Date Study Started: May, 1982

Date Study Completed: Not provided.

Animals: Female (23-33 g; ages were not provided) Chinese hamsters.

Methods: Four groups of 6 hamsters each were orally administered 0, 200, 400 and 800 mg/kg ursodeoxycholic acid (UDCA) twice within 24 h. (A dose-ranging study was mentioned, but details of the study were not provided). Vehicle was 0.8% aqueous hydroxypropylmethylcellulose; dosing volume was 10 mg/kg. A positive control group was orally administered cyclophosphamide (10 ml/kg; 64 mg/kg). At 6 h after the last treatment, animals were sacrificed and slides of bone marrow smears were prepared.

Cells in metaphases were examined under low magnification and classified as either having a normal metaphase, having chromosomes with 1-2 breaks and/or inter- or intrachanges, having metaphases with multiple aberrations, or having pulverized metaphases. Data were statistically analyzed with analyses of variance and student's t-tests.

Results: UDCA did not produce treatment-related chromosomal aberrations. The positive control did produce treatment-related chromosomal aberrations. Thus, UDCA was not mutagenic in Chinese hamster bone marrow cells.

REPRODUCTIVE TOXICOLOGY:

1. Segment I Oral Fertility and Reproductive Performance Study of Ursodeoxycholic Acid (UDCA) in Rats (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Sponsor provided a letter from [redacted] dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to regulations of the FDA at that time.

Study Started: January 1978

Study Completed: August 5, 1981

Animals: Male (241-278 g; mean age of 66 days) and female (165-182 g; mean age of 66 days) Sprague-Dawley rats.

Methods: Rationale for selection of doses was not provided. Four groups of 24 males each were orally administered UDCA (0, 300, 900 and 2700 mg/kg/day) by stomach tube for 10 weeks prior to mating and four groups of 24 females each were orally administered UDCA (0, 300, 900 and 2700 mg/kg/day) for 2 weeks prior to mating. Mating was monogamous. Treatment in males was discontinued after the mating phase (additional 2 weeks). Females were treated until either weaning their offspring (3 weeks postpartum) or laparotomy (Day 20 of gestation). Vehicle was 0.8% aqueous hydroxypropylmethylcellulose solution; dosing volume was 10 ml/kg.

Animals were observed daily for clinical signs of toxicity. Body weights were recorded weekly, except during pregnancy in females, where body weights were recorded daily. Food consumption was recorded daily. Males were sacrificed after 12 weeks of treatment with UDCA and subjected to gross pathological examinations.

One subgroup of dams (12 per treatment group) were allowed to spontaneously deliver pups. The dams were evaluated for clinical signs of toxicity, number of pregnant animals, number of copulation attempts, and mean duration of pregnancy. Pups were examined for number alive and dead, number of stillbirths, number of runts, number with malformations, body weight at birth and at 7, 14 and 21 days after birth, physiological maturation, and behavioral and sensory functioning. After 3 weeks of lactation, dams were sacrificed and number of implantations were determined; animals were subjected to gross pathological examinations; pups were sacrificed and subjected to gross pathological examinations.

A second subgroup of dams (12 per treatment group) were sacrificed on day 20 of gestation. Dams were subjected to gross pathological examinations. Ovaries and uteri were removed and examined for number of corpora lutea, number of implantations, number of fetuses, number of fetuses with malformations, number and weight of placentae, and number of resorptions. Fetuses were evaluated for number dead, number of runts, number of malformations, body weight, pre-implantation loss, and post-implantation loss. Fetuses were sacrificed and examined for visceral variations and skeletal variations.

Data were evaluated with analyses of variance and student's t-tests.

Results:

I. Groups in Which Dams Were Allowed to Spontaneously Deliver Pups

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity in either males or females.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weights of control males were 253.0 and 374.0 g during Weeks 1 and 12 of UDCA treatment, respectively. Mean body weights of control females were 174.8 and 184.8 g during Weeks 1 and 12 of UDCA treatment, respectively. There were no treatment-related effects on body weight.

Mean body weights of control lactating females were 238.1 and 255.5 g during Weeks 1 and 3 of lactation, respectively. There were no treatment-related effects on body weight.

4. Food Consumption: Mean food consumption data were presented in figures; quantitative data were not provided. There were no treatment-related effects on food consumption.

5. Gross Pathology: There were no gross pathological lesions in either males or females.

6. Fertility and Mating Performance: As shown in the following table, there were no treatment-related effects on fertility and general reproductive performance in dams that were allowed to spontaneously deliver pups.

Fertility and general reproductive performance of dams that were allowed to spontaneously deliver pups

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 900	UDCA 2700
Females				
# Paired	12	12	12	12
# Died during pregnancy	0	0	0	0
# Pregnant	10	11	11	11
Mean Duration of Pregnancy (Days)	21.8	22.0	21.8	21.9

F_1 Generation

1. Pup data: As shown in the following table, there were no treatment-related effects on dams and pups.

Summary of dam and pup data in a Segment I reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 900	UDCA 2700
# Dams examined	10	11	11	11
# Pups/dam at birth	10.6	10.2	11.7	11.6
# Pups/dam after 3 weeks	9.4	8.8	10.5	10.7
# Stillbirths	0	0	0	0
# Runts/litter	0	0	0	0.1
# Implantations/dam	11.4	11.5	12.1	12.3
# Body Weight of pups at birth and at Days 7, 14, and 21 after birth	5.61 12.67 20.72 35.50	5.59 12.63 20.95 35.64	5.50 12.30 20.50 34.87	5.45 12.19 20.30 35.22

2. Physiological development of pups: There were no treatment-related effects on development of ears, beginning of hair growth, cutting of teeth and opening of eyes.

3. Behavioral and sensory functioning of pups: There were no treatment-related effects on consciousness, emotional behavior, activity and reactivity, central excitation, motor coordination, muscle tone and reflexes.

4. Gross Pathology: There were no gross pathological lesions in any pup in any dose group.

II. Group in Which Dams Were Sacrificed on Day 20 of Gestation

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity in either males or females.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weights of control pregnant rats were 194.5 and 283.5 g on Days 0 and 20 of pregnancy, respectively. There were no treatment-related effects on body weight.

4. Food Consumption: Mean food consumption data were presented in figures; quantitative data were not provided. There were no treatment-related effects on food consumption.

5. Gross Pathology: There were no gross pathological lesions.

6. Fertility and Mating Performance: As shown in the following table, there were no treatment-related effects on fertility and general reproductive performance in dams that were sacrificed on Day 20 of gestation.

Fertility and general reproductive performance of dams that were sacrificed on Day 20 of gestation

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 900	UDCA 2700
Females				
# Paired	12	12	12	12
# Died during pregnancy	0	0	0	0
# Pregnant	11	10	12	11

F_1 Generation

1. Dam and fetus data: As shown in the following table, there were no treatment-related effects on dams and fetuses.

Summary of maternal and fetal data in a Segment I reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 900	UDCA 2700
# Dams examined	11	10	12	11
# Corpora lutea/dam	12.9	13.1	13.5	13.2
# Implantations/dam	12.3	12.5	12.8	12.6
# Resorptions/dam	0.6	0.6	0.8	0.7
# Fetuses/litter	11.6	11.9	12.1	11.9
Mean placenta weight (g)	0.55	0.55	0.54	0.55
# Runts/litter	0	0	0.1	0
Mean fetal weight (g)	3.51	3.53	3.50	3.47

2. Fetal anomalies and variations: Data were not specifically described. Data were not provided for individual litters. As shown in the following table, there were no treatment-related effects on external, visceral and skeletal variations.

Summary of external, visceral, and skeletal variations in a Segment I reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 900	UDCA 2700
# Litters examined	11	10	12	11
# Fetuses examined	128	119	145	131
External variations (# of fetuses)				
Unspecified	2	1	4	2
# Litters examined	11	10	12	11
# Fetuses examined	121	119	145	131
Visceral variations (# of fetuses)				
Dislocation of testis	1	0	0	0
# Litters examined	11	10	12	11
# Fetuses examined	163	179	171	171
Skeletal variations (# of fetuses)				
Skull (unspecified)	4	1	3	4
Sternebrae	26	30	31	36
Ribs and vertebral body	8	12	6	13

In summary, UDCA did not affect fertility and general reproductive performance of the F_0 generation.

2. Segment I Oral Fertility and Reproductive Performance Study of Ursodeoxycholic Acid (UDCA) in Rats (Report No. was not provided.

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Male (approximately 110 g; 6 weeks of age) and female (135-145 g; 8 weeks of age) Wistar rats.

Methods: In a dose-ranging study of UDCA, 3 groups of 5 non-pregnant females each were orally administered 1, 2, and 4 g/kg/day of UDCA for 11 days. There were no treatment-related effects on mortality and body weight gain, and there were no treatment-related anomalies. Thus, in the present study, 4 groups of 10 males each were orally administered UDCA (0, 250, 1000 and 2000 mg/kg/day, respectively) for 63 days prior to mating and 4 groups of 30 females each were orally administered UDCA (0, 250, 1000 and 2000 mg/kg/day, respectively) for 14 days prior to mating. Vehicle was 0.5% gum arabic solution; dosing volume was 2 ml/kg. When mating was initiated, UDCA treatment in females was continued for up to 7 more days. However, if sperm were found in vaginal smears, UDCA treatment was stopped in the female and the male was caged with another female rat. Each male was mated with 3 different females. If a female mated but did not become pregnant, the female was subsequently mated with a male with proven conception capabilities.

Pregnant dams were observed daily for clinical signs of toxicity. Body weights were measured weekly prior to mating; body weights of pregnant dams were measured daily. Water intake was measured weekly before mating; water intake of pregnant dams was measured on Days 3, 8, 12, 18 and 20 of gestation. Food consumption was not measured.

Pregnant dams were subjected to Caesarian section on Day 20 of gestation. Dams were sacrificed and examined for number of corpus lutea, implantations, live and dead fetuses, and resorbed embryos. Mean fetal weights were measured, and fetuses were examined for external anomalies and variations. Fetuses were sacrificed and two-thirds were examined for skeletal anomalies and variations. The remaining one-third of the fetuses were examined for visceral anomalies and variations. Dams were also subjected to gross pathological examination and absolute weights of liver, kidneys, spleen, heart, ovaries and uteri were determined.

Results:

F_0 Generation:

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weight data were presented in figures; quantitative data were not provided. Prior to mating, there were no treatment-related effects on body weight in males. However, body weights of females during UDCA treatment prior to mating were decreased by approximately 17% (% of difference from

control) in the 250 and 1000 mg/kg/day groups, and by approximately 40% in the 2000 mg/kg/day group. Body weights continued to be suppressed by approximately 20% in the 2000 mg/kg/day group.

4. Water Intake: Data for water intake were not provided. It was reported that there were not treatment-related effects on water intake.

5. Organ Weights: There were on treatment-related effects.

6. Gross Pathology: There were no treatment-related gross pathological lesions.

7. Fertility and Mating Performance: As shown in the following table, there was a reduction in the number of pregnant dams at the 2000 mg/kg/day dose.

Fertility and general reproductive performance of dams

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 250	UDCA 1000	UDCA 2000
Females				
# Paired	30	30	30	30
# Died during pregnancy	0	0	0	0
# Mated	30	27	26	25
# Pregnant	29	27	24	18*

* Significantly different from controls.

F_1 Generation

1. Dam and fetus data: As shown in the following table, there was a significant decrease in the number of live fetuses/litter at the 2000 mg/kg/day dose

Summary of maternal and fetal data in a Segment I reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 250	UDCA 1000	UDCA 2000
# Dams examined	30	30	30	30
# Pregnant animals	29	27	24	18
# Implantations/dam	10.86	10.04	8.46	9.36
# Early resorptions	1	2	0	2
# Late resorptions	0	1	0	1
# Live fetuses/ litter	10.45	9.63	8.61	6.61*
Mean fetal weight (g)	4.31	4.36	4.21	4.02

*Significantly different from controls

2. Fetal anomalies and variations: Data were only provided for external anomalies and skeletal variations. Sponsor reported that there were no visceral and skeletal anomalies. Data were not provided for individual litters. As shown in the following table, there were no treatment-related effects on external anomalies. However, skeletal ossification was generally retarded at the 1000 and 2000 mg/kg/day doses

Summary of external anomalies and skeletal variations in a Segment I reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 250	UDCA 1000	UDCA 2000
# Litters examined	29	27	24	18
# Fetuses examined	201	173	132	80
External anomalies (# of fetuses)				
Complex malformation of exencephaly, open eyelids, cleft vertebrae	0	1	0	0
# Litters examined	29	27	24	18
# Fetuses examined	201	173	132	80
Skeletal variations (# of fetuses)				
No. ossified:				
Palatine	149 (74%)	107 (62%)*	74 (56%)*	45 (56%)*
Centra of 3rd cervical vertebra	73 (36%)	74 (43%)*	17 (13%)*	18 (23%)*
Centra of 4th cervical vertebra	124 (62%)	121 (33%)	43 (33%)*	33 (41%)*
Centra of 5th cervical vertebra	152 (76%)	134 (78%)	61 (46%)*	42 (53%)*
Centra of 6th cervical vertebra	172 (86%)	145 (84%)	83 (63%)*	49 (61%)*
Centra of 7th cervical vertebra	191 (95%)	162 (94%)	108 (82%)*	61 (76%)*
Centra of 5th caudal vertebra	146 (73%)	115 (67%)	59 (45%)*	41 (51%)*
Centra of 6th caudal vertebra	60 (30%)	33 (19%)*	16 (12%)*	13 (16%)*
Arch of 3rd caudal vertebra	145 (72%)	106 (61%)	82 (62%)	44 (45%)*
1st Distal metacarpal	42 (21%)	13 (8%)*	1 (1%)*	2 (3%)*
2nd Distal metacarpal	46 (23%)	16 (9%)*	7 (5%)*	9 (11%)*
3rd Distal metacarpal	175 (87%)	129 (75%)*	90 (68%)*	46 (58%)*
4th Distal metacarpal	166 (83%)	119 (69%)*	77 (58%)*	33 (41%)*
5th Distal metacarpal	43 (21%)	15 (9%)*	4 (3%)*	2 (3%)*

In summary, UDCA significantly reduced the number of pregnant females in the 2000 mg/kg/day dosage group. Moreover, UDCA produced a decrease in the number of live fetuses/litter at the 2000 mg/kg/day dose and a dose-related retardation of fetal skeletal ossification. Since UDCA produced dose-related decreases in body weights of dams, the effects of UDCA on number of pregnant females in the 2000 mg/kg/day dosage group and the toxic effects of UDCA on fetuses are probably related to general UDCA-induced toxicity.

3. Segment II I.P. Teratology Study of Ursodeoxycholic Acid (UDCA) in Mice (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Female (body weights were not provided; approximately 8 weeks of age) pregnant dd mice.

Methods: In a preliminary study, an i.p. UDCA dose of 150 mg/kg/day for 1 week produced minimal depression of weight gain, while 300 mg/kg/day for 1 week produced moderate loss of body weight. Thus, in the present Segment II study, 3 groups of 20-21 female pregnant mice each were intraperitoneally administered 0, 30 and 200 mg/kg/day of UDCA, respectively, from Day 7 through Day 12 of gestation. Organogenesis in mice occurs from approximately Day 6 through Day 15 of pregnancy; thus, dosing in the present study did not completely cover the period of organogenesis. Vehicle was 1% carboxymethyl cellulose solution; dosing volume was 0.2 ml/20 g body weight.

Dams were observed daily for clinical signs of toxicity. Body weights were measured every other day during pregnancy, beginning on Day 0 of pregnancy. Approximately three-fourths of the dams were sacrificed by ether overdosage on Day 18 of gestation and number of implantations, live fetuses, and resorptions were counted. Live fetuses were removed from the uterus, weighed, and examined for external anomalies. Fetuses were sacrificed and examined for visceral anomalies. Fetuses were then prepared for and subjected to skeletal examination.

Five pregnant dams in each dose group were allowed to deliver their litters spontaneously. At birth, live and dead pups were counted, and examined for external anomalies. Pups were weighed weekly. Pups were assessed for eye opening, visual supporting reflex, pinna reflex, vertical screen test, and righting reflex. Pups were sacrificed at 3 weeks following birth and examined for external, visceral and skeletal anomalies.

Results:

I. Groups in Which Dams Were Sacrificed on Day 18 of Gestation

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weight of control dams on Day 0 and Day 18 of gestation were 24.2 and 44.3 g, respectively. There were no treatment-related effects on body weight.

F_1 Generation

1. Dam and fetus data: As shown in the following table, there were no treatment-related effects on fetuses.

Summary of maternal and fetal data in a Segment II reproductive toxicity study in mice

Treatment Dose (mg/kg, i.p.)	Vehicle 0	UDCA 30	UDCA 200
# Dams examined	16	16	15
# Implantations/dam	9.8	9.9	9.7
Resorptions (%)	5.1	6.3	8.9
# Live fetuses/ litter	9.3	9.3	8.9
Mean fetal weight (g)	1.19	1.19	1.12

2. Fetal anomalies and variations: Detailed data were not provided. Sponsor reported that there were no external and visceral anomalies in any fetus. Undifferentiated 13th ribs were observed in 4, 2 and 4 fetuses in the 0, 30 and 200 mg/kg/day groups, respectively. Thus, there were no treatment-related external and visceral anomalies, and skeletal variations in fetuses.

II. Groups in Which Dams Were Allowed to Spontaneously Deliver Pups

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

F_1 Generation

1. Dam and pup data: As shown in the following table, there were no treatment-related effects on pups. Furthermore, there were no treatment-related effects on physiological responses in the various tests.

Summary of maternal and fetal data in a Segment II reproductive toxicity study in mice

Treatment Dose (mg/kg, i.p.)	Vehicle 0	UDCA 30	UDCA 200
# Dams examined	5	5	5
# Implantations/dam	9.6	9.6	9.6
# Live pups/litter	8.0 (Week 1)	8.6	8.4
during Weeks 1, 2	7.4 (2)	8.2	7.8
and 3	7.2 (3)	8.0	7.8
Mean fetal weight	3.55 (Week 1)	3.55	3.55
(g) during Weeks 1,	6.00 (2)	5.95	5.80
2 and 3	9.40 (3)	9.35	9.40

In summary, intraperitoneally administered UDCA was not teratogenic in mice.

4. Segment II Oral Teratology Study of Ursodeoxycholic (UDCA) in Mice (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Female (body weights were not provided; approximately 8 weeks of age) pregnant dd mice.

Methods: In a preliminary study in mice, an oral UDCA dose of 1,250 mg/kg/day for 1 week produced no treatment-related effects, while 2,500 mg/kg/day for 1 week decreased body weight and produced deaths. Thus, in the present study, 3 groups of 21-22 female pregnant mice each were orally administered 0, 300 and 1,500 mg/kg/day of UDCA, respectively, from Day 7 through Day 12 of gestation. Vehicle was 1% carboxymethylcellulose solution; dosing volume was 0.2 ml/20 g body weight.

Dams were observed daily for clinical signs of toxicity. Body weights were measured every other day during pregnancy, beginning on Day 0 of pregnancy. Approximately three-fourths of the dams were sacrificed by ether overdosage on Day 18 of gestation and number of implantations, live fetuses, and resorptions were counted. Live fetuses were removed from the uterus, weighed, and examined for external anomalies. Fetuses were sacrificed and examined for visceral anomalies. Fetuses were then prepared for and subjected to skeletal examination.

Five pregnant dams in each dose group were allowed to deliver their litters spontaneously. At birth, live and dead pups were counted, and examined for external anomalies. Pups were weighed weekly. Pups were assessed for eye opening, visual supporting reflex, pinna reflex, vertical screen test and righting reflex. Pups were sacrificed at 3 weeks following birth and examined for external, visceral and skeletal anomalies.

Results:

I. Groups in Which Dams Were Sacrificed on Day 18 of Gestation

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weight of control dams on Day 0 and Day 18 of gestation were 23.2 and 42.0 g, respectively. There were no treatment-related effects on body weight.

F_1 Generation

1. Dam and fetus data: As shown in the following table, there were no treatment-related effects on fetuses.

Summary of maternal and fetal data in a Segment II reproductive toxicity study in mice

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 1,500
# Dams examined	16	16	17
# Implantations/dam	10.0	10.2	9.8
Resorptions (%)	5.0	4.3	7.2
# Live fetuses/ litter	9.5	9.8	9.1
Mean fetal weight (g)	1.20	1.18	1.06

2. Fetal anomalies and variations: Detailed data were not provided. Sponsor reported that there were no external and visceral anomalies in any fetus. Undifferentiated 13th ribs were observed in 3, 4 and 4 fetuses in the 0, 30 and 200 mg/kg/day groups, respectively. Thus, there were no treatment-related external and visceral anomalies, and skeletal variations in fetuses.

II. Groups in Which Dams Were Allowed to Spontaneously Deliver Pups

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

F_1 Generation

1. Dam and pup data: As shown in the following table, there were no treatment-related effects on pups. Furthermore, there were no treatment-related effects on physiological responses in the various tests

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Summary of maternal and fetal data in a Segment II reproductive toxicity study in mice

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 1,500
# Dams examined	5	5	5
# Implantations/dam	9.4	9.6	9.6
# Live pups/litter during Weeks 1, 2 and 3	7.8 (Week 1) 7.2 (2) 7.2 (3)	8.2 7.0 7.0	7.6 7.4 7.0
Mean fetal weight (g) during Weeks 1, 2 and 3	3.75 (Week 1) 6.05 (2) 9.00 (3)	3.75 6.00 9.10	3.70 6.25 9.60

In summary, orally administered UDCA was not teratogenic in mice.

5. Segment II I.P. Teratology Study of Ursodeoxycholic Acid (UDCA) in Rats (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Female (body weights were not provided; approximately 12 weeks of age) pregnant Wistar rats.

Methods: In a preliminary study, an i.p. UDCA dose of 125 mg/kg/day for 1 week produced no treatment-related effects, while 250 mg/kg/day for 1 week decreased body weight and produced deaths. Thus, in the present study, 3 groups of 20-21 female pregnant rats each were intraperitoneally administered 0, 30 and 200 mg/kg/day of UDCA, respectively, from Day 9 through Day 14 of gestation. Since organogenesis in rats occurs from approximately Day 6 through Day 17 of pregnancy, dosing in this study did not completely cover the period of organogenesis. Vehicle was 1% carboxymethylcellulose solution; dosing volume was 0.5 to 4 ml/200 g body weight.

Dams were observed daily for clinical signs of toxicity. Body weights were measured every other day during pregnancy, beginning on Day 0 of pregnancy. Approximately three-fourths of the dams were sacrificed by ether overdosage on Day 19 of gestation and number of implantations, live fetuses, and resorptions were

counted. Live fetuses were removed from the uterus, weighed, and examined for external anomalies. Fetuses were sacrificed and examined for visceral anomalies. Fetuses were then prepared for and subjected to skeletal examination.

Five pregnant dams in each dose group were allowed to deliver their litters spontaneously. At birth, live and dead pups were counted, and examined for external anomalies. Pups were weighed weekly. Pups were assessed for eye opening, visual supporting reflex, pinna reflex, vertical screen test and righting reflex. Pups were sacrificed at 3 weeks following birth and examined for external, visceral and skeletal anomalies.

Results:

I. Groups in Which Dams Were Sacrificed on Day 19 of Gestation

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weight of control dams on Day 0 and Day 19 of gestation were 204.9 and 310.4 g, respectively. Mean body of the 200 mg/kg/day group on Day 19 of gestation was decreased by 9.6% (% of difference from control).

F_1 Generation

1. Dam and fetus data: As shown in the following table, mean fetal weight in the 200 mg/kg/day group was decreased by 9.5% (% of difference from control). Since the mean body weight of the dams in the 200 mg/kg/day group was also decreased, the decreased mean body weight of the pups is most likely related to maternal toxicity.

Summary of maternal and fetal data in a Segment II reproductive toxicity study in rats

Treatment Dose (mg/kg, i.p.)	Vehicle 0	UDCA 30	UDCA 200
# Dams examined	16	15	15
# Implantations/dam	11.6	11.6	11.3
Resorptions (%)	9.2	10.3	16.0
# Live fetuses/ litter	10.5	10.5	9.5
Mean fetal weight (g)	2.87	2.73	2.60*

*Significantly different from control.

2. Fetal anomalies and variations: Detailed data were not provided. Sponsor reported that there were no external and visceral anomalies in any fetus. Undifferentiated 13th ribs were observed in 6, 5 and 5 fetuses in the 0, 30 and 200 mg/kg/day groups, respectively. Thus, there were no treatment-related external and visceral anomalies, and skeletal variations in fetuses.

II. Groups in Which Dams Were Allowed to Spontaneously Deliver Pups

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

1. Dam and pup data: As shown in the following table, there were no treatment-related effects on pups. Furthermore, there were no treatment-related effects on physiological responses in the various tests.

Summary of maternal and fetal data in a Segment II reproductive toxicity study in rats

Treatment Dose (mg/kg, i.p.)	Vehicle 0	UDCA 30	UDCA 200
# Dams examined	5	5	5
# Implantations/dam	11.4	11.6	9.6
# Live pups/litter during Weeks 1, 2 and 3	10.2 (Week 1) 9.8 (2) 9.6 (3)	8.0 7.6 7.4	8.0 8.0 7.2
Mean fetal weight (g) during Weeks 1, 2 and 3	11.60 (Week 1) 22.20 (2) 30.60 (3)	12.45 21.30 29.55	11.55 21.85 31.15

In summary, intraperitoneally administered UDCA was not teratogenic in rats.

6. Segment II Oral Teratology Study of Ursodeoxycholic Acid (UDCA) in rats (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Female (body weights were not provided; approximately 12 weeks of age) pregnant Wistar rats.

Methods: In a preliminary study in rats, an oral UDCA dose of 4,000 mg/kg/day was determined to be the maximal practicable dose. Thus, in the present study, 3 groups of 20-22 female pregnant rats each were orally administered 0, 300 and 4,000 mg/kg/day of UDCA, respectively, from Day 9 through Day 14 of gestation. Vehicle was 1% carboxymethylcellulose solution; dosing volume was 0.5 to 4 ml/200 g body weight.

Dams were observed daily for clinical signs of toxicity. Body weights were measured every other day during pregnancy, beginning on Day 0 of pregnancy. Approximately three-fourths of the dams were sacrificed by ether overdosage on Day 19 of gestation and number of implantations, live fetuses, and resorptions were counted. Live fetuses were removed from the uterus, weighed, and examined for external anomalies. Fetuses were sacrificed and examined for visceral anomalies. Fetuses were then prepared for and subjected to skeletal examination.

Five pregnant dams in each dose group were allowed to deliver their litters spontaneously. At birth, live and dead pups were counted, and examined for external anomalies. Pups were weighed weekly. Pups were assessed for eye opening, visual supporting reflex, pinna reflex, vertical screen test and righting reflex. Pups were sacrificed at 3 weeks following birth and examined for external, visceral and skeletal anomalies.

I. Groups in Which Dams Were Sacrificed on Day 19 of Gestation

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weight of control dams on Day 0 and Day 19 of gestation were 211.4 and 315.2 g, respectively. There were no treatment-related effects on body weight.

F_1 Generation

1. Dam and fetus data: As shown in the following table, mean fetal weight in the 4,000 mg/kg/day groups was decreased by 9.5% (% of difference from control).

Summary of maternal and fetal data in a Segment II reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 4,000
# Dams examined	16	17	15
# Implantations/dam	12.7	12.6	13.7
Resorptions (%)	3.4	1.9	5.3
# Live fetuses/ litter	12.0	12.4	13.7
Mean fetal weight (g)	2.90	2.80	2.52*

*Significantly different from control group.

2. Fetal anomalies and variations: Detailed data were not provided. Sponsor reported that there were no external and visceral anomalies in any fetus. Undifferentiated 13th ribs were observed in 6, 6, and 8 fetuses in the 0, 300 and 4,000 mg/kg/day groups, respectively. Thus, there were no treatment-related external and visceral anomalies, and skeletal variations in fetuses.

II. Groups in Which Dams Were Allowed to Spontaneously Deliver Pups

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

F_1 Generation

1. Dam and pup data: As shown in the following table, there were no treatment-related effects on pups. Furthermore, there were no treatment-related effects on physiological responses in the various tests

Summary of maternal and fetal data in a Segment II reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 300	UDCA 4,000
# Dams examined	5	5	5
# Implantations/dam	13.6	13.0	13.2
# Live pups/litter during Weeks 1, 2 and 3	9.8 (Week 1) 9.2 (2) 9.2 (3)	9.8 9.4 9.4	11.4 10.0 10.0
Mean fetal weight (g) during Weeks 1, 2 and 3	12.05 (Week 1) 22.55 (2) 30.40 (3)	12.60 23.25 24.70	11.50 21.50 31.25

In summary, orally administered UDCA was not teratogenic in rats.

7. Segment II Oral Teratology Study of Ursodeoxycholic Acid (UDCA) in Rats (Study No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Female (225-230 g; 11-12 weeks of age) pregnant Wistar rats.

Methods: Dosage selection was based upon the results of the Segment I study that was previously reviewed (See Study 2 in this REPRODUCTIVE TOXICOLOGY section). Thus, 4 groups of rats were orally administered 0, 250, 1000 and 2000 mg/kg/day of UDCA on Days 7 through 17 of gestation. Vehicle was 0.5% gum arabic solution; dosing volume was 2 ml/kg. In laparotomized groups, dams were sacrificed on Day 20 of gestation; there were 20 rats each in the 0, 250 and 1000 mg/kg/day dosage groups and 30 rats in the 2000 mg/kg/day dosage group. In lacteal groups, dams were sacrificed on Day 21 of lactation; there were 20 rats each in the 0, 250, 1000 and 2000 mg/kg/day dosage groups.

In the dams of the laparotomized groups, body weights, clinical signs of toxicity, food consumption, and water consumption were measured daily. On Day 20 of gestation, blood samples were obtained from dams for assessments of hematology and blood chemistry. After sacrifice of dams On Day 20, total number of

implantations, number of live and dead fetuses, body weight of live fetuses, and external anomalies of fetuses were determined. One-third of the fetuses were sacrificed and fixed in Bouin solution and anomalies of brain, thoracic organs and other organs were assessed. In the remaining two-thirds of the sacrificed fetuses, skeletal anomalies and variations were assessed using the Dawson method. Furthermore, organ weights were determined in the sacrificed dams.

In the dams of the lacteal groups, body weights, clinical signs of toxicity, food consumption, and water consumption were measured daily. On the 21st day after delivery, dams were sacrificed, subjected to a gross pathological examination including measurements of organ weights, and examined for implantation sites.

Number of live pups was determined at birth. Pup body weights were determined at birth and during Weeks 1, 2, 3 and 4 after birth. Postnatal development was assessed daily. Organ weights were determined in 5-7 sacrificed pups at 4 weeks after birth and in 3-16 sacrificed pups at 11 weeks after birth.

Results:

I. Groups in Which Dams Were Sacrificed on Day 20 of Gestation

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weights of control dams on Days 1 and 17 of gestation were 230.4 and 272.7 g, respectively. Mean body weight of the 2000 mg/kg/day dosage group on Day 17 of gestation was reduced by 14% (% of difference from control).

4. Food Consumption: Data were presented in a figure; quantitative data were not provided. There were no treatment-related effects on food consumption.

5. Hematology: There were no treatment-related effects.

6. Blood Chemistry: There were no treatment-related effects.

7. Organ Weights: There were no treatment-related effects.

F_1 Generation

1. Dam and fetus data: As shown in the following table, there were no treatment-related effects on fetuses.

Summary of maternal and fetal data in Segment II reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 250	UDCA 1000	UDCA 2000
# Dams examined	23	19	21	30
# Implantations/dam	13.6	12.5	13.1	13.2
# Resorptions/dam	1.4	1.9	1.0	2.8
# Live fetuses/ litter	12.09	10.42	11.76	10.27
Mean fetal weight (g)	4.82	4.48	4.66	4.52

2. Fetal anomalies and variations: Data were not provided for individual litters. External and visceral variations were not specifically identified. As shown in the following table, there were no treatment-related effects on external, visceral and skeletal variations

Summary of external, visceral, and skeletal variations in a Segment II reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 250	UDCA 1000	UDCA 2000
# Litters examined	23	19	21	30
# Fetuses examined	278	198	247	308
External variations (# of fetuses)				
Unspecified	0	0	0	5
# Litters examined	23	19	21	30
# Fetuses examined	278	198	247	308
Visceral variations (# of fetuses)				
Unspecified	0	0	0	0
# Litters examined	23	19	21	30
# Fetuses examined	216	149	176	221
Skeletal variations (# of fetuses)				
Asymmetry of sternebrae	0	0	1	0
Lumbar rib variation	0	0	0	3
Others (unspecified)	4	1	3	1
Fused rib	0	0	0	1
Fused sternebrae	0	0	1	0
Fused vertebrae	0	0	0	3
Others (unspecified)	0	0	0	1

II. Groups in Which Dams Were Allowed to Spontaneously Deliver Pups

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Mean body weights of control dams on the day of delivery was 318.7 g. There were no treatment-related effects on mean body weight on the day of delivery.

4. Food Consumption: Data were presented in a figure; quantitative data were not provided. There were no treatment-related effects on food consumption.

F_1 Generation

1. Dam and pup data: As shown in the following table, there were no treatment-related effects on dams and pups.

Summary of maternal and pup data in a Segment II reproductive toxicity study in rats

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 250	UDCA 1000	UDCA 2000
# Dams examined	20	21	23	23
# Pups/dam at birth	11.2	11.0	10.4	10.5
# Implantations/dam	12.3	12.7	13.4	13.1
Mean Body Weight of pups at birth and at Weeks 1 and 4 after birth	4.95 (Birth) 11.1 (Week 1) 49.0 (Week 4)	5.25 10.7 46.7	4.85 10.0 49.8	5.00 9.7 45.8

2. Organ Weights: There were no treatment-related effects.

3. Postnatal Development: There were no treatment-related effects on time to separation of ear auricle, eruption of incisor, emergence of abdominal hair, separation of eyelid, descent of testis and opening of vagina.

In summary, orally administered UDCA was not teratogenic in rats.

8. Segment II Oral Teratology Study of Ursodeoxycholic Acid (UDCA) in Rabbits (Study No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practice and Quality Assurance Requirements: Sponsor provided a letter from [redacted] dated May 7, 1996, stating that studies performed at the above laboratory were conducted according to regulations of the FDA at that time.

Study Started: Not provided.

Study Completed: January 28, 1981

Animals: Female (2.1-2.5 kg; 8-9 months of age) pregnant White Russian rabbits.

Methods: During preliminary dose ranging experiments, toxic reactions were expected after oral administration of 300 mg/kg/day of UDCA in pregnant animals. Thus, 4 groups of 12 rabbits each were orally administered 0, 33, 100 and 300 mg/kg/day of UDCA, respectively, from Day 6 through Day 18 of gestation. Vehicle was 0.8% hydroxypropylmethylcellulose gel; dosing volume was 3 ml/kg.

Does were examined daily for clinical signs of toxicity. Body weights and food consumption were measured daily. On Day 29 of gestation, does were rendered unconscious by a blow on the nape. The uterus was removed and prepared for examination. Following exsanguination, does were subjected to gross pathological examination.

Number of implantations, corpora lutea and resorptions per dam were determined. Live fetuses/litter were counted, mean fetal weights were determined, and fetuses were examined for external anomalies. Approximately two-thirds of the fetuses were sacrificed and examined for visceral anomalies. The remaining one-third were sacrificed and examined for skeletal variations according to the method of Dawson.

Results:

F_0 Generation

1. Observed Effects: All animals in the 100 and 300 mg/kg/day dosage groups displayed constant mild inhibition of motor activity. There were no treatment-related effects in the 0 and 33 mg/kg/day dosage groups.

2. Mortality: Four animals in the 300 mg/kg/day dosage group died on Days 15, 15, 17, and 17 of gestation, respectively. In these animals, inhibition of motor activity progressed to coma and death.

3. Body Weight: Mean body weights of control does were 2.2, 2.3, 2.5 and 2.7 kg on Days 0, 6, 18 and 29 of gestation, respectively. Mean body weights of does in the 100 mg/kg/day dosage group were reduced by -20% (% of difference from control) and -22% on Days 18 and 29 of gestation. Mean body weights of does in the 300 mg/kg/day dosage group were reduced by -32% and -26% on Days 18 and 29 of gestation.

4. Food Consumption: Mean food consumption of control does was 46, 39, 44 and 43 g/kg on Days 0, 6, 18 and 29 of gestation, respectively. Mean food consumption of does in the 100 mg/kg/day dosage group was reduced by -10% (% of difference from control), -59% and -19% on Days 6, 18 and 29 of gestation, respectively. Mean food consumption of does in the 300 mg/kg/day dosage group was reduced by -38%, -68% and -19% on Days 6, 18 and 29 of gestation, respectively.

5. Gross Pathology: Detailed data were not provided by the sponsor. Sponsor reported that animals in the 33, 100 and 300 mg/kg/day dosage groups displayed a dose-related marked demarcation of liver lobules. In addition, animals in the 300 mg/kg/day dosage group had pale livers and kidneys.

F_1 Generation

1. Doe and fetal data: As shown in the following table, the 300 mg/kg/day dose of UDCA produced a reduction in live fetuses/dam and an increase in resorptions/dam. Since the 300 mg/kg/day dose produced maternal toxicity, these effects in fetuses were most likely mediated by the maternal toxicity.

Summary of maternal and fetal data in Segment II reproductive toxicity study in rabbits

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 33	UDCA 100	UDCA 300
# Does examined	12	12	12	12
# Pregnant animals	12	12	12	12
# Implantations/dam	8.3	8.3	8.7	9.3
# Corpora lutea/dam	8.8	9.0	9.3	8.1
# Resorptions/dam	0.7	0.6	2.2	6.5*
# Live fetuses/ litter	7.6	7.8	6.3	1.6*
Mean fetal weight (g)	31.1	30.5	31.3	27.2

*Significantly difference from control group.

2. Fetal anomalies and variations: Sponsor did not provide detailed data for fetal anomalies and variations. Sponsor reported that there were no fetal malformations and macroscopic variations in any dosage group. There were 37, 36, 35 and 10 unspecified skeletal variations seen in the 0, 33, 100 and 300 mg/kg/day dosage groups, respectively.

In summary, orally administered UDCA was not teratogenic in rabbits. However, UDCA did produce maternal toxicity. The 100 and 300 mg/kg/day doses reduced maternal body weight and food consumption; the 300 mg/kg/day dose was lethal.

9. Segment II Oral Teratology Study of Ursodeoxycholic Acid (UDCA) in Rabbits (Study No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Female (3.0-3.5 kg; ages were not provided) pregnant New Zealand white rabbits.

Methods: Dosage selection was based upon the results of preliminary dose ranging studies. A UDCA dose of 80 mg/kg was lethal, while 40 mg/kg reduced body weight and caused 1 death; 20 mg/kg appeared to be a maximal tolerated dose. Thus, 4 groups of 11 pregnant rabbits each were orally administered 0, 5, 10 and 20 mg/kg/day of UDCA, respectively, from Day 6 through Day 18 of gestation. Vehicle was 0.5% gum arabic solution; dosing volume was 1 mg/kg.

Observations for clinical signs of toxicity were done during and following administration of UDCA; details were not provided. Body weights and food consumption of does were measured daily.

All rabbits were subjected to Caesarian section on Day 29 of gestation. Blood samples were obtained from an ear vein for hematological examination on the day that does were subjected to Caesarian section; organ weights were determined.

Number of live fetuses, number of resorptions, and fetal weights were determined. Fetuses were examined for external anomalies, visceral anomalies, and skeletal variations (detailed methodology was not provided).

Results:

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Data were provided in a figure; quantitative data were not provided. There were no treatment-related effects on body weight.

4. Food Consumption: No data were provided. Sponsor stated that there were no treatment-related effects on food consumption.

5. Hematology: There were no treatment-related effects.

6. Organ Weights: There were no treatment-related effects.

F_1 Generation

1. Maternal and fetal data: As shown in the following table, there were no treatment-related effects on does and fetuses.

Summary of maternal and fetal data in a Segment II reproductive toxicity study in rabbits

Treatment Dose (mg/kg, p.o.)	Vehicle 0	UDCA 5	UDCA 10	UDCA 20
# Does examined	11	11	11	11
# Implantations/dam	8.8	8.0	8.1	8.6
# Resorptions/dam	0	0.3	0	0.1
# Live fetuses/ litter	8.45	7.64	8.09	8.45
Mean fetal weight (g)	42.0	42.9	44.6	43.9

2. Fetal anomalies and variations: Sponsor reported that there were no external and visceral anomalies; data were not provided.

In summary, orally administered UDCA was not teratogenic in rabbits.

10. Segment III Perinatal and Postnatal Reproductive Toxicity Study of Ursodeoxycholic Acid (UDCA) in Rats (Report No. was not provided).

Testing Laboratory: [redacted]

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Statements of compliance were not provided.

Study Started: Not provided.

Study Completed: Not provided.

Animals: Female (210-220 g; 11-12 weeks of age) Wister rats.

Methods: Dosage selection was based upon the results of the Segment I study that was previously reviewed (See Study 2 in this REPRODUCTIVE TOXICOLOGY section). Thus, 4 groups of 11 pregnant female rats each were orally administered 250, 1000 and 2000 mg/kg/day via stomach intubation, respectively, from Day 17 of gestation through Day 21 of lactation. Vehicle was 5% gum arabic solution; dosing volume was 2 ml/kg.

Dams were observed daily for clinical signs of toxicity. Body weights were measured on Days 0, 4, 8, 12, 16 and 21 of gestation and daily during 21 days of lactation. Food consumption was measured on Days 0, 3, 6, 9, 12, 15, 18 and 21 of gestation and daily during 21 days of lactation. On Day 21 of lactation, hematology (RBC and WBC counts, hemoglobin levels, hematocrits) and blood chemistry (GOT, Al-p, blood glucose, total albumin, BUN, bilirubin) evaluations were done. Dams were sacrificed, organ weights were measured, and gross pathological examinations were conducted.

On the 4th day following parturition, each litter (F_1) was culled to 8-10 pups (1:1 ratio of males and females when possible). Pups were observed daily for clinical signs of toxicity. Body weights were measured on Days 0, 4 and 7 after birth and once weekly from Week 2 through Week 11. Food consumption was measured weekly for 11 weeks after birth.

Three weeks after parturition, 3 males and 3 females of the F_1 generation were removed from their litters; the remaining pups were sacrificed and subjected to gross pathological, hematological, and blood chemistry assessments. The 3 males and 3 females that were removed were observed for general behavior, general differentiation and learning abilities; their reproductive capabilities were studied at 11 weeks after parturition.

Results:

F_0 Generation

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There were no deaths.

3. Body Weight: Body weight gain from Day 17 of gestation to Day 21 of gestation in the control group was 9.62 g. Mean body weights during lactation were provided in a figure; quantitative data were not provided. There were no treatment-related effects on body weight.

4. Food Consumption: Data were not provided. Sponsor reported that there were no treatment-related effects on food consumption.

5. Hematology: There were no treatment-related effects.

6. Blood Chemistry: There were no treatment-related effects.

7. Organ Weights: There were no treatment-related effects.

8. Gross Pathology: There were no treatment-related effects.