AR226-0276

Oral Teratology Study of T-2999CoC in Rabbits

Experiment No.:	0681TB0212
Conducted At:	Safety Evaluation Laboratory Riker Laboratories, Inc. St. Paul, Minnesota
Dosing Period:	May 10, 1981 through June 26, 1981
Study Director:	E. G. Gortner

E. B. Gortner 1-6-82
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Senior Research Technologist
Animal Teratology Reproduction

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Safety Evaluation Laboratory

Summary

The oral administration of T-2999CoC at 0, 15, 5 and 1.5 mg/kg/day to pregnant New Zealand White/Minikin rabbits during gestational days 6 through 18 (period of organogenesis) was not teratogenic. Compound-related teratogenic effects were not seen on gross, internal or skeletal examinations of rabbit fetuses.

T-2999CoC was embryotoxic at the 15 mg/kg/day level. This embryotoxicity was seen as a high incidence of abortions or total resorptions, increased numbers of resorption sites and low numbers of viable fetuses. The 15 mg/kg/day fetuses experienced poor neonatal survival during a 24 hour incubation. The 5 and 1.5 mg/kg/day dose levels were free from the embryotoxicity and poor neonatal survival seen in the higher dose level.

T-2999CoC caused maternal toxicity of failure to gain net mean body weight between the initiation of dosing and the termination of the study only in the 15 mg/kg/day level. No compound-related clinical signs were unique to T-2999CoC treatment of pregnant rabbits.

Fetal rabbit lenses from all dose groups were evaluated histologically. T-2999CoC did not cause lens abnormalities in fetal rabbits exposed to the compound in utero.

Introduction

This teratology study[a] in rabbits was conducted to evaluate the embryotoxic and teratogenic effects of orally administered T-2999CoC[b]. The study was sponsored by 3M Commercial Chemical Division, St. Paul, Minnesota and was conducted by the Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minesota. Two sets of compound administration groups were dosed between May 10 and June 26, 1981. The protocol and list of the principal participants and supervisory personnel can be found in Appendices I and II respectively.

All portions of this study were conducted according to the Good Laboratory Practice (GLP) regulations and the Safety Evaluation Laboratory Standard Operating Procedures (see Appendix III for Quality Assurance Unit statement). The storage location for specimens, raw data and a copy of the final report is maintained in the Safety Evaluation Laboratory's record archives.

Methods

Sexually mature New Zealand White/Minikin female rabbits were obtained from Dutchland Laboratories, Inc., Denver, PA, and assigned cages according to a computer-generated random numbers table. The rabbits, ranging in weight from 1601 to 2917 grams, were than divided into four groups of 18 animals each. The rabbits were housed individually in stainless steel cages in a temperature and humidity controlled room. Food[c] and water were available ad libitum. The lights were on a 12 hour light/dark cycle. Each female rabbit was injected with 3 mg of pituitary luteinizing hormone via the ear vein before breeding. The does were then artificially inseminated with 0.5 ml of pooled diluted semen. The day of insemination was designated day 0 of pregnancy.

The animals were observed daily from days 3 through 29 of gestation for abnormal clinical signs. Body weights were recorded on gestational days 3, 6, 9, 12, 15, 18 and 29. The four groups were dosed with T-2999CoC suspended daily in corn oil at 0, 15, 5 and 1.5 mg/kg/day. The suspensions were administered daily using a constant dose volume of 1 ml/kg by oral intubation with a syringe and rubber catheter on gestational days 6 through 18. T-2999CoC characterization was provided by 3M Commercial Chemical Division, St. Paul, Minnesota and corn oil clearance was provided by Analytical Chemistry, Riker Laboratories, Inc., St. Paul, Minnesota (Appendix IV).

All surviving animals were euthanatized on gestational day 29. The ovaries and uterus, including its contents, were examined immediately to determine the following: number of corpora lutea, number of viable fetuses, number of resorption sites, fetal weights and sex, and gross fetal abnormalities. The fetuses were than placed in an incubator at 37° C for a 24-hour incubation period. Following the incubation period, all fetuses were killed and examined for internal abnormalities. The fetuses were then preserved in alcohol for clearing and staining of the skeleton with alizarin red to detect skeletal abnormalities. A total of 458 fetal rabbit eyes were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5-6 microns, stained with hematoxylin and eosin and evaluated histologically. This was done because a

[a] Riker Experiment Number 0681TB0212
[b] FM-3924 (technical grade FM-3422)
[c] Purina Rabbit Chow, Ralston Purina Co., St. Louis, MO

lens artifact in rat teratology studies was being evaluated concurrently with this study. The rabbit was used as a second species in case the lens finding was a true abnormality in the rat.

Results and Discussion

The oral administration of T-2999CoC to pregnant rabbits during the period of organogenesis caused maternal toxicity of low mean body weight gain. The high (15 mg/kg/day) dose does lost significantly more weight than the controls (0 mg/kg/day) between days 6 and 9 of the study (Table 1, Appendix V). They also failed to gain net body weight between the first day of dosing and the end of the study. The mid (5 mg/kg/day) and low (1.5 mg/kg/day) dose group mean maternal body weight gains and losses were never significantly different from the controls group. No compound-related clinical signs were unique to T-2999CoC treatment of rabbits.

Maternal deaths occurred during the study. No deaths could be attributed directly to compound-related toxicity, even though the compound caused maternal toxicity of low body weight gain.

A compound-related high incidence and percentage of abortions or total resorptions occurred only in the high dose group rabbits; however, the increased incidence was not statistically significant (Table 2). The incidence and percentage of abortions or total resorptions of the mid and low dose groups were not different from the control group.

T-2999CoC was embryotoxic at the high dose level. The T-2999CoC treated high dose does had a significantly lower number of viable fetuses and significantly higher number of resorption sites than the control group (Table 3, Appendix VI). The numbers of viable fetuses and resorption sites of the mid and low dose groups were not significantly different from the controls. The following litter data measurements were not affected by compound administration to the does: number of dead fetuses, number of implantation sites, number of corpora lutea and mean fetus weight.

The fetuses of T-2999CoC treated high dose does experienced poor neonatal survival. The high dose fetuses had a significantly higher mortality rate than control fetuses (Table 2) during a 24 hour incubation. Survival of mid and low dose fetuses was comparable to controls.

No major gross malformations were observed to be compound-related. Two fetuses from separate control dose group does had clubbed forepaws (Table 4, Appendix VII). Small and runted fetuses were seen in the control, mid and low dose groups, but not in the high dose group. No internal abnormalities were found.

The incidences of skeletal findings among fetuses of rabbits receiving T-2999CoC were generally not different from the findings for the control fetuses. The only exception was a significantly higher incidence of fetuses

with 13 ribs spurred in the low dose group than in the control group (Table 5, Appendix VIII). The oral administration of T-2999CoC to pregnant rabbits was not teratogenic at the dose levels tested.

Nine abnormal lenses were found on histological examination of hematoxylin and eosin stained fetal rabbit eyes. All nine abnormal lenses were from fetuses of control doe N1B1196. The remainder of the fetal lenses either had no significant changes, were not readable, or had autolysis. T-2999CoC did not cause lens abnormalities in fetal rabbits exposed to the compound in utero.

Table 1

Oral Teratology Study of T-2999CoC in Rabbits

Mean Body Weight Gain or Loss (g) of Pregnant Rabbits Between Weighings with Standard Deviations

	DAY	6	9	12	15	18	29
0 mg/kg/day	MEAN	83	-36	-9	8	30	234
	STAN. DEV	47.8	60.4	75.0	68.4	92.3	110.6
15 mg/kg/day	MEAN	56	-120[a]	-50	1	10	159
	STAN. DEV	31.5	67.7	63.4	73.5	72.4	137.8
5 mg/kg/day	MEAN	47	-48	-24	-23	5	244
	STAN. DEV	49.3	48.2	75.1	68.2	66.0	113.3
1.5 mg/kg/day	MEAN	63	-28	1	21	50	178
	STAN. DEV	25.9	77.0	55.7	60.1	62.1	59.1

[a] Significantly lower than the control (Dunnett's t test $p < 0.05$)

Table 2

Oral Teratology Study of T-2999CoC in Rabbits

Reproductive Performance and Fetal Survival

Dose Group	Number of Animals	Conception Rate No.	%	Incidence of Total Abortions or Resorptions	%	24 Hour Incubation Mortality Rate	%
0 mg/kg/day	18	15	83	2/15	13	16/81	20
15 mg/kg/day	18	16	89	7/16	44	14/28[a]	50
5 mg/kg/day	18	16	89	1/16	6	14/79	18
1.5 mg/kg/day	18	14	78	2/14	14	14/58	24

[a] Significantly higher than the control (Chi-square with Yates correction $p < 0.05$)

Table 3

Oral Teratoloby Study of T-2999CoC in Rabbits

Mean Litter Data and Fetal Weights with Standard Deviations

GRP	NO. OF ANIMALS	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPORA LUTEA	MEAN WT. FETUS(G)
0 mg/kg/day	12	4.0	2.7	6.7	0.1	0.2	7.1	7.4	38.0
STAN DEV		1.8	1.7	2.3	0.3	0.9	2.1	1.7	5.5
15 mg/kg/day	8	2.0[a]	1.5	3.5[a]	0.4	1.5[a]	5.4	6.5	34.8
STAN DEV		1.2	1.3	1.8	0.7	1.5	2.1	1.6	7.1
5 mg/kg/day	14	2.9	2.8	5.6	0.0	0.6	6.2	6.5	36.0
STAN DEV		1.3	1.3	1.8	0.0	0.8	2.2	1.5	5.0
1.5 mg/kg/day	10	3.2	2.6	5.8	0.0	0.5	6.3	6.7	36.9
STAN DEV		1.4	1.3	2.3	0.0	1.0	2.1	1.3	5.2

[a] Significantly different than the control (Dunnett's t test $p < 0.05$)

Table 4

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses with Gross Findings[a]

Findings	0 mg/kg/day	15 mg/kg/day	5 mg/kg/day	1.5 mg/kg/day
Total Fetuses examined	81	28	79	58
Small	4 (5)		2 (3)	3 (5)
Runted	1 (1)		2 (3)	2 (4)
Clubbed right forepaw	2 (3)			
Total normal fetuses	74 (91)	28 (100)	75 (95)	53 (91)
Total abnormal fetuses	7 (9)	0 (0)	4 (5)	5 (9)

[a] Treatment groups were not significantly different from the control group (Chi-square with Yates correction $p \leq 0.05$)
( ) = percent of total examined

Table 5

Oral Teratology Study of T-2999CoC in Rabbits

Fetal Skeletal Findings

Findings	0 mg/kg/day	15 mg/kg/day	5 mg/kg/day	1.5 mg/kg/day
Fontanel not closed	14 (17)	4 (14)	16 (20)	7 (12)
Frontal not ossified	10 (12)	7 (25)	5 (6)	8 (14)
Parietal not ossified	10 (12)	7 (25)	5 (6)	8 (14)
Interparietal not ossified	2 (3)			
Holes in both parietals	3 (4)	2 (7)	1 (1)	2 (4)
Holes in one parietal	3 (4)	2 (7)	4 (5)	
Sternebrae not ossified	23 (28)	6 (21)	15 (19)	16 (28)
Sternebrae asymmetrical	7 (9)	2 (7)	5 (6)	1 (2)
Sternebrae bipartite	3 (4)	1 (4)	4 (5)	
Sternebrae fused	4 (5)		2 (3)	1 (2)
Sternebrae scrambled	2 (3)		1 (1)	
Sternebrae misshapen	4 (5)		1 (1)	
One sternebrae missing	1 (1)	1 (4)		
Extra sternebrae	2 (3)		5 (6)	4 (7)
Extra sternebrae not ossified			3 (4)	
13 ribs	9 (11)	4 (14)	18 (23)	7 (12)
13 ribs spurred	6 (7)	5 (18)	7 (9)	13[a] (22)
13 ribs floating		1 (4)	1 (1)	
Ribs forked			1 (1)	
Ribs fused	5 (6)			
10 ribs	1 (1)			
Extra ribs	1 (1)			
Ribs misshapen	1 (1)			
Vertebrae scrambled	1 (1)			
Vertebrae fused	1 (1)			
Vertebrae misshapen	1 (1)			
Caudal vertebrae scrambled	1 (1)			
Total Normal Fetuses	25 (31)	8 (29)	29 (37)	24 (41)
Total Abnormal Fetuses	56 (69)	20 (71)	50 (63)	34 (59)
Total Number Fetuses	81	28	79	58

[a] Significantly higher than the control (Chi-square with Yates correction $p < 0.05$)
( ) = percent of total examined

Appendix I

TITLE: Protocol for Oral Teratology Study of T-2999CoC[a] in Rabbits (Riker Experiment Number 0681TB0212).

OBJECTIVE: A teratology study will be used to evaluate the embryotoxic and teratogenic effects of orally administered T-2999CoC to pregnant rats during the period of organogenesis. The procedure complies with the general recomendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study will be conducted according to the 1978 Good Laboratory Practice Regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

SPONSOR: 3M Commercial Chemical Division, St. Paul, Minnesota.

TESTING FACILITY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota.

STUDY DIRECTOR: E. G. Gortner

START OF DOSING: May, 1981.

TEST SYSTEM: Seventy-two sexually mature New Zealand White/Minikin rabbits from Dutchland Laboratories, Inc., will be housed in stainless steel cages with wire mesh floors in a temperature and humidity controlled room. This strain of rabbit will be used because historical control data is available. Purina Rabbit Chow and water will be available ad libitum. The lights will be on a 12 hour light/dark cycle.

TEST SYSTEM IDENTIFICATION: Each animal will be ear tagged and that number will be indicated on the outside of the cage.

RANDOMIZATION: The animals will be assigned cages according to a computer-generated random numbers table.

CONTROL ARTICLE: Corn oil.

TEST ARTICLE: T-2999CoC.

ANALYTICAL SPECIFICATIONS: The test article composition and purity will be determined by the Sponsor (3M Commercial Chemical group) prior to the start of the study and at the end of dosing. The sponsor will be responsible for retention of test article sample as required in GLP regulations.

[a] FM-3924 (technical grade FM-3422)

DOSAGE LEVELS AND EXPERIMENT DESIGN: The test article will be suspended in corn oil daily. The test article suspension and control article will be administered by oral intubation to the rabbits on days 6 through 18 of gestation according to the following:

Dose Level	Group Size
15 mg/kg/day	18
5 mg/kg/day	18
1.5 mg/kg/day	18
0 mg/kg/day	18

The oral route of administration will be used because toxicity has been defined by this route in a rangefinder study. No dietary contaminants are known to interfere with the test article.

On the day of breeding, each female will be given an intravenous injection of 1 mg of pituitary luteinizing hormone to induce ovulation. The does will then be artificially inseminated with 0.5 ml of pooled diluted semen collected from New Zealand White/Minikin male rabbits.

A blood sample will be taken pre-dose, day 18 and day 29 of gestation from the same six rabbits at each dose level. The samples will be labeled, frozen and transferred to the sponsor for blood levels analyses. The sponsor will be responsible for the analyses of the samples.

The animals will be observed daily from day 3 through day 29 of gestation for abnormal clinical signs. Body weights will be recorded on days 3, 6, 9, 12, 15, 18 and 29 of pregnancy and the rabbits dosed accordingly using a constant dose volume of 1 ml/kg of body weight.

The females will be killed on day 29 and the ovaries, uterus and its contents will be examined to determine: number of corpora lutea, number of fetuses (live and dead), number of resorption sites, number of implantation sites, pup weight and gross abnormalities. The pups will be placed in an incubator at 37° C for a 24 hour survival check. The following day the pups will be terminated and their viscera examined for any internal abnormalities. The eyes will be removed, fixed in buffered formalin for possible processing and histologic examination. The pups will then be fixed in ethyl alcohol for subsequent skeletal examination after clearing and staining with alizarin red.

DATA ANALYSIS AND FINAL REPORT: The proposed statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities. The proposed date for the final report is 2-3 months after detailed pup examinations have been completed (approximately fourth quarter, 1981).

Appendix II

List of Principal Participating Personnel

NAME	FUNCTION
Edwin G. Gortner	Study Director
Elden G. Lamprecht	Veterinary Pathologist
Gary C. Pecore	Supervisor - Animal Care
Bud G. Schmidt	Riker - Analytical
Loren O. Wiseth	Technician

APPENDIX III

STATEMENT OF QUALITY ASSURANCE

STUDY NUMBER: 0681TB0212

TITLE: Oral Teratology Study of T 2999CoC in Rabbits

Audits and/or inspections were performed by the Riker Compliance Audit unit for the above titled study, and reported to the study director and to management as follows:

Date Performed	Date Reported
14 and 19 May 1981	21 May 1981
2 June 1981	3 June 1981
12 June 1981	12 June 1981
18 June 1981	25 June 1981
7 July 1981	9 July 1981
7-8 July 1981	16 July 1981
28 December 1981	4 January 1982
30 December 1981	4 January 1982

D.P. Goenner

Compliance Audit
Riker Laboratories, Inc.

1/4/82
Date

Test and/or Control Article Characterization

for

T-2999CoC

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 6 Jan 81 *.

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes X no ____

3. The stability of the test and/or control substances have been determined or will be determined as of Completion of the Study.

The above information and documentation are located in the sponsor's records.

D. R. Jin JAN/16/81

Sponsor Date

* CCD Analytical Reg. No. 16474 October 28, 1980

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Appendix IV (Continued)

Oral Teratology Study of T-2999CoC in Rabbits
Prestudy and Poststudy Analysis of the Corn Oil Control Article

Analyses	Prestudy	Poststudy
Peroxide Value	1.2 meq/kilo	1.8 meq/kilo
Refractive Index	1.4734	1.4740

Appendix V

Oral Teratology Study of T-2999CoC in Rabbits

Individual Body Weights (g) with Mean Body Weights and Standard Deviations for Pregnant Animals

DAY	3	6	9	12	15	18	29
0 MG/KG/DAY							
N1B	1178	2237	2367	2313	2369	2450	2399	2661
N1B	1179	2279	2447	2359	2392	2292	2474	2758
N1B	1180	2483	2484	2421	2439	2306	2420	2695
N1B	1181	2015	2140	2025	2014	2016	2075	2374
N1B	1194	1781	1820	1813	1842	1922	1970	2149
N1B	1195	1710	1768	1578	1517	1522	1543	1706
N1B	1196	1842	1899	1928	1966	1942	1920	2198
N1B	1197	2917	3033	2973	2731	2705	2560	2939
N1B	1374	2248	2334	2316	2272	2338	2407	2671
N1B	1375	2364	2443	2410	2411	2345	0[a]	0
N1B	1390	2461	2607	2625	2594	2654	2689	2882
N1B	1391	2136	2156	2155	2233	2325	2209	2460
N1B	1392	1880	1977	1979	1994	2020	2092	2008
N1B	1393	1935	2011	2049	2006	2061	2071	2256
N1B	1406	2086	2130	2112	2121	2117	2267	2612
MEAN	2158	2241	2204	2193	2201	2221	2455
STAN. DEV	320.	8337.	9342.	8314.	0304.	1301.	3355.	9
NON PREGNANT ANIMALS							
N1B	1210	2038	2015	1971	2084	2117	2139	2277
N1B	1376	1763	1908	1881	1717	1702	1738	1791
N1B	1377	2028	2116	2165	2198	2174	2201	2401

[a] Animal died - intubation error

Appendix V (Continued)

Oral Teratology Study of T-2999CoC in Rabbits

Individual Body Weights (g) with Mean Body Weights and Standard Deviations for Pregnant Animals

DAY	3	6	9	12	15	18	29
15 MG/KG/DAY							
01B	1183	1765	1834	1769	1763	1716	1827	2036
01B	1184	2209	2282	2048	2131	2219	2212	2419
01B	1185	2127	2177	1991	1884	1850	1798	1594
01B	1198	1890	1999	1899	1901	1829	1789	1914
01B	1199	1826	1890	1737	1686	1762	1823	2002
01B	1200	2055	2102	1967	2008	2084	2152	2152
01B	1201	1896	1928	1671	1561	1613	1580	1753
01B	1211	1903	2018	1943	1940	1988	1992	2205
01B	1378	2316	2338	2158	2030	1953	1851	2175
01B	1379	1931	2006	1864	1824	1811	1863	1970
01B	1380	1805	1898	1797	1686	1712	1840	2087
01B	1381	1958	2001	1945	1870	1706	0[a]	0
01B	1394	2008	2028	1921	1789	1796	1676	1995
01B	1395	2001	2005	1926	1835	1947	1949	2216
01B	1396	1953	1993	1935	1868	1826	1896	2109
01B	1407	1724	1770	1758	1745	1732	1742	1754
MEAN	1960	2017	1896	1845	1847	1866	2025
STAN. DEV	158.	8150.	6125.	3143.	5157.	2163.	3211.	1
NON PREGNANT ANIMALS							
01B	1182	1987	2105	1849	1928	1932	1908	2106
01B	1397	1690	1788	1638	1559	1519	1531	1752

[a] Animal died

Appendix V (Continued)

Oral Teratology Study of T-2999CoC in Rabbits

Individual Body Weights (g) with Mean Body Weights and Standard Deviations for Pregnant Animals

DAY	3	6	9	12	15	18	29
5 MG/KG/DAY							
P1B	1186	2147	2266	2216	2087	2122	2163	2494
P1B	1187	2607	2673	2526	2596	2459	2447	2841
P1B	1188	2367	2388	2297	2344	2327	2278	2603
P1B	1189	1879	1950	1884	1948	1944	1996	2196
P1B	1203	2351	2394	2379	2266	2260	2359	2603
P1B	1205	1783	1824	1795	1795	1885	1881	2056
P1B	1212	2119	2206	2183	2223	2273	2301	2528
P1B	1382	2107	2165	2051	2011	1969	1917	2157
P1B	1383	2158	2200	2106	2001	1871	1732	1690
P1B	1384	2042	2065	2029	2018	2006	2091	2329
P1B	1385	2147	2178	2165	2130	2106	2118	2351
P1B	1398	1703	1782	1770	1739	1724	1793	2041
P1B	1399	2168	2183	2137	2061	1988	1917	0[b]
P1B	1400	1829	1913	1905	1868	1750	1820	2157
P1B	1401	2521	2591	2505	2364	2306	2243	2650
P1B	1408	1868	1763	1808	1909	1985	2000	2107
MEAN	2112	2159	2110	2085	2061	2066	2320	
STAN. DEV	260.3	270.7	240.3	227.8	215.0	217.5	301.6	
NON PREGNANT ANIMALS							
P1B	1202	2073	2147	0[a]	0	0	0	0
P1B	1204	2252	2287	0[a]	0	0	0	0

[a] Animal died - intubation error
[b] Animal died

Appendix V (Concluded)

Oral Teratology Study of T-2999CoC in Rabbits

Individual Body Weights (g) with Mean Body Weights and Standard Deviations for Pregnant Animals

DAY	3	6	9	12	15	18	29
1.5 MG/KG/DA							
Q1B	1190	2054	2120	2092	2066	2046	2130	2332
Q1B	1191	1821	1839	1858	1825	1895	1914	2157
Q1B	1192	1653	1706	1744	1769	1806	1793	2014
Q1B	1206	1737	1798	1772	1796	1863	0[a]	0
Q1B	1207	1986	2106	2106	2182	2226	2250	2501
Q1B	1208	1870	1910	1917	1918	1961	2032	2103
Q1B	1213	2111	2162	2084	2079	1952	2028	2216
Q1B	1386	1871	1943	1746	1613	0[b]	0	0
Q1B	1387	2109	2210	2210	2186	2132	2080	2193
Q1B	1388	2176	2210	2151	2151	2186	2232	2422
Q1B	1389	2428	2501	2333	2434	2519	2548	2742
Q1B	1402	1822	1897	1963	1967	2006	2088	2273
Q1B	1403	2673	2732	2699	0[a]	0	0	0
Q1B	1405	2176	2234	2291	2299	2332	2519	2615
MEAN	2035	2098	2069	2022	2077	2147	2324
STAN. DEV	276.7	280.5	266.3	233.8	210.0	230.7	224.6
NON PREGNANT ANIMALS							
Q1B	1193	1961	1990	1932	1994	1988	1970	2067
Q1B	1209	2342	2437	2410	2371	2295	2225	2590
Q1B	1404	1857	1892	1856	1873	1883	1912	2093
Q1B	1409	1601	1702	1703	1700	1697	1669	1760

[a] Animal died - intubation error
[b] Animal died

Appendix VI

Oral Teratology Study of T-2999CoC in Rabbits

Individual Litter Data with Mean Fetus Weights

0 mg/kg/day

ANIMAL		VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESOR PTION SITES	IMPLAN TATION SITES	CORPRA LUTEA	MEAN AVG
N1B	1178	LITTER TOTALLY REABSORBED							
N1B	1179	4	1	5	0	0	5	5	44.1
N1B	1180	4	2	6	0	0	6	11	40.0
N1B	1181	7	1	8	1	0	9	7	29.0
N1B	1194	3	2	5	0	0	5	9	38.8
N1B	1195	3	0	3	0	3	6	8	36.0
N1B	1196	2	4	6	0	0	6	6	38.9
N1B	1197	4	5	9	0	0	9	8	34.9
N1B	1210	NOT PREGNANT							
N1B	1374	6	4	10	0	0	10	6	35.3
N1B	1375	DEAD							
N1B	1376	NOT PREGNANT							
N1B	1377	NOT PREGNANT							
N1B	1390	5	2	7	0	0	7	7	42.3
N1B	1391	6	4	10	0	0	10	6	28.6
N1B	1392	ABORTED							
N1B	1393	1	3	4	0	0	4	9	42.4
N1B	1406	3	5	8	0	0	8	7	46.1

Appendix VI (Continued)

Oral Teratology Study of T-2999CoC in Rabbits

Individual Litter Data with Mean Fetus Weights

15 mg/kg/day

ANIMAL		VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESOR PTION SITES	IMPLAN TATION SITES	CORPRA LUTEA	MEAN AVG
01B	1182	NOT PREGNANT							
01B	1183	2	2	4	0	1	5	6	42.4
01B	1184	2	4	6	0	3	9	6	32.4
01B	1185	ABORTED							
01B	1198	ABORTED							
01B	1199	2	0	2	0	2	4	6	42.4
01B	1200	ABORTED							
01B	1201	LITTER TOTALLY REABSORBED							
01B	1211	0	2	2	2	0	4	7	39.1
01B	1378	LITTER TOTALLY REABSORBED							
01B	1379	LITTER TOTALLY REABSORBED							
01B	1380	LITTER TOTALLY REABSORBED							
01B	1381	DEAD							
01B	1394	2	0	2	0	2	4	5	40.1
01B	1395	4	2	6	0	0	6	7	28.9
01B	1396	3	1	4	0	4	8	10	28.8
01B	1397	NOT PREGNANT							
01B	1407	1	1	2	1	0	3	5	24.2

Appendix VI (Continued)

Oral Teratology Study of T-2999CoC in Rabbits

Individual Litter Data with Mean Fetus Weights

5 mg/kg/day

ANIMAL		VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESOR PTION SITES	IMPLAN TATION SITES	CORPRA LUTEA	MEAN AVG
P1B	1186	4	4	8	0	0	8	8	27.7
P1B	1187	4	4	8	0	2	10	9	36.1
P1B	1188	3	4	7	0	1	8	6	38.1
P1B	1189	1	3	4	0	0	4	6	38.7
P1B	1202	DEAD							
P1B	1203	1	1	2	0	0	2	6	46.2
P1B	1204	DEAD							
P1B	1205	3	3	6	0	1	7	6	35.8
P1B	1212	4	3	7	0	2	9	6	35.5
P1B	1382	4	3	7	0	0	7	8	26.2
P1B	1383	LITTER TOTALLY REABSORBED							
P1B	1384	3	3	6	0	0	6	8	35.4
P1B	1385	1	2	3	0	0	3	4	39.9
P1B	1398	4	1	5	0	0	5	5	35.1
P1B	1399	DEAD							
P1B	1400	3	2	5	0	1	6	6	34.3
P1B	1401	1	5	6	0	0	6	8	40.3
P1B	1408	4	1	5	0	1	6	5	34.4

Appendix VI (Concluded)

Oral Teratology Study of T-2999CoC in Rabbits

Individual Litter Data with Mean Fetus Weights

1.5 mg/kg/day

ANIMAL		VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPRA LUTEA	MEAN AVG
Q1B	1190	2	1	3	0	0	3	5	40.0
Q1B	1191	2	1	3	0	3	6	6	40.9
Q1B	1192	3	2	5	0	0	5	7	35.0
Q1B	1193	NOT PREGNANT							
Q1B	1206	DEAD							
Q1B	1207	3	5	8	0	0	8	8	34.1
Q1B	1208	4	4	8	0	1	9	9	35.0
Q1B	1209	NOT PREGNANT							
Q1B	1213	4	2	6	0	0	6	7	35.4
Q1B	1386	DEAD							
Q1B	1387	LITTER TOTALLY REABSORBED							
Q1B	1388	6	3	9	0	0	9	7	28.9
Q1B	1389	4	4	8	0	0	8	7	40.2
Q1B	1402	1	2	3	0	1	4	5	47.2
Q1B	1403	DEAD							
Q1B	1404	NOT PREGNANT							
Q1B	1405	3	2	5	0	0	5	6	32.6
Q1B	1409	NOT PREGNANT							

Appendix VII

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses by Dam with Gross Findings

0 mg/kg/day Dam No.	1179	1180	1181	1194	1195	1196	1197	1374	1390	1391	1393	1406
Findings												
Total Fetuses examined	5	6	8	5	3	6	9	10	7	10	4	8
Small										4		
Runted				1								
Clubbed right forepaw		1			1							
Total normal fetuses	5	5	8	4	2	6	9	10	7	6	4	8
Total abnormal fetuses	0	1	0	1	1	0	0	0	0	4	0	0

Appendix VII (Continued)

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses by Dam with Gross Findings

15 mg/kg/day Dam No.	1183	1184	1199	1211	1394	1395	1396	1407
Findings								
Total fetuses examined	4	6	2	2	2	6	4	2
Total normal fetuses	4	6	2	2	2	6	4	2
Total abnormal fetuses	0	0	0	0	0	0	0	0

Appendix VII (Continued)

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses by Dam with Gross Findings

5 mg/kg/day Dam No.	1186	1187	1188	1189	1203	1205	1212	1382	1384	1385	1398	1400	1401	1408
Findings														
Total fetuses examined	8	8	7	4	2	6	7	7	6	3	5	5	6	5
Small							1	1						
Runted		1					1							
Total normal fetuses	8	7	7	4	2	6	5	6	6	3	5	5	6	5
Total abnormal fetuses	0	1	0	0	0	0	2	1	0	0	0	0	0	0

Appendix VII (Concluded)

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses by Dam with Gross Findings

1.5 mg/kg/day Dam No.	1190	1191	1192	1207	1208	1213	1388	1389	1402	1405
Findings										
Total Fetuses examined	3	3	5	8	8	6	9	8	3	5
Small						1	1			1
Runted			1		1					
Total normal fetuses	3	3	4	8	7	5	8	8	3	4
Total abnormal fetuses	0	0	1	0	1	1	1	0	0	1

Appendix VIII

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses by Dam with Skeletal Findings

0 mg/kg/day — Dam No.	1179	1180	1181	1194	1195	1196	1197	1374	1390	1391	1393	1406
Fontanel not closed			3			4		4		3		
Frontal not ossified			2	1		2		2		3		
Parietal not ossified			2	1		2		2		3		
Interparietal not ossified			1	1								
Sternebrae not ossified			6				4	3	5	3	1	1
Sternebrae asymmetrical	1		3		1		2					
Sternebrae bipartite			2				1					
Sternebrae fused			1		1	1	1					
Sternebrae scrambled			1		1							
Sternebrae misshapen			2	1	1							
One sternebrae missing												1
Extra sternebrae					1					1		
13 ribs			3	1	1		1			1		2
13 ribs spurred	1		1				1		1		2	
Ribs fused			1		3		1					
10 ribs			1									
Extra ribs					1							
Ribs misshapen					1							
Vertebrae scrambled					1							
Vertebrae fused					1							
Vertebrae misshapen					1							
Caudal vertebrae scrambled			1					3				
Holes in both parietals								3				
Holes in one parietal												
Total normal fetuses	3	6	0	3	0	0	3	1	1	2	2	4
Total abnormal fetuses	2	0	8	2	3	6	6	9	6	8	2	4
Total number fetuses	5	6	8	5	3	6	9	10	7	10	4	8

Appendix VII (Continued)

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses by Dam with Skeletal Findings

15 mg/kg/day Dam No.	1183	1184	1199	1211	1394	1395	1396	1407
Fontanel not closed	1							
Frontal not ossified						3		
Parietal not ossified					1		4	2
Sternebrae not ossified					1		4	2
Sternebrae asymmetrical		2					2	2
Sternebrae bipartite					1			1
One sternebrae missing							1	
13 ribs							1	
13 ribs spurred		1			1	2		
13 ribs floating		3			1			1
Holes in both parietals		1						
Holes in one parietal					1		1	
						1	1	
Total normal fetuses	3	0	2	2	0	1	0	0
Total abnormal fetuses	1	6	0	0	2	5	4	2
Total number fetuses	4	6	2	2	2	6	4	2

Appendix VIII (Continued)

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses by Dam with Skeletal Findings

5 mg/kg/day Dam No.	1186	1187	1188	1189	1203	1205	1212	1382	1384	1385	1398	1400	1401	1408
Fontanel not closed	2	2				3	2	2				1	3	1
Frontal not ossified						1		2	1			1		
Parietal not ossified						1		2	1			1		
Sternebrae not ossified		1		1		1	2	3	1	3	1	1	1	
Sternebrae asymmetrical	2	2					1							
Sternebrae bipartite	1	1					1					1		
Sternebrae fused	1	1												
Sternebrae scrambled		1										1		
Sternebrae misshapen												1		
Extra sternebrae	4	4	1	1			2		2		1	2	2	
13 ribs						1		2	1		1	1	1	
13 ribs spurred									1					
13 ribs floating											1			
Ribs forked												1		
Holes in both parietals	2							1					1	
Holes in one parietal														3
Extra sternebrae not ossified														
Total normal fetuses	2	3	6	2	2	1	2	3	2	0	2	1	1	2
Total abnormal fetuses	6	5	1	2	0	5	5	4	4	3	3	4	5	3
Total number fetuses	8	8	7	4	2	6	7	7	6	3	5	5	6	5

Appendix VIII (Concluded)

Oral Teratology Study of T-2999CoC in Rabbits

Number of Fetuses by Dam with Skeletal Findings

1.5 mg/kg/day Dam No.	1190	1191	1192	1207	1208	1213	1388	1389	1402	1405
Fontanel not closed	1									
Frontal not ossified							2	2	1	1
Parietal not ossified					1	2	5			
Sternebrae not ossified	1		1	3	1	2	5			
Sternebrae asymmetrical					2	2		3	1	3
Sternebrae fused					1					
Extra sternebrae					1					
13 ribs	1							3	1	
13 ribs spurred					1	1	1	1	2	
Holes in both parietals				1	2	1	3	2		4
						2				
Total normal fetuses	1	3	4	5	4	2	2	1	1	1
Total abnormal fetuses	2	0	1	3	4	4	7	7	2	4
Total number fetuses	3	3	5	8	8	6	9	8	3	5

cc: M. T. Case
E. G. Gortner (original +1)
F. Keller
E. G. Lamprecht
W. C. McCormick
R. A. Prokop
V. Pothapragada/L. Winter
L. O. Wiseth

TITLE: Protocol for Oral Teratology Study of T-2999CoC[a] in Rabbits (Riker Experiment Number 0681TB0212).

OBJECTIVE: A teratology study will be used to evaluate the embryotoxic and teratogenic effects of orally administered T-2999CoC to pregnant rats during the period of organogenesis. The procedure complies with the general recomendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study will be conducted according to the 1978 Good Laboratory Practice Regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

SPONSOR: 3M Commercial Chemical Division, St. Paul, Minnesota.

TESTING FACILITY: Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota.

STUDY DIRECTOR: E. G. Gortner

START OF DOSING: May, 1981.

TEST SYSTEM: Seventy-two sexually mature New Zealand White/Minikin rabbits from Dutchland Laboratories, Inc., will be housed in stainless steel cages with wire mesh floors in a temperature and humidity controlled room. This strain of rabbit will be used because historical control data is available. Purina Rabbit Chow and water will be available ad libitum. The lights will be on a 12 hour/dark cycle.

TEST SYSTEM IDENTIFICATION: Each animal will be ear tagged and that number will be indicated on the outside of the cage.

RANDOMIZATION: The animals will be assigned cages according to a computer-generated random numbers table.

CONTROL ARTICLE: Corn oil.

TEST ARTICLE: T-2999CoC.

ANALYTICAL SPECIFICATIONS: The test article, composition and purity will be determined by the Sponsor (3M Commercial Chemical group) prior to the start of the study and at the end of dosing. The sponsor will be responsible for retention of test article sample as required in GLP regulations.

DOSAGE LEVELS AND EXPERIMENT DESIGN: The test article will be suspended in corn oil daily. The test article suspension and control article will be administered by oral intubation to the rabbits on days 6 through 18 of gestation according to the following:

Dose Level	Group Size
15 mg/kg/day	18
5 mg/kg/day	18
1.5 mg/kg/day	18
0 mg/kg/day	18

The oral route of administration will be used because toxicity has been defined by this route in a rangefinder study. No dietary contaminants are known to interfere with the test article.

On the day of breeding, each female will be given an intravenous injection of 1 mg of pituitary luteinizing hormone to induce ovulation. The does will then be artificially inseminated with 0.5 ml of pooled diluted semen collected from New Zealand White/Minikin male rabbits.

A blood sample will be taken pre-dose, day 18 and day 29 of gestation from the same six rabbits at each dose level. The samples will be labeled, frozen and transferred to the sponsor for blood levels analyses. The sponsor will be responsible for the analyses of the samples.

The animals will be observed daily from day 3 through day 29 of gestation for abnormal clinical signs. Body weights will be recorded on days 3, 6, 9, 12, 15, 18 and 29 of pregnancy and the rabbits dosed accordingly using a constant dose volume of 1 ml/kg of body weight.

The females will be killed on day 29 and the ovaries, uterus and its contents will be examined to determine: number of corpora lutea, number of fetuses (live and dead), number of resorption sites, number of implantation sites, pup weight and gross abnormalities. The pups will be placed in an incubator at 37 C for a 24 hour survival check. The following day the pups will be terminated and their viscera examined for any internal abnormalities. The eyes will be removed, fixed in buffered formalin for possible processing and histologic examination. The pups will then be fixed in ethyl alcohol for subsequent skeletal examination after clearing and staining with alizarin red.

A ANALYSIS AND FINAL REPORT: The proposed statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities. The proposed date for the final report is 2-3 months after detailed pup examinations have been completed (approximately fourth quarter, 1981).

G. Gortner 4-29-81
G. Gortner Date
ior Research Technologist
nal Teratology-Reproduction
dy Director

Elden G Lamprecht 5-4-81
E. G. Lamprecht, DVM, PhD Date
Research Veterinary Pathologist

Larry T Case 4/29/81
. Case, DVM, PhD Date
ager, Pathology-Toxicology
ety Evaluation Laboratory

William C McCormick 4/30/81
W. C. McCormick, MS Date
Toxicologist
Sponsor Representative

SAFETY EVALUATION LABORATORY
ANIMAL TOXICITY STUDY PROPOSAL

A oral rabbit teratology study is to be conducted on
(type of study)
T-2999 CoC.
(compound or material)
The attached protocol details the various aspects of the study and the study will be conducted according to Good Laboratory Practice regulations. The estimated total cost of the study is $ 31,000 and if dosing starts May 1981, the estimated cost by calendar year is $ 31,000 in 1981. However, it is understood that the sponsoring division will be charged actual time and materials via the 3M recharge system on project number 8900 3106. The estimated time from the initiation to completion and reporting of the study is 7-9 months (NOTE: time estimate includes, as applicable period between ordering and receipt of animals, prestudy conditioning and examinations of animals, compound dosing interval, preparation of tissue slides, microscopic examination of tissues, preparation of fetuses, detailed examination of fetuses, compilation and analyses of data, drafting a report, review and final typing of report). Once the study is approved and the compound, with appropriate analytical data, has been received, the animals will be ordered. After this point, cancellation of the study will pose multiple problems because time, space and people have been committed to complete the study. Written confirmation will be necessary for any cancellation and a charge of up to 25% of the remaining estimated cost could be assessed to compensate the testing laboratory.

Testing Laboratory Signatures

E. J. Bottomley 4-29-81
Study Director Date

Marvin T Case 4/29/81
Safety Evaluation Laboratory Date

Approval Signatures

William McConnick 4/30/81
Sponsor Representative Date

J. A. Trokop 5-5-81
Division Date

004228