UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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CASWELL FILE

JAN 17 1996
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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: TELONE II (1,3-Dichloropropene) - A Review of a Chronic/ Carcinogenicity Rat Study Submitted Under Section 6(a)(2) of FIFRA

PC Code: 029001
DP Barcode: D219112
Case: 818694
Action: 625 6(a)(2)
Caswell No.: 324A
MRID No.: 43763501
Submission: S493560

FROM: Alan C. Levy, Ph.D., Toxicologist Alan C. Levy 12-19-95
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Health Effects Division (7509C)

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Special Review and Reregistration Division (7508W)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head Y M Ioannou 12/19/95
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

and

Stephanie Irene, Ph.D., Acting Branch Chief
Toxicology Branch II
Health Effects Division (7509C) Stephanie P Irene 12/20/95

REQUEST: Review a chronic/carcinogenicity rat study with TELONE II [6(a)(2)]

Registrant: DowElanco, Indianapolis, IN

EXECUTIVE SUMMARY:

In a chronic/carcinogenicity study **(MRID No. 43763501)**, TELONE II (96.0% purity, cis/trans isomers) as microcapsules was administered by dietary admix to Charles River Fischer 344 rats (60/sex/group with 10/sex/group sacrificed at 12 months) at doses of 0, 2.5, 12.5 and 25 mg/kg body weight/day for 2 years.

No test article effects were noted regarding the following parameters: survival, clinical signs, ophthalmoscopy, hematology, blood chemistry, urinalysis or organ weights.

Body weight gains were decreased for males (8 and 21%) and females (15 and 25%) at 12.5 and 25 mg/kg/day compared to controls. Food consumption was decreased in females at 25 mg/kg/day. There was an increase in liver masses/nodules in males only at 12.5 and 25 mg/kg/day. There was an increased incidence of basal cell hyperplasia of the nonglandular mucosa of the stomach of both sexes at the 12- and 24-month sacrifices at 12.5 and 25 mg/kg/day. The number of males with primary hepatocellular adenomas was increased over controls at 12.5 and 25 mg/kg/day (2/50, 1/50, 6/50 and 9/50 for the control, low, mid and high-dose groups, respectively) with the number of females being increased only at 25 mg/kg/day (0/50, 0/50, 0/50 and 4/50 for the control, low, mid and high-dose groups, respectively).

The Systemic Toxicity NOEL = 2.5 mg/kg/day

The Systemic Toxicity LOEL = 12.5 mg/kg/day based on a decrease in body weight gain and an increase in the incidence of basal cell hyperplasia of the nonglandular mucosa of the stomach

TELONE II MAY CAUSE AN INCREASE IN THE NUMBER OF MALES AT 12.5 AND 25 MG/KG/DAY AND THE NUMBER OF FEMALES AT 25 MG/KG/DAY WITH PRIMARY HEPATOCELLULAR ADENOMAS.

The highest dose tested in this study (25 mg/kg/day) was considered adequate to assess the carcinogenic potential of Telone II in rats.

This study is **Acceptable** and satisfies the data requirement §83-5) for a chronic/carcinogenicity study in rats.

The RfD/Peer Review Committee will determine if the HED Cancer Peer Review Committee will consider the carcinogenic potential of TELONE II based on the results of this study.

EPA Reviewer: Alan C. Levy, Ph.D. Alan C. Levy, Date 12-19-95
Review Section I, Toxicology Branch II (7509C)

EPA Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou
Review Section I, Toxicology Branch II (7509C) Date 12-19-95

DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic Toxicity/Carcinogenicity - Dietary Admix in Rats OPPTA 870.4300 (§83-5)

EPA IDENTIFICATION: **PC Code:** 029001 **MRID No.:**43763501
DP Barcode: D219112 **Submission Code:** S493560
Tox. Chem. No.: 324A

TEST MATERIAL: Telone II (microcapsules); 1,3-dichloropropene (cis, trans); purity = 96.0% (50.7% cis/45.1% trans)

SYNONYMS: 1,3-D; DCP

TESTING FACILITY: The Toxicology Research Laboratory
The Dow Chemical Company

TITLE OF REPORT: Telone® II Soil Fumigant: Two-Year Chronic Toxicity/ Oncogenicity Study in Fischer 344 Rats

AUTHORS: W.T. Stott, K.A. Johnson, T.K. Jeffries, et al

REPORT DATA: August 15, 1995

LABORATORY REPORT NO.: M-003993-031

SPONSOR: DowElanco, Indianapolis, IN

EXECUTIVE SUMMARY:

In a chronic/carcinogenicity study, Telone II (96.0% purity, cis/trans isomers) as microcapsules was administered by dietary admix to Charles River Fischer 344 rats (60/sex/group with 10/sex/group sacrificed at 12 months) at doses of 0, 2.5, 12.5 and 25 mg/kg body weight/day for 2 years.

No test article effects were noted regarding the following parameters: survival, clinical signs, ophthalmoscopy, hematology, blood chemistry, urinalysis or organ weights.

Body weight gains were decreased for males (8 and 21%) and females (15 and 25%) at 12.5 and 25 mg/kg/day compared to controls. Food consumption was decreased in females at 25 mg/kg/day. There was an increase in liver masses/nodules in males only at 12.5 and 25 mg/kg/day. There was an increased incidence of basal cell hyperplasia of the nonglandular mucosa of the stomach of both sexes at the 12- and 24-month sacrifices at 12.5 and 25 mg/kg/day. The number of males with primary hepatocellular adenomas was increased over controls at 12.5 and 25 mg/kg/day (2/50, 1/50, 6/50 and 9/50 for the control, low, mid and high-dose groups, respectively) with the number of females being increased only at 25 mg/kg/day (0/50, 0/50, 0/50 and 4/50 for the control, low, mid and high-dose groups, respectively).

The Systemic Toxicity NOEL = 2.5 mg/kg/day

The Systemic Toxicity LOEL = 12.5 mg/kg/day based on a decrease in body weight gain and an increase in the incidence of basal cell hyperplasia of the nonglandular mucosa of the stomach

TELONE II MAY CAUSE AN INCREASE IN THE NUMBER OF MALES AT 12.5 AND 25 MG/KG/DAY AND THE NUMBER OF FEMALES AT 25 MG/KG/DAY WITH PRIMARY HEPATOCELLULAR ADENOMAS.

The highest dose tested in this study (25 mg/kg/day) was considered adequate to assess the carcinogenic potential of Telone II in rats.

This study is **Acceptable** and satisfies the data requirement (§83-5) for a chronic/carcinogenicity study in rats.

COMPLIANCE:

A signed and dated Good Laboratory Practice Compliance statement, a Quality Assurance statement and a list of Quality Assurance inspections were included in the Report.

A signed and dated statement of no confidentiality claim was provided.

A signed and dated "Flagging Statement" was included: "I have applied the criteria of 40 CFR 158.34 for flagging studies of potential adverse effects to the results of the attached study. This study meets or exceeds the criteria numbered 1." [An incidence of neoplasms in male or female animals which increases with dose]

I. MATERIALS AND METHODS

A. Materials

1. TEST MATERIAL:

Name: Telone II; 1,3-dichloropropene (cis and trans isomers); AGR 0295646

Physical State: solid

Description: Telone II in 80% starch/20% sucrose microcapsules 100-400 μm in diameter

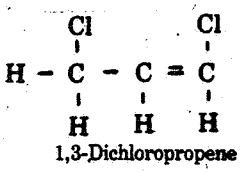
Lot/Batch No.: 9359-1B

Purity: prior to study start, 96.0% (50.7% cis/45.1% trans); "loaded microcapsules were determined to contain 38.7% Telone by weight (Shabrang, 1992)" [Report page 17]

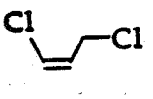
Stability: Report page 29: This was determined in a prior subchronic study and was shown to be stable for at least 21 days at concentrations comparable to those used in the present study (Haut, et al., 1992).

CAS No.: 542-75-6

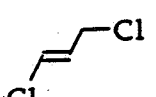
Structure:



1,3-Dichloropropene



cis-



trans-

2. VEHICLE/CONTROL: Control rats received placebo starch/ sucrose microcapsules (Lot No. 9359-1 PB)

3. TEST ANIMALS

Species: rat
Strain: Fischer 344
Age/Weight at Study Initiation: age not stated; group mean weights of 158 g for males and 120 g for females
Source: Charles River Laboratories, Kingston, NY
Housing: males: 2/cage for the first 62 weeks and 1/cage for the remainder of the study; females: 2/cage for the entire study
Diet: Purina Certified Chow #5002 ad libitum
Water: tap, ad libitum
Environmental Conditions: temperature = 17.7-24.3°C; humidity = 36-73%; 12 hour light/dark cycle; air flow = 10-12 changes/hour
Acclimation Period: at least 14 days

B. Study Design

1. IN-LIFE DATES:

Start of dosing: July 2, 1992
12-month interim sacrifice: July 7, 1993
Terminal sacrifice: July 5, 6, 7 and 8, 1994.

2. ANIMAL ASSIGNMENT: Computer randomization based on body weights

Table 1

STUDY DESIGN FOR A TWO-YEAR STUDY IN RATS WITH MICROCAPSULATED TELONE II ADMINISTERED BY DIETARY ADMIX

Test Group	Dose to Rats (mg/kg/day)	Number of Rats/Group			
		Main Study-24 Months		Interim Sacrifice-12 Months	
		Males	Females	Males	Females
Control	0	50	50	10	10
Low	2.5	50	50	10	10
Mid	12.5	50	50	10	10
High	25	50	50	10	10

Data extracted from the Report text, page 19.

3. DOSE SELECTION

Oral LD50 - males = 300 and females = 224 mg/kg [Report page 15]

Two-Week Dietary Probe Study - Male and female Fischer 344 rats were administered microencapsulated Telone II in the diet at targeted doses of 0, 10, 25, 50 and 100 mg/kg/day. Statistically significant "decreases in body weight" in males and females at 50 and 100 mg/kg/day were reported. There was a microscopic thickening of the mucosa of the nonglandular portion of the stomach in both males and females administered 50 and 100 mg/kg/day (also, one 25 mg/kg/day male). The NOEL was 10 mg/kg/day for males and 25 mg/kg/day for females. [Report page 15]

13-Week Study - Fischer 344 rats (10/sex/dose) were given Telone II as a dietary admix at targeted doses of 0, 5, 15, 50 and 100 mg/kg/day. "Body weights of both sexes of rats ingesting Telone II were decreased relative to controls." Basal cell hyperplasia and hyperkeratosis of the non-glandular portion of the stomach were reported for, "a number of males and females" given ≥ 15 mg/kg/day. "The no-observed-adverse-effect level (NOAEL) in males and the NOEL in female rats was 5 mg/kg/day Telone II." [Report page 15]

Chronic Study - In a 1985 NTP study, Telone II, in a corn oil vehicle, was administered at 25 or 50 mg/kg/day (epoxide stabilized formulation) to male and female Fischer 344 rats, 3 days/week, by gavage for up to two years. Hyperplasia and tumors of the nonglandular portion of the stomach were reported for males and females in addition to benign tumors of the liver in males. [Report page 16]

4. DIET PREPARATION AND ANALYSIS

The test article was administered in the diet by mixing a microencapsulated formulation in basal feed. The Report stated (page 20) that concentration checks confirmed the delivery of Telone II to the rats and that, when ingested, the absorption of the microencapsulated material was at least as great as the neat material (W. Stott et al, report in preparation).

Report page 61 (Report Table 3) presented test article purity analyses results. For the three intervals, the percent purity (cis + trans isomers) were: 95.8, 96.9 and 97.6.

Homogeneity of the test article in the diet was determined for the 2.5 mg/kg/day females at four intervals throughout the study. At each interval, samples were analyzed from the top, middle and bottom of side 1, center and side 2. The data were presented in Report Table 4, page 62. Means ± standard deviations (S.D. and % relative S.D.) for the four intervals were: 0.00244 ± 0.000313 (12.83%), 0.00256 ± 0.0000385 (15.04%), 0.00405 ± 0.000377 (9.31%) and 0.00509 ± 0.000504 (9.90%). **Homogeneity data are considered to have been within acceptable limits.**

Concentrations of the test article in the diets were determined at 11 intervals for all three dose levels for males and females as well as for the premix. The mean % of target for all determinations was 96-103. [Report Table 5, pages 63-65] **Concentration data are considered to have been with acceptable limits.**

Appropriate diets were made by combining the premix with basal diet. Based on stability, premixes were mixed at least every two weeks. Premixes were adjusted for 38.7% microencapsulation loading and diet concentrations were calculated on a mg/kg body weight/day basis. Based on the most recent body weight and food consumption, the diets were prepared each week for the first 13 weeks and then adjusted every four weeks thereafter. The control group received the starch/sucrose encapsulation matrix mixed with basal diet in an amount equal to the vehicle in the high-dose group (mixed every four weeks throughout the study).

5. ANIMALS RECEIVED FRESH DIET: weekly

6. STATISTICS (Report pages 27 and 28)

Means and standard deviations were reported for food consumption and leukocyte differential counts.

Body weights, organ weights, clinical chemistry, urine specific gravity and appropriate hematology data were evaluated by Bartlett's test for equality of variances. Dependent upon the result of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance (ANOVA) followed by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons. Statistical outliers were identified by a sequential test, but were routinely excluded only from feed consumption data.

The nominal alpha levels were:

Bartlett's test = 0.01
Parametric ANOVA = 0.10
Nonparametric ANOVA = 0.10
Dunnett's test = 0.05, two-sided
Wilcoxon Rank-Sum test = 0.05, two-sided
Outlier test = 0.02, two-sided

Only the two-year interval histopathological diagnoses were statistically analyzed. The incidences of specific observations, for tissues where all rats in all groups were scheduled to be examined, were first tested for deviation from linearity using ordinal spacings of the doses. If linearity was not rejected, the data were tested for linear trend by the Cochran-Armitage Trend test. If the trend was significant, or if significant deviation from linearity was noted, incidences for each treated group were compared to the control group using a pairwise chi-square test with Yate's continuity correction.

For tissues evaluated from all control and high-dose animals, but only from selected rats in the low- and/or mid-dose groups, statistical analysis consisted of pairwise chi-square test with Yate's continuity correction.

The Gehan-Wilcoxon procedure was used to test for differences in mortality patterns for all rats scheduled for terminal sacrifice. No mortality adjustments were made in the statistical analysis as no association between mortality and dose level was noted.

C. Methods

1. OBSERVATIONS: Cageside observations were made at least twice each day for morbidity, moribundity, death and clinical signs. Weekly clinical examinations were performed throughout the study.

2. BODY WEIGHTS: These were recorded prestudy, weekly for the first 13 weeks and at about monthly intervals until study termination.

3. FOOD CONSUMPTION: This was calculated prior to the start of the study, weekly for 13 weeks and for a one-week period each month for the remainder of the study. Values were expressed as g/rat/day. Compound intake (mg/kg/day and ppm) as well as feed efficiency (g of feed/kg body weight/day) were calculated.

4. OPHTHALMOSCOPIC EXAMINATION: Examinations were conducted on all rats prior to the start of the study (penlight illumination) and at the 1-year and 2-year scheduled sacrifices (moistened slide/fluorescent light technique).

5. BLOOD COLLECTION: Samples were obtained from the orbital sinus of fasted rats lightly anesthetized with methoxyflurane. Hematology and clinical chemistry parameters were examined at about 6 and 12 months from the 10/sex/group scheduled for the 12-month sacrifice and from the first 10 and 20/sex/group from those scheduled for the 24-month sacrifice (10/sex/group after about 18 months; 20/sex/group at about 24 months).

a. Hematology

xHematocrit (HCT)*
xHemoglobin (HGB)*
xLeukocyte count (WBC)*
xErythrocyte count (RBC)*
xPlatelet count*
-Blood clotting (thromboplastin time, clotting time, prothrombin time)
xLeukocyte differential count*
-Mean corpuscular HGB (MCH)
-Mean corpus. HGB conc. (MCHC)
-Mean corpus. volume (MCV)
-Reticulocyte count

* = EPA Guideline Requirement - = Not examined

b. Clinical Chemistry

Electrolytes
xCalcium*
xChloride*
-Magnesium
xPhosphorus*
xPotassium*
xSodium*

Enzymes
xAlkaline phosphatase (ALK)
-Cholinesterase (ChE)
xCreatine phosphokinase*
-Lactic acid dehydrogenase (LDH)
xSerum alanine aminotransferase (SGPT)*
xSerum aspartate aminotransferase (SGOT)*
-Gamma glutamyl transferase (GGT)
-Glutamic dehydrogenase

Other
xAlbumin*
xBlood creatinine
xBlood urea nitrogen*
xTotal cholesterol*
xGlobulins
xGlucose*
xTotal bilirubin*
xTotal serum protein (TP)*
xTriglycerides
-Serum protein electrophoresis

* = EPA Guideline Requirement - = Not examined

6. URINALYSIS: Samples were obtained by manual compression of the abdomen of nonfasted rats. Urine was examined from the 12-month sacrifice animals, 10/sex/group after about 6 and 12 months. For those animals scheduled for the 24-month sacrifice, urine was obtained after about 18 months from 10/sex/group and after about 24 months from 20/sex/group.

xApppearance*	xGlucose*
-Volume*	xKetones*
xSpecific gravity*	xBilirubin
xpH	xBlood*
xSediment (microscopic)*	-Nitrate
xProtein*	xUrobilinogen

* = EPA Guideline Requirement - = Not examined

7. SACRIFICE AND PATHOLOGY: Necropsies were performed on methoxyflurane anesthetized rats (overnight fast) scheduled for sacrifice at 12 months (10/sex/group) and at 24 months (all survivors). All found dead and moribund sacrifice animals were also necropsied. Organ weights were expressed as absolute and relative-to-body weight. The following tissues were examined (x) and organs were weighed (xx):

Digestive	Respiratory	Urogenital
xTongue	xTrachea*	xxKidneys*
xSalivary glands*	xLungs*	xUrinary bladder*
xEsophagus*	xNose	xxTestes*
xStomach*	-Pharynx	xEpididymides*
xDuodenum*	xLarynx	xProstate*
xJejunum*		xSeminal vesicles*
xIleum*	Cardiov/Hemat	xxOvaries*
xCecum*	xAorta*	xUterus*
xColon*	xxHeart*	xCervix
xRectum*	xBone marrow*	xOviducts
xxLiver*	xLymph nodes*	xVagina
xPancreas*	xSpleen*	
	xThymus*	

Neurologic	Glandular	Other
xxBrain*	xxAdrenals*	xBone*
xPeripheral nerve*	xLacrimal gland	xSkeletal muscle*
xSpinal cord (3 levels)*	xMammary gland*	xSkin*
xPituitary*	xParathyroids*	xAll gross lesions and masses*
xEyes (optic n.)*	xThyroids*	xCoagulating glands
		xOral tissue

* = EPA Guideline Requirement

II. RESULTS

A. Observations

1. TOXICITY (Report Tables 9 and 10, pages 70-72)

There were no clinical signs which appeared to be related to test article administration.

2. MORTALITY (Report Tables 6 and 7, pages 66 and 67)

Of the 50 rats/sex/group scheduled for the 24-month terminal sacrifice, the following mortality occurred:

Table 2

MORTALITY IN A 24-MONTH DIETARY ADMIX RAT STUDY WITH TELONE II

Males (mg/kg/day)				Females (mg/kg/day)			
0	2.5	12.5	25	0	2.5	12.5	25
13(26)a	16(32)	16(32)	16(32)	11(22)	15(30)	19(38)	11(22)

a = number died out of 50 (% mortality)
Data extracted from Report Tables 6 and 7, pages 66 and 67.

Mortality for males was similar for all treated and control groups (26-32%). The 12.5 mg/kg/day female group had a greater mortality than did the control group (38% versus 22%). However, at 25 mg/kg/day, mortality was the same as in the control group.

B. Body Weight

There were statistically significant ($p<0.05$) lower group mean body weights in males at 12.5 and 25 mg/kg/day throughout the study. Body weight gains for this sex were decreased 8% at 12.5 mg/kg/day and 21% ($p<0.05$) at 25 mg/kg/day over the 24-month period of dosing.

In females, there was a lower group mean at 12.5 mg/kg/day during the latter 6 months of the study. The 25 mg/kg/day rats of this sex had group means less than controls ($p<0.05$) throughout the study. For the 24 months, the 12.5 and 25 mg/kg/day females had body weight gains 15 and 25% less ($p<0.05$) than controls, respectively.

Table 3

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A 24-MONTH DIETARY ADMIX RAT STUDY WITH TELONE II

Week	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2.5	12.5	25	0	2.5	12.5	25
BODY WT-g								
0a	158	158	158	157	120	121	120	120
13	307	304	297*	280*	185	184	181	174*
25	357	350	336*	318*	203	198	196	188*
53	416	412	390*	355*	219	220	216	204*
65	419	414	390*	360*	233	232	230	215*
77	423	418	390*	363*	259	256	253	232*
93	405	401	375*	352*	283	278	264*	244*
104	384	374	364	335*	285	281	260*	244*
B.W. gain								
0-13 g	150	146	140*	123*	65	64	61	54*
%	-	-3	-7	-18	-	-2	-6	-17
0-53 g	258	254	232*	198*	99	99	96	84*
%	-	-2	-10	-23	-	0	-3	-15
0-104 g	227	216	208	179*	165	159	141*	124*
%	-	-5	-8	-21	-	-4	-15	-25

Number of rats/sex/group: weeks 0-53 = 60; weeks 53-104 = 50 ("52" week interim sacrifice of 10/sex/group)
a = week 0 is day -3
Data extracted from Report Tables 11-14, pages 73-90.

C. Food Consumption and Compound Intake

1. FOOD CONSUMPTION (Report Tables 15 and 16, pages 91-96)

Report page 32 (Feed Consumption and Dosages Delivered) stated, "In general, feed consumption values for high dose group male and female rats were decreased relative to controls consistent with their lower body weights." For males, during the first 60 weeks (418 days) of the study, the group mean food consumption for rats in the 25 mg/kg/day group was similar to the control value. During the remaining 44 weeks, the 25 mg/kg/day group mean food consumption was similar to the first 60 weeks; whereas, the control group mean was greater during the last 44 weeks than during the previous 60 weeks. In 25 mg/kg/day females, food consumption was less than in the control group throughout most of the study. Report page 32 also indicated that the 25 mg/kg/day males and females, "... were noted to waste (scratch) feed to a greater extent than controls, sporatically, over much of the dosing period."

2. COMPOUND CONSUMPTION

Report Table 17, pages 97-99, presented group mean mg/kg/day of test article consumption for all three dose levels (males and females) during weeks 1-13, and every four weeks thereafter (104 weeks). Group means (target of 2.5, 12.5 and 25 mg/kg/day) for the entire study were as follows (mg/kg/day): males = 2.5, 12.7 and 25.4; females = 2.5, 12.7 and 24.8.

Report Table 18, pages 100 and 101, presented group mean ppm. For the 2.5, 12.5 and 25 mg/kg/day dose levels, the ppm ranges were as follows: males = 33-65, 163-323 and 319-622; females = 25-59, 145-283 and 281-578.

3. FOOD EFFICIENCY (Report Tables 19 and 20, pages 102-107)

For the 25 mg/kg/day males, the food efficiency values (g feed/kg body weight/day) were generally higher than the controls (lower body weight gain). Female food efficiency values for all four groups were similar throughout the study.

D. Ophthalmoscopic Examination (Report Table 8, pages 68 and 69)

There were no findings which appeared to have been due to test article administration.

E. Blood Work

1. HEMATOLOGY (Report Tables 21-44, pages 108-143)

There were no parameters at any intervals of either sex which were considered to have been effected by the administration of Telone II.

2. CLINICAL CHEMISTRY (Report Tables 61-76, pages 169-184)

The only parameter which may have been altered by test article administration was group mean triglyceride levels which were below control values. Although there were decreases ($p<0.05$) in group mean values at 25 mg/kg/day for both males and females at more than one interval, the toxicological significance of these observations is unclear and may have been due to decreased body weight gain.

Table 4

GROUP MEAN TRIGLYCERIDE LEVELS (mg/dl) IN A 24-MONTH DIETARY ADMIX RAT STUDY WITH TELONE II

Interval (months)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2.5	12.5	25	0	2.5	12.5	25
6	109±26a	135±31*	96±15	98±17	84±18	79±12	71±10	66±13*
12	75±19	63±14	64±16	48±11*	60±31	46±6	43±8	47±13
18	111±43	107±38	171±258b	82±42	177±87	143±53	113±43*	74±34*
24	152±62	176±119	116±60*	112±67*	257±83	255±165	236±115	148±74*

a = group mean ± S.D.
Number of rats = months 6, 12 and 18 is 10/sex/group; month 24 is 20/sex/group
Statistical Significance: * = $p<0.05$
b = one value of 898; all others, 31-148
Data extracted from Report Tables 61-68, pages 169-176.

F. Urinalysis (Report Tables 45-60, pages 144-168)

There were no apparent test article effects on any of the parameters examined.

G. Sacrifice and Pathology

1. ORGAN WEIGHTS (Report Tables 77-80, pages 185-188)

12-Month Sacrifice: The only effect appeared to be an increase in some relative organ weights in the 25 mg/kg/day rats of both sexes. This is considered to have been due to lower group mean body weights at this dose.

24-Month Sacrifice: There were increases in some relative organ weights over respective control values for both males and females administered the test article at 25 mg/kg/day. This is probably due to lower terminal body weights.

2. GROSS PATHOLOGY (Report Tables 81 and 82, pages 189-210)

The only tissue which may have had lesions at a greater incidence than control was the liver.

Table 5

INCIDENCE OF GROSSLY OBSERVABLE LIVER LESIONS IN A 24-MONTH DIETARY ADMIX RAT STUDY WITH TELONE II

Observation	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2.5	12.5	25	0	2.5	12.5	25
Number of Rats Examined	50	50	50	50	50	50	50	50
Mass/Nodule (one)	2	2	4	7	0	0	1	1
(two)	0	1	2	0	0	0	0	0
(three) ...	0	0	0	1	0	0	0	0
Total	2	3	6	8	0	0	1	1

Data extracted from Report Table 82, page 202.

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3. MICROSCOPIC PATHOLOGY

a) Non-Neoplastic

(1) 12-Month Interim Sacrifice (Report Table 83, pages 211-224)

Basal cell hyperplasia of the nonglandular mucosa of the stomach was the only finding which was considered to have been related to test article administration. This observation was absent in controls of both sexes but was present in males and females at 12.5 and 25 mg/kg/day.

Table 6

THE INCIDENCE OF BASAL CELL HYPERPLASIA OF THE NONGLANDULAR MUCOSA OF THE STOMACH AT THE 12-MONTH INTERIM SACRIFICE IN A 24-MONTH DIETARY ADMIX RAT STUDY WITH TELONE II

Observation	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2.5	12.5	25	0	2.5	12.5	25
Number of Rats Examined	10	10	10	10	10	10	10	10
Very slight	0	1	5	6	0	0	2	7
Slight	0	0	2	4	0	0	1	2
Any severity	0	1	7	10	0	0	3	9

Data extracted from Report Table 83, page 222.

(2) 24-Month Terminal Sacrifice (Report Table 84, pages 225-270)

The incidence of basal cell hyperplasia of the nonglandular mucosa of the stomach was increased over controls in both sexes at 12.5 and 25 mg/kg/day.

Table 7

THE INCIDENCE OF BASAL CELL HYPERPLASIA OF THE NONGLANDULAR MUCOSA OF THE STOMACH AT THE 24-MONTH TERMINAL SACRIFICE IN A 24-MONTH DIETARY ADMIX RAT STUDY WITH TELONE II

Observation	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2.5	12.5	25	0	2.5	12.5	25
Number of rats examined	50	50	50	50	50	50	50	50
Very slight	3	3	19*T	18*T	0	1	19*T	33*T
Slight	0	0	1	12*	0	0	1	4
Any severity	3	3	20*	30*T	0	1	20*	37*T

* = Statistically identified difference from control mean by Yate's Chi-Square pairwise test, Alpha = 0.10, two-sided. Alpha = 0.05, one-sided
T = Linear trend by Cochran-Armitage Trend test, Alpha = 0.02,two-sided, Alpha = 0.01, one-sided.
Data extracted from Report Table 84, page 264.

In the liver, the incidence of eosinophilic and/or basophilic foci appeared to be effected by Telone II administration.

Table 8

THE INCIDENCE OF EOSINOPHILIC AND/OR BASOPHILIC FOCI (OF ALTERED CELLS) IN THE LIVER AT THE 24-MONTH TERMINAL SACRIFICE IN A 24-MONTH DIETARY ADMIX RAT STUDY WITH TELONE II

Observation	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2.5	12.5	25	0	2.5	12.5	25
Number of rats examined	50	50	50	50	50	50	50	50
Basophilic, very slight	24	23	28	29	8	9	13	7
slight	13	11	4*	1*T	19	19	21	19
moderate	0	1	0	0	16	14	9	14
any severity	37	35	32	30	43	42	43	40
Eosinophilic, very slight	29	25	18*	11*T	12	24*	20	32*T
slight	3	11*	23*	24*T	0	3	3	1
moderate	0	0	2	1	0	0	0	0
any severity	32	36	43	36	12	27*	23*	33*T

* = Statistically identified difference from control mean by Yate's Chi-Square pairwise test, Alpha = 0.10, two-sided, Alpha = 0.05, one-sided.
T = Linear trend by Cochran-Armitage Linear Trend test, Alpha = 0.02, two-sided, Alpha = 0.01, one-sided.
No. of foci: very slight = 1-5; slight = 6-15; moderate = >15
Data extracted from Report Table 84, pages 239 and 240.

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b) Neoplastic

(1) 12-Month Interim Sacrifice (Report Table 85, page 271)

There were no increased incidences over controls in the number of rats with neoplasms in Telone II treated groups.

(2) 24-Month Terminal Sacrifice (Report Table 86, pages 272-281)

The incidence of hepatocellular adenomas appeared to be increased in males at 12.5 and 25 mg/kg/day and in females at 25 mg/kg/day. [One 25 mg/kg/day male had a primary hepatocellular carcinoma.]

No historical control data were provided for comparison.

Table 9

THE INCIDENCE OF PRIMARY HEPATOCELLULAR ADENOMAS AT THE 24-MONTH TERMINAL SACRIFICE IN A 24-MONTH DIETARY ADMIX RAT STUDY WITH TELONE II

Observation	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2.5	12.5	25	0	2.5	12.5	25
Number of rats examined	50	50	50	50	50	50	50	50
(one)	1	1	3	8*T	0	0	0	4
(two)	1	0	2	1	0	0	0	0
(three)	0	0	1	0	0	0	0	0
Total	2	1	6	9*T	0	0	0	4T

* = Statistically identified difference from control mean by Yate's Chi-Square Pairwise test, Alpha = 0.10, two-sided, Alpha = 0.05, one-sided.
T = Linear trend by Cochran-Armitage Linear Trend test, Alpha = 0.02, tow-sided, Alpha = 0.01, one-sided.
Data extracted from Report Tables 84 and 86, pages 242 and 273.

III. DISCUSSION

Analytical data for test article stability, homogeneity and concentration were considered to have been within acceptable limits.

No clinical signs were attributed to test article administration.

For males, mortality was similar for all four groups (26% for controls and 32% for all three treated groups). Regarding females, the percent mortality was as follows (mg/kg/day): 0 = 22, 2.5 = 30, 12.5 = 38 and 25 = 22. It is not considered that the administration of Telone II had an effect on mortality.

Group mean body weight gains over the 2-year period were decreased in males administered 12.5 and 25 mg/kg/day (-8 and -21%, respectively). In females, there were decreases in group mean weight gains of 15 and 25% (for the 2-year period) for the 12.5 and 25 mg/kg/day groups, respectively. For both males and females administered 2.5 mg/kg/day, the group means were not greater than -5% from respective control means.

The Study Authors considered food consumption to be reduced for both sexes at 25 mg/kg/day which was said to be consistent with decreased body weights (compared with controls). For the first 60 weeks, consumption by 25 mg/kg/day males was similar to controls; whereas, during the last 44 weeks, it was less than controls, but similar to consumption during the first 60 weeks. This appeared to be due to an increase in food consumption by control males during this 44-week period. For 25 mg/kg/day females, consumption was less than controls throughout the 104 weeks. In addition, the Report noted that 25 mg/kg/day rats of both sexes wasted ("scratched") feed sporadically over the course of the study.

Group mean compound intakes (mg/kg/day) over the entire 104-week dosing period for the 2.5, 12.5 and 25 mg/kg/day groups were as follows: males = 2.5, 12.7 and 25.4; females = 2.5, 12.7 and 24.8. PPM ranges for the above dose groups were: males = 33-65, 163-323 and 319-622; females = 25-59, 145-283 and 281-578.

Food efficiency (g of feed/kg body weight/day) for 25 mg/kg/day males was generally higher than in the control group, probably due to a decrease in body weight gain. For females, all four groups had similar food efficiency values for the entire study.

There were no apparent effects of test article administration on ophthalmoscopic parameters.

No hematology parameters were considered to have been altered by test article administration.

Although group mean triglyceride levels were below respective control values for the 12.5 and 25 mg/kg/day rats of both sexes, the toxicological significance is not clear and may have been due to a decrease in body weight gain.

There were no apparent test article effects on any urinalysis parameters.

Some relative organ weights in 25 mg/kg/day group males and females were greater than respective control values at both the 12- and 24-month sacrifices. These were considered to have been due to lower group mean body weights.

The only grossly observable change at the terminal sacrifice (50/sex/group) was an increase in the incidence of males with liver masses/nodules in groups administered 12.5 and 25 mg/kg/day. Animals were designated as having one, two or three masses/nodules. The control male group had two rats with this observation; whereas, at 2.5 12.5 and 25 mg/kg/day, the numbers of rats were 3, 6 and 8, respectively. For females, the 0, 2.5, 12.5 and 25 mg/kg/day groups had 0, 0, 1 and 1 animal with this finding.

Microscopic tissue examination showed an increase in incidence and severity (absence, very slight or slight) of basal cell hyperplasia of the nonglandular mucosa of the stomach at both sacrifices (both sexes). At the 12-month interim sacrifice, the "any severity" incidences (out of 10/sex/group) for the 0, 2.5, 12.5 and 25 mg/kg/day groups were: males = 0, 1, 7 and 10; females = 0, 0, 3 and 9. No statistical significance was indicated in the Report. At the 24-month sacrifice, the "any severity" incidences (50/sex/group) for the dose groups were: males = 3, 3, 20 and 30; females = 0, 1, 20 and 37. Statistical significance was reported for the 12.5 and 25 mg/kg/day groups of both sexes. The severity (very slight and slight) was the same at both the 12- and 24-month sacrifices.

The hyperplasia of the non-glandular mucosa of the stomach, as reported in this study, was similar to the findings in an NTP gavage study and 2- and 13-week studies conducted by the Registrant (Report page 50). In this current study (dietary admix, microencapsulated), no stomach tumors were reported, as compared with the NTP gavage study.

There were statistically significant differences in the incidence of males and females at the 24-month terminal sacrifice regarding eosinophilic and/or basophilic foci of altered liver cells. Report pages 45 and 46 present comments pertaining to these foci:

> "Foci of altered cells in the liver are very common in untreated aged Fischer rats where the incidence may approach 100% of rats after 24 months on study (Eustis et al., 1990). Stereologic techniques indicate that control F344 rats of this age have highly variable numbers of foci that range from 431 to 1865 for male livers and 727 to 1654 for female livers (Popp et al., 1985). The relationship of foci of altered cells to neoplasia is unclear. Foci are sometimes increased in short term studies of potent hepatocarcinogens and are considered by some to be preneoplastic lesions. However, other studies indicate that they are reversible lesions while still others suggest that they are non-neoplastic end stage lesions (Goodman et al., 1994). The liver foci noted in the present study appear to be late events. As expected, few foci were present at the 12-month interim necropsy; in fact, treated rats had fewer foci than controls at that time."

"In the study reported herein, liver foci were categorized and sub-categorized per tinctorial staining properties and numerical semi-quantitation. Those sub-categories that showed a statistical increase at all three dose levels - namely eosinophilic altered foci of hepatocytes - slight (males) or all combined subcategories (females) were considered of equivocal toxicologic significance for the following reasons:

a) There is a wide variation in the historical background occurrence of this type of finding in the F344 rat liver, with an incidence approaching 100% in aged rats,

b) The method used for categorization and sub-categorization of this finding is necessarily arbitrary and subject to differing methodology,

c) There was an unusually low incidence rate noted for this finding in the concurrent control group of female rats (12/50 vs. 29/50 in a control group of a contemporary study),

d) This finding of foci of altered hepatocytes, when categorized and sub-categorized per tinctorial appearance and numerical count had some categories showing statistical increases and other categories showing statistical decreases,

e) There was a lack of any clear treatment related effect in those severity categories (i.e., moderate or greater) that would indicate the occurrence of a definitive finding."

No increased incidences in neoplastic findings over respective control values were reported for treated rats (10/sex/group) sacrificed after 12 months. The number of 24-month sacrifice males and females (50/sex/group) with primary hepatocellular adenomas (one/rat, two, three and total number) was statistically significantly greater (Yate's Chi Square and Linear Trend for males and Linear Trend for females) in the 25 mg/kg/day groups (9 total for males versus 2 for controls and 4 total for females versus 0 for controls) compared with respective controls. Although not statistically significant, males at 12.5 mg/kg/day had a larger number of rats with this observation (total) than did the control group (6 versus 2). No females at the 0, 2.5 or 12.5 mg/kg/day doses had these tumors.

Report pages 43,44 and 45 present comments pertaining to these findings:

"Male rats ingesting the high dose level of 25 mg/kg/day TELONE II had a slight increase in lesions diagnosed as benign liver tumors, hepatocellular adenomas. These lesions, categorized as benign tumors, consisted of slow growing groups of well-differentiated hepatocytes that were difficult to separate from the non-neoplastic lesions. Most hepatocellular adenomas were noted as incidental observations in rats euthanized after 24-months on study. All of the hepatocellular adenomas present in rats dying spontaneously were also regarded as incidental tumors, not contributory to death. A single hepatocellular carcinoma was observed in this study. It was noted as a nonfatal tumor in a male rat from the group ingesting 25 mg/kg/day TELONE II Soil Fumigant that survived to the termination of the 24-month study."

"Pairwise statistical analysis indicated that the incidence of hepatocellular adenoma was increased only in the group of males ingesting the high dose level of 25 mg/kg/day TELONE II Soil Fumigant. The incidence of hepatocellular adenoma noted in the males at the intermediate dose level of 12.5 mg/kg/day was not statistically different from the concurrent control group, but the incidence (6/50 vs. 2/50 in the controls) suggests a possible effect at this dose level. At the low dose level of 2.5 mg/kg/day TELONE II in the males, the incidence was 1/50 vs. 2/50 in the controls, indicating the absence of any treatment related increase in hepatocellular adenomas."

"The incidence of hepatocellular adenomas in the female rats ingesting 0, 2.5, 12.5 or 25 mg/kg/day TELONE II was 0/50, 0/50, 0/50 and 4/50. Although a linear trend test indicated a statistically significant increase, there was no statistical difference by pairwise comparison. The historical background incidence for hepatocellular adenomas in recent studies conducted at the same testing facility with F344 female rats varies from 0 to 6%. Thus, the response in the female rat liver is considered equivocal."

"These tumor results are similar to those reported previously for rats gavaged with an older formulation of TELONE II (NTP, 1985). In the NTP study, rats were gavaged three times per week with either 25 mg/kg or 50 mg/kg TELONE II in corn oil. Thus the targeted (total) weekly doses were 75 and 150 mg/kg TELONE II in the NTP study as compared to 87.5 mg/kg and 175 mg/kg TELONE II in the current dietary study reported herein. In the NTP study, 1/52 (1.9%) control male rats had a hepatic neoplastic nodule; 6/52 (11.5%) males administered the lower dose had a neoplastic nodule; and 7/52 (13.5%) of the rats administered the high dose had a neoplastic nodule with an additional rat having an hepatocellular carcinoma. The incidence at both dose levels was statistically identified as increased. Neoplastic nodule was a term in use at the time of the NTP study based upon a scheme of classification of proliferative liver lesions (Squire and Levitt, 1975). It was basically the diagnostic equivalent of a hepatocellular adenoma (Goodman et al., 1994) although there may have been some confusion with nodular, non-neoplastic proliferative lesions of the liver secondary to atrophy, degeneration or necrosis. In the current study, nodular non-neoplastic lesions in livers damaged by other processes (i.e., leukemic infiltrate or heart failure) were termed regenerative hyperplasia. In the NTP study, the report states that similar lesions were classified as nodular hyperplasia to distinguish them from neoplastic nodules. However, the term nodular hyperplasia could not be found in the tabulated data from the NTP study."

"As in the current study, female rats given the high dose level of 50 mg/kg TELONE II three times weekly in the NTP study had a slightly increased incidence of hepatic neoplastic nodules but this was not statistically identified and was considered not to be related to TELONE II ingestion."

IV. CONCLUSIONS

In a chronic/carcinogenicity study, Telone II (96.0% purity, cis/trans isomers) as microcapsules was administered by dietary admix to Charles River Fischer 344 rats (60/sex/group with 10/sex/group sacrificed at 12 months) at doses of 0, 2.5, 12.5 and 25 mg/kg body weight/day for 2 years. The following parameters were examined: survival, clinical signs, body weight, food consumption, food efficiency, ophthalmoscopic, hematology, blood chemistry, urinalysis, macroscopic pathology, organ weights and microscopic pathology.

No test article effects were noted regarding the following parameters: survival, clinical signs, ophthalmoscopy, hematology, blood chemistry, urinalysis or organ weights.

Body weight gains were decreased for males (8 and 21%) and females (15 and 25%) at 12.5 and 25 mg/kg/day compared to controls. Food consumption was decreased in females at 25 mg/kg/day. There was an increase in liver masses/nodules in males only at 12.5 and 25 mg/kg/day. There was an increased incidence of basal cell hyperplasia of the nonglandular mucosa of the stomach of both sexes at the 12- and 24-month sacrifices at 12.5 and 25 mg/kg/day. The number of males with primary hepatocellular adenomas was increased over controls at 12.5 and 25 mg/kg/day (2/50, 1/50, 6/50 and 9/50 for the control, low, mid and high-dose groups, respectively) with the number of females being increased only at 25 mg/kg/day (0/50, 0/50, 0/50 and 4/50 for the control, low mid and high-dose groups, respectively).

The Systemic Toxicity NOEL = 2.5 mg/kg/day

The Systemic Toxicity LOEL = 12.5 mg/kg/day based on a decrease in body weight gain and an increase in the incidence of basal cell hyperplasia of the nonglandular mucosa of the stomach

TELONE II MAY CAUSE AN INCREASE IN THE NUMBER OF MALES AT 12.5 AND 25 MG/KG/DAY AND THE NUMBER OF FEMALES AT 25 MG/KG/DAY WITH PRIMARY HEPATOCELLULAR ADENOMAS.

The highest dose tested in this study (25 mg/kg/day) was considered adequate to assess the carcinogenic potential of Telone II in rats.

This study is **Acceptable** and satisfies the data requirement (§83-5) for a chronic/carcinogenicity study in rats.