Joe McLaughlin (8-3-72)

Final report-Teratologic Evaluation of FDA 71-7 (**Sodium Nitrate**) in Mice, Rats, Hamsters & Rabbits 6/20/72

19.

FOOD AND DRUG LABORATORIES, INC.

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

#28

FINAL

June 20, 1972

Teratologic Evaluation of FDA 71-7

(Sodium Nitrate)

in

Mice, Rats, Hamsters and Rabbits

N22

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 June 20, 1972

Laboratory No. 0728 j
Contract No. FDA 71-260

Sample: White crystalline material

Marking: FDA 71-7 (Sodium nitrate)

Examination Requested: Teratologic evaluation of FDA 71-7 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 400 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."

Comment: Attention is called to the fact that this is the fifth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs: each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

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: 41 through 46

Material: FDA 71-7

Date April 30, 1972

Laboratory No. 0728 j

Table 1

Fate Summary
(Mice)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
41	Sham	0	24	21	23	20
42	Aspirin*	150	22	20	17	15
43	FDA 71-7	4	25	23	25	23
44	FDA 71-7	20	25	24	25	24
45	FDA 71-7	100	25	23	25	23
46	FDA 71-7	400	25	25	23	23

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 41 through 46

Material: FDA 71-7

Date April 30, 1972

Laboratory No. 0728 j

Table 2
Reproduction Data
(Mice)

Group:	41	42	43	44	45	46
Dose (mg/kg):	Sham	Aspirin**	4	20	100	400
Pregnancies						
Total No.	21	20	23	24	23	25
Died or Aborted (before Day 17)	1	5	0	0	0	2
To term (on Day 17)	20	15	23	24	23	23
Live litters						
Total No. *	20	15	23	24	22	23
Implant sites						
Total No.	232	191	276	291	274	279
Average/dam *	11.6	12.7	12.0	12.1	11.9	12.1
Resorptions						
Total No. *	8	9	7	5	20	17
Dams with 1 or more sites resorbed	6	6	5	4	12	10
Dams with all sites resorbed	0	0	0	0	1	0
Per cent partial resorptions	30.0	40.0	21.7	16.7	52.2	43.5
Per cent complete resorptions	-	-	-	-	4.35	-
Live fetuses						
Total No.	222	180	269	284	253	261
Average/dam *	11.1	12.0	11.7	11.8	11.0	11.3
Dead fetuses						
Total No. *	2	2	0	2	1	1
Dams with 1 or more dead	2	2	-	2	1	1
Dams with all dead	0	0	-	0	0	0
Per cent partial dead	10.0	13.3	-	8.33	4.35	4.35
Per cent all dead	-	-	-	-	-	-
Average fetus weight, g	0.91	0.80	0.95	0.94	0.97	1.02

* Includes only those dams examined at term.
** Positive control: 150 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46 | Table 3 | Laboratory No. 0728 j

Material FDA 71-7 | Date April 30, 1972

Summary of Skeletal Findings

(Mice)

Findings	Group No. / Dose (mg/kg)	41 Sham	42 Aspirin**	43 4	44 20	45 100	46 400
Live Fetuses Examined (at term)		151/20	126/15	186/23	189/24	176/21***	192/23
Sternebrae							
Incomplete oss.		43/15	84/15	58/17	26/11	31/12	20/7
Scrambled						1/1	
Bipartite		6/6	8/6	7/5	5/5		8/5
Fused		2/2		1/1			
Extra			1/1				
Missing		15/6	40/11	16/8	5/3	2/2	3/1
Other							
Ribs							
Incomplete oss.							
Fused/split							
Wavy			3/2	2/2			
Less than 12							
More than 13		7/5	1/1	4/4	7/6		6/5
Other							
Vertebrae							
Incomplete oss.							
Scrambled							
Fused							
Extra ctrs. oss.							
Scoliosis							
Tail defects							
Other							
Skull							
Incomplete closure							
Missing							
Craniostosis							
Other							
Extremities							
Incomplete oss.		7/2					
Missing							
Extra							
Miscellaneous							
Hyoid; missing		29/10	33/13	25/15	19/11	18/10	8/4
Hyoid; reduced		11/7	27/12	32/15	10/8	5/3	8/7

* Numerator=Number of fetuses affected; Denominator=Number of litters affected
** Positive control: 150 mg/kg
*** 1 set of litters unreadable.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46 Date April 30, 1972

Material FDA 71-7 Laboratory No. 0728 j

Table 3-a

Summary of Soft Tissue Abnormalities

(Mice)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
44	FDA 71-7	20	J 8055	1	Meningoencephalocele

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46

Species Mice

Date April 30, 1972

Laboratory No. 0728 j

Table 4

Average Body Weights *

Group	Material	Dose Level	Day 0	6	11	15	17	**
		mg/kg	g					
41	Sham	0	27.4	30.0	34.5	44.1	50.3	(20)
42	Aspirin ***	150	30.3	31.9	35.5	44.1	48.6	(15)
43	FDA 71-7	4	27.2	30.1	34.6	44.5	50.3	(23)
44	FDA 71-7	20	26.6	30.2	35.0	45.8	51.6	(24)
45	FDA 71-7	100	26.0	29.3	34.1	44.0	49.4	(23)
46	FDA 71-7	400	27.1	30.1	35.1	45.6	52.6	(23)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 150 mg/kg

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were individually 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 intubation; 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 41

Material Sham

Dose 0

Appendix II

Reproduction Data in Mice (Individual)

Date April 30, 1972

Laboratory No. 0728

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
S 8121	P	13	11		2	0.64	
S 8122	P	13	12		1	0.60	
S 8123	P	12	12			0.91	
S 8124	P	15	15			1.02	
S 8125	P	15	15			0.90	
S 8126	P	12	11		1	0.88	
S 8127	P	11	11			0.94	
S 8128	P	10	10			0.90	
S 8129	P	11	11			0.98	
S 8130	P	5	5			1.20	
S 8131	NP	0				----	
S 8132	P	7	5		2	0.65	
S 8133	NP	0				----	
S 8134	P	13	11	1	1	0.83	
S 8135	NP	0				----	
S 8136	P	12	11		1	0.77	
S 8137	P	12	12			0.87	
S 8138	P	11	11			1.04	
S 8139	P	11	11			----	Died Day 14
S 8140	P	10	10			1.05	
S 8141	P	11	11			1.05	
S 8142	P	12	12			0.95	
S 8143	P	12	12			1.05	
S 8144							Not assigned
S 8145	P	15	14	1		0.94	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 42 | Appendix II | Date April 30, 1972

Material Aspirin | Reproduction Data in Mice (Individual) | Laboratory No. 0728

Dose 150 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
A 8121	P	14	14			0.83	
A 8122	P	13	12		1	0.68	
A 8123	P	13	11		2	0.96	
A 8124	P	10	9		1	0.73	
A 8125	P	7	7			0.92	
A 8126	P	11	11			0.81	
A 8127	P	15	14		1	0.91	
A 8128	P	18	17		1	0.75	
A 8129	P	14	14			0.72	
A 8130	P	12	9		3	0.77	
A 8131	NP					----	
A 8132	--					----	Number not assigned.
A 8133	P	12	11	1		0.52	
A 8134	P	13	13			0.83	
A 8135	P	11	11			----	Died Day 13
A 8136	NP					----	
A 8137	P	13	12	1		0.90	
A 8138	P	14	14			0.71	
A 8139	P	12	12			----	Died Day 12
A 8140	P	12	12			0.95	
A 8141							Not assigned
A 8142							Not assigned
A 8143	P	11	11			----	Died Day 8
A 8144	P	13	13			----	Died Day 10
A 8145	P	10	10			----	Died Day 10

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 43 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 j

Dose 4 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 8001	P	14	14			0.80	
J 8002	NP					----	
J 8003	P	10	10			0.74	
J 8004	P	13	12		1	0.80	
J 8005	NP					----	
J 8006	P	13	13			0.84	
J 8007	P	14	14			0.75	
J 8008	P	12	10		2	0.96	
J 8009	P	14	14			0.88	
J 8010	P	10	10			0.90	
J 8011	P	11	11			0.87	
J 8012	P	10	10			0.88	
J 8013	P	12	12			1.01	
J 8014	P	10	10			1.03	
J 8015	P	11	11			1.19	
J 8016	P	12	10		2	1.02	
J 8017	P	13	12		1	1.00	
J 8018	P	13	13			1.07	
J 8019	P	12	12			1.13	
J 8020	P	11	11			0.99	
J 8021	P	13	13			0.93	
J 8022	P	13	12		1	1.04	
J 8023	P	13	13			0.87	
J 8024	P	10	10			0.90	
J 8025	P	12	12			1.36	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 44 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 j

Dose 20 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 8031	P	9	9			0.85	
J 8032	P	12	12			0.97	
J 8033	P	14	14			0.91	
J 8034	P	14	14			0.99	
J 8035	P	13	13			1.02	
J 8036	P	11	9		2	0.78	
J 8037	P	15	14		1	0.80	
J 8038	P	12	12			1.00	
J 8039	P	11	11			0.78	
J 8040	P	12	12			0.90	
J 8041	P	10	10			0.96	
J 8042	P	11	11			0.89	
J 8043	P	12	12			0.89	
J 8044	P	11	11			1.13	
J 8045	P	8	8			1.06	
J 8046	P	10	8	1	1	0.98	
J 8047	P	14	14			0.93	
J 8048	P	13	13			1.03	
J 8049	P	15	15			0.86	
J 8050	P	14	14			1.02	
J 8051	NP					----	
J 8052	P	10	10			0.95	
J 8053	P	13	12		1	1.00	
J 8054	P	14	14			0.97	
J 8055	P	13	12	1		0.86	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 45 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 j

Dose 100 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 8061	P	12	12			0.95	
J 8062	P	11	11			1.13	
J 8063	P	12	12			1.02	
J 8064	P	11	10		1	0.89	
J 8065	P	13	11		2	0.87	
J 8066	P	13	13			0.94	
J 8067	P	6			6	----	
J 8068	P	14	13		1	0.87	
J 8069	P	11	10		1	1.04	
J 8070	P	12	11		1	0.86	
J 8071	P	12	12			0.92	
J 8072	P	11	11			0.76	
J 8073	P	11	8	2	2	0.96	
J 8074	P	13	13			1.00	
J 8075	P	12	11		1	0.91	
J 8076	P	9	9			1.05	
J 8077	P	10	8		2	1.11	
J 8078	P	12	12			0.97	
J 8079	P	14	13		1	1.08	
J 8080	NP					----	
J 8081	P	13	13			0.96	
J 8082	P	17	16		1	0.98	
J 8083	P	13	12		1	1.05	
J 8084	NP					----	
J 8085	P	12	12			1.05	

* P= Pregnant; NP= Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 46 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Mice (Individual) | Laboratory No. 0728 j

Dose 400 mg/kg

Dam No.	Fate *	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 8091	P	16	16			0.99	
J 8092	P	19	18	1		0.94	
J 8093	P	15	15			0.93	
J 8094	P	12	12			0.99	
J 8095	P	12	10		2	1.04	
J 8096	P	11	7		4	0.86	
J 8097	P	5			5	----	Died Day 17
J 8098	P	14	12		2	0.98	
J 8099	P	13	12		1	0.91	
J 8100	P	12	11		1	0.95	
J 8101	P	11	11			0.95	
J 8102	P	13	13			0.90	
J 8103	P	15	15			0.98	
J 8104	P	13	13			----	Died Day 13
J 8105	P	10	10			1.04	
J 8106	P	11	11			1.04	
J 8107	P	13	13			0.98	
J 8108	P	11	10		1	0.97	
J 8109	P	14	14			1.07	
J 8110	P	12	11		1	1.11	
J 8111	P	11	10		1	1.08	
J 8112	P	11	9		2	1.15	
J 8113	P	11	9		2	1.02	
J 8114	P	10	10			1.10	
J 8115	P	2	2			1.57	

* 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 June 20, 1972

Laboratory No. 0729 j
Contract No. FDA 71-260

Sample: White crystalline material

Marking: FDA 71-7 (Sodium nitrate)

Examination Requested: Teratologic evaluation of FDA 71- 7 in rats.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 250 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."

Comment: Attention is called to the fact that this is the fifth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

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: 41 through 46

Material: FDA 71-7

Date April 30, 1972

Laboratory No. 0729 j

Table 1

Fate Summary
(Rats)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
41	Sham	0	23	20	23	20
42	Aspirin*	250	26	21	26	21
43	FDA 71-7	2.5	23	23	23	23
44	FDA 71-7	12	23	20	22	19
45	FDA 71-7	54	23	20	23	20
46	FDA 71-7	250	22	22	22	22

* Positive Control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 41 through 46

Material: FDA 71-7

Date April 30, 1972

Laboratory No. 0729 j

Table 2
Reproduction Data
(Rats)

Group:	41	42	43	44	45	46
Dose (mg/kg):	Sham	Aspirin**	2.5	12.0	54	250
Pregnancies						
Total No.	20	21	23	20	20	22
Died or Aborted (before Day 20)	0	0	0	1	0	0
To term (on Day 20)	20	21	23	19	20	22
Live litters						
Total No. *	20	20	23	19	20	22
Implant sites						
Total No.	245	235	272	217	214	233
Average/dam *	12.3	11.2	11.8	11.4	10.7	10.6
Resorptions						
Total No. *	18	34	5	8	14	6
Dams with 1 or more sites resorbed	6	10	2	4	5	5
Dams with all sites resorbed	0	1	0	0	0	0
Per cent partial resorptions	30.0	47.6	8.70	21.1	25.0	22.7
Per cent complete resorptions	-	4.76	-	-	-	-
Live fetuses						
Total No.	227	194	266	209	200	227
Average/dam *	11.4	9.24	11.6	11.0	10.0	10.3
Dead fetuses						
Total No. *	0	7	1	0	0	0
Dams with 1 or more dead	-	3	1	-	-	-
Dams with all dead	-	0	0	-	-	-
Per cent partial dead	-	14.3	4.35	-	-	-
Per cent all dead	-	-	-	-	-	-
Average fetus weight, g	3.73	2.72	3.68	3.71	3.77	3.75

* Includes only those dams examined at term.

** Positive control, 250 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46 | Laboratory No. 0729 j

Table 3

Material FDA 71-7 | Date April 30, 1972

Summary of Skeletal Findings*

(Rats)

Findings	Group No.	41	42	43	44	45	46
	Dose (mg/kg)	Sham	Aspirin**	2.5	12	54	250
Live Fetuses Examined (at term)		147/18[a]	109/16[b]	167/22	139/19	134/19[c]	154/21
Sternebrae							
Incomplete oss.		26/10	75/16	36/12	25/10	56/14	55/17
Scrambled		1/2	5/3	2/1		1/1	1/1
Bipartite		1/1	22/10	4/4	3/3		
Fused			1/1				
Extra			2/2				
Missing		9/6	82/15	26/10	17/7	25/11	21/10
Other							
Ribs							
Incomplete oss.					2/1	2/2	1/1
Fused/split			1/1				
Wavy		1/1	13/7	13/6	4/3	3/3	
Less than 12							
More than 13		11/7	40/13	5/4	10/5	6/4	12/7
Other							
Vertebrae							
Incomplete oss.		3/2	68/14	1/1	1/2	5/3	1/1
Scrambled			1/1				
Fused							
Extra ctrs. oss.							
Scoliosis							4/3
Tail defects							
Other							
Skull							
Incomplete closure		13/7	2/1	6/3	9/4	9/4	3/1
Missing							
Craniostosis				1/1		1/1	2/1
Other; occipitals; incomplete			21/6	1/1	2/1	1/1	1/1
Extremities							
Incomplete oss.							
Missing							
Extra							
Miscellaneous							
Hyoid; missing		15/9	38/12	19/10	15/6	16/7	11/5
Hyoid; reduced		5/5	16/6	6/7	6/4	7/5	8/5

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

** Positive control: 250 mg/kg

a/ 2 litters lost
b/ 3 litters lost
c/ 1 litter lost

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46 Date April 30, 1972

Material FDA 71-7 Laboratory No. 0729 j

Table 3-a

Summary of Soft Tissue Abnormalities

(Rats)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
42	Aspirin *	250	A 9124	2	Umbilical Hernia
				1	Encephalomyelocele
			A 9130	3	Meningoencephalocele
			A 9145	4	Encephalomyelocele
				4	Umbilical Hernia
43	FDA 71-7	2.5	J 9021	3	Subcutaneous cranial hematoma
45	FDA 71-7	54.0	J 9068	1	Hematoma - hind limb
46	FDA 71-7	250	J 9105	1	Hematoma - hind limb

* Positive control

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46

Species Rats

Date April 30, 1972

Laboratory No. 0729 j

Table 4

Average Body Weights *

Group	Material	Dose Level	Day 0	6	11	15	20	**
		mg/kg	g					
41	Sham	0	214	233	243	269	332	(20)
42	Aspirin***	250	208	224	236	260	300	(21)
43	FDA 71-7	2.5	215	230	244	263	326	(23)
44	FDA 71-7	12	215	228	244	263	323	(19)
45	FDA 71-7	54	215	232	246	268	324	(20)
46	FDA 71-7	250	204	220	233	249	307	(22)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control

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 intubation; the controls were sham treated.

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 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 41 | Appendix II | Date April 30, 1972

Material Sham | Reproduction Data in Rats (Individual) | Laboratory No. 0729

Dose 0

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
S 9121	P	15	12		3	3.14	
S 9122	P	17	15		2	3.36	
S 9123	P	11	11			4.07	
S 9124	NP	0				----	
S 9125	P	12	12			3.90	
S 9126	P	11	10		1	3.75	
S 9127	P	11	11			3.37	
S 9128	P	9	9			3.73	
S 9129	P	12	12			3.64	
S 9130	P	11	11			3.96	
S 9131	P	15	14		1	3.63	
S 9132	P	14	14			3.99	
S 9133	NP	0				----	
S 9134	P	12	12			3.45	
S 9135	P	11	11			3.35	
S 9136	P	13	5		8	3.72	
S 9137	P	11	11			4.00	
S 9138	P	10	10			3.98	
S 9139	P	17	14		3	3.61	
S 9140	P	11	11			3.90	
S 9141	NP	0				----	
S 9142	P	12	12			4.29	
S 9143	P	10	10			3.75	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 42 | Appendix II | Date April 30, 1972

Material Aspirin | Reproduction Data in Rats (Individual) | Laboratory No. 0729

Dose 250 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
A 9121	P	12	11		1	3.61	
A 9122	P	11	9	1	1	2.69	
A 9123	NP	0				----	
A 9124	P	11	9		2	3.46	
A 9125	P	11			11	----	
A 9126	P	14	7		7	2.31	
A 9127	P	12	12			2.59	
A 9128	P	14	14			2.50	
A 9129	NP	0				----	
A 9130	P	12	12			2.64	
A 9131	P	8	4	2	2	1.93	
A 9132	P	13	13			2.62	
A 9133	P	11	9		2	1.25	
A 9134	P	12	12			2.52	
A 9135	NP	0				----	
A 9136	NP	0				----	
A 9137	P	10	9		1	2.96	
A 9138	P	12	11		1	2.77	
A 9139	P	9	3		6	3.41	
A 9140	P	10	10			3.25	
A 9141	P	11	11			3.21	
A 9142	P	12	12			2.95	
A 9143	NP	0				----	
A 9144	P	8	8			2.93	
A 9145	P	11	7	4		2.03	
A 9146	P	11	11			2.75	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 43 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 j

Dose 2.5 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 9001	P	11	11			3.10	
J 9002	P	14	13	1		3.55	
J 9003	P	11	11			3.85	
J 9004	P	14	14			3.77	
J 9005	P	12	12			4.03	
J 9006	P	13	13			4.23	
J 9007	P	12	12			3.71	
J 9008	P	13	13			3.57	
J 9009	P	12	12			5.14	
J 9010	P	8	8			3.33	
J 9011	P	13	13			3.69	
J 9012	P	13	13			3.85	
J 9013	P	14	14			3.54	
J 9014	P	12	12			2.22	
J 9015	P	13	13			3.54	
J 9016	P	12	12			3.90	
J 9017	P	13	13			3.83	
J 9018	P	10	10			3.96	
J 9019	P	12	12			3.64	
J 9020	P	7	7			4.43	
J 9021	P	12	8		4	1.98	
J 9022	P	11	10		1	3.93	
J 9023	P	10	10			3.89	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 44 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 j

Dose 12.0 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 9031	P	13	10		3	3.46	
J 9032	P	12	12			3.51	
J 9033	NP	0				----	
J 9034	P	11	11			3.84	
J 9035	P	11	9		2	**	
J 9036	P	14	13		1	3.09	
J 9037	P	7	7			3.64	
J 9038	P	9	9			3.85	
J 9039	NP	0				----	
J 9040	P	13	13			3.70	
J 9041	P	12	12			3.70	
J 9042	P	16	16			3.80	
J 9043	P	13	7		6	----	Aborted Day 18.
J 9044	P	10	10			3.69	
J 9045	P	13	13			3.71	
J 9046	P	12	12			3.71	
J 9047	P	14	12		2	3.65	
J 9048	P	12	12			3.84	
J 9049	P	9	9			3.89	
J 9050	P	12	12			3.92	
J 9051	P	9	9			3.86	
J 9052	NP	0				----	
J 9053	P	8	8			3.88	

* P = Pregnant; NP = Not Pregnant

** No weights

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 45 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 j

Dose 54.0 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 9061	P	8	7		1	3.20	
J 9062	P	11	5		6	3.79	
J 9063	P	13	9		4	3.50	
J 9064	P	12	12			3.83	
J 9065	NP	0				----	
J 9066	P	11	11			3.89	
J 9067	P	11	11			3.97	
J 9068	P	10	10			3.77	
J 9069	P	9	9			3.60	
J 9070	P	9	9			3.70	
J 9071	P	15	15			3.60	
J 9072	P	14	14			3.56	
J 9073	P	12	12			2.64	
J 9074	P	11	11			3.96	
J 9075	P	14	13		1	3.79	
J 9076	NP	0				----	
J 9077	P	9	9			3.68	
J 9078	NP	0				----	
J 9079	P	1	1			3.45	
J 9080	P	10	10			3.88	
J 9081	P	12	12			3.96	
J 9082	P	15	15			4.03	
J 9083	P	7	5		2	5.60	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 46 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Rats (Individual) | Laboratory No. 0729 j

Dose 250. mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 9091	P	9	7		2	3.67	
J 9092	P	11	11			3.59	
J 9093	P	14	14			4.12	
J 9094	P	11	11			4.08	
J 9095	P	13	12		1	3.60	
J 9096	P	11	10		1	3.17	
J 9097	P	8	8			3.75	
J 9098	P	10	10			3.68	
J 9099	P	9	8		1	3.85	
J 9100	P	9	9			4.08	
J 9101	P	14	14			3.81	
J 9102	P	12	12			3.47	
J 9103	P	7	7			3.49	
J 9104	P	12	12			4.01	
J 9105	P	9	9			3.75	
J 9106	P	10	10			3.35	
J 9107	P	8	7		1	3.52	
J 9108	P	10	10			3.87	
J 9109	P	10	10			3.61	
J 9110	P	13	13			3.92	
J 9111	P	10	10			3.99	
J 9112	P	13	13			4.10	

* P = Pregnant; NP = Not Pregnant

HAMSTERS

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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 20, 1972

Laboratory No. 0730 j
Contract No. FDA 71-260

Sample: White crystalline material

Marking: FDA 71-7 (Sodium nitrate)

Examination Requested: Teratologic evaluation of FDA 71-7 in hamsters

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 400 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."

Comment: Attention is called to the fact that this is the fifth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

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: 41 through 46

Material: FDA 71-7

Date April 30, 1972

Laboratory No. 0730 j

Table 1

Fate Summary
(Hamsters)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
41	Sham	0	25	24	25	23
42	Aspirin *	250	29	28	27	26
43	FDA 71-7	4	27	26	27	26
44	FDA 71-7	20	25	23	25	23
45	FDA 71-7	100	25	24	24	22
46	FDA 71-7	400	25	24	25	24

* Positive Control

FOOD AND DRUG RESEA[illegible]H LABORATORIES, INC.

Group: 41 through 46

Material: FDA 71-7

Date April 30, 1972

Laboratory No. 0730 j

Table 2
Reproduction Data
(Hamsters)

Group:	41	42	43	44	45	46
Dose (mg/kg):	Sham	Aspirin**	4	20	100	400
Pregnancies						
Total No.	24	28	26	23	24	24
Died or Aborted (before Day 14)	1	2	0	0	2	0
To term (on Day 14)	23	26	26	23	22	24
Live litters						
Total No. *	23	26	26	23	21	24
Implant sites						
Total No.	303	323	315	302	272	299
Average/dam *	13.2	12.4	12.1	13.1	12.4	12.5
Resorptions						
Total No. *	24	10	19	9	25	22
Dams with 1 or more sites resorbed	5	7	12	8	11	11
Dams with all sites resorbed	0	0	0	0	0	0
Per cent partial resorptions	21.7	26.9	46.2	34.8	50.0	45.8
Per cent complete resorptions	-	-	-	-	-	-
Live fetuses						
Total No.	278	312	295	286	236	277
Average/dam *	12.1	12.0	11.3	12.4	10.7	11.5
Dead fetuses						
Total No. *	1	1	1	7	11	0
Dams with 1 or more dead	1	1	1	3	2	-
Dams with all dead	0	0	0	0	1	-
Per cent partial dead	4.35	3.85	3.85	13.0	9.09	-
Per cent all dead	-	-	-	-	4.55	-
Average fetus weight, g	1.81	1.82	1.84	1.80	1.81	1.76

* Includes only those dams examined at term.

** [illegible]

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46 | Laboratory No. 0730 j

Table 3

Material FDA 71-7 | Date April 30, 1972

Summary of Skeletal Findings *
(Hamsters)

Findings / Group No. / Dose (mg/kg)	41 Sham	42 Aspirin**	43 4	44 20	45 100	46 400
Live Fetuses Examined (at term)	194/23	215/26	203/26	196/23	164/21 ***	191/24
Sternebrae						
Incomplete oss.	97/23	98/23	65/19	76/19	77/19	47/18
Scrambled	2/2	2/2				
Bipartite	38/15	26/16	33/14	19/10	21/9	29/15
Fused						
Extra		7/4	9/3		10/5	11/10
Missing	19/10	31/12	32/14	21/13	15/7	31/11
Other						
Ribs						
Incomplete oss.						
Fused/split						
Wavy						
Less than 12						
More than 13	72/12	50/19	58/21	43/15	57/15	34/16
Other (missing)		2/2				
Vertebrae						
Incomplete oss.		1/1				
Scrambled		1/1				
Fused						
Extra ctrs. oss.	1/1					
Scoliosis						
Tail defects						
Other						
Skull						
Incomplete closure			1/1			
Missing						
Craniostosis						
Other						
Extremities						
Incomplete oss.				1/1		4/1
Missing						
Extra						
Miscellaneous						
Hyoid; missing	2/1	1/1	2/1	1/1		6/2
Hyoid; reduced	3/2	5/3		2/1	1/1	

* Numerator=Number of fetuses affected; Denominator=Number of litters affected
** Positive control: 250 mg/kg.
*** 1 litter lost.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46 Date April 30, 1972

Material FDA 71-7 Laboratory No. 0730 j

Table 3-a

Summary of Soft Tissue Abnormalities

(Hamsters)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
41	Sham	0	S 0122	1	Meningoencephalocele
42	Aspirin	250	A 0125	1	Meningoencephalocele
				1	Hydrocephalus

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46

Species Hamsters

Date April 30, 1972

Laboratory No. 0730 j

Table 4

Average Body Weights *

Group	Material	Dose Level	Day 0	6	8	10	14 **
		mg/kg	g				
41	Sham	0	102.6	108.4	112.9	123.5	146.8 (23)
42	Aspirin***	250	102.0	104.6	106.8	118.1	140.7 (26)
43	FDA 71-7	4	101.7	106.8	111.6	121.9	142.3 (26)
44	FDA 71-7	20	100.3	106.4	110.2	120.2	142.7 (23)
45	FDA 71-7	100	97.5	103.2	107.5	117.9	140.0 (22)
46	FDA 71-7	400	92.8	100.5	104.2	115.5	136.0 (24)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control

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.

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 sketal abnormalities.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 41 | Appendix II | Date April 30, 1972

Material Sham | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730

Dose 0

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
S 0121	P	10	10			1.82	
S 0122	P	12	11	1		2.01	
S 0123	P	14	14			1.84	
S 0124	P	13	13			1.94	
S 0125	P	12	2		11	1.91	
S 0126	P	17	17			1.67	
S 0127	P	13	11		2	1.83	
S 0128	P	15	13		2	1.88	
S 0129	P	12	12			1.89	
S 0130	P	12	12			1.94	
S 0131	P	20	20			1.94	
S 0132	P	16	16			1.61	
S 0133	P	10	10			1.95	
S 0134	P	13	13			1.77	
S 0135	P	13	13			1.87	
S 0136	P	14	14			1.44	
S 0137	P	13	12		1	1.90	
S 0138	P	14	14			1.82	
S 0139	P	12	12			1.67	
S 0140	NP	0				----	
S 0141	P	10	10			1.85	
S 0142	P	14	14			1.80	
S 0143	P	12	11	1		----	Aborted Day 13.
S 0144	P	12	12			1.96	
S 0145	P	11	3		8	1.26	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 42 | Appendix II | Date April 30, 1972

Material Aspirin | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730

Dose 250 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
A 0121	P	10	9		1	1.92	
A 0122	P	9	7		2	2.04	
A 0123	P	7	7			1.95	
A 0124	P	10	10			1.98	
A 0125	P	13	13			1.90	
A 0126	NP	0				----	
A 0127	P	13	12		1	1.73	
A 0128	P	11	10		1	1.82	
A 0129	P	10			10	----	Died Day 8
A 0130	P	20	18		2	1.49	
A 0131	P	19	19			1.58	
A 0132	P	12	12			1.57	
A 0133	P	13	13			1.97	
A 0134	P	14	14			1.96	
A 0135	P	11	11			2.05	
A 0136	P	13	13			1.77	
A 0137	P	10	9		1	1.75	
A 0138	P	13	12	1		1.71	
A 0139	P	11	11			1.86	
A 0140	P	12	12			1.85	
A 0141	P	13	13			1.62	
A 0142	P	14	14			1.74	
A 0143	P	10	10			1.92	
A 0144	P	12	12			1.67	
A 0145	P	15	15			1.75	
A 0146	P	14	14			----	Died Day 10.
A 0147	P	13	13			1.73	
A 0148	P	11	11			1.77	
A 0149	P	14	12		2	1.92	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 43 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 j

Dose 4 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 0001	P	11	10		1	2.13	
J 0002	P	10	10			1.97	
J 0003	NP	0				----	
J 0004	P	12	12			2.11	
J 0005	P	13	12		1	1.62	
J 0006	P	13	13			1.87	
J 0007	P	15	14		1	1.75	
J 0008	P	11	11			1.91	
J 0009	P	16	13		3	1.71	
J 0010	P	8	8			2.33	
J 0011	P	13	10		3	1.56	
J 0012	P	19	19			1.64	
J 0013	P	12	10		2	1.73	
J 0014	P	11	11			1.57	
J 0015	P	9	9			1.97	
J 0016	P	11	11			1.75	
J 0017	P	7	7			2.41	
J 0018	P	9	9			1.70	
J 0019	P	14	13		1	1.76	
J 0020	P	10	9		1	1.65	
J 0021	P	13	13			1.81	
J 0022	P	11	11			2.09	
J 0023	P	13	11	1	1	1.86	
J 0024	P	11	9		2	1.77	
J 0025	P	17	16		1	1.74	
J 0026	P	15	13		2	1.74	
J 0027	P	11	11			1.90	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 44 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 j

Dose 20 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 0031	P	14	9	5		2.00	
J 0032	NP	0				----	
J 0033	P	10	10			1.44	
J 0034	P	13	13			1.75	
J 0035	P	13	13			1.99	
J 0036	P	16	16			1.87	
J 0037	P	13	12		1	1.68	
J 0038	P	11	11			1.82	
J 0039	P	13	13			1.61	
J 0040	NP	0				----	
J 0041	P	15	14		1	1.75	
J 0042	P	18	17		1	1.70	
J 0043	P	8	8			1.83	
J 0044	P	14	14			1.62	
J 0045	P	14	14			1.74	
J 0046	P	12	12			2.15	
J 0047	P	12	12			2.02	
J 0048	P	14	13		1	1.78	
J 0049	P	13	11	1	1	1.95	
J 0050	P	12	12			1.86	
J 0051	P	11	10		1	1.93	
J 0052	P	13	12	1		1.72	
J 0053	P	13	13			1.89	
J 0054	P	14	12		2	1.73	
J 0055	P	16	15		1	1.60	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 45 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 j

Dose 100 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 0061	P	11	8		3	2.32	
J 0062	NP					----	
J 0063	P	10	9		1	1.65	
J 0064	P	13	13			1.96	
J 0065	P	9	9			1.70	
J 0066	P	10	10			----	Aborted Day 14
J 0067	P	13	13			1.85	
J 0068	P	13	13			1.88	
J 0069	P	13	5		8	1.59	
J 0070	P	10		10		----	
J 0071	P	19	17		2	1.69	
J 0072	P	14	13		1	1.84	
J 0073	P	19	17		2	1.79	
J 0074	P	8	7		1	1.66	
J 0075	P	10	10			1.78	
J 0076	P	12	12			1.71	
J 0077	P	17	16	1		1.51	
J 0078	P	11	8		3	1.68	
J 0079	P	14	13		1	1.92	
J 0080	P	9	9			1.81	
J 0081	P	14	14			2.01	
J 0082	P	11	9		2	1.93	
J 0083	P	10	9		1	2.08	
J 0084	P	10		10		----	Died Day 14
J 0085	P	12	12			1.66	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 46 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Hamsters (Individual) | Laboratory No. 0730 j

Dose 400 mg/kg

Dam No.	Fate*	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 0091	P	12	11		1	1.93	
J 0092	P	11	11			2.03	
J 0093	P	13	13			2.02	
J 0094	NP	0				----	
J 0095	P	14	14			1.72	
J 0096	P	14	14			1.77	
J 0097	P	12	12			1.94	
J 0098	P	15	15			1.77	
J 0099	P	13	13			1.83	
J 0100	P	11	11			1.98	
J 0101	P	15	13		2	1.84	
J 0102	P	16	14		2	1.24	
J 0103	P	9	7		2	1.86	
J 0104	P	11	11			1.67	
J 0105	P	14	9		5	1.77	
J 0106	P	12	8		4	1.23	
J 0107	P	12	10		2	1.53	
J 0108	P	11	11			1.50	
J 0109	P	15	14		1	1.89	
J 0110	P	13	13			1.84	
J 0111	P	13	13			1.96	
J 0112	P	12	12			1.71	
J 0113	P	10	9		1	1.66	
J 0114	P	10	9		1	1.80	
J 0115	P	11	10		1	1.65	

* P = Pregnant; NP = Not Pregnant

RABBITS

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 June 20, 1972

Laboratory No. 0731 j
Contract No. FDA 71-260

Sample: White crystalline material

Marking: FDA 71-7 (Sodium nitrate)

Examination Requested: Teratologic evaluation of FDA 71- 7 in rabbits .

Procedure: (See Appendix I)

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination of the data, it is tentatively concluded that:

"The administration of up to 250 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."

Comment: Attention is called to the fact that this is the fifth of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 36 compounds will have been tested in 18 pairs: each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

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: 41 through 46

Material: FDA 71-7

Date April 30, 1972

Laboratory No. 0731 j

Table 1

Fate Summary
(Rabbits)

Group	Material	Dose mg/kg	Total		At Term	
			Mated	Pregnant	Surviving (Total)	Number Pregnant
41	Sham	0	15	12	15	12
42	6 - AN*	2.5	15	13	13	11
43	FDA 71-7	2.5	15	11	14	10
44	FDA 71-7	12.0	15	13	15	13
45	FDA 71-7	90.0	15	10	14	9
46	FDA 71-7	250.0	15	10	13	9

* Positive Control : 6 - Amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 41 through 46 Date April 30, 1972

Material: FDA 71-7 Laboratory No. 0731 j

Table 2
Reproduction Data
(Rabbits)

Group:	41	42	43	44	45	46
Dose (mg/kg):	Sham	6 AN**	2.5	12.0	90.0	250
Pregnancies						
Total No.	12	13	11	13	10	10
Died or aborted (before Day 29)	0	2	1	0	1	2
To term (on Day 29)	12	11	10	13	9	9
Corpora lutea						
Total No.	236	173	137	130	112	155
Average/dam mated	15.7	11.5	9.13	8.67	7.47	11.3
Live litters						
Total No.*	8	9	8	9	3	5
Implant sites						
Total No. (at term)	58	57	44	51	28	31
Average/dam*	4.83	5.18	4.40	3.92	3.11	3.44
Resorptions						
Total No.*	9	17	5	11	8	8
Dams with 1 or more sites resorbed	4	9	4	6	7	5
Dams with all sites resorbed	4	2	2	4	6	4
Per cent partial resorptions	33.3	81.8	40.0	46.2	77.8	55.6
Per cent complete resorptions	33.3	18.2	20.0	30.8	66.7	44.4
Live fetuses						
Total No. (at term)	48	39	39	40	20	23
Average/dam*	4.00	3.55	3.90	3.08	2.22	2.56
Dead fetuses						
Total No.*	1	1	0	0	0	2
Dams with 1 or more dead	1	1	-	-	-	1
Dams with all dead	0	0	-	-	-	0
Per cent partial dead	8.33	9.09	-	-	-	11.1
per cent all dead	-	-	-	-	-	0
Average fetus weight, g	37.0	28.3	40.6	37.4	35.8	45.6

* Includes only those dams examined at term.

** Positive control: 2.5 mg/kg 6-amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46 | Laboratory No. 0731 j

Table 3

Material FDA 71-7 | Date April 30, 1972

Summary of Skeletal Findings*
(Rabbits)

Findings / Group No. / Dose (mg/kg)	41 Sham	42 6-AN**	43 2.5	44 12.0	45 90.0	46 250.0
Live Fetuses Examined (at term)	47/8	32/9	27/6[a]	34/7[a]	20/3	23/5
Sternebrae						
Incomplete oss.	6/3	16/8	3/2	8/3	1/1	
Scrambled						
Bipartite		3/3		1/1	1/1	
Fused	4/2	12/5		1/1		
Extra	1/1	1/1				1/1
Missing	1/1	2/1		1/1		
Other						
Ribs						
Incomplete oss.	1/1		2/2	1/1		1/1
Fused/split		12/6				1/1
Wavy						
Less than 12	1/1					
More than 13		2/2	1/1	2/2	2/2	
Other; missing	2/2		4/2	2/2	1/1	
Vertebrae						
Incomplete oss.		1/1				
Scrambled		11/5				
Fused						
Extra ctrs. oss.						
Scoliosis		6/3				
Tail defects		32/9				
Other						
Skull						
Incomplete closure						
Missing						
Craniostosis		9/3	2/2	1/1	2/1	3/1
Other; Incomplete eyes		29/7				
Extremities						
Incomplete oss.						
Missing						
Extra						
Miscellaneous						
Dyschondroplasia		2/2				
Club feet		16/7				
Cleft palate		26/6				

* Numerator=Number of fetuses affected; Denominator=Number of litters affected
** Positive control: 2.5 mg/kg 6-amino nicotinamide dosed on Day 9.
a) 2 litters lost.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46 Date April 30, 1972

Material FDA 71-7 Laboratory No. 0731 j

Table 3-a

Summary of Soft Tissue Abnormalities

(Rabbits)

Group	Material	Dose level mg/kg	Dam	Number of Pups	Description
42	6 AN *	2.5	Z 1061	1	Anopia
				1	Club feet
			Z 1062	1	Anopia
				1	Club feet
				1	Fetal monster
			Z 1063	6	Anopia
				6	Hair lip
				6	Medial Rotation Hind Limbs
			Z 1065	3	Anopia
				3	Club feet
			Z 1066	8	Anopia
				4	Club feet
			Z 1068	7	Anopia
				7	Club feet
			Z 1072	9	Anopia
				3	Club feet
				8	Cleft Palate
			Z 1073	1	Anopia
				1	Club feet
				1	Cleft Palate
			Z 1075	1	Anopia
				1	Club feet
				1	Cleft Palate

* Positive control: 2.5 mg/kg 6 - Amino nicotinamide dosed on Day 9.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 41 through 46

Species Rabbits

Date April 30, 1972

Laboratory No. 0731 j

Table 4

Average Body Weights *

Group	Material	Dose Level	Day 0	6	12	18	29	**
		mg/kg	kg					
41	Sham	0	2.52	2.55	2.58	2.60	2.68	(12)
42	6 - AN***	2.5	2.60	2.63	2.67	2.65	2.88	(11)
43	FDA 71-7	2.5	2.26	2.31	2.35	2.33	2.37	(10)
44	FDA 71-7	12.0	2.37	2.38	2.38	2.37	2.41	(13)
45	FDA 71-7	90.9	2.38	2.41	2.45	2.39	2.58	(9)
46	FDA 71-7	250.0	2.16	2.29	2.22	2.25	2.33	(9)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 6-amino nicotinamide 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 20×10^6 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.

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 41 | Appendix II | Date April 30, 1972

Material Sham | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731

Dose 0

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
S 1061	P	7	1			1	----	
S 1062	P	7	2			2	----	
S 1063	P	8	4			4	----	
S 1064	P	36	4	4			41.4	
S 1065	P	13	2			2	----	
S 1066	NP	5	0				----	
S 1067	P	11	6	6			32.0	
S 1068	NP	7	0				----	
S 1069	P	19	1	1			41.3	
S 1070	P	21	7	7			37.2	
S 1071	NP	3	0				----	
S 1072	P	30	11	11			33.5	
S 1073	P	9	2	2			40.0	
S 1074	P	33	10	10			36.7	
S 1075	P	27	8	7	1		34.1	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 42 | Appendix II | Date April 30, 1972

Material 6 - AN | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731

Dose 2.5 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
Z 1061	P	8	3	2		1	32.8	
Z 1062	P	20	3	2		1	18.9	
Z 1063	P	12	7	6		1	25.8	
Z 1064	NP	5	0				----	
Z 1065	P	11	5	3		2	34.2	
Z 1066	P	14	9	8		1	30.8	
Z 1067	P	21	4			4	----	Died Day 13
Z 1068	P	17	7	7			25.0	
Z 1069	P	11	3			3	----	
Z 1070	NP	0	0				----	
Z 1071	P	10	2		1	1	----	Aborted Day 29
Z 1072	P	14	9	9			31.6	
Z 1073	P	8	6	1		5	20.7	
Z 1074	P	5	1	0		1	----	
Z 1075	P	17	4	1	1	2	35.2	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 43 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 j

Dose 2.5 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 1001	P	16	7	7			39.4	
J 1002	P	12	5	5			41.8	
J 1003	NP	7	0				----	
J 1004	P	12	8	7		1	42.2	
J 1005	P	6	2			2	----	
J 1006	NP	5	0				----	
J 1007	P	4	1			1	----	
J 1008	NP	2	0				----	
J 1009	P	15	5	5			39.0	
J 1010	P	11	2	2			43.9	
J 1011	NP	7	0				----	
J 1012	P	10	4	4			----	Died Day 19.
J 1013	P	6	4	4			40.4	
J 1014	P	14	4	4			36.6	
J 1015	P	10	6	5		1	41.5	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 44 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 j

Dose 12.0 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 1016	P	13	5	5			42.0	
J 1017	P	10	7	7			36.0	
J 1018	P	12	6	6			37.9	
J 1019	P	7	2			2	----	
J 1020	P	17	9	6		3	43.7	
J 1021	NP	6	0				----	
J 1022	P	11	1			1	----	
J 1023	P	2	1			1	----	
J 1024	P	6	3			3	----	
J 1025	NP	4	0				----	
J 1026	P	13	4	4			37.7	
J 1027	P	9	3	3			34.5	
J 1028	P	7	2	2			43.0	
J 1029	P	6	5	5			34.0	
J 1030	P	7	3	2		1	27.6	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 45 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 j

Dose 90 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 1031	P	5	1			1	----	
J 1032	P	7	1			1	----	
J 1033	NP	2	0				----	
J 1034	P	10	8	8			36.9	
J 1035	P	17	6	6			38.8	
J 1036	P	12	1				----	Died Day 22
J 1037	NP	2	0				----	
J 1038	P	7	1			1	----	
J 1039	P	5	1			1	----	
J 1040	P	5	1			1	----	
J 1041	NP	11	0				----	
J 1042	P	12	7	6		1	31.8	
J 1043	NP	4	0				----	
J 1044	P	8	2			2	----	
J 1045	NP	5	0				----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 46 | Appendix II | Date April 30, 1972

Material FDA 71-7 | Reproduction Data in Rabbits (Individual) | Laboratory No. 0731 j

Dose 250 mg/kg

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Resorption Sites	Average Fetus Weight (g)	Remarks
J 1046	P	20	8	6		2	46.6	
J 1047	NP	1	0				----	
J 1048	P	14	7	7			42.3	
J 1049	P	13	3	3			47.5	
J 1050	P	16	4	4			47.1	
J 1051	P	13	3	3			44.5	
J 1052	P	12	1			1	----	
J 1053	NP	3	0				----	
J 1054	NP	5	0				----	
J 1055	P	12	2		2		----	Died Day 25.
J 1056	P	10	1			1	----	
J 1057	P	12	3			3	----	
J 1058	P	5	1			1	----	
J 1059	NP	8	0				----	
J 1060	NP	11	0				----	Died Day 10 -- accident.

* P = Pregnant; NP = Not Pregnant