AD 6

Two Week Oral Rangefinding Toxicity Study
of T-2509CoC in Rats

Experiment No.:	179RR023
Dosing Started:	October 3, 1979
Recovery Intervals Completed:	November 15, 1979
Study Director:	G. R. Steffen, BS
Testing Facility:	Safety Evaluation Laboratory Riker Laboratories, Inc., St. Paul, Minnesota
Sponsor:	W. C. McCormick Toxicology Services 3M Company St. Paul, Minnesota

G. R. Steffen, BS — 2/22/80 — Date
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Summary

The oral rangefinder toxicity study of T-2509CoC in rats was completed in two parts. In Part I the acute oral ALD50 was defined in male CD rats. Six groups having six rats/group, 51-59 days of age when dosed, were administered single doses ranging from 250-10,000 mg/kg. In Part II, T-2509CoC was administered by daily gastric intubation to male and female CD rats. Twelve rats/sex/group, 51 days of age on dose day 1, were dosed at 200 or 75 mg/kg/day. Dosing continued for five days at the high level (200 mg/kg/day) and for nine days at the low level (75 mg/kg/day). All surviving high dose animals and half of the low dose group had a 14-15 day recovery interval following dosing. Selected low dose animals were euthanatized on day 10. All surviving animals in both groups were euthanatized at the end of the recovery interval. Brain, stomach, liver, adrenal and kidney tissues were evaluated microscopically.

The acute oral ALD50 of T-2509CoC in male rats was 878 mg/kg with 95% confidence limits of 558-1399 mg/kg. Gastrointestinal irritation was the most common effect caused by high acute doses. Handling-precipitated convulsions occurred in some dose groups 2-7 days following dosing. The lowest dose group (250 mg/kg) was free of treatment-related effects.

Daily gastric intubation of rats with T-2509CoC resulted in treatment-, dosage- and sex-related effects. The primary effects were gastric irritation, handling-precipitated convulsions and microscopic liver changes. These effects contributed to dosage-related deaths. Secondary effects at both levels were a tucked-up appearance, an unkempt appearance, soft feces, body weight loss during the dosing period, head tremors and hyperactivity. The high dose group, but not the low dose group, had histologic evidence of gastric irritation. There were no microscopic changes in the brain sections from either level to correlate with the CNS effects. The treatment-related liver effects consisted of focal necrosis and fatty changes. The males generally had earlier occurring deaths, more convulsive episodes and more animals with liver changes.

Reversal of the toxic effects occurred after dosing stopped. Severe delayed CNS effects did not occur during recovery. The gastric lesions and other secondary effects subsided during recovery. All recovery animals had weight gain. The recovery animals did have mild fatty liver changes but not liver necrosis.

Introduction

The oral rangefinder toxicity study of T-2509CoC in rats was completed in two parts. Part I evaluated the acute oral ALD50 in male rats. Part II evaluated the toxic effects and recovery following daily oral dosing in male and female rats using dose levels selected from the results in Part I.

Toxicology Services, 3M Company, St. Paul, Minnesota was the study sponsor. The study was conducted by the Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota. Acute dosing started on October 3, 1979 and the repeat dose portion was completed on November 15, 1979. The protocol with amendments and a list of principal participants and supervisory personnel are found in Appendices I and II respectively.

Since the study was a rangefinder experiment, it was excluded from Good Laboratory Practice (GLP) regulations, however, the study was conducted in accordance with our Standard Operating Procedures which incorporate GLP requirements. The storage location for specimens, raw data and final report is maintained in the record archives for the Safety Evaluation Laboratory.

Methods

Part I: The approximate oral ALD50 was determined in male CD rats obtained from Charles River Laboratories, Inc. The rats were conditioned in-house for approximately one week prior to dosing. They were housed six/wire-top cage with food and water available ad libitum. Animal identification was by individual numbers and a color coding system. The animal housing room was temperature and humidity controlled and had a 12 hour light/dark cycle.

Six rats/dose group were selected from the conditioned animals. The animals were 51-59 days old and weighed 179-262 grams when dosed. A constant dose volume of 10 ml/kg was used at each level. Distilled water was used to dilute the test article to the appropriate concentrations.

The animals were observed 3-6 hours following dosing and daily thereafter for 14 to 20 days. Body weights were obtained just prior to dosing and on the day of necropsy. Gross necropsy abnormalities were recorded for all animals that died or were killed at the end of the observation period but tissues were not saved.

Part II: Male and female CD rats were obtained from Charles River Laboratories, Inc. and conditioned in-house for two weeks prior to dosing. The animals were individually housed in hanging cages having wire mesh fronts and bottoms. Food and water were available ad libitum. Animal identification was by individual ear tags and cage cards. The room was temperature and humidity controlled and had a 12 hour light/dark cycle.

Twelve rats/sex/level were selected from the conditioned animals and assigned to the 200 or 75 mg/kg/day group. On day 1 the animals were 51 days old and the males weighed 154-211 grams and the females weighed 144-185 grams. The test article solutions were prepared daily using distilled water as the diluent. A constant dose volume of 10 ml/kg was used for each dose level. Dosing continued for five days at the high dose level (200 mg/kg/day) and for nine days at the low dose level (75 mg/kg/day). The low dose group had six animals/sex retained for recovery after dose day 9 and the remaining animals were euthanatized on day 10. All surviving animals in the high dose group were retained for recovery after dosing stopped. The recovery interval was 14 days for the low dose group and 15 days for the high dose group.

The animals were observed daily for signs of toxicity. Body weights were obtained predose, biweekly during dosing and once or twice a week during recovery. All animals that died or were euthanatized were subjected to a gross necropsy and tissues were saved for histopathological evaluation.

The dose levels for Part II were selected at the completion of Part I. The 75 mg/kg/day level for the repeat dose portion of the study was selected to deliver a cummulative 14 day dose approximately equal to the ALD50 value. The 200 mg/kg/day level was selected to be close to the acute no-effect level and to deliver a cummulative 14 day dose at least two times greater than the ALD50 value.

Results

Part I: The acute oral ALD50 of T-2509CoC in male rats was 878 mg/kg having 95% confidence limits of 558 to 1399 mg/kg.

Dosage-related gastrointestinal toxicity was the primary T-2509CoC effect. Clinical signs included a tucked-up appearance, soft feces/diarrhea and hypo-reactivity/hypoactivity (Table 1). Most deaths at the higher levels occurred overnight on the day of dosing (found dead on study day 2) while those at the lower levels were delayed through study day 8 (Table 2). Most animals found dead had gross evidence of reddened or hemorrhagic gastrointestinal mucosa (Table 3).

Delayed CNS toxicity was evident at some dosage levels. One animal at each of the 5000, 2000 and 1000 mg/kg levels had clonic/tonic convulsions on days 6, 2 or 7 respectively. Each episode was precipitated by handling and the animals were found dead within 24 hours. Hypereactivity occurred three to nine days following dosing at the 2000, 1000 and 500 mg/kg levels but these animals recovered. The observation period was extended to 20 days at the 1000 and 500 mg/kg levels because of the delayed deaths but no additional effects occurred.

The lowest dose group (250 mg/kg) was free of treatment-related effects and these animals showed normal weight gain. All animals at each dose level that survived for the duration of the study had acceptable weight gain and were free of obvious gross lesions at necropsy.

Table 1

Acute Oral Toxicity Study
of T-2509CoC in Male Rats
10,000 mg/kg Group
Number of Animals Affected

Observation	Observation Period																
	Hours				Days												
	0-1	1-2	2-4	4-6	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared normal	2	3	2	NE	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	3	2	NE	NE	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	NE	2	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Hyporeactive	NE	2	4	NE	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	NE	NE	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	NE	NE	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Chromodacryorrhea	NE	NE	1	NE	-	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactive	NE	NE	3	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Tucked-up abdomen	NE	NE	NE	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Deaths	0	0	1	0	5 (overnight)												

NE = Not evident

Table 1 (continued)

Acute Oral Toxicity Study
of T-2509CoC in Male Rats
5000 mg/kg
Number of Animals Affected

Observation	Observation Period																
	Hours				Days												
	0-1	1-2	2-4	4-6	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared normal	4	4	1	1	2	2	2	2	1	NE	-	-	-	-	-	-	-
Salivation	3	1	NE	1	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-
Bloody nares & mouth	2	NE	NE	1	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-
Hyporeactive	2	1	5	5	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-
Dyspnea	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-
Soft feces	NE	1	5	5	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-
Ptosis	NE	1	1	1	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-
Tucked-up abdomen	NE	NE	2	3	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-	-
Clonic/tonic convulsion	NE	NE	NE	NE	NE	NE	NE	NE	1[a]	NE	-	-	-	-	-	-	-
Deaths	0	0	0	0	3	1	0	0	1	1							

NE = Not evident

[a] Precipitated by handling

Table 1 (continued)

Acute Oral Toxicity Study
of T-2509CoC in Male Rats
2000 mg/kg
Number of Animals Affected

Observation	Observation Period																
	Hours				Days												
	0-1	1-2	2-4	4-6	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared normal	6	6	6		NE	2	2	NE	NE	NE	NE	-	-	-	-	-	-
Hyporeactive	NE	NE	1		4	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-
Dyspnea	NE	NE	1		NE	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-
Bloody nares	NE	NE	NE		2	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-
Tucked-up abdomen	NE	NE	NE		3	NE	NE	1	1	1	NE	-	-	-	-	-	-
Clonic/tonic convulsion	NE	NE	NE		1[a]	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-
Soft feces	NE	NE	NE		1	NE	NE	NE	NE	NE	NE	-	-	-	-	-	-
Ptosis	NE	NE	NE		NE	NE	NE	1	NE	NE	NE	-	-	-	-	-	-
Hypereactive	NE	NE	NE		NE	1	NE	NE	NE	NE	NE	-	-	-	-	-	-
Thin	NE	NE	NE		NE	NE	NE	2	1	1	NE	-	-	-	-	-	-
Hypoactive	NE	NE	NE		NE	NE	NE	1	NE	NE	NE	-	-	-	-	-	-
Deaths	0	0	0		3	0	1	0	1	0	1						

NE = Not evident

[a] Precipitated by handling

Table 1 (continued)

Acute Oral Toxicity Study
of T-2509CoC in Male Rats
1000 mg/kg
Number of Animals Affected

Observation	Observation Period																
	Hours				Days												
	0-1	1-2	2-4	4-6	2	3	4	5	6	7	8	9	10	11	12	13	14-20
Appeared normal	6	6	6	6	6	6	6	5	4	3	NE	NE	3	3	3	3	3
Clonic/tonic convulsion	NE	NE	NE	NE	NE	NE	NE	NE	NE	1[a]	NE	NE	NE	NE	NE	NE	NE
Thin	NE	NE	NE	NE	NE	NE	NE	NE	NE	1	NE	NE	NE	NE	NE	NE	NE
Hypereactive	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	3	3	NE	NE	NE	NE	NE
Tremors	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	1	1	NE	NE	NE	NE	NE
Deaths	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0

NE = Not evident

[a] Precipitated by handling

Table 1 (concluded)

Acute Oral Toxicity Study
of T-2509CoC in Male Rats
500 mg/kg
Number of Animals Affected

Observation	Observation Period																
	Hours				Days												
	0-1	1-2	2-4	4-6	2	3	4	5	6	7	8	9	10	11	12	13	14-20
Appeared normal	6	6	6	6	6	6	6	6	5	5	NE	NE	5	5	5	5	5
Hypereactive	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	5	5	NE	NE	NE	NE	NE
Deaths	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

NE = Not evident

250 mg/kg
Number of Animals Affected

Observation	Observation Period																
	Hours				Days												
	0-1	1-2	2-4	4-6	2	3	4	5	6	7	8	9	10	11	12	13	14-20
Appeared normal	6	6	6		6	6	6	6	6	6	6	6	6	6	6	6	6
Deaths	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2

Acute Oral Toxicity Study
of T-2509CoC in Male Rats
Death Pattern

Dose Level	Deaths/Day(s) 1	2	3	4	5	6	7	8	9-Term	Total Deaths No. Dosed
10000 mg/kg[a]	1	5	---	---	---	---	---	---	---	6/6
5000 mg/kg[a]	0	3	1	0	0	1	1	---	---	6/6
2000 mg/kg	0	3	0	1	0	1	0	1	---	6/6
1000 mg/kg	0	0	0	0	1	1	0	1	0	3/6
500 mg/kg	0	0	0	0	0	1	0	0	0	1/6
250 mg/kg	0	0	0	0	0	0	0	0	0	0/6

ALD50 = 878 mg/kg
95% Confidence Limits = 558 to 1399 mg/kg[b]

[a] Data from this level not used for ALD50 calculations
[b] Computations by Duluth Probit Analysis, Environmental Research Laboratory Program

Table 3

Acute Oral Toxicity Study
of T-2509CoC in Male Rats
Necropsy Results
Number of Animals Affected

Gross Observation	Dose Group (mg/kg)					
	10,000	5000	2000	1000	500	250
No. Dead/No. Dosed	6/6	6/6	6/6	3/6	1/6	0/6
No Visible Lesions						
Euthanatized	0	0	0	3	5	6
Found Dead	0	2	1	1	0	0
Reddened Stomach Mucosa	4	2	2	0	0	0
Hemorrhagic Stomach Mucosa	2	2	1[a]	1	1	0
Hemorrhagic Small Intestinal Mucosa	0	0	2	0	0	0
Cannabilized	0	0	0	1	0	0

[a] With possible ulcer

Part II: Treatment- and dosage-related gastric irritation was the most prominant effect caused by daily oral administration of T-2509CoC. The high dose group, but not the low dose group, had histologic evidence of gastric irritation. This irritation was present in 11 of 12 males and 8 of 12 females (Table 4). In some instances early hemorrhagic gastric ulcers were found. Clinical signs associated with gastric irritation were present in both dose groups and included a dosage-related tucked-up appearance, dosage-related soft feces for the male only and an unkempt appearance more common in the low dose group than the high dose group (Tables 5 and 6). Better survival at the low dose level no doubt allowed greater expression of a secondary effect, such as an unkempt appearance. Abdominal pain (tucked-up appearance) continued for the first few days of recovery. The surviving high dose animals and some low dose animals appeared unkempt throughout the recovery interval.

Dosage-related CNS effects occurred at both treatment levels. These effects were expressed as convulsions and isolated instances of head tremors and hyperactivity. Histopathological brain lesions were not found in animals at either level. The brain sections were obtained at the fore-, mid and hind brain levels. Tonic or clonic/tonic convulsions occurred in twice the number of males compared to females: six high dose males, three high dose females, two low dose males and one low dose female. The less severe CNS effects, head tremors or hyperactivity, occurred in only one animal in each dose group except that no high dose males had these signs. One low dose female continued to be hyperactive during the recovery interval.

The convulsive episodes were generally precipitated by handling and did not increase in frequency after dosing was stopped. Handling-precipitated convulsions started on dose day 5 at the high dose level and each animal that convulsed was dead within 24 hours. The low dose female convulsed on study day 9 and survived. The low dose males convulsed during handling just prior to planned necropsies on days 8 and 10. One high dose male convulsed the third day (day 8) after dosing was stopped but the frequency of convulsive episodes were not increasing compared to the rats dosed acutely in Part I. All animals were handled (picked up) as much during recovery as they were while being dosed.

Treatment- and dosage-related deaths resulted from the treatment-related toxicities. Deaths occurred earlier in the study for the high dose males than for the high dose females. Seven of 12 males and two of 12 females were dead by day 6 and by day 8 all the males and seven of 12 females were dead. The high dose females continued to die through day 12 (recovery day 7) with two surviving throughout the recovery interval. The only treatment-related death at the low dose level was a female on day 9.

Treatment-related microscopic liver toxicity showed evidence of reversal following the recovery interval. The liver effects consisted of fatty changes and focal areas of necrosis. Minimal to moderately severe fatty

changes were present in five of 12 males and three of 12 females at the high dose level and three of five males at the low dose level. Focal areas of necrosis occurred in two of 12 males and one of 12 females at the high dose level and three of five males and two of five females at the low dose level. These low dose effects reflect only those rats killed for the interim necropsy. The last three high dose females that died during the recovery interval (died on days 9, 11 or 12) had no histopathological changes. The two females in the high dose group that survived had one animal with only fatty changes at necropsy. The low dose recovery animals did not have focal liver necrosis but they still had minimal to mild fatty changes in five of six males and one of six females. The greater number of low dose rats compared to high dose rats with liver changes reflected the better survival rate at the low dose level and the subchronic nature of the liver response.

Treatment-related body weight loss occurred during dosing and body weight gain occurred during the recovery interval. The weight loss was similar at both dose levels but the females lost more weight than the males (Table 7). Animals in both dose groups also appeared thin. The surviving high dose females and many of the low dose recovery animals continued to appear thin during the recovery interval even though they were gaining weight. Food consumption for all animals appeared normal throughout the study.

The only other treatment-related histopathological change was congested and hemorrhagic adrenals in one of 12 males and two of 12 females at the high dose level. The adrenal effect was probably due to stress caused by one or more of the other treatment-related toxicities. An infrequent treatment-related clinical effect was urinary incontinence in four high dose males. The incontinence occurred for several days prior to death but was not associated with microscopic kidney changes. One low dose male was euthanatized on day 8 having a soft subcutaneous lump caused by an intubation-induced perforated esophagus.

Table 4

Gross and Microscopic Tissue Observations

NOTE: Following complete gross necropsy, histologic examination was done on brain, liver, stomach, adrenals and kidneys. If a tissue is not listed, no significant changes were found, ie only abnormalities are listed.

Dose Group and Rat No.	Organ	Gross Observations	Microscopic Observations
200 mg/kg Males			
9R17285 (died day 6)	Stomach	Small hemorrhagic areas (ulcers?)	At the outer regions of the gastric mucosa there were scattered focal areas of congestion and hemorrhage. These hemorrhagic foci were not associated with any inflammation nor with any particular necrosis of the gastric mucosa.
	Liver	No significant changes	Moderately severe fatty changes were present, as approximately one-third of the hepatocytes had cytoplasmic fat vacuoles. The involved cells sometimes, but not always, had a central lobular distribution.
9R17286 (died day 8)	Stomach	Small black areas (hemorrhages?)	Scattered foci of congestion and hemorrhage were present in the outer regions of the gastric mucosa.
	Adrenals	Dark (hemorrhagic?)	Rather marked congestion was present, particularly in the medulla but in the cortex as well.
9R17287 (died day 6)	Stomach	Large hemorrhagic areas (ulcers?)	Scattered foci of congestion and hemorrhage were present in the gastric mucosa.
9R17288 (died day 7)	Stomach	Large black spots (hemorrhages?)	Scattered foci of congestion and hemorrhage were present in the gastric mucosa. At one of these foci there was some necrosis of the gastric epithelium (development of a shallow hemorrhagic ulcer).
9R17289 (died day 8)	Adrenals	Dark (hemorrhagic?)	No significant changes
	Small Intestine	Reddened areas	Tissue not processed for examination
	Liver	No significant changes	Mild fatty changes were indicated as approximately 10-15% of the hepatocytes had cytoplasmic fat vacuoles.
9R17290 (died day 6)	Stomach	Dark lines (hemorrhages?)	Focal areas of congestion and hemorrhage were present in the gastric mucosa. In one instance this was associated with some necrosis of the gastric epithelium (development of an early hemorrhagic ulcer).
	Liver	No significant changes	Minimal fatty changes were present as approximately 5% of the hepatocytes had cytoplasmic fat vacuoles.
9R17291 (died day 6)	Stomach	Small black specks (hemorrhages?)	No significant changes
9R17292 (died day 7)	Stomach	Red lines	Focal areas of congestion and hemorrhage were present in the gastric mucosa.
	Liver	No significant changes	Mild fatty changes were present as approximately 10-15% of the hepatocytes had fine cytoplasmic fat vacuoles.
9R17293 (died day 6)	Stomach	Long black lines (hemorrhagic?)	Focal areas of congestion and hemorrhage were present in the gastric mucosa.

Table 4 (continued)

Gross and Microscopic Tissue Observations

Dose Group and Rat No.	Organ	Gross Observations	Microscopic Observations
200 mg/kg Males (con't)			
9R17294 (died day 7)	Stomach	Large black spots (hemorrhages?)	There were focal areas of congestion and hemorrhage in the gastric mucosa.
	Liver	No significant changes	In one area there were several small foci of coagulation necrosis.
9R17295 (died day 5)	Stomach	No significant changes	There were focal areas of congestion and hemorrhage in the gastric mucosa.
	Liver	White band on left lateral lobe	The area observed -rossly appeared to be a longitudinal area of coagulation necrosis. In addition, there were minimal fatty changes as approximately 5% of the hepatocytes had fine cytoplasmic fat vacuoles.
	Spleen	Appears pale	Tissue not processed for examination.
9R17296 (died day 6)	Stomach	Large black spots (hemorrhages?)	Focal areas of congestion and hemorrhage were present in the gastric mucosa and at several areas were associated with necrosis of the gastric epithelium (a developing hemorrhagic gastric ulcer)
200 mg/kg Females			
9R17343 (died day 8)	Stomach	Large dark area (hemorrhages?)	A hemorrhagic ulcer was present with localized necrosis of the gastric epithelium.
	Adrenals	Dark (hemorrhagic?)	Rather extensive congestion and some areas of hemorrhage were present.
9R17344 (died day 7)	Stomach	Small black spots (hemorrhages?)	Foci of congestion and hemorrhage were present in the upper portion of the gastric mucosa.
	Liver	No significant changes	Scattered foci of coagulation necrosis were present. Infiltration of mononuclear cells were present within some of these necrotic foci.
9R17345 (died day 7)	Stomach	Dark red lines	No significant changes
	Liver	No significant changes	Mild fatty changes were present as approximately 10% of the hepatocytes had cytoplasmic fat vacuoles
9R17346 (died day 11)		(none)	
9R17347 (died day 7)	Stomach	Dark red streaks	Foci of congestion and hemorrhage were present in the upper regions of the gastric mucosa.
9R17348 (died day 6)	Stomach	No significant changes	A small, shallow hemorrhagic ulcer was present.
9R17349 (died day 7)	Stomach	Large black spots (hemorrhages?)	Small focal areas of congestion and hemorrhage were present in the gastric mucosa.
9R17350 (died day 6)	Stomach	Thin dark lines (hemorrhages?)	No significant changes

Table 4 (continued)

Gross and Microscopic Tissue Observations

Dose Group and Rat No.	Organ	Gross Observations	Microscopic Observations
200 mg/kg Females (con't)			
9R17351 (on compound 5 days, terminated day 20)	Liver	No significant changes	Fatty changes were present as approximately 30-40% of the hepatocytes had cytoplasmic fat vacuoles.
9R17352 (died day 9)	Stomach	Black lines (hemorrhages?)	No significant changes
	Adrenals	Enlarged and dark (hemorrhagic)	Areas of congestion and hemorrhage were present.
	Liver	No significant changes	Mild fatty changes were present as about 10% of the hepatocytes had cytoplasmic fat vacuoles.
9R17353 (on compound 5 terminated day 20)		(none)	
9R17354 (died day 12)		(none)	
75 mg/kg Males			
9R17297 (on compound 9 days, terminated day 23)	Liver	No significant changes	Mild fatty changes were present as approximately 10-20% of the hepatocytes had cytoplasmic fat vacuoles.
9R17298 (on compound 9 days, terminated day 23)	Liver	No significant changes	Mild fatty changes were present as approximately 10% of the hepatocytes had cytoplasmic fat vacuoles.
9R17299 (on compound 9 days, terminated day 10)	Liver	No significant changes	Moderately severe fatty changes were present as approximately 50% of the hepatocytes had cytoplasmic fat vacuoles. In addition, in one section there were several small, focal areas of older coagulation necrosis with infiltration of numerous mononuclear cells cleaning up the cellular debris.
9R17300 (on compound 9 days, terminated day 10)	Liver	Pale streaks and foci	The lesions observed grossly were focal areas of coagulation necrosis. A limited amount of acute to subacute inflammatory response was present adjacent to and within some of these foci.
	Adrenals	Dark, enlarged?	Areas of congestion and hemorrhage were present in the cortex.
9R17301 (on compound 9 days, terminated day 10)	Adrenals	Enlarged?	No significant changes
	Liver	Pale streaks	The areas observed grossly were areas of coagulation necrosis, frequently accompanied by a limited amount of acute to subacute inflammatory response.
9R17302 (on compound 9 days, terminated day 10)	Liver	No significant changes	Approximately 10-20% of the hepatocytes had fatty changes as indicated by cytoplasmic fat vacuoles.

Table 4 (continued)

Gross and Microscopic Tissue Observations

Dose Group and Rat No.	Organ	Gross Observations	Microscopic Observations
75 mg/kg Males (con't)			
9R17303 (on compound 9 days, terminated day 23)	Liver	No significant changes	Minimal fatty changes were present as approximately 1-2% of the hepatocytes had cytoplasmic fat vacuoles
9R17304 (on compound 9 days, terminated day 23)	Liver	No significant changes	Minimal fatty changes were present as 1-2% of the hepatocytes had cytoplasmic fat vacuoles.
9R17305 (on compound 9 days, terminated day 23)	Liver	No significant changes	Approximately 1-2% of the hepatocytes had cytoplasmi fat vacuoles indicating mild fatty changes.
9R17306 (euthanatized day 8)	Esophagus	Puncture hole just anterior to thoracic inlet; leads to large necrotic subcutaneous area in left axilla - intubation error	No tissue saved
	Adrenals	Dark (hemorrhagic?), enlarged	Congestion and hemorrhage were present.
9R17307 (on compound 9 days, terminated day 23)		(none)	
9R17308 (on compound 9 days, terminated day 10)	Liver	No significant changes	Mild fatty changes were indicated as approximately 10% of the hepatocytes had cytoplasmic fat vacuoles.
75 mg/kg Females			
9R17355 (on compound 9 days, terminated day 10)		(none)	
9R17356 (died day 10)	Adrenals	Enlarged?	Congestion and focal hemorrhages were present in the cortex and medulla.
9R17357 (on compound 9 days, terminated day 10)	Liver	Possible pale area	No significant changes
9R17358 (on compound 9 days, terminated day 10)	Liver	Pale streaks	The lesions observed grossly were focal areas of coagulation necrosis accompanied by infiltration of an acute to subacute inflammatory exudate.
9R17359 (on compound 9 days, terminated day 23)		(none)	

Table 4 (concluded)

Gross and Microscopic Tissue Observations

Dose Group and Rat No.	Organ	Gross Observations	Microscopic Observations
75 mg/kg Females (con't)			
9R17360 (on compound 9 days, terminated day 10)	Liver	Pale streak	There were focal areas of coagulation necrosis which were usually accompanied by a subacute inflammatory exudate.
	Adrenals	Appear enlarged	No significant changes
9R17361 (on compound 9 days, terminated day 23)	Liver	No significant changes	There were minimal fatty changes as approximatelv 1-2% of the hepatocytes had cytoplasmic fat vacuoles.
9R17362 (on compound 9 days, terminated day 23)		(none)	
9R17363 (on compound 9 days, terminated day 10)		(none)	
9R17364 (on compound 9 terminated day 23)		(none)	
9R17365 (on compound 9 terminated day 23)		(none)	
9R17366 (on compound 9 terminated day 23)		(none)	

Table 5

Two Week Oral Rangefinding Study
of T-2509CoC in Rats
Repeat Dose Effects
Animals Affected/Day

Effect	Study Day																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
200 mg/kg/day Males																						
Appeared normal	12	12	3	4	NE	NE	NE	NE														
Soft feces	NE	NE	7	NE	NE	4	1	1														
Tucked-up appearance	NE	NE	4	8	10	5	2	1														
Thin	NE	NE	NE	1	NE	5	2	1														
Urinary incontinence	NE	NE	NE	1	NE	NE	NE	NE														
Tonic convulsion[a]	NE	NE	NE	NE	5	NE	NE	1														
Unkempt appearance	NE	NE	NE	NE	NE	2	1	1														
Deaths	0	0	0	0	1	6	3	2														
200 mg/kg/day Females																						
Appeared normal	12	12	11	6	NE	NE	NE	NE	NE	NE	NE	NE	1	1	1	NE	NE	NE	NE	NE		
Tucked-up appearance	NE	NE	1	6	12	11	6	4	1	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE		
Thin	NE	NE	NE	NE	NE	11	6	5	4	4	3	2	1	1	1	2	2	2	2	2		
Urinary incontinence	NE	NE	NE	NE	NE	2	1	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE		
Tonic convulsion[a]	NE	NE	NE	NE	1	2	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE		
Unkempt appearance	NE	NE	NE	NE	NE	1	NE	NE	1	1	NE	NE	1	1	1	1	1	1	1	1		
Bloody nose and mouth	NE	NE	NE	NE	NE	NE	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE		
Hyperactive	NE	NE	NE	NE	NE	NE	NE	NE	NE	1	1	NE	NE	NE	NE	NE	NE	NE	NE	NE		
Deaths	0	0	0	0	0	2	4	1	1	0	1	1	0	0	0	0	0	0	0	0		
75 mg/kg/day Males																						
Appeared normal	12	12	8	9	9	7	5	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Soft feces	NE	NE	2	NE	NE	1	1	NE	1	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Tucked-up appearance	NE	NE	NE	NE	3	2	4	5	3	3	1	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE

NE = Not evident

[a] Convulsive episodes generally occurred during handling (dosing, weighing, etc.)

Table 5 (concluded)

Two Week Oral Rangefinding Study
of T-2509CoC in Rats
Repeat Dose Effects
Animals Affected/Day

Effect	Study Day																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
75 mg/kg/day Males (con't)																						
Respiratory congestion	NE	NE	3	3	NE	2	3	3	3	3	NE	NE	1	1	2	2	2	2	2	2	2	2
Thin	NE	NE	NE	NE	NE	NE	2	5	5	5	2	2	3	3	3	3	3	3	3	NE	NE	NE
Tonic convulsions[a]	NE	NE	NE	NE	NE	NE	NE	1	NE	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Unkempt Appearance	NE	NE	NE	NE	NE	NE	NE	NE	7	7	4	4	5	5	5	5	5	5	5	5	5	5
Bloody nose and mouth	NE	NE	NE	NE	NE	NE	NE	NE	1	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Head tremors	NE	NE	NE	NE	NE	NE	NE	NE	NE	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Lump on left side	NE	NE	NE	NE	NE	1	1	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Deaths	0	0	0	0	0	0	0	1[b]	0	5[b]	0	0	0	0	0	0	0	0	0	0	0	0
75 mg/kg/day Females																						
Appeared normal	12	12	12	12	12	9	7	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2
Tucked-up appearance	NE	NE	NE	NE	NE	3	4	5	6	5	1	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Thin	NE	NE	NE	NE	NE	NE	4	7	8	7	4	4	2	2	2	2	2	2	2	2	2	2
Respiratory congestion	NE	NE	NE	NE	NE	NE	NE	NE	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Clonic/tonic convulsion[a]	NE	NE	NE	NE	NE	NE	NE	NE	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Unkempt appearance	NE	NE	NE	NE	NE	NE	NE	NE	5	4	2	2	4	4	4	4	4	4	4	4	4	4
Hyperactive	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	1	1	1	1	1	1	1	NE	NE	NE
Hair loss on abdomen	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	1	1	1	1	1	1	1	1	1	1	1	1
Deaths	0	0	0	0	0	0	0	0	0	6[c]	0	0	0	0	0	0	0	0	0	0	0	0

NE = Not evident

[a] Convulsive episodes generally occurred during handling (dosing, weighing, etc.)
[b] Euthanatized
[c] Five euthanatized, one found dead

Table 6

Two Week Oral Rangefinding Study
of T-2509CoC in Rats
Summary of Clinical Observations
Number Affected/Group

Clinical Sign	Dose Group (mg/kg/day)			
	200 ♂	200 ♀	75 ♂	75 ♀
No signs through study	0/12	0/12	1/12	1/12
Treatment-related deaths	12/12	10/12	0/12	1/12
Soft feces	8/12	0/12	2/12	0/12
Tucked-up appearance	11/12	12/12	6/12	6/12
Thin	6/12	11/12	8/12	8/12
Unkempt appearance	4/12	3/12	8/12	6/12
Tonic or clonic/tonic convulsions	6/12	3/12	2/12	1/12
Hyperactive	0/12	1/12	0/12	1/12
Head tremors	0/12	0/12	1/12	0/12
Urinary incontinence	1/12	3/12	0/12	0/12
Respiratory congestion	0/12	0/12	7/12	1/12
Bloody nose and mouth	0/12	1/12	1/12	0/12
Lump on left side	0/12	0/12	1/12	0/12
Hair loss on abdomen	0/12	0/12	0/12	1/12

Table 7

Two Week Oral Rangefinding Study
of T-2509CoC in Rats
Group Mean Body Weights

Dose Group	Study Day							% Change from Day +1	
	-6	+1	6	9	13	20	23	End of Dosing	End of Recovery
200 mg/kg/day ♂	138.5 ± 9.21	177.8 ±13.33	134.2[a] ±19.03	---	---	---	---	-25%	---
200 mg/kg/day ♀	133.0 ± 5.43	165.0 ± 9.28	114.1[a] ±10.25	124.8 ±10.05	154.0 ±14.4	186.5 ±0.71	---	-31%	+13%
75 mg/kg/day ♂	141.3 ± 7.52	174.8 ±13.91	170.9 ±20.23	165.5[b] ±19.39	175.3 ±16.33	224.7 ±17.19	245.7 ±18.58	- 5%	+41%
75 mg/kg/day ♀	133.5 ± 7.69	163.7 ±10.63	143.0 ± 8.32	129.5[b] ±15.16	148.3 ±16.58	182.4 ± 9.03	190.7 ±13.80	-21%	+16%

[a] Dosing discontinued following day 5
[b] Dosing discontinued following day 9

Discussion

Daily gastric intubation of rats with T-2509CoC resulted in treatment-, dosage- and sex-related effects. The primary effects were gastric irritation, handling-induced convulsions and microscopic liver changes. Dosage-related responses to the gastric effects were a tucked-up appearance, an unkempt appearance and soft feces; only the males had soft feces. Treatment-related responses to the gastric effects were thinness and body weight loss during dosing. The females lost more weight than the males. Gross and microscopic stomach irritation which included a few early hemorrhagic gastric ulcers, was present in the high dose group only. Convulsive episodes (CNS stimulation) occurred in more animals in the high dose group than the low dose group and more males than females had convulsions. Less frequent treatment-related CNS effects were head tremors and hyperactivity. Dosage- and sex-related liver toxicity occurred in the form of focal necrosis and fatty changes. The liver changes occurred in more males than females.

Dosage-related deaths were caused by T-2509CoC treatment. More high dose males died than high dose females and the males started dying earlier in the study.

The recovery interval resulted in reversal or cessation of the toxic effects. Gastric lesions were not present in the two surviving high dose females. Gastric pain (tucked-up appearance) continued for the first few days of recovery and a few animals continued to appear unkempt and thin. All recovery animals, however, had body weight gain throughout the recovery interval. The later-occurring deaths in the high dose female group were probably due to a generalized debilitated condition because none of these animals had microscopic changes. Severe delayed convulsions did not occur during recovery. Focal liver necrosis was not present in the recovery animals. The low dose recovery group had more males than females with mild fatty changes in the liver.

Appendix I

Two Week Oral Rangefinding Toxicity Study
of T-2509CoC in Rats
Protocol

TITLE: Protocol for a Two Week Oral Rangefinding Toxicity Study in Rats (Study Number 179RR023)

TEST ARTICLE: A clear, pale yellow solution identified as T-2509CoC. This chemical will be supplied by Toxicology Services, 3M Company, St. Paul, Minnesota.

OBJECTIVE: The primary objective will be to identify any major toxic effects in rats during two weeks of daily gastric intubation of T-2509CoC. Before the doses will be selected to complete the above objective however, the acute oral ALD50 of T-2509CoC in rats will need to be determined. This acute portion will use males only from the same supplier, strain and age range of rats as described below for the repeat dose portion. The general procedures for a Riker Safety Evaluation Method 605A will be used for the acute dosing except that six males/level will be dosed. The sponsor and study director will use the acute data to select the dosage levels for the two week repeat dose study.

At the conclusion of the two week dosing interval, the sponsor and the study director have the option of continuing the observation period for about one-half the males and females at each level. The extended observation period will be two weeks in length and dosing will not occur during that interval.

CONTROL ARTICLE: None

SPONSOR: Toxicology Services, 3M Company, St. Paul, Minnesota

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

DOSING INTERVAL: September-October, 1979

DOSAGE LEVELS, ROUTE, GROUP, SIZE, ETC.: Oral gastric intubation will be used because it appears to be an appropriate route for evaluating the systmic toxicity in rats. The rats will be dosed daily for two weeks (1).

A constant dose volume of 10 ml/kg will be used for each dosage level. Distilled water will be used for all necessary dilutions of the test article.

Appendix I (continued)

Dose Group	Dosage Levels (2)	Group Size
High	(to be selected after the acute	12 ♂, 12 ♀
Low	portion has been completed)	12 ♂, 12 ♀

ANALYTICAL CHARACTERIZATIONS: The sponsor has on file one or more of the following or other pertinent characterizations of the test article: identity, synthesis, strength, purity, stability.

Preparation and measurement records for the various dilutions of the test article will be considered adequate verification of content and concentration of the dosing solutions for this rangefinding study.

TEST SYSTEM: Twenty-four male and 24 female Charles River CD rats, 45-55 days (3) old on dose day 1, will be used for the repeat dose study. The animals will be housed individually in hanging stainless steel cages with wire mesh floors and fronts. The room will be temperature and humidity controlled with the lights on a 12 hour light/dark cycle.

JUSTIFICATION FOR SELECTION OF TEST SYSTEM: Charles River CD rats will be used because of historical in-house data on the strain.

TEST SYSTEM IDENTIFICATION: Each animal will be assigned a number which will be indicated on the outside of the cage and on an individual ear tag.

RANDOMIZATION OF TEST SYSTEM: The animals will be indiscriminately removed from the shipping boxes by Animal Care personnel and placed in the rack of cages from left to right starting at the top and working down. The study director will assign dose groups by vertical rows.

DIET SPECIFICATIONS: Purina Laboratory Chow and water will be available ad libitum throughout the study.

BIOAVAILABILITY OF TEST ARTICLE: During previous acute toxicity studies in rats, systemic absorption of the test article appears to have occurred in the form of delayed CNS effects.

CLINICAL OBSERVATIONS: The animals will be observed daily throughout the dosing interval for evidence of treatment-related toxicity. Body weights will be recorded approximately one week prior to dose day 1, on dose day 1 and biweekly thereafter. Food consumption will be estimated weekly throughout the dosing interval. If animals are retained for two additional weeks following dosing, they will be observed and weighed as indicated above (4).

TISSUE PATHOLOGY: Gross necropsies will be conducted on all rats which die during the study. In animals with autolysis, as a minimum, samples of lung, liver, kidney, brain and any gross lesion will be fixed in formalin. Microscopic examination will determine if the tissues are satisfactory for histologic diagnosis. Any rat in a moribund condition should be terminated to insure satisfactory tissue samples. Approximately 24 hours following the last dose, the selected survivors will be killed for gross necropsy examination. The animals selected for additional observation without dosing, if any are selected, will be killed for gross necropsy examination at the end of that interval. Samples to be fixed in 10% buffered formalin are listed below.

1. Mammary Gland (female)
2. Eyes (2)
3. Thymus
4. Thyroid/Parathyroid/Trachea/Esophagus
5. Lung
6. Heart
7. Liver
8. Adrenals (2)
9. Kidneys (2)
10. Urinary Bladder
11. Testes/Epididymis or Ovaries
12. Uterus or Prostate
13. Spleen
14. Pancreas
15. Stomach
16. Small Intestine (at least two areas)
17. Large Intestine
18. Mesenteric Lymph Node
19. Sciatic Nerve
20. Spinal Cord/Bone Marrow (cervical and thoracic)
21. Brain
22. Pituitary
23. Any Gross Lesion

The tissues to be processed and examined histologically will be selected by the pathologist at the conclusion of the gross necropsy. The tissue selections will be reviewed with the sponsor prior to processing (5).

DATA ANALYSIS AND FINAL REPORT: The appropriate statistical analyses, if any, will be selected at the conclusion of the study. The proposed date for the final report is 1-2 months after slide reading is completed (approximately 2-5 months after necropsy).

Appendix I (concluded)

AMENDMENTS:

1. The last day of dosing for the high dose group (200 mg/kg/day) was day 5 and the last dose day for the low dose group (75 mg/kg/day) was day 9. All surviving high dose animals were observed for approximately two weeks following the last dose. Following completion of dosing at the low dose level the appropriate number of males and females were killed to reach a total of 6/sex dead. The remaining 6/sex were observed for approximately two more weeks.

2. The selected high dose level was 200 mg/kg/day and the selected low dose level was 75 mg/kg/day.

3. Age range for the acute rats extended to 59 days old on the day of dosing.

4. Body weights obtained once or twice a week during recovery rather than biweekly.

5. The tissues processed for histopathological evaluation were liver, adrenals, stomach, kidneys and brain.

Appendix II

Two Week Oral Rangefinding Toxicity Study
of T-2509CoC in Rats
List of Principal Participating Personnel

Name	Function
Marvin T. Case	Veterinary Pathologist
James D. Henderson, Jr.	Staff Veterinarian
Cathy E. Ludemann	Coordinator - Clinical Pathology
Gary C. Pecore	Coordinator - Animal Care
Inara Porietis	Histopathology Technician
Jan H. Skroms	Toxicology Technician
G. Ray Steffen	Study Director

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