquid

Marking: FDA 71-30 (Oil of clove)

Examination Requested: Teratologic evaluation of FDA 71-30 in hamsters

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 177.0 mg/kg (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor of any members of its staff, may be used in connection with the advertising or sale of any product or process without written authorization.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 221 & 222; 227 through 230

Material: FDA 71-30

Date: March 16, 1973

Laboratory No.: 1575 c

Table 1

Fate Summary
(Hamsters)

Group	Material	Dose ** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
221	Sham	0.0	27	21	27	21
222	Aspirin*	250.0	25	25	24	24
227	FDA 71-30	1.8	25	22	25	22
228	FDA 71-30	8.2	26	21	26	21
229	FDA 71-30	38.2	28	20	28	20
230	FDA 71-30	177.0	25	21	25	21

* Positive Control: 250.0 mg/kg
** Administered as a corn oil solution (See Appendix I)
1) Includes all dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230 Laboratory No. 1575 c

Table 3

Material FDA 71-30 Date March 16, 1973

Summary of Skeletal Findings *

(Hamsters)

Findings / Group No.: / Dose (mg/kg):	Sham	Aspirin**	1.8	8.2	38.2	177.0
Live Fetuses Examined (at term)	184/21	244/24	195/22	178/20	179/19	186/21
Sternebrae						
Incomplete oss.	70/17	160/23	91/20	95/20	86/18	101/18
Scrambled						
Bipartite	19/13	31/8	24/17	27/14	29/13	20/12
Fused		1/1				
Extra	2/2	1/1	3/2		9/6	
Missing	25/9	56/17	27/10	19/8	22/10	30/8
Other						
Ribs						
Incomplete oss.						
Fused/split						1/1
Wavy						
Less than 12						
More than 13	68/18	55/19	86/20	55/14	45/16	52/18
Other						
Vertebrae						
Incomplete oss.	7/4		3/2	1/1	3/2	7/2
Scrambled						
Fused	1/1					
Extra ctrs. oss.						
Scoliosis		2/2				
Tail defects						
Other						
Skull						
Incomplete closure		1/1			1/1	
Missing						
Craniostosis						
Other						
Extremities						
Incomplete oss.	6/3	12/7	8/4	4/2	4/2	11/4
Missing						
Extra						
Miscellaneous						
Hyoid; missing	2/2	2/2	3/1			4/1
Hyoid; reduced	8/5	7/6	6/5	6/4	4/4	11/5

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230 Date March 16, 1973

Material FDA 71-30 Laboratory No. 1575 c

Table 3-a

Summary of Soft Tissue Abnormalities

(Hamsters)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
221	Sham	0.0	S 5691	1	Anasarca
228	FDA 71-30	8.2	C 5050	5	Anasarca

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230

Date March 16, 1973

Table 4

Species Hamsters

Laboratory No. 1575 c

Average Body Weights*

Group	Material	Dose Level	Day 0	6	8	10	14**
		mg/kg	g				
221	Sham	0.0	122.1	122.2	126.6	136.4	157.7 (21)
222	Aspirin***	250.0	127.8	128.4	132.6	144.2	168.6 (24)
227	FDA 71-30	1.8	120.0	118.6	120.1	131.5	154.5 (22)
228	FDA 71-30	8.2	122.5	122.1	124.5	134.7	154.0 (21)
229	FDA 71-30	38.2	122.6	123.6	125.7	136.7	157.3 (20)
230	FDA 71-30	177.0	119.5	121.3	124.5	135.0	154.8 (21)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 250.0 mg/kg

Appendix I

Teratology Study in Hamsters

Virgin adult female golden hamsters from an outbred strain were individually housed in mesh bottom cages in temperature and humidity controlled quarters with free access to food and fresh tap water at all times. They were mated (1 to 1) with mature males and the appearance of motile sperm in the vaginal smear was considered as Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, the indicated dose levels of the test material were administered by oral intubation. The controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose. The test material was prepared and doses calculated according to the following table:

Dosage (mg/kg)	Dose (ml/kg)	Concentration (mg/ml)
≦ 250	1	≦ 250
251 - 500	2	125 - 250
501 - 750	3	133 - 250
751 - 1000	4	187 - 250
1001 - 1250	5	200 - 250
1251 - 1500	6	208 - 250
1501 - 1600	6.4	235 - 250

Body weights were recorded on Days 0, 8, 10, and 14 of the gestation period. All animals were observed daily for appearance and behavior with particular attention to food consumption in order to better recognize any abnormalities resulting from anorexic effects in the pregnant animal.

On Day 14, all animals were subjected to Caesarian section under deep anesthesia and the numbers of implantation sites, resorption

sites, live and dead fetuses were recorded. All live pups were weighed and the genital tract of each dam was examined for any anatomical abnormalities.

All fetuses were examined grossly for the presence of external congenital defects and one-third of each litter underwent detailed visceral examination under 10X magnification. The remaining two-thirds of the pups were cleared in potassium hydroxide, stained with alizarin red dye, and examined for the presence of skeletal abnormalities.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 221

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date March 16, 1973

Laboratory No. 1575

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 5666	NP		0						----	
S 5667	NP		0						----	
S 5668	P		14	14		9	5		1.65	
S 5669	P		12	12		5	7		1.54	
S 5670	P		12	12		4	8		1.39	
S 5671	P		13	13		3	10		1.52	
S 5672	NP		0						----	
S 5673	P		14	14		6	8		1.78	
S 5674	P		13	11		7	4	2	1.97	
S 5675	P		11	10		3	7	1	1.46	
S 5676	P		15	15		6	9		1.92	
S 5677	P		13	13		3	10		1.71	
S 5678	P		14	14		3	11		1.82	
S 5679	NP		0						----	
S 5680	P		10	10		4	6		1.88	
S 5681	NP		0						----	
S 5682	P		15	15		7	8		1.90	
S 5683	P		12	12		7	5		1.88	
S 5684	P		13	13		7	6		1.91	
S 5685	P		13	13		6	7		1.91	
S 5686	P		12	12		3	9		1.88	
S 5687	P		10	9		2	7	1	1.83	
S 5688	P		14	14		5	9		2.08	
S 5689	P		15	15		6	9		1.78	
S 5690	NP		0						----	
S 5691	P		11	10	1	0	10		1.58	
S 5692	P		14	14		4	10		2.00	

* P = Pregnant; NP = Not Pregnant

RABBITS

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date June 1, 1973

Laboratory No. 1576 c
Contract No. FDA 71-260

Sample: Clear yellow liquid

Marking: FDA 71-30 (Oil of clove)

Examination Requested: Teratologic evaluation of FDA 71-30 in rabbits

Procedure: (See Appendix I)

Results: See Tables 1 through 4 and Appendix II

Conclusion:

On the basis of the data presented herein, the following conclusion appears to be warranted:

"The administration of up to 172 mg/kg (body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

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Vice President

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 221 & 222; 227 through 230

Date: April 30, 1973

Material: FDA 71-30

Laboratory No.: 1576 c

Table 1

Fate Summary
(Rabbits)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
221	Sham	0.0	11	11	11	11
222	6-AN*	2.5	12	12	11	11
227	FDA 71-30	1.72	13	13	12	12
228	FDA 71-30	7.99	12	10	12	10
229	FDA 71-30	37.1	13	12	13	12
230	FDA 71-30	172.0	15	12	15	12

* Positive Control: 6-amino nicotinamide dosed on Day 9
** Administered as a corn oil solution (See Appendix I)
1) Includes all dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 221 & 222; 227 through 230

Material FDA 71-30

Table 2
Reproduction Data
(Rabbits)

Date: April 30, 1973

Laboratory No.: 1576 c

Group:	221	222	227	228	229	230
Dose (mg/kg):	Sham	6-AN**	1.72	7.99	37.1	172.0
Pregnancies						
Total No.	11	12	13	10	12	12
Died or Aborted (before Day 29)	0	1	1	0	0	0
To term (on Day 29)	11	11	12	10	12	12
Corpora Lutea						
Total No.	140	179	156	146	174	209
Average/dam mated	12.7	14.9	13.0	12.2	13.4	13.9
Live Litters						
Total No.*	10	11	12	9	12	12
Implant Sites						
Total No.	50	81	70	57	77	64
Average/dam*	4.55	7.36	5.83	5.70	6.42	5.33
Resorptions						
Total No.*	5	5	5	7	2	4
Dams with 1 or more sites resorbed	4	3	3	3	2	4
Dams with all sites resorbed	1	0	0	1	0	0
Per cent partial resorptions	36.4	27.3	25.0	30.0	16.7	33.3
Per cent complete resorptions	9.09	--	--	10.0	--	--
Live Fetuses						
Total No.	45	76	65	50	75	60
Average/dam*	4.09	6.91	5.42	5.00	6.25	5.00
Sex ratio (M/F)	1.37	0.90	0.76	1.08	1.03	1.73
Dead Fetuses						
Total No.*	0	0	0	0	0	0
Dams with 1 or more dead	--	--	--	--	--	--
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	--	--	--	--	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	40.0	32.8	39.9	40.6	40.2	40.4

* Includes only those dams examined at term.

** Positive Control: 2.5 mg/kg of 6-aminonicotinamide dosed on Day 9

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230 Laboratory No. 1576 c

Table 3

Material FDA 71-30 Date April 30, 1973

Summary of Skeletal Findings*
(Rabbits)

Findings / Group No.:	221	222	227	228	229	230
Dose (mg/kg):	Sham	6-AN**	1.72	7.99	37.1	172.0
Live Fetuses Examined (at term)	45/10	76/11	65/12	50/9	75/12	60/12
Sternebrae						
Incomplete oss.		4/3	5/4	3/3	3/3	2/2
Scrambled						
Bipartite				2/2	2/2	1/1
Fused		4/3	4/2		2/2	
Extra		4/2	3/2		1/1	5/4
Missing	1/1		1/1			
Other						
Ribs						
Incomplete oss.						
Fused/split		11/4	1/1			
Wavy						
Less than 12						
More than 13						
Other						
Vertebrae						
Incomplete oss.						
Scrambled		13/4	1/1			
Fused						
Extra ctrs. oss.						
Scoliosis		17/7	1/1			
Tail defects		68/11				
Other						
Skull						
Incomplete closure			1/1	1/1		
Missing						
Craniostosis		2/2				
Other						
Extremities						
Incomplete oss.						
Missing						
Extra						
Miscellaneous						

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 6-aminonicotinamide dosed on Day 9

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230 Date April 30, 1973

Material FDA 71-30 Laboratory No. 1576 c

Table 3-a
Summary of Soft Tissue Abnormalities
(Rabbits)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
222	6-AN*	2.5	Z 6353	1	Medial rotation hind limbs
			Z 6355	6	Medial rotation hind limbs; Short tail
			Z 6361	2	Anopia; medial rotation hind limbs
				1	Anopia; medial rotation hind limbs; short tail; lobster claw
			Z 6362	3	Anopia
				3	Anopia; medial rotation hind limbs
				1	Anopia; medial rotation hind limbs; short tail
			Z 6363	5	Anopia
			Z 6364	5	Anopia
				2	Anopia; medial rotation hind limbs
			Z 6367	7	Anopia
			Z 6368	9	Anopia
228	FDA 71-30	2.17	C 6034	1	Craniostenosis; medial rotation hind limbs

* Positive Control: 6-aminonicotinamide dosed on Day 9

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 221 & 222; 227 through 230

Date April 30, 1973

Table 4

Species Rabbits

Laboratory No. 1576 c

Average Body Weights*

Group	Material	Dose Level	Day 0	Day 6	Day 12	Day 18	Day 29**
		mg/kg	kg	kg	kg	kg	kg
221	Sham	0.0	2.17	2.30	2.27	2.31	2.41 (11)
222	Aspirin***	2.5	2.28	2.42	2.43	2.52	2.63 (11)
227	FDA 71-30	1.72	2.36	2.50	2.51	2.58	2.61 (12)
228	FDA 71-30	7.99	2.09	2.12	2.10	2.12	2.31 (10)
229	FDA 71-30	37.1	2.09	2.41	2.40	2.46	2.62 (12)
230	FDA 71-30	172.0	2.16	2.29	2.30	2.34	2.48 (12)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 2.5 mg/kg of 6-aminonicotinamide dosed on Day 9

Appendix I

Teratology Study in Rabbits

Virgin, adult, Dutch-belted female rabbits were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. On Day 0, each doe was given an injection of 0.4 ml of human chorionic gonadotropin (400 IU) via the marginal ear vein. Three hours later, each doe was inseminated artificially with 0.3 ml of diluted semen from a proven donor buck using approximately 20 x 10^6 motile sperm according to the procedure described by Vogin et al (Pharmacologist 11, 282 (1969)). Beginning on Day 6 and continuing daily through Day 18 the females were dosed with the indicated dosages by oral intubation. The controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose. The test material was prepared and doses calculated according to the following table:

Dosage (mg/kg)	Dose (ml/kg)	Concentration (mg/ml)
≦ 250	1	≦ 250
251 - 500	2	125 - 250
501 - 750	3	133 - 250
751 - 1000	4	187 - 250
1001 - 1250	5	200 - 250
1251 - 1500	6	208 - 250
1501 - 1600	6.4	235 - 250

Body weights were recorded on Days 0, 6, 12, 18, and 29 of gestation. All animals were observed daily for appearance and behavior, with particular attention to food consumption and body weight in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 29 all does were subjected to Caesarean section under surgical anesthesia, and the numbers of corpora lutea, implantation sites, resorption sites and live and dead fetuses were recorded. Body weights of the live pups were also recorded. The urogenital tract of each animal was examined in detail for normality. In addition all fetuses underwent a detailed gross examination for the presence of external congenital abnormalities. The live fetuses of each litter were then placed in an incubator for 24 hours for the evaluation of neonatal survival. All surviving pups were sacrificed, and all pups examined for visceral abnormalities (by dissection). All fetuses were then cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 221

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date April 30, 1973

Laboratory No. 1576

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 6352	P	8	5	4		2	2	1	42.9	
S 6353	P	11	4	4		3	1		31.9	
S 6354	P	17	7	6		3	3	1	29.8	
S 6355	P	5	2					2	----	
S 6360	P	18	2	2		2	0		45.6	
S 6361	P	12	5	5		4	1		46.6	
S 6362	P	8	4	4		1	3		31.8	
S 6363	P	9	6	6		4	2		40.9	
S 6365	P	20	7	6		3	3	1	37.3	
S 6366	P	15	5	5		3	2		45.1	
S 6367	P	17	3	3		1	2		47.7	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 222

Material 6-AN

Dose 2.5 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date April 30, 1973

Laboratory No. 1576

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Z 6351	P	12	9	9		5	4		33.1	Two Neonatal Deaths
Z 6353	P	10	8	8		5	3		32.7	One Neonatal Death
Z 6354	P	16	9	7		4	3	2	32.8	Two Neonatal Deaths
Z 6355	P	13	8	6		4	2	2	29.5	Six Neonatal Deaths
Z 6358	P	12	8	8		3	5		30.4	Four Neonatal Deaths
Z 6359	P	--	--	--		--	--		----	Aborted Day 16
Z 6361	P	20	3	3		1	2		28.9	Three Neonatal Deaths
Z 6362	P	23	7	7		4	3		28.0	Four Neonatal Deaths
Z 6363	P	20	5	5		1	4		34.3	Two Neonatal Deaths
Z 6364	P	15	8	7		2	5	1	35.2	Two Neonatal Deaths
Z 6367	P	17	7	7		2	5		41.7	
Z 6368	P	21	9	9		5	4		33.8	Three Neonatal Deaths

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 227

Material FDA 71-30

Dose 1.72 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date April 30, 1973

Laboratory No. 1576 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 6001	P	9	5	4		1	3	1	42.2	
C 6002	P	--	--	--		--	--		----	Aborted Day 17
C 6003	P	14	8	8		4	4		42.1	
C 6006	P	10	5	4		1	3	1	48.1	
C 6007	P	12	8	8		3	5		32.3	Five Neonatal Deaths
C 6008	P	11	3	3		2	1		36.5	
C 6009	P	16	6	6		3	3		48.6	
C 6010	P	10	7	7		3	4		38.7	One Neonatal Death
C 6011	P	8	2	2		1	1		30.2	One Neonatal Death
C 6012	P	15	7	7		2	5		42.7	One Neonatal Death
C 6015	P	24	6	6		3	3		33.8	One Neonatal Death
C 6017	P	11	4	1		1	0	3	46.8	
C 6018	P	16	9	9		4	5		36.3	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 228

Material FDA 71-30

Dose 7.99 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date April 30, 1973

Laboratory No. 1576 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 6021	NP	9	0						----	
C 6022	P	10	5	5		3	2		43.2	One Neonatal Death
C 6023	NP	8	0						----	
C 6024	P	13	6	6		4	2		45.2	
C 6025	P	13	7	6		5	1	1	46.2	
C 6027	P	14	6	6		2	4		35.5	
C 6028	P	10	5	5		3	2		37.5	One Neonatal Death
C 6029	P	7	3	2		0	2	1	42.4	
C 6030	P	17	5	5		4	1		40.3	
C 6031	P	11	5					5	----	
C 6033	P	18	6	6		1	5		47.0	
C 6034	P	16	9	9		4	5		28.5	One Neonatal Death

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 227

Material FDA 71-30

Dose 1.8 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date March 16, 1973

Laboratory No. 1575 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 5001	NP		0						----	
C 5002	NP		0						----	
C 5003	P		14	14		7	7		1.80	
C 5004	P		12	12		2	10		1.57	
C 5005	P		15	15		9	6		1.54	
C 5006	P		11	10		4	6	1	1.74	
C 5007	P		12	12		7	5		1.70	
C 5008	NP		0						----	
C 5009	P		13	12		6	6	1	1.85	
C 5010	P		17	17		10	7		1.72	
C 5011	P		12	12		2	10		1.54	
C 5012	P		14	12		5	7	2	1.64	
C 5013	P		16	16		12	4		1.91	
C 5014	P		16	16		7	9		1.76	
C 5015	P		12	12		5	7		1.80	
C 5016	P		11	9		5	4	2	1.36	
C 5017	P		12	12		4	8		1.77	
C 5018	P		12	12		4	8		1.88	
C 5019	P		16	16		5	11		2.08	
C 5020	P		14	14		7	7		1.93	
C 5021	P		13	13		7	6		1.90	
C 5022	P		16	16		8	8		1.93	
C 5023	P		12	12		4	8		1.64	
C 5024	P		12	12		3	9		1.85	
C 5025	P		10	10		3	7		1.75	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 228

Material FDA 71-30

Dose 8.2 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date March 16, 1973

Laboratory No. 1575 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 5031	P		12	12		6	6		1.37	
C 5032	P		12	12		4	8		1.75	
C 5033	P		12	12		3	9		1.86	
C 5034	P		5	5		3	2		1.91	
C 5035	P		12	12		6	6		1.80	
C 5036	P		15	15		8	7		1.54	
C 5037	NP		0						----	
C 5038	P		14	13		6	7	1	1.84	
C 5039	P		16	16		11	5		1.84	
C 5040	P		12	12		7	5		1.78	
C 5041	NP		0						----	
C 5042	P		8	5		1	4	3	2.03	
C 5043	P		15	15		6	9		1.82	
C 5044	P		16	16		6	10		1.51	
C 5045	P		13	13		9	4		1.91	
C 5046	NP		0						----	
C 5047	NP		0						----	
C 5048	P		18	17	1	11	6		1.89	
C 5049	P		14	14		6	8		2.03	
C 5050	P		10		5			5	----	
C 5051										Not Assigned
C 5052	P		19	19		11	8		1.66	
C 5053	P		11	11		5	6		1.70	
C 5054	P		17	17		5	12		1.60	
C 5055	NP		0						----	
C 5056	P		15	15		5	10		1.82	
C 5057	P		10	9	1	5	4		1.90	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 229

Material FDA 71-30

Dose 38.2 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date March 16, 1973

Laboratory No. 1575 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 5061	P		13	13		3	10		1.68	
C 5062	P		8					8	----	
C 5063	P		15	15		3	12		1.65	
C 5064	NP		0						----	
C 5065	P		17	17		10	7		1.44	
C 5066	P		16	14	1	7	7	1	1.36	
C 5067	P		13	12		6	6	1	1.82	
C 5068	P		11	10	1	6	4		1.47	
C 5069	P		13	13		8	5		1.73	
C 5070	NP		0						----	
C 5071	P		14	13	1	3	10		1.87	
C 5072	NP		0						----	
C 5073	P		17	16		6	10	1	1.57	
C 5074	P		14	14		8	6		2.05	
C 5075	P		14	13		3	10	1	----**	
C 5076	NP		0						----	
C 5077	NP		0						----	
C 5078	NP		0						----	
C 5079	P		15	15		6	9		2.14	
C 5080	P		16	16		5	11		1.72	
C 5081	P		12	12		6	6		2.10	
C 5082	P		13	13		7	6		2.02	
C 5083	P		12	12		6	6		1.91	
C 5084	P		14	14		4	10		1.83	
C 5085	NP		0						----	
C 5086	P		16	15		5	10	1	1.68	
C 5087										Not Assigned
C 5088	P		15	14		9	5	1	1.68	
C 5089	NP		0						----	

* P = Pregnant; NP = Not Pregnant

** Not Weighed

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 230

Material FDA 71-30

Dose 177.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date March 16, 1973

Laboratory No. 1575 c

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
C 5091	P		9	9		3	6		1.95	
C 5092	NP		0						----	
C 5093	P		11	11		2	9		1.49	
C 5094	P		16	16		9	7		1.46	
C 5095	P		7	6		4	2	1	1.69	
C 5096	P		15	15		7	8		1.70	
C 5097	P		11	10		5	6	1	2.02	
C 5098	P		13	13		5	8		1.76	
C 5099	P		12	10	1	5	5	1	1.91	
C 5100	NP		0						----	
C 5101	P		11	11		3	8		1.84	
C 5102	P		13	13		5	8		1.52	
C 5103	P		13	13		5	8		1.72	
C 5104	P		13	13		4	9		1.77	
C 5105	P		13	13		5	8		1.98	
C 5106	NP		0						----	
C 5107	P		17	17		8	9		1.69	
C 5108	P		17	17		6	11		1.72	
C 5109	P		13	13		6	7		2.03	
C 5110	P		12	12		4	8		1.99	
C 5111	P		13	13		--**	--**		2.02	
C 5112	P		14	14		7	7		1.89	
C 5113	NP		0						----	
C 5114	P		14	14		3	11		1.91	
C 5115	P		13	13		6	7		1.88	

* P = Pregnant; NP = Not Pregnant

** Not Recorded