GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
100 mg/kg/day				
60280	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60281	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60282	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60283	F	S	MACROSCOPIC: Lymph Node	- Iliac, enlarged, mild.
60284	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
0 to Termination F0 Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
100 mg/kg/day				
60287	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60288	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60291	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60292	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60293	F	S	MACROSCOPIC: Stomach, Glandular	- Mucosa, erosions, 1 mm, mild.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Records: Pathology
0 to Termination FO Adults: Rats

GROUP, ANIMAL NUMBER	SEX	DATE[1]	TISSUE:	OBSERVATIONS:
100 mg/kg/day				
50295	F	S	MACROSCOPIC, All Tissues	- Within normal limits.
50298	F	S	MACROSCOPIC, All Tissues	- Within normal limits.
50299	F	S	MACROSCOPIC, All Tissues	- Within normal limits.
50300	F	S	MACROSCOPIC, All Tissues	- Within normal limits
50302	F	S	MACROSCOPIC, All Tissues	- Within normal limits.

[1]DATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
0 to Termination FD Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
100 mg/kg/day				
60303	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60304	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60306	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60308	F	S	MACROSCOPIC: Stomach, Glandular	- Mucosa, ulcer, focal, mild
60309	F	S	MACROSCOPIC: Stomach, Glandular	- Mucosa, thickened, mild.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Records: Pathology
0 to Termination FO Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
100 mg/kg/day				
60308	F	S	MACROSCOPIC: Skin	- Hair thinned,generalized,moderate.

297-1388

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
D to Termination FD Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
47485	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47486	M	S	MACROSCOPIC: Oral Tissues	- Teeth broken, upper incisors.
47487	M	S	MACROSCOPIC: Lung Stomach, Glandular Thymus	- Dark red area, 0.8 x 0.6 cm, left, moderate. - Focus, Mucosa, dark red, 0.3 x 0.2 cm, mild Foci, light red, 2 mm, mild.
47488	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47489	M	S	MACROSCOPIC: All Tissues	- Within normal limits.

181-1356

[1]FATE CODES: S-SCHEDULED SACRIFICE E-SACRIFICED IN EXTREMIS D-DIED ON STUDY

Individual Animal Data Record: Pathology
0 to Termination FO Adults, Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
47480	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47481	M	S	MACROSCOPIC: Oral Tissues	- Teeth malaligned, incisors.
47482	M	S	MACROSCOPIC: Stomach Thymus	- Mucosa, focl, red, pinpoint, x2, trace, pyloric area. - Enlarged, 2.0 x 2.0 x 1.5 cm, moderate.
47483	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47484	M	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S-SCHEDULED SACRIFICE E-SACRIFICED IN EXTREMIS D-DIED ON STUDY

Individual Animal Data Record: Pathology
0 to Termination F0 adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
47495	M	S	MACROSCOPIC: Oral Tissues	- Teeth broken, upper incisors.
47498	M	S	MACROSCOPIC: Oral Tissues	- Teeth missing,upper incisors.
47497	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47498	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47499	M	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
0 to Termination CD Adults, Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
47600	M	D	MACROSCOPIC: Liver Stomach, Glandular	- Pale, generalized, mild. - Mucosa, foci, dark red, pinpoint, mild.
47601	M	E	MACROSCOPIC: All Tissues	- Within normal limits.
47602+	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47603	M	S	MACROSCOPIC: Thymus	- Foci, red, pinpoint to 1 mm, mild.
47604	M	S	MACROSCOPIC: All Tissues	- Within normal limits.

300

191-1286

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
D (a Termination FO Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
47505	M	S	MACROSCOPIC: Kidney,	- hydronephrosis, right, mild.
47506	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47507	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47508	M	S	MACROSCOPIC: Kidney	- Hydronephrosis, right, mild.
47509	M	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S-SCHEDULED SACRIFICE E-SACRIFICED IN EXTREMIS D-DIED ON STUDY

Individual Animal Data Record: Pathology
0 to Termination F0 Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
47510	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47511	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47512	M	S	MACROSCOPIC: Lymph Node, Mandibular	- Enlarged,mild, right.
47513	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47514	M	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record, Pathology
0 to Termination FD adults: Rats

GROUP, ANIMAL NUMBER	SEX	DATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
47615	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47616	M	D	MACROSCOPIC: Lung Trachea	- Hemorrhage, ecchymotic, severe, all lobes. - Filled with frothy fluid, severe.
47617	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47618	M	S	MACROSCOPIC: All Tissues	- Within normal limits.
47619	M	S	MACROSCOPIC: Oral Tissues	- Tooth missing,upper right incisor.

[1]DATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
0 to Termination FO Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
60311	F	E	MACROSCOPIC: All Tissues	- Within normal limits.
60312	F	S	MACROSCOPIC: Skin	- Hair thinned,generalized,mild.
60318	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60310	F	S	MACROSCOPIC: Liver Stomach, Glandular	- Adhesions to diaphragm,mild, median lobe. - Mucosa, erosions, 1 mm, mild.
60319	F	S	MACROSCOPIC: Eye	- Cornea cloudy, right, severe.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
D to Termination PO Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
50321	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50322	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50325	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50326	F	S	MACROSCOPIC: Lungs	- Congestion, moderate, right cranial and middle lobes.
50327	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

TBT-1386

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
60328	F	S	MACROSCOPIC: Stomach, Glandular	- Mucosa, ulcer, focal, mild.
60329	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60330	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60331	F	S	MACROSCOPIC: Lung	- Foci,red, 1 mm, multilobar,mild.
60332	F	S	MACROSCOPIC: Stomach, Glandular	- Mucosa, foci, black, 1 to 3 mm, moderate; Mucosa, thickened, moderate.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
♀ 1a Termination FO Adults: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
50333	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50334	F	S	MACROSCOPIC: Kidney	- Mottled, capsule, left, mild.
			Stomach, Glandular	- Mucosa, ulcer, multifocal, mild.
50335	F	S	MACROSCOPIC: Uterus	- Cyst,multifocal,bilateral,mild
50337	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50338	F	S	MACROSCOPIC: Liver	- Foci, red, 1 to 2 mm, multilobar,mild.

307

151-1296

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE.	OBSERVATIONS:
200 mg/kg/day				
50338	F	S	MACROSCOPIC, All Tissues	- Within normal limits.
50341	F	S	MACROSCOPIC, All Tissues	- Within normal limits.
50342	F	S	MACROSCOPIC, Lung	- Focus, red, 1 mm, mild, left lobe.
50344	F	S	MACROSCOPIC, All Tissues	- Within normal limits.
50348	F	S	MACROSCOPIC, All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E-SACRIFICED IN EXTREMIS DURING ON STUDY

Individual Animal Data Record: Pathology
13 Day Uterine Examination, FO Female: Rats

GROUP, ANIMAL NUMBER	SEX	DATE[1]	TISSUE:	OBSERVATIONS:
0 mg/kg/day (Control)				
50208	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50210	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50211	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50213	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50222	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

T01-1385

[1]DATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
13 Day Uterine Examination, FO Female: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
0 mg/kg/day (Control)				
60224	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60225	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60228	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60232	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60235	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS O=DIED ON STUDY

Individual Animal Data Record: Pathology
13 Day Uterine Examination, F0 Female; Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
10 mg/kg/day				
60243	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60249	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60251	F	S	MACROSCOPIC: Skin	- Scab, 0.2 cm, mild, head.
60254	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60258	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
13 Day Uterine Examination, PO Female: Data

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
10 mg/kg/day				
60288	F	S	MACROSCOPIC: Liver	- Foci, white, 0.1 to 0.2 cm, multifocal, mild.
60260	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60271	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60273	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60274	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
13 Day Uterine Examination, FD Female Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
100 mg/kg/day				
50288	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50289	F	S	MACROSCOPIC: Eye	- Cataract, right, severe.
50290	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50292	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
50294	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
13 Day Dietary Examination, F0 Females: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
100 mg/kg/day				
60297	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60298	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60301	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60307	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60310	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]FATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
13 Day Dietary Examination, FD Female: Rats

GROUP, ANIMAL NUMBER	SEX	FATE[1]	TISSUE:	OBSERVATIONS:
250 mg/kg/day				
60313	F	S	MACROSCOPIC: Lung Skin	- Fact, red, pinpoint to 1 mm, mild. - Hair loss, mild, ventral abdomen.
60314	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60315	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60317	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
60320	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

181-1385

[1]FATE CODES: S=SCHEDULED SACRIFICE F=SACRIFICED IN EXTREMIS D=DIED ON STUDY

Individual Animal Data Record: Pathology
13 Day Uterine Examination, FO Female, Rats

GROUP, ANIMAL NUMBER	SEX	DATE[1]	TISSUE:	OBSERVATIONS:
280 mg/kg/day				
80823	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
80824	F	S	MACROSCOPIC: All Tissues	- Within normal limits
80386	F	S	MACROSCOPIC: All Tissues	- Within normal limits.
80340	F	S	MACROSCOPIC: Kidney	- Hydronephrosis, right, mild.
80343	F	S	MACROSCOPIC: All Tissues	- Within normal limits.

[1]DATE CODES: S=SCHEDULED SACRIFICE E=SACRIFICED IN EXTREMIS D=DIED ON STUDY

APPENDIX C

Historical Control Data

Individual studies have been subjected to Quality Assurance audit; the following compilation of data has not.

HDC Historical Control Data: Rat Cesarean Section
Source: Charles River Strain: CD®SD CD®

Individual Studies: Maternal and Fetal Observations at Cesarean Section

Study No. / Date:	1 10/83	2 11/83	3 12/83	4 2/84	5 3/84	6 3/84	7 5/84	8 5/84	9 5/84	10 5/84	11 6/84	12 6/84	13 7/84	14 8/84
No. of animals in control group:	30	31	29	29	26	26	22	25	29	25	29	25	25	29
No. of animals that died:	0	1	0	0	0	0	0	0	0	0	0	0	0	0
No. of animals examined at Cesarean section:	30	30	29	29	29	26	22	25	29	25	29	25	25	29
No. nongravid:	0	0	1	2	6	9	3	1	2	0	0	1	5	0
No. gravid:	30	30	28	27	23	17	19	24	27	25	29	24	20	29
No. of dams with resorptions only:	0	0	0	1	0	0	1	0	0	0	0	0	0	0
No. of dams with live fetuses:	30	30	28	27	23	17	18	24	27	25	29	24	20	29
No. of live fetuses/dam:	15.1	13.7	15.3	12.1	13.1	14.5	12.1	14.9	14.6	14.0	13.9	14.5	13.6	14.2
No. late resorptions/dam:	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. early resorptions/dam:	0.9	1.3	0.9	1.3	0.9	0.7	0.9	0.7	0.9	0.9	1.2	0.9	1.0	1.0
No. postimplantation losses/dam:	0.9	1.3	0.9	1.3	0.9	0.7	0.9	0.8	0.9	0.9	1.2	0.9	1.0	1.0
No. implantations/dam:	16.0	15.0	15.8	15.4	13.9	15.8	13.1	15.6	15.5	14.9	15.1	15.4	14.6	15.2
No. corpora lutea/dam:	17.0	16.3	16.4	15.9	16.5	16.4	15.9	16.9	18.0	15.6	16.3	17.2	17.6	18.6
Fetal sex ratio: Male:Female	143: 158	157: 117	129: 124	132: 148	110: 130	119: 113	110: 120	173: 184	169: 186	182: 168	172: 179	162: 166	140: 132	176: 178
Mean fetal body weight (g):	5.5	5.4	5.1	5.4	5.5	5.5	5.6	5.6	5.4	5.4	5.6	5.6	5.7	5.5
Mean adjusted[a] maternal body weight change (g):	NA	NA	NA	NA	NA	NA	NA	71	69	NA	NA	NA	NA	NA
Mean unadjusted[b] maternal body weight	144	127	153	126	144	141	115	152	146	142	153	146	142	139
Mean Placental weight (g)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a]Weight change of dam minus the weight of the uterus
[b]Weight change of dam including uterus
NA Not Applicable
The historical control data updated November 1987 includes data from studies terminating October 1983 to April 1987.

IRDC Historical Control Data: Rat Cesarean Section
Source: Charles River Strain: CD®

Individual Studies; Maternal and Fetal Observations at Cesarean Section

Study No. / Date:	15 9/84	16 9/84	17 10/84	18 12/84	19 1/85	20 2/85	21 3/85	22 5/85	23 6/85	24 6/85	25 6/85	26 8/85	27 8/85	28 8/85
No. of animals in control group:	25	25	27	25	25	25	20	25	25	25	25	25	25	25
No. of animals that died:	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No. of animals examined at Cesarean section:	25	25	27	25	25	25	20	25	25	25	25	25	25	25
No. nongravid:	1	3	3	2	4	4	0	0	0	3	3	4	4	4
No. gravid:	24	22	24	23	21	21	20	25	25	23	22	21	21	21
No. of dams with resorptions only:	0	0	0	0	0	0	0	0	0	0	0	1	0	0
No. of dams with live fetuses:	24	22	24	23	21	21	20	25	25	23	22	20	21	21
No. of live fetuses/dam:	14.4	14.4	13.7	14.7	12.7	13.8	14.2	14.5	13.7	14.9	13.3	12.7	13.9	14.3
No. late resorptions/dam:	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. early resorptions/dam:	0.8	1.0	1.0	1.2	0.7	0.9	1.0	0.7	0.8	0.8	1.0	1.9	1.3	1.0
No. postimplantation losses/dam:	0.8	1.0	1.0	1.2	0.7	0.9	1.0	0.7	0.8	0.8	1.0	1.9	1.3	1.0
No. implantations/dam:	15.2	15.4	13.8	15.8	13.4	14.6	15.1	15.2	14.5	15.3	16.3	14.6	14.8	15.3
No. corpora lutea/dam:	16.4	16.2	15.4	16.4	14.8	15.5	16.1	16.2	16.3	16.6	17.7	18.6	16.7	17.0
Fetal sex ratio: Male:Female	170: 176	152: 165	156: 149	177: 160	138: 129	140: 149	144: 159	165: 198	176: 166	159: 175	160: 177	132: 138	141: 142	167: 155
Mean fetal body weight (g):	3.6	3.6	3.4	3.4	3.3	3.4	3.4	3.4	3.3	3.4	3.4	3.2	3.2	3.6
Mean adjusted[a] maternal body weight	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	48	44	NA
Mean unadjusted[b] maternal body weight	134	141	115	138	117	150	132	142	147	149	149	107	113	141
Mean Placental Weight (g)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a]Weight change of dam minus the weight of the uterus
[b]Weight change of dam including uterus
NA Not Applicable

IRDC Historical Control Data: Rat Cesarean Section
Source: Charles River Strain: CD®® CD®

Individual Studies: Maternal and Fetal Observations at Cesarean Section

Study No. / Date:	29 9/85	30 9/85	31 10/85	32 11/85	33 11/85	34 11/85	35 11/85	36 12/85	37 2/86	38 2/86	39 3/86	40 3/86	41 3/86	42 4/86
No. of animals in control group:	25	29	25	25	25	25	25	25	30	20	25	20	25	25
No. of animals that died:	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No. of animals examined at Cesarean section:	25	25	25	25	25	25	25	25	30	20	25	20	25	25
No. nongravid:	3	2	2	1	2	2	1	4	2	0	1	0	3	3
No. gravid:	22	23	23	24	23	23	24	21	28	20	24	20	23	22
No. of dams with resorptions only:	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No. of dams with live fetuses:	22	23	23	24	23	23	24	21	28	20	24	20	23	22
No. of live fetuses/dam:	15.4	12.3	13.7	15.0	15 4	13.9	14.2	15.9	13.5	14.3	13.1	13.9	14.3	14.0
No. late resorptions/dam:	0.8	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. early resorptions/dam:	1.3	0.8	1.7	0.8	0.9	1.1	1.0	0.9	1.3	1.1	1.7	0.8	1.7	0.9
No. postimplantation losses/dam:	1.4	0.8	1.7	0.8	0.9	1.1	1.1	0.9	1.3	1.2	1.7	0.8	1.7	0.9
No. implantations/dam:	16.8	13.1	14.9	15.8	16.3	15.0	15.3	16.3	14.6	15.9	14.8	14.7	16.0	14.8
No. corpora lutea/dam:	17.7	14.5	16.4	17.5	18.2	16.3	15.9	17.8	16.0	17.5	15.6	16.1	17.5	16.8
Fetal sex ratio: Male:female	166: 173	150: 132	152: 164	184: 175	204: 191	174: 185	169: 176	158: 166	190: 182	162: 144	152: 163	142: 135	181: 167	199: 150
Mean fetal body weight (g):	3.3	3.4	3.3	3.7	3.6	3.5	3.4	3.5	3.5	3.4	3.4	3.6	3.6	3.7
Mean adjusted[C] maternal body weight	NA	NA	NA	NA	NA	NA	NA	NA	63	NA	NA	NA	66	NA
Mean unadjusted[D] maternal body weight	130	135	119	160	164	126	134	148	136	122	122	135	149	149
Mean Placental Weight (g)	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.45	NA	0.46	NA	0.48

[C]Weight change of dam minus the weight of the uterus
[D]Weight change of dam including uterus
NA Not Applicable

Individual Studies: Maternal and Fetal Observations at Cesarean Section

Study No.	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Date:	4/86	5/86	6/86	6/86	8/86	10/86	10/86	11/86	11/86	11/86	1/87	1/87	2/87	2/87
No. of animals in control group:	25	25	25	25	25	20	25	25	25	25	25	25	25	25
No. of animals that died:	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No. of animals sacrificed at Cesarean section:	25	25	25	25	25	20	25	25	25	25	25	25	25	25
No. nongravid:	3	3	1	8	1	0	3	2	3	1	3	0	5	4
No. gravid:	22	22	24	17	24	20	22	23	22	24	22	25	20	21
No. of dams with resorptions only:	0	0	0	0	0	0	0	0	0	0	0	0	0	1
No. of dams with live fetuses:	22	22	24	17	24	20	22	23	22	24	22	25	20	20
No. of live fetuses/dam:	13.7	13.7	13.9	12.6	14.0	14.6	9.1	15.1	15.1	14.2	14.0	12.5	12.8	12.2
No. late resorptions/dam:	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
No. early resorptions/dam:	0.7	0.8	1.0	0.6	1.1	1.1	0.7	0.9	0.9	1.0	1.2	1.9	1.1	0.8
No. postimplantation losses/dam:	0.7	0.8	1.0	0.8	1.1	1.1	0.7	0.9	0.9	1.0	1.2	1.6	1.2	0.8
No. implantations/dam:	14.4	14.5	14.9	13.2	15.1	15.7	9.8	16.0	16.0	15.3	15.2	14.0	14.0	13.0
No. corpora lutea/dam:	16.5	16.5	15.8	14.4	16.3	16.9	11.5	16.8	17.4	17.2	16.9	15.6	15.2	16.2
Fetal sex ratio: Male:Female	153: 149	136: 166	171: 163	116: 98	171: 188	162: 129	95: 97	146: 155	169: 165	198: 182	190: 158	181: 151	144: 112	120: 137
Mean fetal body weight (g):	3.5	3.7	3.5	3.4	3.6	3.7	3.5	3.5	3.4	3.4	3.5	3.3	3.5	3.4
Mean adjusted[a] maternal body weight	NA	NA	NA	NA	NA	NA	50	56	61	67	NA	45	57	NA
Mean unadjusted[b] maternal body weight	148	159	143	125	157	131	79	128	143	140	142	109	126	124
Mean Placental Weight (g)	NA	NA	NA	NA	NA	0.48	NA	NA	NA	NA	NA	NA	NA	NA

[a]Weight change of dam minus the weight of the uterus
[b]Weight change of dam including uterus
NA Not Applicable

IRDC Historical Control Data: Rat Caesarean Section
Source: Charles River Strain: CDB$® CD®

Individual Studies: Maternal and Fetal Observations at Caesarean Section

Study No.	57	58	59										TOTAL
Date:	3/87	4/87	6/87										
No. of animals in control group:	20	25	25										1447
No. of animals that died:	0	0	0										1
No. of animals examined at Caesarean section:	20	25	25										1446
No. nongravid:	0	5	2										137
No. gravid:	20	20	23										1309
No. of dams with resorptions only:	0	0	0										4
No. of dams with live fetuses:	20	20	23										1305
No. of live fetuses/dam:	14.6	7.3	13.8										13.9
No. late resorptions/dam:	0.0	0.0	0.0										0.0
No. early resorptions/dam:	0.8	0.6	1.2										1.0
No. postimplantation losses/dam:	0.8	0.6	1.2										1.0
No. implantations/dam:	15.4	7.9	15.0										14.7
No. corpora lutea/dam:	16.2	12.4	17.2										16.2
Fetal sex ratio: Male:Female	145: 147	69: 76	175: 182										9008: 8881
Mean fetal body weight (g):	3.5	3.1	3.5										3.5
Mean adjusted[a] maternal body weight	NA	35	NA										35
Mean unadjusted[b] maternal body weight	129	70	130										135
Mean Placental Weight (g)	0.46	NA	0.44										0.48

[a]Weight change of dam minus the weight of the uterus
[b]Weight change of dam including uterus
NA Not Applicable

191-1395

FILE #42
IRDC REPRODUCTIVE HISTORICAL CONTROL DATA
Charles River CD®SD CD® Rats

Sex, (When Applicable) Reproductive/Litter Parameter		Number	Value	Range
MALE				
Fertility Index	Fertile Males / Total Males Mated	1057 / 1200	88.1%	(80.0% - 100.0%)
FEMALE				
Fertility Index	No. of Females Pregnant / No. of Females Mated	1396 / 1584	88.1%	(80.5% - 100.0%)
Copulatory Index	Females with Evidence of Copulation / Total Females Mated	942 / 974	96.7%	(76.0% - 100.0%)
Copulatory Interval (Days)	Total No. of Days / No. of Females Mated	3382 / 1096	3.1	(2.6 - 4.9)
Gestation Length (Days)	Total No. of Days / No. of Pregnant Dams	27294 / 1238	22.0	(21.7 - 27.6)
No. Implantation Sites at Weaning/Dam	Total No. of Implantation Sites / No. of Pregnant Dams	10986 / 767	14.3	(11.0 - 15.3)
Postimplantation Loss/Dam	No. of Implantation Sites at Weaning - Total No. Pups at Birth / No. of Pregnant Dams	850 / 765	1.1	(0.6 - 1.9)
LITTER SIZE				
Viable Pups/Litter at Day 0	Total No. of Viable Pups / No. of Pregnant Dams	16949 / 1280	13.2	(6.0 - 14.7)
Stillborn Pups/Litter	Total No. of Stillborn Pups / No. of Pregnant Dams	259 / 1206	0.2	(0.0 - 0.5)
PUP SEX DISTRIBUTION AT WEANING	Total No. of Males: Total No. of Females	Total No. of Males: Total No. of Females 4690:4632	%Male:%Female 50.3:49.7	%Male:%Female - %Male:%Female (45.6:41.0 - 59.0:55.4)

The historical control data compiled June, 1988 includes data from studies terminating January, 1984 to May, 1987.

Sex, (When Applicable) Reproductive/Litter Parameter		Number	Value	Range
PUP SURVIVAL AT BIRTH				
Gestation Survival Index	Total No. of Viable Pups at Birth / Total No. of Pups Born	16675 / 16950	98.5%	(95.7% - 100.0%)
PUP SURVIVAL INDICES THROUGH WEANING				
Lactation Day 4 (Before Reduction)	Total No. of Viable Pups on Day 4 (B.R.) / Total No. of Viable Pups on Day 0	16366 / 16610	98.5%	(86.8% - 99.9%)
Lactation Day 7	Total No. of Viable Pups on Day 7 / Total No. of Viable Pups on Day 4 (A.R.)	10953 / 11014	99.4%	(95.5% - 100.0%)
Lactation Day 14	Total No. of Viable Pups on Day 14 / Total No. of Viable Pups on Day 4 (AR), 5 (AR) or 7	10692 / 10761	99.9%	(99.0% - 100.0%)
Lactation Day 21	Total No. of Viable Pups on Day 21 / Total No. of Viable Pups on Day 12 or 14	10782 / 10789	99.9%	(99.1% - 100.0%)
PUP BODY WEIGHT (g) AT BIRTH				
Litter Weight (Males and Females)	Total Mean Viable Litter Weight at Birth / Total No. of Litters	8798.2 / 1418	6.2 g	(5.9 g - 6.7 g)
PUP BODY WEIGHT (g) THROUGH WEANING				
Lactation Day 4 (Before Reduction)				
Litter Weight (Males and Females)	Total Mean Viable Litter Weight on Day 4 (B.R.) / Total No. of Litters on Day 4 (B.R.)	12510.2 / 1266	9.9 g	(9.2 g - 11.4 g)
Litter Weight (Males and Females)	Total Mean Viable Litter Weight on Day 4 (A.R.) / Total No. of Litters on Day 4 (A.R.)	12392.7 / 1241	10.0 g	(9.2 g - 11.4 g)
Lactation Day 7				
Litter Weight (Males and Females)	Total Mean Viable Litter Weight on Day 7 / Total No. Litters on Day 7	21088.8 / 1341	15.7 g	(14.3 g - 17.5 g)

Sex, (When Applicable) Reproductive/Litter Parameter		Number	Value	Range
Lactation Day 14				
Litter Weight (Males and Females)	Total Mean Viable Litter Weight on Day 14	44421.2	34.8 g	(26.5 g - 33.2 g)
	Total No. Litters on Day 14	1277		
Lactation Day 21				
Male	Total Mean Viable Mal. Pup Weight on Day 21	57752.8	49.8 g	(36.1 g - 53.6 g)
	Total No. of Litters on Day 21	1160		
Female	Total Mean Viable Female Pup Weight on Day 21	55706.7	47.8 g	(34.8 g - 52.0 g)
	Total No. of Litters on Day 21	1165		

CONTROL HEMATOLOGICAL VALUES

Rats (4-11 Months of Age)

Test Name	Units	Male			Female		
		Mean	± S.D.	N	Mean	± S.D.	N
Leukocytes	$X10^3$/cmm	10.1	5.1-15.1	497	6.9	3.8-10.0	494
Erythrocytes	$X10^6$/cmm	7.72	5.36-9.08	510	7.29	6.00-8.38	522
Hemoglobin	g/dl	13.8	13.4-18.2	508	15.5	13.7-17.3	534
Hematocrit	%	42	32-52	504	46	38-54	525
MCV	μ^3	60	51-71	508	64	54-74	525
MCH	uug	21	17-25	508	22	18-26	525
MCHC	g/dl	34	25-43	508	34	27-41	525
Reticulocytes	/100 RBC	3.3	0.9-5.7	277	2.0	0.6-3.4	273
Platelets	$X10^3$/cmm	816	608-1024	508	797	517-1077	526
Seg. Neutrophils	/100 WBC	16	2-30	456	15	4-28	494
Band Neutrophils	/100 WBC	0	0-1	508	0	0-1	528
Lymphocytes	/100 WBC	80	67-93	483	79	64-94	501
Eosinophils	/100 WBC	2	0-4	506	2	0-4	528
Monocytes	/100 WBC	2	0-4	453	2	0-5	468
Basophils	/100 WBC	0	0-0	508	0	0-0	528

International Research & Development Corporation

CONTROL BIOCHEMICAL VALUES

Rats (3-5 Months of Age)

Test Name	Units	Male Mean	Male ± S.D.	Male N	Female Mean	Female ± S.D.	Female N
Alkaline Phosphatase	IU/l	90	34-146	54	48	19-79	54
AST (SGOT)	IU/l	93	47-139	54	75	41-109	53
ALT (SGPT)	IU/l	30	19-42	53	35	18-52	54
CPK	IU/l	417	126-708	70	421	84-758	70
Glucose	mg/dl	106	81-131	54	107	88-126	54
Cholesterol	mg/dl	75	39-111	50	94	55-132	50
Urea Nitrogen	mg/dl	17.4	11.1-23.6	54	20.1	12.4-27.8	54
Creatinine	mg/dl	0.6	0.3-0.9	54	0.6	0.3-0.8	54
Calcium	mg/dl	10.3	9.1-11.4	50	10.8	9.9-11.7	50
Phosphorus	mg/dl	7.7	5.9-9.5	54	6.8	4.5-9.1	54
Sodium	meq/l	147	140-154	50	148	140-155	50
Potassium	meq/l	7.0	5.7-8.2	50	6.4	5.0-7.8	50
Chloride	meq/l	105	101-109	50	106	102-111	50
Total Protein	g/dl	6.3	5.5-7.1	50	6.8	5.8-7.8	50
Albumin	g/dl	3.5	3.0-3.9	50	4.0	3.3-4.7	50
GGTP	IU/l	1.0	<1.0-2.0	55	1.0	<1.0-2.0	55
LDH	IU/l	807	162-1512	62	825	153-1496	61
OCT	U/l	5.5	<0.1-11.0	45	5.3	<0.1-10.6	45
Uric Acid	mg/dl	NDA	NDA	NA	NDA	NDA	NA
Triglyceride	mg/dl	NDA	NDA	NA	NDA	NDA	NA
Phospholipid	mg/dl	NDA	NDA	NA	NDA	NDA	NA
Total Bilirubin	mg/dl	0.2	<0.1-0.3	50	0.2	<0.1-0.4	49

APPENDIX E

Protocol and Protocol Addenda

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSIN# G0539.03)

Protocol Sheet No. 1 Study No. 191-1295 (IRDC# 954764)

TITLE: SEGMENT I STUDY OF FERTILITY IN RATS

ITEM	JUSTIFICATION
1	Correction of animal conditioning.
2	Identification of test article.
3	Study initiation.

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	EXPERIMENTAL DESIGN (p. 3): Females will not be received as weanlings. They will be approximately 85 days old at the start of dosing. Baseline blood collection for the females will be conducted 5 days after receipt of the animals. However, dosing cannot start within seven days of the baseline blood collection since animals are randomized after the baseline blood collection and then animals must be smeared for 10 days prior to test material administration.
2	Test article is identified as IRDC# 954128.
3	Initiate study in accordance with attached protocol.

Study Director Dr. Kit A. Keller

Signature

3/09/88

Date

IR90-49-5

PROTOCOL

SEGMENT I STUDY OF FERTILITY IN RATS

SPONSOR: The Procter & Gamble Company
Cincinnati, Ohio 45247

ISSUE DATE: 2/4/78 BAH

TEST SUBSTANCE IDENTIFICATION NUMBER (TSIN): G0539.03

DIVISIONAL REQUEST DOCUMENT NUMBER (DRD): BY0764$ AAA 2/22/81

TEST FACILITY: International Research and Development Corporation
500 North Main Street
Mattawan, Michigan 49071

STUDY NUMBER: 191-1395

PURPOSE: The objective of the study is to determine the effect of the test material on fertility, parturition, neonatal viability, and reproductive performance in rats. This study design utilizes treatment of both sexes with the test material.

TEST MATERIAL DATA:

Identification:	G05397.03
Purity:	100%
Physico-chemical properties:	White Powder
Stability:	Expiration Date: 3/89
Storage Conditions:	Room Temperature
Safety Precautions:	May be an eye and skin irritant. For eye contact, flush promptly and thoroughly with water. For skin contact, flush with water.

DOSING SUSPENSION PREPARATION: Appropriate dosing suspensions will be prepared fresh on a weekly basis following the directions outlined in Appendix I and IV (added as a protocol addendum following range finding study). Doses will be administered at a constant volume of 2.5 ml/kg.

TEST ANIMALS:

Species	Rat
Strain	Charles River COBS® CD®
Source	The Charles River Breeding Laboratories, Inc. 9801 Shaver Road Portage, Michigan 49081
Age at Start of Study:	Males (minimum of 40 days of age) Females (minimum of 40 days of age)
Body Weight at Start of Study:	Males: Approximately 130-170g Females: Approximately 110-160g
Method of Identification:	Each parental rat will be identified by cage, group and individually by a Monel metal ear tag bearing its animal number. The individual animal number plus the IRDC study number will comprise a unique identification number for each animal.
Number on Study:	140 males and 140 females.

TEST ANIMALS - continued

Reason for Selection: The rat is an acceptable model for reproduction studies. This laboratory has historical control data on the fertility, neonatal survival indices and growth rates, and the reproductive performance in rats of this strain from this source.

ROUTE OF ADMINISTRATION:

Parenal by gavage. The anticipated major route of exposure by humans is dermal. The oral route was chosen to maximize systemic exposure.

METHOD AND FREQUENCY OF ADMINISTRATION OF THE TEST MATERIAL:

Gavage. Animals will receive the test material by gavage as a single daily dose (seven days a week) in the afternoon at least six hours after illumination of the housing area. When administered in vehicle, the dose will be at a constant volume of 2.5 ml/kg/day. Administration of both the test and control materials to the parental males will begin 60 days prior to mating and to the parental females 14 days prior to mating and continue until sacrifice. Individual dosages will be based on most recent body weights.

EXPERIMENTAL DESIGN:

Animal Communication and Selection

One hundred sixty weanling (21-23 days of age) Charles River CCBS^{R}, CD^{R} rats of each sex will be received from Charles River Breeding Laboratories, Portage, MI. The rats will be randomly removed from the shipping cartons and examined for evidence of disease and/or poor physical condition. If acceptable, they will be weighed and housed in individual suspended wire cages. Five days after receipt, blood samples will be collected into collection tubes containing EDTA anti-coagulant, following the approved Standard Operating Procedures of the Test Facility. The method used will be specified. Hemoglobin, hematocrit, white blood cell count, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, and differential white blood cell will be determined. Those animals judged to have an abnormal hemogram as defined by the Standard Operating Procedures of the Test Facility will be eliminated. Dosing will be started within seven days of the baseline blood collections. The remaining animals will then be given a detailed physical examination and those considered suitable for the study will be randomly distributed into the group indicated using the procedure described below.

Housing

All animals will be housed in an environmentally controlled room with a temperature controlled to 23 ± 2°C and humidity controlled to 50 ± 15 percent. The temperature and humidity will be recorded. Fluorescent lighting will provide illumination 12 hours per day. The lighting cycle will be maintained so that dosing occurs at least six hours after illumination of the housing area. During the study, rats will be housed individually in suspended wire-mesh cages except during mating, gestation and lactation. On gestation day 0, the females will be housed in plastic cages with ground corn cob bedding until sacrifice.

Randomization (Procedure A)

Animal numbers and corresponding body weights are entered onto magnetic tape which is used as the data source for the randomization procedure. The mean body weight and standard deviation are calculated by sex and a computer program lists those animals whose body weights are within ± 1.5 standard deviations of the mean. From the qualifying animals, the randomization procedure selects and assigns the required number of animals. Bartlett's Chi-square test for homogeneity of variances is applied to the groups. If the groups are not judged to be homogeneous, new randomizations are applied until homogeneity is established. At this time (prior to dosing), females will be randomly selected for a 13-day uterine examination.

Dosage Levels

The dosage levels will be determined from a pilot study and will be agreed to by the Divisional Toxicologist and Study Director. The dosage levels will be attached as a protocol addendum (Appendix IV). There will be three dose levels and a vehicle control.

	Number of Parental Animals			
Number	Male	Female	Dosage	Dose Volume ml/kg
1	35	35	0 (vehicle control)	2.5
2	35	35	Low Dose	2.5
3	35	35	Mid Dose	2.5
4	35	35	High Dose	2.5

Basal Laboratory Diet

1. Basal Diet: Certified Rodent Chow® #5002, Ralston Purina Company. Diet will be available ad libitum to all animals.

2. Identification: Each lot utilized will be identified and recorded.

3. Contaminant Levels: The diet used will be a certified diet with specified analyses for contaminants performed by the supplier.

Drinking Water

The drinking water used for test animals at IRDC will be monitored for contaminants at periodic intervals according to IRDC Standard Operating procedures. Water will be available ad libitum for all animals.

OBSERVATIONS AND EXAMINATIONS

A. Parental Observations

1. Signs: All parental animals will be observed for mortality and signs of overt toxicity twice each day (morning and afternoon, seven days a week). Should mortality or such signs of overt toxicity be observed, these will be recorded on the day observed. Examples of overt toxicity include tremors, convulsions, loss of righting reflex, excessive blood loss and moribundity. An exception to this would be a recurring, frequently observed reaction for which a general statement would suffice. During treatment a detailed clinical observation of each animal will be performed daily and the findings recorded.

2. Body Weights: Males will be weighed every three days for the first three weeks of treatment and then weekly until sacrifice. Females will be weighed weekly during treatment until evidence of copulation is observed. Females to be sacrificed on gestation day 13 will be weighed on gestation days 0,7,13. Females allowed to deliver will be weighed on gestation days 0,7,13 and 20 and on days 0,7,14 and 21 of lactation.

3. Mortality: Any rat showing sign of severe debility or toxicity, particularly if death appears imminent, will be killed to prevent loss of tissues through autolysis. All rats killed in extremis or found dead will be subjected to a routine necropsy procedure. The disposition of all animals and intact fetuses or pups is described in the Necropsy/Pathology Section of this protocol (page 9).

4. Abortion: Any female showing sign of abortion or premature delivery will remain on study and be necropsied on the day of scheduled sacrifice.

5. Females Which Fail to Deliver: On the 25th day after termination of mating, a gross necropsy will be performed on all females where evidence of mating was detected but which failed to deliver. On the 25th day after separation from the male, this same procedure will be performed on all females where no evidence of mating was detected and which did not deliver. Uteri from females that appear nongravid will be opened and placed in 10% ammonium sulfide solution for determination of pregnancy status[1]. Any condition which could have prevented pregnancy will be recorded.

6. Estrous Cycle Determinations: Ten days prior to test article administration and until evidence of copulation is observed or the mating period is ended, the females will have vaginal smears taken daily to establish estrous cycles.

7. Food Consumption: Individual twenty-four hour food consumption will be measured weekly for all adults up to the mating period and after the mating period for males and non-bred females. During mating, individual food consumption will not be measured, since the animals will be cohabitating at this time. During gestation and lactation, food consumption will be measured for the females on the specified body weight days.

B. Breeding Procedures

After 60 days of exposure for the F_0 males and 14 days of exposure for the F_0 females, one male will be randomly assigned and housed with one female of the same treatment group in a plastic cage with corn cob bedding for mating.

The maximum mating period will be 20 days. The females will remain with the same males for 10 days or until evidence of copulation is observed. If no evidence of copulation is obtained in 10 days, the female will be placed with another male (sperm positive in the initial 10 days) in the same treatment group for the remaining 10 days. After the 20-day mating period, the males will be returned to suspended wire-mesh cages until sacrifice. Male/female pairings and the occurrence of copulation will be recorded.

The occurrence of copulation will be established by daily inspection for a copulatory plug or vaginal smear for sperm. A positive finding is considered day "0" of gestation. Each female is then returned to an individual plastic cage.

[1] Kopf,R.,Lorenz, D., and Salewski,E. (1964). The effects of thalidomide on the fertility of rats studied in two generations. Naunyn Schmiedebergs Arch. Pharmacol., 247:121-135.

C. Females Scheduled for 13-day Uterine Examination

Prior to the 13th day of gestation a blood sample will be drawn from the 10 previously randomly selected females chosen for sacrifice. The blood sample will be taken and analyzed as described in the Animal Conditioning and Selection section of this protocol (page 3). On the 13th day of gestation, approximately 10 previously randomly selected females in each group will be sacrifice by CO_2 inhalation and undergo a gross necropsy. The tissues listed in Appendix II will be taken. The disposition of all animals is described in the Necropsy/Pathology section of this protocol (page 9). During the necropsy the uterus and ovaries will be exposed by a mid-abdominal incision. The location of viable and nonviable embryos, early resorptions, and the number of total implantations and corpora lutea will be recorded. The thoracic and abdominal cavities and organs of the dams will be examined for grossly evident morphological changes. Maternal tissues will be preserved in 10% neutral buffered formalin for microscopic examination as described in the Necropsy/Pathology section of this protocol (page 9).

Uteri from females that appear nongravid will be opened and placed in 10% ammonium sulfide solution for determination of pregnancy status.

D. Females Scheduled to Deliver

The remaining females from each group will be allowed to deliver. Toward the end of the gestation period, females will be examined twice daily for signs of parturition. The bred females will be allowed to give birth (F_1). The duration of gestation will be calculated and any difficulties observed at parturition will be recorded. The day on which all pups have been delivered will be defined as day 0 of lactation. The litters will be examined as soon as possible after delivery for litter size, stillborns, live births and gross anomalies. Litter size will be reduced to ten (10) pups of equal sex distribution, whenever possible, on lactation day 4. The culled pups will be examined externally and discarded. Litters will be caged with their mothers for 3 weeks after birth. The dams and pups will be observed daily for survival, behavioral abnormalities in nesting and nursing and presence of dead pups will be recorded. Any intact dead pups found will be necropsied, examined for anomalies and discarded. The heart will be dissected by a modification of the method described by Staples[2]. Pups will be weighed individually on days 0,4,7,14 and 21 of lactation. The number of males and females per litter will be recorded on day 21 of lactation.

[2] Staples, R.E. (1974). Detection of visceral alterations in mammalian fetuses. Teratology, 9: A37-A38.

B. Termination of the Study

At weaning, all remaining dams will be observed externally, sacrificed and subjected to a gross necropsy. The number of uterine implantation sites will be recorded. If abnormalities are noted in any pup at weaning, these pups will be necropsied. If a disparity in body weight is present at weaning for any groups of otherwise normal appearing pups, all normal appearing pups from all groups will be retained for an additional 4-week observation period (if needed), all pups will be examined externally, sacrificed and discarded. Tissues will be preserved in 10% neutral buffered formalin for histopathological examination only if abnormalities are observed during the gross examination.

All males will be necropsied after completion of 13-day uterine examinations in the females, and tissues preserved as designated in the Necropsy/Pathology section of this protocol (page 9).

If evidence of reduced fertility is observed, the males reproductive tissues will be examined histopathologically.

Prior to necropsy of the females and males, a blood sample will be drawn and analyzed as described in the Animal Conditioning and Selection section of this protocol (page 3).

F. Disposition of Pups and Dead or Moribund Adults

1. Adults

A gross necropsy will be performed on all adult rats not surviving to scheduled sacrifice in an attempt to determine the cause of death. The tissue specimens listed in Appendix II will be taken (see Necropsy/Pathology).

2. Fetuses

The fetuses from dams found dead during gestation will be examined externally to the fullest possible extent and discarded.

3. Pups

If a dam dies during gestation, the pup will be externally examined, sacrificed, and necropsied. If a pup is found dead, the pup will be externally examined and necropsied. The thoracic and abdominal cavities and organs of the pups will be examined for grossly evident morphological changes. Any macroscopic lesions will be saved in 10% neutral buffered formalin for histopathological examination.

C. Necropsy/Pathology

Prior to necropsy, obtain blood samples and analyze as described in the Animal Conditioning and selection section of this protocol (page 3). Weigh all animals on the day of necropsy for use in assessing in-life body weight changes. Obtain a body weight at the time of euthanasia for use in establishing relative organ weights. Sacrifice the animals by CO_2 inhalation. Exsanguinate prior to cessation of heart beat following the appropriate Standard Operating Procedure of the Test Facility. Necropsy should be started within ten (10) minutes after euthanasia. Necropsy all surviving animals and record all gross observations for all organs following the Standard Operating Procedure of the Test Facility. Lungs shall be perfused with fixative intratracheally at a pressure of approximately 40 cm of water. Take tissue specimens given in Appendix II. These tissues will be preserved in 10% neutral buffered formalin for possible future histopathological examination (additional cost). Special attention will be given to the reproductive organs.

ovaries	testes - with epididymis
prostate	uterus
seminal vesicles	vagina
	all gross lesions

Any deviations from the protocol such as missing tissues, hematology results, etc., should be noted in the raw data. All scheduled necropsy room assistants will be trained and qualified.

Determine absolute weights of liver and kidneys and calculate organ to body weight ratios.

STUDY DURATION:

The duration of the study from initiation of treatment of the F_0 animals through lactation of the pups is approximately 4 months. A study schedule will be issued prior to study initiation that will include the following information: dates for animal arrival, mating initiation and termination, administration of the test and control articles (initiation and termination), 13-day uterine examination, parturition, lactation and sacrifice. If at weaning a weight disparity is present among the pups an additional 4-week observation period will be added.

STATISTICAL ANALYSIS:

All statistical analyses will compare the treatment groups to the control groups with the level of significance at p 0.05 and p 0.01. Body weight gain, hematology results, and pathology findings will be compared to judge significance of difference.

A. 13-Day Sacrifice Data

The proportions of resorbed and dead fetuses and postimplantation losses will be compared by the Mann-Whitney U-test, as described by Siegel[3] and Weil[4], to judge significance of differences. Mean number of corpora lutea, total implantation and viable embryos will be compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variance and the appropriate t-test (for equal and unequal variances) as described by Steel and Torrie[5], using Dunnett's multiple comparison tables[6] to judge significance of differences.

B. Litter Data

The male and female fertility indices will be compared using the Chi-square test criterion with Yates' correction for 2 x 2 contingency tables and/or Fisher's exact probability test as described Siegel[3] to judge significance of differences. Pup survival indices will be compared by the Mann-Whitney U-test as described by Siegel[3] and Weil[4] to judge significance of differences.

Mean number of live pups per litter at birth, mean pup body weights taken on lactation days 0, 4, 7, 14, and 21 (and days 28, 35, 42, and 49 if needed), and parental body weights will be compared by analysis of variance (one-way classification), a Bartlett's test for homogeneity of variances and the appropriate t-test (for equal and unequal variances), as described by Steel and Torrie[5] using Dunnett's multiple comparison tables[6] to judge significance of differences.

[3] Siegel, S. (1956). Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York, N.Y.

[4] Weil, C.S. (1970). Selection of the valid number of sampling units considering of their combination in toxicological studies involving reproduction. teratogenesis or carcinogenesis. Food Cosmet. Toxicol. 8: 177-182.

[5] Steel, R. G. D. and Torrie, J. H. (1960). Principles and Procedures of Statistics. McGraw-Hill, New York, N.Y.

[6] Dunnett, C.W. (1964). New tables for multiple comparisons with a controlled Biometrics, 31: 482-491.

REPORT

A comprehensive report will be prepared upon completion of the study. The final report will contain a summary, experimental design and methods, evaluation of the test results, and data and measurements required by this protocol. The report will include the following:

Text

1. Experimental design
2. Survival data
3. Results of significant daily observations
4. Body weights
5. Hematology findings
6. Results of mating including male and female breeding success
7. Necropsy and histopathologic findings
8. Results of 13-day uterine examinations
9. Results of parturition and litter observations

Tables

1. Estrous cycle determinations (individual)
2. F_0 male weekly body weights and survival (summary and individual)
3. Maternal body weights during gestation and lactation
4. Hematology determinations (summary and individual)
5. Gross Pathology Findings (summary and individual)
6. 13-day uterine examination data (summary and individual)
7. Gestation and lactation data (summary and individual)
8. Uterine observations at weaning (individual)
9. Food consumption (summary and individual)

Appendices

1. Test [illegible] data (if available)
2. Dates of Quality Assurance inspection and report of significant deviations from protocol
3. Historical control data

The final report shall conform to all requirements outlined in Section 58.185 Subject J, Good Laboratory Practices regulations.

DATA AND SAMPLE RETENTION

All data, including reports from this study, will be retained for at least 10 years after completion of the study and stored in the IRDC Archives and will be made available for inspection upon request by authorized personnel of the Sponsor. An appropriate sample of the test article will be retained for 10 years following completion of the study.

Fetal skeletons and any wet tissues, blocks and slides collected for histopathology should be returned to the Sponsor for retention following acceptance of the final report. Fetal soft tissue specimens should be discarded, except those specimens in which a malformation has been observed. In such cases, the Study Director should contact the Sponsor (Divisional Toxicologist) for instructions. Typically, the written description of the malformation in the Study Report will be sufficient documentation of the effect, and the specimen should be discarded. However, in extraordinary circumstances (e.g., an unusual abnormality, or frequent occurrence of a specific malformation) the Sponsor may direct the Study Director to photograph the malformation, and to include the photograph in the report. The specimen should be discarded after a satisfactory photograph has been made.

The Study Director will be notified in writing when the report has been accepted. Instructions for returning specimens are given in Appendix III.

RECORDS TO BE MAINTAINED

A. Protocol and protocol addenda
B. Study schedule
C. Pre-initiation data
D. Technical personnel list with signatures
E. Source, purchase order, shipping labels and age for animals
F. Sex verification
G. Diet lot numbers and contaminant analyses
H. Animal identification (ear tag)
I. Room humidity and temperature
J. General procedures
K. Scale and balance accuracy verification
L. Daily observations (room check)
M. Daily detailed observations for pharmacotoxic signs, and general appearance and behavior
N. Body weights
O. Test material data
P. Test material preparation instructions
Q. Test material preparation
R. Test material administration
S. Data and type of termination for each animal in the study, i.e., spontaneous death, accidental death, sacrificed in extremis (unscheduled sacrifice), or scheduled sacrifice (late tables)
T. Necropsy
U. Randomization data
V. Quality Assurance inspections
W. Drinking water analysis
X. Blood draws
Y. Food consumption data
Z. Miscellaneous study data

QUALITY ASSURANCE AND GOOD LABORATORY PRACTICES:

This nonclinical laboratory study will be conducted in accordance with the Good Laboratory Practice Regulations.

The study will be subjected to periodic inspections, and the final report will be reviewed by IRDC's Quality Assurance Department in accordance with IRDC's Standard Operating Procedures. Study quality assurance inspection records will be made available to the Sponsor during Sponsor visits to IRDC.

STATEMENT OF COMPLIANCE:

The final report will include a statement signed by the Study Director that the Final Report Accurately Reflects the raw data obtained during the performance of the study and that there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of the study. If deviations are encountered that will affect the quality or integrity of the study, each deviation will be described in detail.

ALTERATION OF DESIGN:

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such change will be honored by IRDC. However, it then becomes the responsibility of the Sponsor to follow such verbal changes with a written verification. All protocol modifications will be signed by the Study Director.

APPROVED BY SPONSOR:

Divisional Toxicologist Gregory S. Allgood, Ph.D. Date 2/4/88

To be completed by the Test Facility

SCHEDULE:

Proposed Starting Date of Study: April 11, 1988

Proposed Completion Date of Study: August 16, 1988

Proposed Date of Final Report: January 20, 1989

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

Study Director: Dr. Kit A. Keller 3/9/88

Study Cost: _____

Protocol - Appendix I

INSTRUCTION FOR THE PREPARATION AND HANDLING OF TEST SUBSTANCE - VEHICLE MIXTURES

1. Test substance - vehicle mixture should be prepared:

   ( ) daily; (X) weekly; ( ) see Special Instructions on page 2

2. Mixtures that are not true solutions should be mixed by use of a magnetic stirring bar while dosing. All mixtures should be shaken or stirred before use.

3. A homogeneity determination (X) will; ( ) will not be required. If a homogeneity determination is required, sample the mixture from the top, middle, and bottom, while stirring , and:

   (X) Return all three samples, appropriately labeled, to the Sponsor's Divisional Toxicologist for analysis.

   Shipping Instructions

   Send approximately 20 ml. Send ( ) frozen; (X) under ambient conditions; ( ) other _____.

   ( ) Analyze all three samples using the analytical method in Appendix _____.

4. A concentration and/or stability analysis of the mixture (X) will; ( ) will not be required. If an analysis is required:

   (X) Prepare a sufficient quantity of the test substance - vehicle mixture so that a portion can be returned to the Sponsor's Divisional Toxicologist. Return parent material at end of study and sample of dosing solutions from week 1, mid-study, and last week of study to Sponsor for analysis.

   ( ) Analyze the test substance - vehicle mixture for test substance concentration using the analytical method in Appendix _____.

5. Special Instructions:

   Dosage preparation instructions will be determined following a pilot study and will be attached as a protocol addendum (Appendix IV).

Protocol 345

TISSUE COLLECTION LIST FOR [illegible] C11 AND C11C

LUNG (2 SECTIONS - LEFT APICAL, RIGHT DIAPHRAGMATIC)	ADRENAL
HEART (3 SECTIONS - LEFT ATRIUM AND VENTRICLE, SEPTUM, RIGHT ATRIUM AND VENTRICLE)	THYMUS/THYMIC REGION
AORTA (THORACIC)	LEFT PSOAS MUSCLE
TONGUE	SPLEEN
TRACHEA, ESOPHAGUS, THYROID/PARATHYROID (IF INCLUDED WITH THYROID)	PANCREAS
SUBMANDIBULAR LYMPH NODE	BONE MARROW (5TH COSTOCHONDRAL JUNCTION
	SKIN (TREATMENT AREA SKIN)
ILEOCECOCOLIC LYMPH NODE	BRAIN (LONGITUDINALLY SECTION - HALF FOR PROCESS, HALF FOR SAVE)
STOMACH (3 SECTIONS - CARDIAC, FUNDIC, PYLORIC REGIONS)	THORACOLUMBAR SPINAL CORD
SUBMANDIBULAR SALIVARY GLAND	LEFT SCIATIC NERVE (MID FEMUR)
[illegible], GALL BLADDER	PITUITARY (IN TOTO)
[illegible]DENUM	EYE
JEJUNUM	
ILEUM	
CECUM	
COLON	LESIONS NOT INCLUDED IN STANDARD SAMPLES CONSIDERED BY THE ATTENDING PATHOLOGIST TO POTENTIALLY REPRESENT TEST SUBSTANCE EFFECTS
RECTUM	
URINARY BLADDER	NOTE: FOR PAIRED ORGANS, EXCEPT THE LUNGS, KIDNEYS, AND UTERINE HORNS, THE LEFT WILL BE TAKEN FOR HISTOPATHOLOGY AND THE RIGHT AS SAVE TISSUE.* EACH KIDNEY, LUNG, AND UTERINE HORN SHOULD BE TRIMMED AS INDICATED WITH DUPLICATE SAMPLES COLLECTED FROM EACH, ONE FOR HISTOPATHOLOGY AND ONE FOR SAVE. FOR OTHER TISSUES, EXCEPT THE PITUITARY, DUPLICATE SAMPLES WILL BE COLLECTED (USING ONE FOR HISTOPATHOLOGY AND ONE FOR SAVE). THE BRAIN IS TAKEN AS INDICATED. FOR LESIONS, SAMPLES WILL BE TAKEN FOR HISTOPATHOLOGY AND, WHEN POSSIBLE, FOR SAVE.
KIDNEY (2 SECTIONS - LEFT LONGITUDINAL, RIGHT CROSS-SECTION)	
MALE	
TESTICLE WITH EPIDIDYMIS	
PROSTATE	
SEMINAL VESICLE	
FEMALE	
OVARY	
UTERUS (CROSS SECTION OF EACH HORN)	
[illegible]VA	*SAVE TISSUE: THOSE TISSUES COLLECTIVELY PLACED IN 10% NBF FOR POSSIBLE FUTURE REFERENCE.

PLACE TISSUES IN 10% NEUTRAL BUFFERED FORMALIN (NBF) AT A DILUTION OF 1 PART TISSUE TO > 10 PARTS FIXATIVE.

Protocol - Appendix III

INSTRUCTIONS FOR RETURN OF TERATOLOGY SPECIMENS, WET TISSUES

PARAFFIN BLOCKS, AND MICROSCOPIC SLIDES TO THE PROCTER & GAMBLE COMPANY

Wet Tissues: must be sealed in plastic bags (preferably heat-sealed). Bags such as "Kapak Pouches" by Skotchpak are excellent and available from most scientific supply houses. Place only one animal's tissues in a bag together with an amount of 10% buffered neutral formalin adequate to thoroughly moisten all tissues. Several seals should be made (with an identifying label written with waterproof ink which includes study number, group, animal number) between the seals.

Paraffin Blocks: should be placed on end (with the label visible) in numerical order by groups in special tray boxes which consists of a cardboard tray 18" x 9" x 2" plus a protective cardboard sleeve. These boxes will accommodate approximately 175 to 200 blocks and are available from John Green, Buckeye Box Co., 601 N. Hague Avenue, Columbus, Ohio 43204 (614-274-8484) (part #UP-1). Label the end of the box to identify the contents. This permanent label should list the study number and groups.

Microscope Slides: labeled to indicate the study number, group and animal number must be packed in numerical order by groups in plastic boxes. Lids should be secured with tape. A packing material such as polyfoam, paper towels, etc. should be placed inside of the lid to prevent breakage. A permanent label should be placed on the outside of the lid identifying the study number and groups.

IMPORTANT

An itemized list should accompany each shipment. This list should indicate the following:

1. The number of units in the shipment; i.e., the number of shipping cartons.

2. The number of slides, blocks and individual containers of wet tissue or teratology specimens in study.

Contract Laboratories: Procter & Gamble will request (in writing) the return of specimens, indicating approximately when they should be shipped. All costs incurred in shipment of these materials to the Sponsor should be included in the fixed study cost. When ready, please notify and send to:

Archive Technician
The Procter & Gamble Company
Miami Valley Laboratories - BM 005216
11810 E. Miami River Road
Ross (Butler County), Ohio 45061
Telephone: (513) 245-2288

CSA/rd4-1/6/88
VAH/02/16

THE PROCTER & GAMBLE COMPANY
MIAMI VALLEY LABORATORIES
P.O. BOX [illegible] CINCINNATI, OHIO 45247

February 25, 1988

RECEIVED
FEB 26 1988
[illegible] RESEARCH AND DEVELOPMENT CORPORATION

Mr. J. L. Schardein
International Research &
Development Corporation
500 North Main St.
Mattawan, Michigan 49071

Dear Dr. Johnson:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Notice: 1) This study is expected to be submitted to the following regulatory agency: Food and Drug Administration. The study should be listed on the Test Facility's master list of regulated studies. The stipulations of the protocol are to be implemented in complete conformance with the appropriate regulations [FDA Good Laboratory Practice Regulations (21 CFR, Part 58) or EPA GLP Regulations (40 CFR, Part 792)].

2) Quality Assurance Inspections:

All critical phases and the final report will be inspected. The critical phases may be defined by the QAU and Study Director.

3) Documentation of the derivation, characterization, and stability testing of the test substance will be the responsibility of the Sponsor.

4) The test substance is to be used for research and development purposes only.

Test: Segment I Study of Fertility in Rats
Protocol No: Special Protocol Dated 2/4/88
Test Substance No: G0539.03 Doc. Req. No.: BT 0764
Physical Form: Powder

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Divisional Toxicologist. All unused samples are to be returned to the Divisional Toxicologist at the following address (the cost of shipment should be included in the study cost). The cost of this study will be shared equally by two P&G Divisions. Please send separate invoices to:

Dr. Greg Allgood Telephone (513) 530-4098
The Procter & Gamble Company
11511 Reed Hartman Highway - Room HB2D39
Cincinnati, Ohio 45241

Dr. Gerald Haase
R&D Labs
Richardson-Vicks
Rusham Park
White Hall Lane
Egham, Surrey
TW20-9NAO, England

The Richardson-Vicks invoice should reference budget code 740-510-01D-8.

Complete both copies of the attached protocol by adding your study number, proposed start and completion dates, study cost and have the Study Director sign and date them. The Study Director should define the start and completion dates on the protocol. Retain one copy and return one copy to me, along with a letter stating that you agree to do the work specified in the attached protocol. Three copies of the draft report are needed as soon as possible, and are to be sent to my attention at the address shown on the letterhead. Also, complete the attached animal accounting form and return it with the final report.

The invoice for this study should be sent to:

Mr. Jim Matthews
The Procter & Gamble Company
11511 Reed Hartman Highway - Room HB2D31
Cincinnati, Ohio 45241

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research & Development Department

G. A. Nixon
Human & Environmental Safety Division

Approved:
R. E. Winters, Ph.D.
Director, Human & Environmental Safety Div.

st
cc: Study File
MVL QAU
G. E. Allgood

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSI# CQ539.03)

Protocol Sheet No. 2 Study No. 191-1395 (DRD# BY0764)

TITLE: SEGMENT I STUDY OF FERTILITY IN RATS

ITEM	JUSTIFICATION
1	Provision for use of 1N NaOH in test article preparation is necessary.

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	1. Appendix II: 5. Adjust the pH of the dosing suspension to 7.0 using 1M citric acid or 1N NaOH as necessary.

Study Director Dr. Kit A. Keller

Signature 3/23/88 Date

IR90-49-5

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSIMP G0539.03)

Protocol Sheet No. 3 Study No. 191-1345 (DRD# BY0764)

TITLE: SEGMENT 1 STUDY OF FERTILITY IN RATS

ITEM	JUSTIFICATION
1	Provision for use of 1N NaOH in test article preparation as necessary (repeat of Protocol Sheet No. 2, but with inclusion of Sponsor signature block).

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	1. Appendix II: 5. Adjust the pH of the dosing suspension to 7.0 using 1M citric acid or 1N NaOH as necessary.

Approved By:

Dr. Greg Allgood 4/5/88
Dr. Greg Allgood Date

Study Director Dr. Kit A. Keller

Signature 3/28/88
Signature Date

IR90-49-5

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSIRD 00539.03)

Protocol Sheet No. 4 Study No. 191-1395 (DRDC BYO764)

TITLE: SEGMENT I STUDY OF FERTILITY IN RATS

ITEM	JUSTIFICATION
1	Inclusion of Sponsor supplied dosing suspension preparation instructions and dosage levels (Appendix IV).
2	Clarification of weekly food consumption measurements.
3	Clarification of animal housing during mating.
4	Clarification of blood sampling time.
5	Inclusion of additional clinical chemistry.

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	Dosing suspension preparation instructions and dosage levels is appended as Appendix IV.
2	Individual twenty-four hour food consumption will be measured on the 1st day of each week for all adults up to the mating period and after the mating period for males and non-bred females. (page 6 of 17).

Study Director Dr. Kit A. Keller

Signature 4/07/88 Date

IR90-49-5

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSI## C0539.03)

Protocol Sheet No. 4 Study No. 191-1395 (DRD# BY0764)

TITLE: SEGMENT I STUDY OF FERTILITY IN RATS

ITEM	PROTOCOL REVISION OR CLARIFICATION
3	Animals will be housed in wire-mesh cages during the mating period to allow for detection of copulatory plugs (page of 17).
4	On the 13th day of gestation, prior to sacrifice, a blood sample will be drawn from the 10 previously randomly selected females chosen for 13-day uterine examination (page 7 of 17).
5	In addition to the clinical testing outlined on page 3 of 17 and 7 of 17 of the protocol the following serum analyses will be conducted on all collected blood samples from females chosen for 13 day uterine examination: Glucose Bun Creatinine Cholesteral Triglycerides Alkaline phosphatase SGOT SGPT Albumin Total protein Ca P K Na Fe

Approved By:

H.G. Allgood 4/12/88

Dr. Greg Allgood Date

Study Director Dr. Kit A. Keller

Signature 4/07/88

Date

IR9D-49-5

Protocol - Appendix IV
Dosing Suspension Preparation Instructions

Dosage Levels Number	Dosage (mg/kg/day)	Dosing Suspension Concentration (mg/ml)	Dose Volume (ml/kg)
1	(vehicle control)	0	2.5
2	10	4.0	2.5
3	100	40.0	2.5
4	250	100.0	2.5

Note: The procedures listed below provide instructions for the preparation of 100 grams of dosing suspensions. The procedures may be scaled up to produce larger quantities of dosing suspensions if necessary. Dosing suspension should be prepared weekly.

1. Prepare an appropriate volume of a solution of 1.0% (w/v) Methylcellulose (USP/FCC grade, 4000 cps) in deionized, distilled water.

2. Add the appropriate amount of the test substance (see below) in 5.0 grams of Polyethylene Glycol 400 (Carbowax PEG 400, NF). The PEG can be warmed to approximately 37 degrees Centigrade to improve the solubility of PEG.

3. Add the PEG solution slowly (under agitation) to the appropriate amount of the Methylcellulose solution (see below) to prepare 100 grams of the final dosing suspension.

Dosing Suspension Concentration (mg/ml)	Test Substance (grams) Added to PEG	PEG 400 (grams)	1% Methylcellulose Solution Needed to Make 100 Grams of Final Dosing Suspension
Placebo Vehicle	0	5.0	95.0
4 mg/ml	0.4	5.0	94.6
40 mg/ml	4.0	5.0	91.0
100 mg/ml	10.0	5.0	85.0

4. Sonicate the dosing suspension for approximately 90 seconds.

5. Adjust the pH of the dosing suspension to 7.0 using 1M citric acid and 1M NaOH.

6. Sonicate the dosing suspensions at the beginning of each day prior to commencement of dosing. Agitate the dosing suspension gently during the dosing procedures with a magnetic stir bar/stirrer.

Note: The components of the PEG:methylcellulose vehicle may be conveniently ordered through Fisher Scientific.

Methylcellulose (4000 centipoise) Fisher Catalog #M-352-500

Carbowax PEG 400 Fisher Catalog #P-167-1

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSI#: G0539.05)

Protocol Sheet No. 5 Study No. 191-1395 (DRD# 8Y0764)

TITLE: SEGMENT I STUDY OF FERTILITY IN RATS

ITEM	JUSTIFICATION
1	Clarification of Appendix II - Tissue Collection List.

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	Delete "skin (treatment area skin)" and "gall-bladder" as this is a gavage administered study in rats and therefore provision for collection of these tissues is not appropriate.

Approved By:

Dr. Greg Allgood 7/12/88
Date

Study Director Dr. Kit A. Keller

Signature 6/30/88
Date

1R90-49-5

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSI№ G0539.03)

Protocol Sheet No. 6 Study No. 191-1395 (IRDC BY0764)

TITLE: SEGMENT I STUDY OF FERTILITY IN RATS

ITEM	JUSTIFICATION
1	Identification of additional test article shipment.

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	Additional shipment of the test article, G0539.03 ~~(Lot No. BY0764)~~, is identified as IRDC No. 9542C.

Approved By:

Dr. Greg Allgood 5/1/88
Dr. Greg Allgood Date

Study Director Dr. Kit A. Keller

Signature 7/18/88
Signature Date

IR90-49-5

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSI# 00539.03)

Protocol Sheet No. 7 Study No. 191-1395 (IRDC# 8X0764)

TITLE: SEGMENT I STUDY OF FERTILITY IN RATS

ITEM	JUSTIFICATION
1	Change in IRDC Personnel Responsibilities due to resignation of Dr. Kit A. Keller.

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	Effective January 16, the Study Director of the study is James L. Schardein, M.S., D.A.T.S.

Study Director James L. Schardein, M.S.

Signature 1/16/89 Date

IR90-49-S

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

(TSIR# G0539.03)

Protocol Sheet No. 8 Study No. 191-1395 (DRD# BY0764)

TITLE: SEGMENT I STUDY OF FERTILITY IN RATS

ITEM	JUSTIFICATION
1	Correction of body weight range for animals used in study.

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	Actual body weight range for animals used in study:
	Males: 189-240 g
	Females: 226-293 g

Approved By:

3/21/89

Sponsor's Representative Date

Study Director James L. Schardein, M.S.

3/09/89

Signature Date

IR90-49-5

I N T E R D E P A R T M E N T A L C O R R E S P O N D E N C E

FROM: R. M. Siconolfi **DATE:** June 3, 1988

TO: Study File **R/L:** NON-DISCRETIONARY

SUBJECT: MONITORING VISIT

The following study was reviewed for data quality:

LABORATORY: IRDC

STUDY NO.: 191-1395

TSIN: G0539.03 (Seg 1 Fert Rats)

DATE OF REVIEW: May 18, 1988

REVIEWED BY: R. M. Siconolfi

st

cc: Divisional Toxicologist - G. S. Allgood
F. E. [illegible]berg

I N T E R D E P A R T M E N T A L C O R R E S P O N D E N C E

FROM: G. A. Nixon DATE: July 27, 1988

TO: Study File R/L: NON-DISCRETIONARY

SUBJECT: MONITORING VISIT

The following study was reviewed for data quality:

LABORATORY: IRDC

STUDY NO.: 191-1395

TSIN: G0539.03 (Seg. I. Pert. Rats)

DATE OF REVIEW: 7/21/88

REVIEWED BY: G. A. Ni..n
G.A. Nixon 7/27/88

st

cc: Divisional Toxicologist - G. S. Allgood
F. E. [illegible]berg

INTERDEPARTMENTAL CORRESPONDENCE

FROM: G. A. Nixon DATE: October 26, 1988

TO: Study File R/L: NON-DISCRETIONARY

SUBJECT: MONITORING VISIT

The following study was reviewed for data quality:

LABORATORY: IRDC

STUDY NO.: 191-1395

TSIN: G0539.03 (Seg. I Fertility in Rats)

DATE OF REVIEW: 10/5/88

REVIEWED BY: G. A. Nixon

G. A. Nixon

st

cc: Divisional Toxicologist - G. S. Allgood
F. E. Freeberg

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

DRD: BYO 764

TSIN: 60539.02

Test Article
(Product/Ingredient): Octopirox®
Physical Form: Solid
Color: Off-White
Storage Conditions:

Brand Notebook Ref: MC-0479-15
pH: 8-10 (1% solution in water)
Expiration Date: ~~5/8/88~~ 2/89
Hazards: corrosive

X Room Temperature _____

___ Other _____

Stability Testing is:

X Completed

___ In Progress

D.O.T. Hazard Classification: Non-hazardous/Not combustible

These chemicals/test articles have been checked against the TSCA inventory list. X Yes ___ No

Formulated Composition

Component[a]	(C.A.S.)[b] (Number)	Target Nominal Component Level	Target Actual Component Level	Measured Component Level	Stock Code No.	Lot Number
Octopirox®	-	100	100	99.5	BX288	2

(a) Use chemical or CTFA names which uniquely identifies each component. Common, non-chemical names such as (Tergitol 15-S-9 or yellow dye #10) should be indicated in parentheses following the chemical name. Non-definitive identification (e.g., BC-Base, Arquad) is not acceptable.

(b) CAS numbers should be included whenever available. They are especially important for main components of the formulation.

DRD: BTD 784

TSIN: G0509.03

ANALYSIS

Date Submitted	Submitter Code Or Lab Notebook	Analysis	(X) *	Measured Value	Limits	Laboratory Testing Or Data Source
5/8/87	85149005	MCT	X	Pass	Pass	SWTC Micr
	88025018	MCT		Pass	Pass	" "
	85149005	Active Assay		99.5%	98.0-101.5%	SWTC Analy
	88025017	Active Assay		99.5%	" "	" "
	85149005 and 88025017	IR		Pass	Must match standard spectrum	SWTC Analy
	85149005 and 87127003	Appearance		Pass	Pass	GMP

* Analysis required by toxicologist or microbiologist

Approvals: The test substance as made and characterized is a representative example of the intended formulation. Making records for plant-made product are being/have been obtained and evaluated by Products Research.

Process Development: Caroline Cardin (Signature) 1/15/88 (Date)

Products Research: N/A (Signature) (Date)

Acceptable Microbial Susceptibility: Jerry J. Kirkland (Signature) 1/26/88 (Date)

GMP Quality Assurance: Thomas R. Johnston (Signature) 2/2/88 (Date)

(Attach batch records for GMP review)

_____ finished products will be retained by Quality Assurance
(# samples)

This substance has been appropriately characterized and is acceptable for testing outlined in the attached DRD.

Division Toxicologist: [signature] (Signature) 2/2/88 (Date)

VA1/005
8/87

INTERDEPARTMENTAL CORRESPONDENCE

FROM: DATE:

TO: Operations Section R/L:

SUBJECT: NONCLINICAL STUDY REGULATORY STATUS

Notification pertaining to: DRD # 8Y0764

TSIN 60539.03

1. The study described in the above protocol is to be considered:

   [X] Safety
   [ ] Regulated Safety
   [ ] Investigational

2. [X] The data is expected to be submitted to the following regulatory agency:

   [X] FDA
   [ ] EPA
   [ ] other _____

   [ ] The data is not expected to be submitted to a regulatory agency. (Boxes #4 and #5 below need not be checked)

3. [X] The test substance has been characterized and results are shown on the test substance characterization report (TSCR).

4. [X] The method of synthesis, fabrication, or derivation of the test related substances has been documented (required for regulated studies).

5. [X] Stability testing has been done or will be done on the test substance (required for regulated studies).

Sponsor's Divisional Toxicologist _____

Date: 2/22/88

bg: REGSTAT
10/23/86