FOOD AND DRUG

LABORATORIES, INC.

FDRL

FINAL

July 31, 1973

Teratologic Evaluation of FDA 71-44

(Caffeine)

in

Rabbits

Final report-Teratologic evaluation of FDA 71-44 (**Caffine**) in Rabbits 7/31/73

RABBITS

FOOD AND DRUG
Research LABORATORIES, INC.
WAVERLY DIVISION
Route 17
P.O. Box 107
Waverly, New York 14892
(607) 565-2931

FINAL REPORT

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

Date July 31, 1973

Laboratory No. 0904 y
Contract No. FDA 71-260

Sample: Fine white powdered material

Marking: FDA 71-44 (Caffeine)

Examination Requested: Teratologic evaluation of FDA 71-44 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 7.0 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."

However, at the highest dose level (32.5 mg/kg), there appeared to be an increase in maternal mortality with a decrease in the fertility rate. There was no clear indication that caffeine was teratogenic even at this level.

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: 171 & 172; 177 through 180

Date: June 28, 1973

Table 1

Material: FDA 71-44

Laboratory No.: 0904 y

Fate Summary
(Rabbits)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
171	Sham	0.0	14	8	12	7
172	6-AN*	2.5	14	9	12	8
177	FDA 71-44	0.325	24	10	20	8
178	FDA 71-44	1.5	23	13	21	11
179	FDA 71-44	7.0	15	11	14	10
180	FDA 71-44	32.5	30	19	24	13

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

** Administered as a water solution (See Appendix I)

1) Includes all dams examined at term

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 171 & 172; 177 through 180

Material: FDA 71-44

Table 2
Reproduction Data
(Rabbits)

Date: June 28, 1973

Laboratory No.: 0904 y

Group:	171	172	177	178	179	180
Dose (mg/kg):	Sham	6-AN**	0.325	1.5	7.0	32.5
Pregnancies						
Total No.	8	9	10	13	11	19
Died or Aborted (before Day 29)	2	1	2	2	1	6
To term (on Day 29)	7	8	8	11	10	13
Corpora Lutea						
Total No.	221	199	262	293	216	409
Average/dam mated	15.8	14.2	10.9	12.7	14.4	13.6
Live Litters						
Total No.*	6	6	7	10	9	9
Implant Sites						
Total No.	47	36	45	68	61	61
Average/dam*	6.71	4.50	5.63	6.18	6.10	4.69
Resorptions						
Total No.*	19	7	11	18	12	23
Dams with 1 or more sites resorbed	4	4	5	6	6	8
Dams with all sites resorbed	1	2	--	1	1	4
Per cent partial resorptions	57.1	50.0	62.5	54.6	60.0	61.5
Per cent complete resorptions	14.3	25.0	--	9.09	10.0	30.8
Live Fetuses						
Total No.	28	29	32	50	48	38
Average/dam*	4.00	3.63	4.00	4.55	4.80	2.92
Sex ratio (M/F)	0.87	0.93	0.78	0.96	0.85	1.00
Dead Fetuses						
Total No.*	--	--	2	--	1	--
Dams with 1 or more dead	--	--	2	--	1	--
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	--	--	25.0	--	10.0	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	35.6	34.9	38.5	39.6	41.2	38.8

* 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 171 & 172; 177 through 180 Laboratory No. 0904 y

Table 3

Material FDA 71-44 Date June 28, 1973

Summary of Skeletal Findings*

(Rabbits)

Findings / Group No.:	171	172	177	178	179	180
Dose (mg/kg):	Sham	6-AN**	0.325	1.5	7.0	32.5
Live Fetuses Examined (at term)	28/6	29/6	32/7	50/10	48/9	34/8[a]
Sternebrae						
Incomplete oss.		1/1	2/1	1/1	2/2	2/1
Scrambled						
Bipartite		2/2				3/2
Fused	2/1	1/1	3/3	1/1		1/1
Extra	1/1			1/1	1/1	
Missing	1/1					
Other						
Ribs						
Incomplete oss.						
Fused/split		7/3				
Wavy						
Less than 12						
More than 13		5/1				
Other						
Vertebrae						
Incomplete oss.						
Scrambled		8/2		1/1		1/1
Fused			1/1			
Extra ctrs. oss.						
Scoliosis		6/2				
Tail defects		12/3		1/1		
Other		1/1				
Skull						
Incomplete closure	1/1		1/1		3/1	
Missing						
Craniostosis		3/1				2/1
Other						
Extremities						
Incomplete oss.						
Missing						
Extra						
Miscellaneous						

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

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

a One litter lost in processing

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 171 & 172; 177 through 180 Date June 28, 1973

Material FDA 71-44 Laboratory No. 0904 y

Table 3-a

Summary of Soft Tissue Abnormalities

(Rabbits)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
171	Sham	0.0	S 4262	1	Encephalocele
172	6-AN*	2.5	Z 4264	1	Cleft palate
				1	Medial rotation; club foot; cleft palate
				1	Medial rotation; short tail
			Z 4267	4	Encephalocele; anopia; club foot
				1	Anopia; club foot; short tail
				1	Anopia; short tail

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

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 171 & 172; 177 through 180

Date June 28, 1973

Table 4

Species Rabbits

Laboratory No. 0904 y

Average Body Weights*

Group	Material	Dose Level	Day 0	Day 6	Day 12	Day 18	Day 29**
		mg/kg	kg				
171	Sham	0.0	2.04	2.11	2.15	2.20	2.26 (7)
172	6-AN***	2.5	2.23	2.26	2.30	2.42	2.53 (8)
177	FDA 71-44	0.325	2.05	2.12	2.15	2.22	2.28 (8)
178	FDA 71-44	1.5	2.39	2.33	2.42	2.47	2.61 (11)
179	FDA 71-44	7.0	2.18	2.23	2.28	2.41	2.44 (10)
180	FDA 71-44	32.5	2.12	2.15	2.18	2.23	2.32 (13)

* 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 171

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date June 28, 1973

Laboratory No. 0904

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 4256	P	17	8	4		1	3	4	32.9	
S 4257	NP	4	0						----	
S 4258	NP	10	0						----	
S 4259	NP	7	0						----	
S 4260	NP	9	0						----	
S 4261	P	19	9					9	----	
S 4262	P	36	9	9		4	5		31.4	
S 4263	NP	7	0						----	Died Day 21
S 4264	P	11	3	--		--	--		----	Died Day 26
S 4265	P	24	5	3		1	2	2	34.2	
S 4266	P	28	5	1		1	0	4	37.3	
S 4267										Not Assigned
S 4268	P	24	8	8		5	3		33.4	
S 4269	NP	3	0						----	
S 4270	P	22	3	3		1	2		44.3	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 172

Material 6-AN

Dose 2.5 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date June 28, 1973

Laboratory No. 0904

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Z 4256	P	19	5	5		3	2		38.5	
Z 4257	NP	17	0						----	
Z 4258	NP	12	0						----	Died Day 16
Z 4259	P	18	5	5		2	3		36.0	
Z 4260	NP	12	0						----	
Z 4261	P	13	6	5		2	3	1	31.0	
Z 4262	P	9	1					1	----	
Z 4263	NP	6	0						----	
Z 4264	P	23	7	3		2	1	4	31.3	Three Neonatal Deaths
Z 4265										Not Assigned
Z 4266	P	23	8	--		--	--		----	Died Day 22
Z 4267	P	14	6	6		3	3		35.7	Four Neonatal Deaths
Z 4268	NP	7	0						----	
Z 4269	P	18	5	5		2	3		36.8	
Z 4270	P	8	1					1	----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 177

Material FDA 71-44

Dose 0.325 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date June 28, 1973

Laboratory No. 0904 y

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Y 4001	P	17	7	5		2	3	2	34.2	
Y 4002	NP	10	0						----	
Y 4003	P	9	3	1		0	1	2	49.1	
Y 4004	P	13	7	7		2	5		24.4	Two Neonatal Deaths
Y 4005	P	13	2	2		1	1		51.1	
Y 4006	NP	0	0						----	
Y 4007	NP	6	0						----	
Y 4008	NP	4	0						----	Died Day 11
Y 4009	NP	2	0						----	
Y 4010	NP	3	0						----	
Y 4011	NP	7	0						----	
Y 4012	NP	3	0						----	
Y 4013	NP	10	0						----	
U 4014	NP	2	0						----	
Y 4015	NP	7	0						----	
Y 4061	P	16	7	6		3	3	1	45.6	
Y 4062	P	21	6		1			5	----	
Y 4063	P	35	6	5	1	2	3		33.0	
Y 4064	NP	12	0						----	
Y 4065	P	21	7	6		4	2	1	32.4	
Y 4082	P	16	6	--		--	--		----	Died Day 11
Y 4083	NP	4	0						----	Died Day 13
Y 4084	P	23	3	--		--	--		----	Accid. Death Day 14
Y 4085	NP	8	0						----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 178

Material FDA 71-44

Dose 1.5 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date June 28, 1973

Laboratory No. 0904 y

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Y 4016	P	8	6					6	----	
Y 4017	NP	3	0						----	
Y 4018	P	21	7	5		4	1	2	40.2	
Y 4019	P	24	6	6		3	3		37.6	
Y 4020	P	26	8	4		1	3	4	41.4	
Y 4021	NP	1	0						----	
Y 4022	NP	9	0						----	
Y 4023	NP	4	0						----	
Y 4024	P	29	11	--		--	--		----	Died Day 5
Y 4025	P	21	5	5		1	4		39.9	One Neonatal Death
Y 4026	P	25	6	3		--**	--**	3	30.6	
Y 4027	NP	5	0						----	
Y 4028	P	27	7	7		4	3		47.2	Two Neonatal Deaths
Y 4029	P	16	5	4		2	2	1	34.0	One Neonatal Death
Y 4030	NP	0	0						----	
Y 4066	NP	0	0						----	
Y 4067	NP	2	0						----	
Y 4068	NP	8	0						----	
Y 4069	NP	4	0						----	
Y 4086	P	14	7	5		1	4	2	26.6	
Y 4087	P	15	7	--		--	--		----	Died Day 13
Y 4088	P	14	5	5		3	2		49.1	
Y 4089	P	17	6	6		4	2		49.2	

* P = Pregnant; NP = Not Pregnant
** Not Recorded

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 179

Material FDA 71-44

Dose 7.0 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date June 28, 1973

Laboratory No. 0904 y

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Y 4031	P	12	5	--		--	--		----	Died Day 17
Y 4032	P	22	9	5		3	2	4	36.6	One Neonatal Death
Y 4033	P	18	7	6		3	3	1	39.7	
Y 4034	P	16	6	5	1	1	4		46.4	Two Neonatal Deaths
Y 4035	P	24	8	6		3	3	2	39.3	
Y 4036	P	26	7	6		3	3	1	35.3	
Y 4037	P	23	4	4		2	2		44.2	
Y 4038	P	12	3					3	----	
Y 4039	P	18	6	5		2	3	1	42.6	
Y 4040	P	16	5	5		2	3		45.3	
Y 4041	NP	9	0						----	
Y 4042	NP	3	0						----	
Y 4043	NP	0	0						----	
Y 4044	NP	1	0						----	
Y 4045	P	16	6	6		3	3		41.5	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 180

Material FDA 71-44

Dose 32.5 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date June 28, 1973

Laboratory No. 0904 y

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Y 4046	NP	10	0						----	
Y 4047	P	5	3					3	----	
Y 4048	P	50	5	--		--	--		----	Died Day 17
Y 4049	NP	7	0						----	
Y 4050	NP	11	0						----	
Y 4051	NP	7	0						----	
Y 4052	NP	10	0						----	
Y 4053	P	22	3	3		1	2		46.6	
Y 4054	NP	10	0						----	
Y 4055	P	12	3					3	----	
Y 4056	P	18	4	3		2	1	1	38.5	
Y 4057	NP	5	0						----	
Y 4058	NP	5	0						----	
Y 4059	NP	9	0						----	
Y 4060	NP	1	0						----	
Y 4070	P	24	8	--		--	--		----	Died Day 17
Y 4071	NP	2	0						----	
Y 4072	P	14	6	--		--	--	1	----	Died Day 25
Y 4073	P	17	4	2		2	0	2	43.4	
Y 4074	P	7	6	6		2	4		31.3	Six Neonatal Deaths
Y 4075	P	10	6	1		1	0	5	50.0	
Y 4076	P	9	7	7		4	3		27.2	Two Neonatal Deaths
Y 4077	P	10	8	8		4	4		32.0	Two Neonatal Deaths
Y 4078	P	32	8	7		--	--	1	----	Sacrificed Day 12
Y 4079	P	15	1					1	----	
Y 4080	P	12	6	5		2	3	1	41.1	One Neonatal Death

Continued on next page

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 180

Material FDA 71-44

Dose 32.5 mg/kg

Appendix II

Reproduction Data in Rabbits (Individual)

Date June 28, 1973

Laboratory No. 0904 y

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Continued										
Y 4081	P	22	4	--		--	--		----	Died Day 13
Y 4090	P	10	3	3		1	2		39.3	
Y 4091	P	24	7	6		--	--	1	----	Died Day 9
Y 4092	P	19	7					7	----	

* P = Pregnant; NP = Not Pregnant