RS-160J
TXR-6591

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

2/11/88 006591

FEB 11 1

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review a 2-Generation Inhalation Reproduction Study in Fisher 344 Rats with Telone II

Caswell No. 324 A Tox. Proj. 7-0987
EPA No. 464-511

FROM: Sidney J. Stolzenberg, Ph.D.
Review Section V
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Lois A. Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Quang Q. Bui, Ph.D., Acting Head
Review Section V
Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Dow Chemical Company
Midland, MI 48640

Action Requested:

Special review of a 2-generation rat reproduction study in support of the use of Telone II as a soil fumigant.

Recommendations:

1. Based on the data in the present study, we are not able to assign a NOEL or LEL for systemic toxicity to F_0 and F_1 parent males and females. In two

1 of 36

previous studies submitted by the applicant, increased liver and kidney weights in rats were observed following only 12 weeks of exposure by inhalation at 5 ppm (LDT) in one study and 50 ppm, in the second. It would therefore have been prudent to measure organ weights in the present 2 generation reproduction study. Liver and kidney weight increases are more sensitive indicators of systemic toxicity than the parameters measured. In a 6-month rat inhalation study submitted by the applicant, cloudy swelling of renal epithelium was seen at a 3 ppm dose. Organ weight data, if available, must be submitted.

2. Tentatively, the NOEL for reproductive effects found in this study was $\geq$ 90 ppm (HDT). Conception indices of females were somewhat low, ranging between 73 and 86 % in the four F_1 and F_2 generations. A table of historical control data on reproductive indices for this strain of rat at the applicant's testing facility would be useful.

3. The applicant concluded that the stomach lesions observed in the 90 ppm group was the result of general stress and was not a compound effect. We do not accept this conclusion.

   In the present 2-generation reproduction study, rats exposed to 10 and 30 ppm as well as controls were subjected to the same stress or handling as the 90 ppm group but failed to develop stomach lesions.

   These stomach lesions were limited entirely to the nonglandular region, mainly the mucosa but it sometimes included the submucosa. In the Introduction, page 8 of the report, the applicant claimed, "Macromolecular binding was noted to occur in the stomach with roughly four- and five-fold less occurring in the liver, kidney, and bladders . . . (of rats)." It has also been reported by the applicant that most binding occurred in the nonglandular stomach, which was about twice as high as that seen for glandular stomach. Data from previous organ distribution studies indicate that in rodents, binding of Telone by the non-glandular stomach is twice as high or greater than that observed in glandular stomach.

Telone II is known to be a dermal and eye irritant and is a highly reactive compound. It should not be surprising that tissues showing preferential uptake and retention of this compound, such as the non-glandular tissue of the stomach, would develop lesions. In fact, with both rat and mouse carcinogenicity tests, the stomach is a highly susceptible target organ for tumor development.

4. In nasal tissue of exposed adults, hyperplasia of respiratory epithelium and focal degeneration of olefactory tissue were seen at 90 ppm (HDT). Males and females were equally sensitive.

5. Decreased body weight gain was seen in males and females exposed at 90 ppm. Males were more sensitive to this effect.

6. There are discrepancies in the data of Tables 17 and 18 in volume 1, pages 50 and 51 of the report. The values for N (number of litters) are surprisingly low for body weight and body weight gain of the dams on day 21 of pregnancy compared to all other time periods. In tables 19 and 20 (pages 52 and 53), for body weight and body weight gain of the same animals during lactation, the values of N are much higher and correspond more to such values during the earlier part of the gestational period seen in Tables 17 and 18. The applicant should be requested to address these discrepancies and submit a full explanation together with data to support the explanation.

Core Classification:

Supplementary.

This classication may be upgraded if the applicant provides an acceptable explanation for the discrepancy cited above.

Background Information:

The following information was obtained from the Introduction and Purpose section of the present submission and from EPA summaries of results of previous studies.

Human exposure limits (American Conference of Government Hygienists) are as follows:

Threshold limit value: 1 ppm
Short-term excursion limit: 10 ppm

Tumors due to oral treatment with telone were seen in forestomach, liver, and urinary bladder of rats and mice, also in adrenal and thyroid of rats, in NTP studies conducted at Frederick Cancer Research Center. Increased incidence of fibrosarcomas was seen in mice after S.C. injection for 538 days. In addition, there is mutagenicity concern based on the outcome of tests with Telone or 1,3-dichloropropene in bacteria, drosophila, and mammalian cells (see report by K.C. Dearfield of November 25, 1987). Increased liver and kidney weights were seen in rats after exposure by inhalation for 13 weeks. Cloudy swelling of renal tubular epithelium was seen in rats, exposed by inhalation at 3 ppm for 6 months. In the 2-year rat carcinogenicity study cited above, increased incidence of kidney nephropathy was seen.

006591

Primary Reviewer: Sidney Stolzenberg, Ph.D.
Section V, Toxicology Branch (TS-769C)
Secondary Reviewer: Quang Q. Bui, Ph.D.
Section V, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Reproduction; Two-Generation, Inhalation

Species: Rat
Guidelines: 83-4

Study Title: Telone II Soil Fumigant: Two-Generation Inhalation Reproduction Study in Fischer 344 Rats

EPA ID Nos.: EPA ID No. 464-511
EPA Accession No. 403124-01
EPA Record No. 202022
Caswell No. 324 A
Project No. 7-0987

Sponsor: Dow Chemical Company
Midland, MI 48674

Testing Laboratory: Mammalian and Environmental Toxicological Research Laboratory
Health and Environmental Sciences
Dow Chemical Company
Midland, MI

Study No.: M-003993-015

Study Date: July 13, 1987

Study Authors: Breslin, W.J.; Kirk, H.D.; Streeter, C.M.; Quast, J.F.; Szabo, J.R.

Quality Assurance:

A statement of compliance with GLP is signed by D.G. Keyes at Dow Chemical Company.

Animal Used:

Fisher 344 from Charles River in Kingston, NY, were delivered at about 4 weeks of age and exposures to Telone II (F_0 parents) started at about 6 weeks of age.

Test Compound:

Telone II soil fumigant, Lot NO. TB831213-4, with 91.2% minimum cis-trans 1,3-dichloropropene. Impurities comprised of

PRODUCT IMPURITY INFORMATION IS NOT 006591 INCLUDED

[redacted]

Vehicle:

None. Liquid Telone II was vaporized by preheating compressed air at somewhat less than 70 °C.

Targeted Dose: 0, 10, 30, and 90 ppm, 6 hours per day. Air flow was about 2500 L/min, air temperature was 21 to 22 °C and humidity 50%, approximately.

Methods:

Dosage: The highest dose of 90 ppm, 6 hours exposure per day selected for this study was based on MTD (decreased weight gain and nasal mucosa pathology changes) determined from ongoing 6-month and 2-year studies with this strain by the same route. However, during the first 7 days, the targeted dose was only 0, 5, 20, and 60 ppm, 6 hours daily exposure but was increased to the experimental doses of 0, 10, 30, and 90 ppm on day 8. Concentration of test substance in each chamber was monitored at least once per hour by infra-red spectrophotometry. Controls were subjected to the same exposure conditions and air flow but no compound. Chamber concentrations actually found and chamber conditions are summarized for both F_0 and F_1 parents in the table which follows. They are based on 193 exposures of F_0 parents and 210 exposures of F_1 parents at each dose level. The analytical concentrations are actually daily time weighted average concentrations in each cage and each day generally determined from 7 to 9 values for each exposure at each level. The nominal concentration was determined from the ratio of Telone II quantity used to total air flow-through each chamber on a daily basis.

Chamber Conditions

Generation	Target Conc. (ppm)	0	10	30	90
F_0	Analytical conc. (ppm)	--	9.8±1.2	29.8±1.9	89.0±6.5
	Range	--	4.8-12.5	19.3-32.6	56.8-98.2
	Nominal conc. (ppm)	--	9.5±1.1	28.7±1.8	83.3±6.5
	Range	--	4.8-12.7	18.8-33.6	56.2-100.8
F_0	Temperature (C)	22-23	22-23	22-23	22-23
	Humidity (%)	60±10	55±7	53±8	51±6

6

Chamber Conditions (cont'd)

Generation	Target Conc. (ppm)	0	10	30	90
F_1	Analytical conc. (ppm)	--	10.1±0.6	30.1±0.9	90.1±1.8
	Range	--	8.7-14.1	25.6-33.1	76.5-94.2
	Nominal conc. (ppm)	--	9.4±0.8	28.2±1.0	84.8±2.2
	Range	--	7.4-13	25.6-32.7	72.2-89.5
	Temperature (C)	22-23	21-22	21-23	22-23
	Humidity (%)	48±9	45±10	44±8	47±7

Experimental Plan:

For the F_0 generation, 30 males and 40 females per group were exposed for 10 weeks at 6 hours per day, 5 days per week prior to breeding but exposure was increased to 7 days per week during breeding (1 male to 1 female) at weeks 11 to 13 (3 weeks), then during gestation and lactation. F_{1a} litters were randomly culled to 8 pups per dam on day 4 of age with half of each sex when possible and weaned on day 28 of age. One week after weaning, breeding for the F_{1b} litter was initiated (one male to one female in similar dose groups) and continued for 3 weeks. For both F_{1a} and F_{1b} generations, the dams were not exposed from day 20 of gestation until day 5 postpartum. F_{1b} litters were also culled on day 4 of age to 8 pups per dam, half of each sex if possible and weaned on day 28. Pups were not exposed during lactation but were separated from their mothers for 6 hours per day during her exposure. Whereas all F_{1a} pups were killed after weaning (no necropsies or pathology performed), 30 male and 30 female pups from each treatment group were selected from F_{1b} offspring as parents for the F_2 generations. 10 of each sex were subjected to gross pathology and the remainder killed without further study.

Exposure of F_1 male and female parents to Telone II began after weaning (around week 32 of the study) and continued for 12 weeks, but for only 5 days per week, 6 hours per day. Mating of 1 male to 1 female from each treatment groups for the F_{2a} litter continued for 3 weeks (weeks 45 to 47). Exposure to Telone II of F_{1b} females was discontinued from days 20 of gestation to day 5 post-partum. F_{2a} litters were culled to 8 (4 of each sex if possible) on day 4, exposure of dams to compound resumed on day 5 of lactation and F_{2a} litters were weaned on day 28, then all were killed. Mating of F_1 parents was resumed a week after weaning for obtaining the F_{2b} offspring. Exposure of F_1 females was discon-

tinued between day 20 of gestation to day 5 of lactation. F_{2b} pups were culled on day 4 of age to 8 per litter and weaned on day 28. Gross pathology was performed on 10 pups of each sex per group and all F_1 adults when the pups were weaned. Limited histopathology described below was performed on the F_1 adults.

It should also be pointed out that litters with less than 8 were left intact. Culling in those with more than 8 was done in a random fashion with no preferential runt culling. During breeding, day 0 is date of vaginal detection sperm. During lactation, individual pup and dam weights were obtained on days 1, 4, 7, 14, 21, and 28. At other times, the animals were weighed weekly, except females after mating were also weighed on day 4 after mating.

Exposure to Telone II of F_0 to F_1 were continued until adults were killed. Pregnant females were not exposed to the fumigant from day 20 of gestation to day 4 postpartum. Pups were not exposed to fumigant but the dams were separated from the pups for 6 hours of exposure per day during lactation days 5 to 28. Nonpregnant females were excluded from exposure for a similar time period to equalize exposure days for pregnant and nonpregnant.

Necropsy of Adults:

F_0 males and females were killed on abou day 218 to 220, which occurred shortly after weaning the F_{1b} pups. F_1 male and female adults were killed after weaning the F_{2b} pups, around 66 weeks after initiation of the study. Eyes were examined by placing a glass microscope slide over the cornea and examining eyes under fluorescent light.

All males and females of F_0 and F_1 rats were subjected to gross pathology, including those found dead if possible and in moribund condition. Tissues listed below (from Table 3 of the report) were collected from F_0 and F_1 adults, then preserved for possible histopathology. However, histopathology was performed on reproductive organs and "potential target organs" listed with one asterisk in the table that follows, only for controls and high dose animals. Nasal and stomach tissues were examined in animals of all 3 treated and control groups. Eyes were examined by passing a glass microscope slide over the cornea and observing eyes under fluorescent light.

TISSUES COLLECTED AND PRESERVED AT NECROPSY

Adrenals	Liver*	Salivary Glands
Aorta	Lungs*	Seminal Vesicles*
Auditory Sebaceous Glands	Mammary Gland	Skeletal Muscle
Bone	Mediastinal Lymph Node	Skin
Bone Marrow	Mediastinal Tissues	Small Intestine
Brain	Mesenteric Lymph Node	Spinal Cord
Cecum	Mesenteric Tissues	Spleen
Cervix*	Nasal Tissues**	Stomach**
Coagulating Glands*	Oral Tissues	Testes*
Epididymides*	Ovaries*	Thymus
Esophagus	Oviducts*	Thyroid Gland
Eyes	Pancreas	Tongue
Heart	Parathyroid Glands	Trachea*
Kidneys*	Peripheral Glands	Urinary Bladder*
Lacrimal/Harderian Glands*	Peripheral Nerve	Uterus*
Large Intestine	Pituitary	Vagina*
Larynx*	Prostate*	

---

* Potential target and reproductive organs from control and high dose adult animals that were routinely processed for histological examination.

** Examined histologically at all dose levels.

Necropsy of Offspring Litters

Gross pathology was performed only on 10 of each sex per group from F_{1b} and F_{2b} litters. Their eyes were also examined by using a glass microscope slide over the cornea and observing under fluorescent light. Tissues were preserved in formalin but no histopathology was performed.

Statistics:

Statistical evaluation of body weight and body weight gain were by Bartlett's test for equality of variance ($\alpha = 0.01$), followed by a parametric or nonparametric ANOVA. If significant with the ANOVA, a Dunnett's test or Wilcoxon's Rank-Sum test with Bonferroni's correction was performed. Gestation length and litter size were analyzed by Wilcoxon Rank-Sum test with Bonferroni's correction.

Fertility indices (mating, conception, and gestation) were evaluated by Fisher Exact test, neonatal sex ratio by the binominal distribution test, neonatal survival indices by the Wilcoxon test as modified by Haseman and Hoel using the litter

as the experimental unit. Because of numerous statistical comparisons and false positive perceived, final interpretations in a number of cases took into consideration other factors such as dose response relationships, other related biologic and pathologic findings.

RESULTS:

F_0 Generation Adults.

F_0 Adult Clinical Observations:

No treatment related effects were noted.

Mortality:

One male on 10 ppm dose died on day 215 of the test (close to expected time of scheduled necropsy), "attributed to food impaction in the esophagus."

Body Weight of Adults:

In males, significantly lower increase at high dose starting at 2 weeks after initiation of exposure and continuing virtually to end of study for F_0 which was day 213 ($p < 0.05$ at all time periods between day 15 to day 192). In females, small decrease at high dose only around day 24 (n.s.), 30 ($p < 0.05$) but tendency to recover afterward and no significant effects to day 71. No effects during F_{1a} gestation and lactation were observed. There were no effects on body weight in groups receiving the 2 lower dose levels with both the males and females.

Gestation Body Weights (g) of F0 Females for F1A Litters

Conc. ppm		Gestation Day 1	4	7	14	21
0	Mean	170.0	174.2	179.5	201.8	251.6
	S.D.	6.9	7.0	7.7	8.3	12.5
	N=	22	22	22	22	22
10	Mean	166.9	171.6	177.4	196.4	244.3
	S.D.	11.8	12.0	12.6	13.9	21.5
	N=	22	22	22	22	20
30	Mean	164.2	169.0	174.5	196.1	249.1
	S.D.	11.3	11.7	12.0	13.4	18.5
	N=	22	22	22	22	20
90	Mean	165.7	170.5	176.2	196.8	249.3
	S.D.	9.2	9.3	9.4	10.6	18.3
	N=	26	26	26	26	26

No values were identified as different from control by Dunnett's test, alpha = 0.05, two-sided.

10

Conc. ppm		Post-Partum Day 1	4	7	14	21	28
0	Mean	187.2	188.2	195.3	204.4	204.7	193.6
	S.D.	8.2	8.4	7.6	7.8	9.2	9.8
	N=	22	22	22	22	22	22
10	Mean	185.0	187.1	194.0	203.8	202.2	193.4
	S.D.	12.8	15.3	13.7	15.2	13.6	12.8
	N=	22	22	22	22	22	22
30	Mean	183.9	187.7	189.7	198.2	201.4	194.7
	S.D.	12.3	13.2	14.1	14.6	11.9	11.9
	N=	22	22	22	22	22	22
90	Mean	180.0	183.3	189.9	197.4	197.0	189.1
	S.D.	11.0	13.7	12.0	12.4	11.0	8.8
	N=	26	26	26	26	26	26

No values were identified as different from control by Dunnett's test, alpha = 0.05, two-sided.

Reproductive Indices for F_0 Adults/F_{1a} Litters

Definitions of all parameters and results are shown in the table which follows. Female mating index, based on vaginal sperm observed, was 90 to 100 percent in control and treated groups with no obvious compound-related effects. If a female had been with a male with which mating did not occur the first week, it was paired with another one during week 2. Male mating index in controls, also based on vaginal sperm detected in its mate, was 93.3 percent but in the treated groups it was 76.7 to 83.3 percent; possibly small decrease but not significant. Female conception index, based on number delivering a litter/number known to have mated, was only 73.3 percent in controls and 75.9 to 89.7 in the 3 treated groups; no compound effect. Male conception index was only 78.6 to 92.0 percent with no significant effect. Gestation index, defined as those delivering a live litter as a percentage of those delivering litter, was 100 percent for controls and all 3 treated groups. Gestation survival index which is percent of newborn pups alive at birth, was greater than 99 percent for all four groups. Survival index during lactation remained 100 percent throughout lactation for controls, low, and mid dose but decreased slightly with time after the fourth day of lactation in the high dose group,but no significant effect. Sex ratio and gestation length were obviously not affected by treatment.

Reproductive Indices - F1 Adults/F1A Litters

	Telone II, ppm			
	0	10	30	90
Number of females	30	30	30	30
Female mating index %[a]	100 (30/30)	96.7 (29/30)	90.0 (27/30)	96.7 (29/30)
Female conception index %[b]	73.3 (22/30)	75.9 (22/29)	81.5 (22/7)	89.7 (26/29)
Male mating index %[c]	93.3 (28/30)	76.7 (23/30)	80.0 (24/30)	83.3 (25/30)
Male conception index %[d]	78.6 (22/28)	78.3 (18/23)	83.3 (20/24)	92.0 (23/25)
Gestation index %[e]	100 (22/22)	100 (22/22)	100 (22/22)	100 (26/26)
Gestation survival index %[f]	99.1 (227/229)	99.5 (216/217)	100 (235/235)	99.7 (297/298
Day 1 survival index %[g]	100 (225/227)	99.5 (215/216)	100 (235/235)	100 (297/297
Day 4 survival index %[g]	99.1 (225/227)	99.5 (215/216)	100 (235/235)	100 (297/297
Day 7 survival index %[h]	100 (173/173)	100 (162/162)	100 (173/173)	97.6 (200/205
Day 14 survival index %[h]	100 (173/173)	100 (162/162)	100 (173/173)	97.6 (200/205
Day 21 survival index %[h]	100 (173/173)	100 (161/161)[1]	100 (173/173)	97.1 (199/205
Day 28 survival index %[h]	100 (173/173)[1]	100 (161/161)	100 (173/173)	96.1 (197/205
Sex ratio on day 1 Male:Female	45:55	49:51	54:46	49:51
Gestation length (days)[k]	22.0 (0.0)	22.0 (0.7)	21.9 (0.3)	22.0 (0.3)

[a]Number of females with a sperm positive vaginal smear expressed as a percentage of the total number of mated females.
[b]Number of females delivering a litter expressed as a percentage of the number of mated (sperm positive) females.
[c]Number of males which mated resulting in a sperm positive vaginal smear/total number of males mated.
[d]Number of males which sired a litter/number of males mated resulting in a sperm positive vaginal smear.
[e]Number of females delivering a live litter expressed as a percentage of the number of females delivering a litter.
[f]Percentage of newborn pups that were alive at birth.
[g]Percentage of liveborn pups that survived for 1 or 4 days respectively.
[h]Percentage of liveborn pups that survived until days 7, 14, 21, or 28 of lactation. All litters were culled to 8 pups, 4 males and 4 females where possible, on day 4 of lactation. Percentages are based on the number of pups after culling.
[k]Mean, (Standard Deviation).
[l]Trauma-related deaths due to animal handling or animal care procedures were not reported.

Live litter size at birth was 10.3 for controls, 9.8 to 11.4 in the 3 treated groups; no compound effect. There was also no increase in dead births due to treatment. Live litter size thought lactation was not affected by treatment.

F1A Litter Size

Conc. ppm		Born Dead	Born Alive	Day 1	Day 4(B)	Day 4(A)	Day 7	Day 14	Day 21	Day 28
0	Mean	0.1	10.3	10.3	10.2	7.9	7.9	7.9	7.9	7.8
	S.D.	0.3	1.8	1.8	1.7	0.6	0.6	0.6	0.6	0.7
	N=	22	22	22	22	22	22	22	22	22
10	Mean	0.0	9.8	9.8	9.8	7.4	7.4	7.4	7.3	7.3
	S.D.	0.2	3.2	3.2	3.2	1.8	1.8	1.8	1.8	1.8
	N=	22	22	22	22	22	22	22	22	22
30	Mean	0.0	10.7	10.7	10.7	7.9	7.9	7.9	7.9	7.9
	S.D.	0.2	1.9	1.9	1.9	0.5	0.5	0.5	0.5	0.5
	N=	22	22	22	22	22	22	22	22	22
90	Mean	0.0	11.4	11.4	11.4	7.7	7.7	7.7	7.7	7.6
	S.D.	0.2	2.0	2.0	2.0	1.6	1.6	1.6	1.6	1.6
	N=	26	26	26	26	26	26	26	26	26

(A) After culling.
(B) Before culling.
No values were identified as different from control by Dunnett's test, alpha = 0.05, two-sided.

No effect of treatment at any dose level was seen on mean body weight or body weight gain of pups throughout lactation.

F1A Mean Pup Body Weights (g)

Conc.	Days Post-Partum							
ppm	1	4(b)	4(a)	7	14	21	28(m)	28(f)
0 Mean	5.5	8.0	8.1	10.1	17.6	25.7	50.7	48.3
S.D.	0.3	0.5	0.5	0.7	0.9	1.7	3.9	3.3
N=	22	22	22	21	22	22	22	22
10 Mean	5.4	7.9	7.9	9.9	17.1	25.6	49.1	47.5
S.D.	0.5	0.7	0.7	0.9	2.1	3.7	6.8	4.1
N=	22	22	22	22	22	22	22	21
30 Mean	5.5	7.9	7.9	10.0	16.9	25.3	49.7	47.2
S.D.	0.4	0.5	0.6	0.9	1.7	2.7	4.5	4.3
N=	22	22	22	22	22	22	22	22
90 Mean	5.3	7.8	7.8	9.9	17.1	25.5	50.4	48.3
S.D.	0.4	0.7	0.7	0.7	1.0	2.3	3.8	2.8
N=	26	26	26	25	25	25	25	25

(a) After culling.
(b) Before culling.
(m) Male.
(f) Female.
No values were identified as different from control by Dunnett's test, alpha = 0.05, two-sided.

Two runts were seen in 2 separate litters only in high dose treated litters. No other external anomalies except that due to injury, were seen in the offspring after birth.

External Alterations - F1A Litters

Observations		0	10	30	90
Total pups examined		229	217	235	298
Total litters examined		22	22	22	26
		Number Affected			
Bruise (tail or nose)	Pups	0	1	0	1
	Litters	0	1	0	1
Broken tail	Pups	0	0	0	1
	Litters	0	0	0	1
Runt	Pups	0	0	0	2
	Litter	0	0	0	2

F_0 Adults/F_{1b} Litters

Body Weight of Dams: No effect on body weight or body weight increase was seen during gestation. A tendency for decrease in body weight was seen during lactation in high-dose group, but significant ($p < 0.05$) only on day 21 (See table which follows)

Gestation Body Weight Gains (g) of F0 Females for F1B Litters

Conc. ppm		Gestation Days 1-4	4-7	7-14	14-21	1-21
0	Mean	4.4	4.9	19.8	45.6	74.3
	S.D.	2.5	2.0	3.5	16.4	16.6
	N=	25	25	25	19	19
10	Mean	3.3	5.1	18.4	40.2	65.3
	S.D.	2.3	2.5	4.7	24.4	26.1
	N=	4	24	24	14	14
30	Mean	4.7	4.6	20.9	50.8	79.3
	S.D.	2.4	2.9	3.5	9.8	10.9
	N=	23	23	23	8	8
90	Mean	3.9	4.2	20.7	46.3	74.7
	S.D.	2.3	2.1	3.7	16.3	18.1
	N=	25	25	25	22	22

No values were identified as different from control by Dunnett's test, alpha = .05, two-sided.

The values given for N (number of litters) in the above table are surprisingly low for days 14-21 and requires an explanation by the applicant. This discrepency is no longer apparent in the table which follows on lactation body weights.

Lactation Body Weights (g) of F0 Females for F1B Litters

Conc. ppm		Post-Partum Days 1	4	7	14	21	28
0	Mean	207.9	212.7	214.9	222.0	222.3	211.1
	S.D.	11.1	11.8	11.0	10.7	10.3	10.2
	N=	24	24	24	24	24	24
10	Mean	205.0	208.9	212.8	220.6	218.9	211.8
	S.D.	12.9	13.0	12.1	14.3	16.0	12.0
	N=	22	23	23	23	23	23
30	Mean	206.8	211.4	215.3	223.2	220.8	207.3
	S.D.	10.3	12.1	12.4	13.0	10.5	14.4
	N=	23	23	23	23	23	23
90	Mean	204.1	209.0	209.0	216.7	212.8*	208.4
	S.D.	9.8	11.6	10.1	10.7	11.6	10.3
	N=	25	25	25	25	25	25

* $P<0.05$ by Dunnett's t test, two sided

16

Reproductive Indices. Female mating index (based on inseminated females) was 90.0 to 100 percent in control or treated, male mating index was 76.7 to 90 percent (also based on inseminated female caged with the male); no effects of treatment. Female conception index was 80.0 to 86.2 percent--no compound effect but still low, even in controls where it was 86.2 percent. Male conception index was 95.8 percent in controls, 85.2 to 95.7 in treated; no compound effect. Gestation index was 100 percent for controls and all treated groups. There was a suggestion of a slight decrease in litter survival index on days 1 and 4 of lactation in the low-dose group only ($p < 0.05$).

Reproductive Indices - F0 Adults/F1B Litters

	Telone II, ppm			
	0	10	30	90
Number of females	30	30	30	30
Female mating index %[a]	96.7 (29/30)	100 (30/30)	90.0 (27/30)	96.7 (29/30)
Female conception index %[b]	86.2 (25/29)	80.0 (24/30)	85.2 (23/27)	86.2 (25/29)
Male mating index %[c]	80.0 (24/30)	90.0 (27/30)	76.7 (23/30)	86.7 (26/30)
Male conception index %[d]	95.8 (23/24)	85.2 (23/27)	95.7 (22/23)	92.3 (24/26)
Gestation index %[e]	96.0 (24/25)	100 (24/24)	100 (23/23)	100 (25/25)
Gestation survival index %[f]	98.1 (259/264)	99.6 (244/245)	99.3 (269/271)	100 (273/273
Day 1 survival index %[g]	100 (259/259)	98.0* (239/244)	99.6 (268/269)	99.6 (272/273
Day 4 survival index %[g]	100 (259/259)	98.0* (239/244)	99.6 (268/269)[1]	99.3 (271/273)
Day 7 survival index %[h]	100 (182/182)	100 (170/170)	100 (173/173)	99.5 (287/188
Day 14 survival index %[h]	100 (182/182)	98.2 (167/170)	100 (173/173)	99.5 (287/188
Day 21 survival index %[h]	100 (182/182)	98.2 (166/169)[1]	100 (173/173)	99.5 (187/188
Day 28 survival index %[h]	99.5 (181/182)	98.2 (165/168)[1]	100 (173/173)	99.5 (185/186
Sex ratio on day 1 Male:Female	54:46	52:48	49:51	51:49
Gestation length (days)[k]	21.7 (0.7)	21.6 (0.6)	21.3* (0.4)	21.8 (0.5)

[1]Trauma-related deaths due to animal handling or animal care procedures were not reported.

* P<0.05

See page 9 of this report for other footnotes and definitions.

Litter size at birth based on live births was 10.4 in controls versus 10.2 to 11.7 in the 3 treated groups (no effect). There were also no effects of treatment at any dose level on number per litter born dead or on live litter size on days 1, 4, 7, 14, 21, or 28 of lactation. Pup weights during lactation were decreased ($p < 0.05$) only on days 7 and 14 in low-dose group, which we tend to agree was a sporadic, not compound-related effect.

F1B Litter Size

Conc. ppm		Born Dead	Born Alive	Day 1	Day 4(B)	Day 4(A)	Day 7	Day 14	Day 21	Day 28
0	Mean	0.2	10.4	10.4	10.4	7.3	7.3	7.3	7.3	7.3
	S.D.	0.6	3.7	3.7	3.7	1.8	1.8	1.8	1.8	1.8
	N=	25	25	25	25	25	25	25	25	25
10	Mean	0.0	10.2	10.0	10.0	7.1	7.1	7.0	6.9	6.9
	S.D.	0.2	3.8	3.8	3.8	2.2	2.2	2.2	2.2	2.2
30	Mean	0.1	11.7	11.7	11.7	8.0	8.0	8.0	8.09	8.0
	S.D.	0.3	2.0	1.9	1.9	0.0	0.0	0.0	0.2	0.2
	N=	23	23	23	23	23	23	23	23	23
90	Mean	0.0	10.9	10.9	10.8	7.5	7.5	7.5	7.5	7.4
	S.D.	0.0	3.2	3.2	3.2	1.3	1.3	1.3	1.3	1.3
	N=	25	25	25	25	25	25	25	25	25

(A) After culling.
(B) Before culling.
No values were identified as different from control by Dunnett's test, alpha = 0.05, two-sided.

There were 3 runts in 3 separate litters which occurred only in high dose treated groups. Other "external alterations," not related to injury, such as microphthalmia and ear hemorrhage are listed but occurred in only a single group in mid and high dose, respectively.

F1B PUP BODY WEIGHTS (G)

Conc. ppm		Days Post-Partum 1	4(b)	4(a)	7	14	21	28(m)	28(f)
0	Mean	5.2	7.7	7.7	10.2	18.5	28.0	51.7	49.6
	S.D.	0.5	1.2	1.2	1.3	1.7	2.7	6.5	6.2
	N=	24	24	24	24	24	24	24	24
10	Mean	5.1	7.2	7.3	9.3*	17.0$	27.2	49.8	47.1
	S.D.	0.5	0.9	1.1	1.4	2.8	3.3	5.2	5.7
	N=	22	23	23	23	23	23	23	22
30	Mean	5.1	7.3	7.3	9.8	18.0	27.7	51.0	48.3
	S.D.	0.4	0.6	0.6	0.7	1.3	2.2	4.7	4.3
	N=	23	23	23	23	23	23	23	23
90	Mean	5.2	7.5	7.5	10.1	18.0	27.1	51.0	48.6
	S.D.	0.6	0.9	0.9	1.0	1.3	3.3	5.5	3.9
	N=	25	25	25	25	25	25	25	25

a After Culling
b Before Culling
f Female.
m Male.
* Significant differnce from control by Dunnett's test, alpha=.05, two sided.

$ Significant difference from control by Wilcoxon's test, alpha=.05, two sided.

External Alterations - F1B Litters

Observations		Telone II, ppm 0	10	30	90
Total pups examined		264	245	271	273
Total litters examined		25	24	23	25
		Number Affected			
Broken tail	Pups	0	1	0	0
	Litters	0	1	0	0
Microphthalmia	Pups	0	0	1	0
	Litters	0	0	1	0
Runt	Pups	0	0	0	3
	Litters	0	0	0	3
Hemorrhage (ear)	Pups	0	0	0	1
	Litters	0	0	0	1

Gross pathology was performed on 10 weanling rats per sex in each dose group. No compound-related effects were seen in the eyes or other organ.

Gross Pathologic Observations - F1B Weanlings

Sex	Males				Females			
Exposure Concentration in ppm	0	10	30	90	0	10	30	90
Numbers of rats examined	10	10	10	10	10	10	10	10
Eyes								
Within normal limits	10	10	9	10	10	10	10	10
Opacity, lens, left:	0	0	1	0	0	0	0	0
All Other Tissues (Complete Necropsy Performed)								
Within normal limits	10	10	10	10	10	10	10	10

Gross Pathology of F_0 Adults

Necropsies were performed on day 215 to 220 of the study with complete gross pathology on each male and female. The only organ showing changes visible in gross pathology that could be attributed to treatment was the stomach and was limited to high dose treated; seven males and eight females. Six of the males had focal roughened surface of the nonglandular mucosa and one had multifocal erosions (possibly ulcers) in nonglandular mucosa. An additional male on low dose had a focal erosion of nonglandular mucosa. Of the eight females on high dose with stomach lesions, three had focal or multifocal cysts of the nonglandular submucosa, three had focal or multifocal areas of roughened surface in the nonglandular submucosa and three had focal or multifocal erosions or ulcers in the nonglandular mucosa.

Microscopic Pathology of F_0 Adult (See table below)

This was limited to selected tissues which included controls and high dose for organs of the male and female reproductive tracts, kidney, liver, lungs, larynx, trachea, and urinary bladder but all three doses and controls for stomach and nasal tissues. The latter two, stomach and nasal tissues from previous studies, are known target organs for compound induced lesions. In male reproductive organs, no effects were seen in the coagulating glands, epididymides, seminal vesicles, prostate, or testes. In female reproductive tract of those on high dose, no effect was seen in cervix, ovaries, oviduct, uterus, or vagina; all were stated to be "within normal limits." In kidneys, larynx, liver, lungs, trachea, and urinary bladder of males and females where high dose was compared to controls, no compound induced lesions were seen. In nasal tissue, hyperplasia of respiratory epithelium described as "slight" was seen in 17 males and 8 females on high dose, focal degeneration of olfactory epithelium was seen in one male and two females on high dose. None of these lesions were seen in nasal tissues of animals at the 2 lower dose levels or in controls. Other lesions, such as inflammation of the respiratory mucosa, tended to occur more often in the high dose group than in controls or lower dose treated groups. In stomach, lesions due to treatment were found in five males and nine females on high dose. Five of each sex on high dose had focal acanthosis of nonglandular mucosa, five high dose females and one low dose male had submucosa edema; subacute nonglandular focal inflammation was seen in one low and four high dose males and in the nine high dose females, focal ulcer of nonglandular mucosa was seen in five females.

In the narrative section of the report, the following statements are made related to these gross and histopathology findings. "An increased incidence of stomach lesions, consistent with active and/or healing ulcers was observed...in the 90 ppm exposure group. The ulcers were considered a result of stress

secondary to exposure...and not a direct effect...." Regarding the nasal mucosa tissue damage it is claimed that the lesions in the respiratory epithelium occurred in the nasal turbinate section at the level of the first ruga palantina which involved about 1 percent of the respiratory mucosa. It is claimed that this type of lesion "consisted of disorganization of the respiratory epithelimum at the tip of the dorsal concha the respiratory hyperplasia was limited to a slight uniform thickening which occasionally produced a pseudo stratified appearance. The olfactory epithelia damage in one male and two females on high dose was characterized by focal degenerative processes in the neuronal layer and loss of sustenticular cell cytoplasm."

Summary of Histopathologic Observations - F0 Adults*

Sex	Males				Females			
Dose ppm	0	10	30	90	0	10	30	90
Number examined	30	30	30	30	30	30	30	30
Stomach (No. Examined)	30	30	30	29	30	30	30	30
Number with lesions	0	1	0	6	0	0	0	9
Acanthosis. nonglandular mucosa. focal	0	0	0	5	0	0	0	5
Edema submucosa	0	1	0	0	0	0	0	5
Inflammation - subacute. nonglandular mucosa. focal	0	1	0	4	0	0	0	9
Ulcer. nonglandular mucosa. focal	0	0	0	0	0	0	0	5
Nasal Tissues (No. Examined)	30	30	30	30	30	30	30	30
Number with lesions	6	1	1	20	5	0	3	14
Degeneration. olfactory epithelium. focal	0	0	0	1	0	0	0	2
Hyperplasia. respiratory epithelium	0	0	0	17	0	0	0	8
Inflammation - subacute. nasolacrimal duct. bilateral. diffuse	0	0	0	0	1	0	0	0
Inflammation - subacute. respiratory mucosa	3	1	0	6	3	0	0	8
Cyst(s) with keratinous debris. nasolacrimal duct	1	0	1	0	1	0	0	2
Foreign body reaction. respiratory mucosa	2	0	0	0	0	0	0	0

*Includes only organs with known compound-related effects.

Organ Weights: None were obtained for both the adult or weanling rats.

F_1 Generation

Number of Adults per Group at Start of F_1 Generation: 30 males and 30 females.

Clinical Observations through the Study: No compound-related effect was noted.

F_1 rats were treated for 12 weeks which started after weaning prior to mating for F_{2a} litters.

Clinical Observations:

No effects were seen in F males or females throughout the study.

Mortality: One low dose female on day 112 after initiation of exposure of F_1 parents, one high dose female on day 134, one low dose male on day 162 of the study. Only the high dose female died of perforating ulcers which may be treatment related although the applicant claims all three deaths were not treatment related.

Body Weight: In male adults, decreased weight gain in high dose group became obvious within 10 days but was significant at numerous time periods ($p < 0.05$) from day 45 and after. In females, before mating, the decrease on body weight of high dose treated was less than in males and occasionally significant ($p < 0.05$) such as on day 10 and 45. During gestation, body weight of high dose was less than controls ($p < 0.05$) only during days 1, 4, and 7 not after, but weight gains were no different at any time, including days 1, 4, and 7. During lactation, body weight of high dose females was consistently lower than controls ($p < 0.05$ on days 14, 21, and 28) but there was no difference in body weight gain at any time.

Reproductive Indices for F_1 Adults/F_{2a} Litters

There were 30 females in each group that were mated but the number that became pregnant and delivered a litter was 24, 23, 19, and 27 for control, low, mid, and high dose, respectively. Female and male mating indices were not affected in any treated group; neither were male or female conception indices affected by the compound at any dose level. It should be noted that female conception indices were 85.7, 79.3, 70.4, and 93.1 percent in the control, low, mid, and high dose groups, male conception indices were 91.3, 83.3, 75.0, and 92.6 for controls to high dose.

Gestation survival index ranged from 98.8 to 100 percent and no compound effect. There were no compound related effects on survival indices of pups at any time period during lactation (very low mortality). Also, no effects or sex ratio on day 1 of lactation or gestation length of dams were seen.

Reproductive Indices - F1 Adults/F2A Litters

	Telone II, ppm			
	0	10	30	90
Number of females	30	30	30	30
Female mating index %[a]	93.3 (28/30)	96.7 (29/30)	90.0 (27/30)	96.7 (29/30)
Female conception index %[b]	85.7 (24/28)	79.3 (23/29)	70.4 (19/27)	93.1 (27/29)
Male mating index %[c]	76.7 (23/30)	80.0 (24/30)	66.7 (20/30)	90.0 (27/30)
Male conception index %[d]	91.3 (21/23)	83.3 (20/24)	75.0 (15/20)	92.6 (25/27)
Gestation index %[e]	100 (24/24)	100 (23/23)	100 (19/19)	100 (27/27)
Gestation survival index %[d]	100 (214/214)	98.8 (245/248)	100 (217/217)	99.6 (277/279)
Day 1 survival index %[e]	99.5 (213/214)	99.6 (244/245)	98.6 (214/217)	99.3 (277/279)
Day 4 survival index %[e]	99.5 (213/214)	99.6 (244/245)	98.2 (213/217)	99.3 (277/279)
Day 7 survival index %[f]	100 (162/162)	100 (177/177)	100 (145/145)	100 (205/205)
Day 14 survival index %[f]	100 (162/162)	100 (177/177)	99.3 (144/145)	99.5 (204/205)
Day 21 survival index %[f]	99.4 (161/162)	100 (177/177)	99.3 (144/145)	99.5 (203/205)
Day 28 survival index %[f]	98.8 (160/162)	100 (177/177)	99.3 (144/145)	99.0 (204/205)
Sex ratio on day 1	47:53	50:50	56:44	47:53
Gestation length (days)[k]	21.5 (0.5)	21.4 (0.5)	21.3 (0.5)	21.4 (0.5)

See page 9 of this report for footnotes and definitions.

Live litter size was increased at birth in the mid dose group ($p < 0.05$) but this was obviously a sporadic effect due to small mean litter size for controls (8.9 pups). Neither the litter size nor mean weight or weight gain of pups from treated groups were affected after culling on day 4. There was also no increase in pups born dead due to treatments in any group. No effect of compound on incidence of external alterations was noted (see tables which follow).

F2A Litter Size

Conc. ppm		Born Dead	Born Alive	Day 1	Day 4(B)	Day 4(A)	Day 7	Day 14	Day 21	Day 28
0	Mean	0.0	8.9	8.9	8.9	6.8	6.8	6.8	6.7	6.7
	S.D.	0.0	3.7	3.8	3.8	2.2	2.2	2.2	2.2	2.2
	N=	24	24	24	24	24	24	24	24	24
10	Mean	0.1	10.7	10.6	10.6	7.7	7.7	7.7	7.7	7.7
	S.D.	0.5	2.3	2.3	2.3	0.8	0.8	0.8	0.8	0.8
	N=	23	23	23	23	23	23	23	23	23
30	Mean	0.0	11.4*	11.3*	11.2*	7.6	7.6	7.6	7.6	7.6
	S.D.	0.0	2.9	2.9	2.9	1.0	1.0	1.0	1.0	1.0
	N=	19	19	19	19	19	19	19	19	19
90	Mean	0.0	10.3	10.3	10.3	7.6	7.6	7.6	7.6	7.5
	S.D.	0.2	2.6	2.6	2.6	1.2	1.2	1.2	1.2	1.2
	N=	27	27	27	27	27	27	27	27	27

(A) After culling.
(B) Before culling.
* Significant difference from control by Dunnett's test, alpha = .05, two-sided.

F2A Pup Body Weights (g)

Conc. ppm		Days Post-Partum 1	4(b)	4(a)	7	14	21	28(m)	28(f)
0	Mean	5.3	7.5	7.5	9.5	16.1	24.4	44.9	43.3
	S.D.	0.6	1.3	1.3	1.7	2.2	2.7	6.8	6.3
	N=	24	24	24	24	24	24	21	23
10	Mean	5.1	7.4	7.3	9.6	16.3	24.6	46.4	43.7
	S.D.	0.4	0.9	0.9	1.1	1.4	2.5	4.9	4.8
	N=	23	23	23	23	23	23	23	23
30	Mean	5.0	7.4	7.4	9.8	17.0	24.8	47.7	46.3
	S.D.	0.5	0.9	0.9	1.0	1.0	1.9	4.4	4.1
	N=	19	19	19	19	19	19	19	19
90	Mean	5.0	7.3	7.4	9.5	16.3	24.3	45.5	43.4
	S.D.	0.5	1.0	1.0	1.0	1.5	2.8	6.6	4.9
	N=	27	27	27	27	27	27	27	26

(a) After culling.
(b) Before culling.
(m) Male.
(f) Female.
No values were identified as different from control by Dunnett's test, alpha = 0.05, two-sided.

F_1 Female Adults/F_{2b} Litters

Body Weight of Adult Females: No effects on body weight or body weight gain were seen during pregnancy or throughout lactation.

Reproductive Indices for F_1 Adults/F_{2b} Litters

Number of females per group was 30, 29, 30, and 29 in control, low, mid, and high dose groups, respectively. No effect of compound treatment on mating index of either sex was evident. Female conception index was 85.2 for controls, 80.0 to 92.6 for the three treated groups, whereas for males, conception index for controls was 76.7 percent and ranging from 63.3 to 83.3 percent in the treated groups. No effect of compound was noted. However, conception rate for both sexes is low for what should be expected in rats.

Gestation index was only 95.7 percent in controls, 100 percent in the three treated groups; no effect. Gestation survival index was only 92.8 percent for controls because of two dead litters but was 98.8 to 100 percent in the three treated groups (no effect). Survival indices for pups remained 98 % or higher for controls and treated groups at all postpartum lactation periods except for mid dose where it fell to 96.9 and 95.9 percent on days 1 and 4, respectively, but no significant effect was evident. There were also no effects on sex ratio observed on day 1 postpartum nor on gestational length.

Reproductive Indices - F1 Adults/F1B Litters

	Telone II, ppm			
	0	10	30	90
Number of females	30	29	30	29
Female mating index %[a]	90.0 (27/30)	93.1 (27/29)	83.3 (25/30)	100 (29/29)
Female conception index %[b]	85.2 (23/27)	92.6 (25/27)	80.0 (20/25)	86.2 (25/29)
Male mating index %[c]	76.7 (23/30)	72.4 (21/29)	63.3 (19/30)	83.3 (25/30)
Male conception index %[d]	87.0 (20/23)	95.2 (20/21)	84.2 (16/19)	92.0 (23/25)
Gestation index %[e]	95.7 (22/23)	100 (25/25)	100 (20/20)	100 (25/25)
Gestation survival index %[f]	92.8 (219/236)	99.1 (227/229)	100 (195/195)	98.8 (248/251)
Day 1 survival index %[g]	99.5 (218/219)	99.6 (226/227)	100 (189/195)	99.6 (247/248)
Day 4 survival index %[g]	98.6 (216/219)	99.1 (225/227)	95.9 (187/195)	99.2 (238/249)
Day 7 survival index %[h]	99.4 (157/158)	100 (179/179)	99.2 (131/132)[l]	100 (172/172)
Day 14 survival index %[h]	98.7 (156/158)	100 (179/179)	98.5 (130/132)	100 (172/172)
Day 21 survival index %[h]	98.7 (156/158)	99.4 (178/179)	98.5 (130/132)	100 (172/172)
Day 28 survival index %[h]	98.7 (156/158)	99.4 (178/179)	98.4 (124/126)[l]	100 (172/172)
Sex ratio on day 1 Male:Female	55:45	47:53	51:49	51:49
Gestation length (days)[k]	21.5 (0.6)	21.3 (0.5)	21.6 (0.5)	21.7 (0.5)

No values were identified as different from controls by the appropriate statistical analysis.

See page 9 of this report for footnotes and definitions

There were no compound related effects on number per litter born dead or alive, no effect of treatment on litter size throughout lactation. There was an increase in mean pup weight in the 10 ppm treated group for both the males and females between days 14-28 of lactation. No increase in numbers of pups with external alterations such as runts or anomalies, were seen.

F2B Litter Size

Conc. ppm		Born Dead	Born Alive	Day 1	Day 4(B)	Day 4(A)	Day 7	Day 14	Day 21	Day 28
0	Mean	0.7	9.5	9.9	9.8	7.2	7.1	7.1	7.1	7.1
	S.D.	3.1	4.1	3.8	3.7	2.1	2.1	2.2	2.2	2.2
	N=	23	23	22	22	22	22	22	22	22
10	Mean	0.1	9.1	9.0	9.0	7.2	7.2	7.2	7.1	7.1
	S.D.	0.4	3.2	3.2	3.2	1.6	1.6	1.6	1.7	1.7
	N=	25	25	25	25	25	25	25	25	25
30	Mean	0.0	9.9	9.9	9.4	6.6	6.6	6.6	6.5	6.2
	S.D.	0.0	3.8	3.7	4.3	2.5	2.7	2.7	2.6	2.9
	N=	20	25	25	20	20	20	20	20	20
90	Mean	0.1	9.9	9.9	9.5	7.0	7.2	7.2	7.2	7.2
	S.D.	0.4	3.8	3.7	3.9	1.9	1.7	1.7	1.7	1.7
	N=	25	25	25	25	25	24	24	24	24

(A) After culling.
(B) Before culling.
No values were identified as different from control by Dunnett's test, alpha = 0.05, two-sided.

F2B Pup Body Weights (g)

Conc. ppm	Days Post-Partum 1	4(b)	4(a)	7	14	21	28(m)	28(f)
0 Mean	5.2	7.5	7.5	9.6	17.7	27.0	50.6	47.8
S.D.	0.4	0.9	0.9	1.4	2.0	2.8	5.7	5.7
N=	22	22	22	22	21	21	21	20
10 Mean	5.2	8.1	8.1	10.5	18.9*	29.1	54.8*	51.7*
S.D.	0.6	1.2	1.2	1.3	1.8	3.6	5.4	5.2
N=	25	25	25	25	25	25	25	25
30 Mean	5.2	7.5	7.5	9.8	17.5	25.9	49.2	46.3
S.D.	0.4	1.1	1.1	1.0	1.5	2.4	5.0	4.7
N=	19	19	19	18	18	18	17	18
90 Mean	5.1	7.4	7.4	9.8	17.7	25.7	48.8	47.6
S.D.	0.5	1.1	1.1	0.8	1.3	3.1	5.8	4.6
N=	25	25	25	24	24	24	23	23

(a) After culling.

(b) Before culling.
(m) Male.
(f) Female.
*Significant difference from control by Dunnett's test, alpha = .05, two-sided.

Ten weanling pups of each sex per dose were selected for complete gross necropsy. No effects were noted.

Gross Pathologic Observations - F2B Weanlings

Sex	Males				Females			
Exposure Concentration in ppm	0	10	30	90	0	10	30	90
Numbers of rats examined	10	10	10	10	10	10	10	10
All Other Tissues (Complete Necropsy Performed								
Within normal limits	10	10	10	10	10	10	10	10

Gross Pathology of F_1 Adults. Complete pathology of all males and females on test was obviously performed. Four of 30 females and no male all on high dose, showed stomach tissues changes such as focal cysts in the nonglandular mucosa, thickened stomach wall, multifocal roughened surface of nonglandular mucosa

or erosion of glandular mucosa. One female at low dose also had multifocal glandular mucosa erosion and one on low dose had focal glandular mucosa erosion. No evidence of damage to other organs was seen.

Microscopic Pathology of F_1 Adults. Although tissues were collected and preserved apparently sufficient for a complete histopathology evaluation, only a few were examined, similar to those selected for histopathology of F_0 adults. For reproductive organs, only those from high dose and control animals were examined. No effects on cervix, uterus, vagina, ovaries, or oviducts in female and no effects prostate, seminal vesicles, epididymides, coagulating glands, or testes in male were seen. Other organs for which high dose in males and females were compared to controls for which no changes were found included kidneys, larynx, liver, lungs, trachea, and urinary bladder. Tissues for which all three treated and control groups were examined included stomach and nasal tissues. In stomach, damage to tissues was found only in eight females, no males, on high dose. This included five with focal acanthosis of nonglandular mucosa but one female and one male control also showed this form of stomach lesion. One female on high dose had focal cyst of nonglandular mucosa, four had focal subacute inflammation of nonglandular mucosa but one male control and one male low dose also had this type of lesion. Two female high dose had submucosa edema but one female control also had this. One female on high dose had a focal ulcer in the nonglandular mucosa; multifocal ulcers of glandular mucosa was seen one female in each of the three treated groups. In the nasal tissues, almost all on high dose showed tissue changes. Respiratory epithelial hyperplasia considered slight, was seen in 29 of the 30 males and in 28 of the 30 females on high dose, none in controls or lower doses. Slight focal degeneration of olfactory epithelium occurred in 2 males and 13 females on high dose. Subacute respiratory mucosa inflammation was seen in 5 low dose, 2 mid dose, and 1 high dose female but this form of lesion was also seen in 1 control female. Cysts with keratinous debris was seen in nasolacrimal duct of at least 1 in each of the 3 treated male groups and 1 female low dose with none in controls. Foreign body reactions were seen in 1 male on mid dose and 1 female on high dose.

Summary of Histopathologic Observations F_1 Adults*

Sex	Males				Females			
Dose (ppm)	0	10	30	90	0	10	30	90
Number Examined	30	30	30	30	30	30	30	30
Stomach (No. Examined)	30	30	30	30	30	30	30	30
Number with Lesions	1	1	0	0	1	0	1	8
Acanthosis, nonglandular mucosa, focal	1	0	0	0	1	0	0	5
Cyst, nonglandular mucosa, focal	0	0	0	0	0	0	0	1
Edema, submucosa	1	0	0	0	0	0	0	2
Inflammation - subacute, nonglandular mucosa, focal	1	1	0	0	0	0	0	4
Ulcer, glandular mucosa, multifocal	0	0	0	0	0	1	1	1
Ulcer, nonglandular mucosa, focal	0	0	0	0	0	0	0	1
Nasal Tissues (No. Examined)	30	30	30	30	30	30	30	30
Number with Lesions	0	1	2	29	4	6	2	28
Degeneration, olfactory epithelium, focal	0	0	0	2	0	0	0	13
Hyperplasia, respiratory epithelium	0	0	0	29	0	0	0	28
Inflammation - subacute, respiratory mucosa	0	0	0	0	1	5	2	1
Cyst(s) with keratinous debris, nasolacrimal duct	0	1	2	1	0	1	0	0
Foreign body reaction, respiratory mucosa	0	0	1	0	0	0	0	1

Includes only organs with known compound-related effects.

SUMMARY AND EVALUATION

Systemic toxicity to the F_0 and F_1 male and female adults was observed almost exclusively in those exposed at 90 ppm, the highest dose tested. Manifestations of toxicity included a decrease in body weight gain, lesions in the stomach and nasal tissues. Stomach lesions were seen in 20 to 30 percent of males or females generally confined to focal or multifocal damage in the nonglandular mucosa. None of these effects were seen at 30 ppm or less.

The applicant claimed that these stomach lesions are "consistent with active and/or healing ulcers. . . considered a result of stress secondary to exposure. . . ." We tend to dismiss their conclusion that the stomach lesions observed was not a compound effect. Stomach lesions were rarely seen in any of F_0 and F_1 males or females treated at lower dose levels or in controls. In their introduction, the applicant pointed out that macromolecular binding of 1,3-DCP in the stomach is known to be 4 to 5 times higher than that observed in other major organs such as liver, kidney, or even urinary bladder. There is also a depression in non-protein sulfhydryl content of the stomach and liver that was dose related, seen in a previous metabolism study with Telone in rats. In pharmacokinetic studies with mice, distribution to the nonglandular stomach region was 15 percent whereas it was only 6 or 7 percent in kidney or liver and only 3 percent in glandular stomach. In a rat pharmacokinetic study, distribution to nonglandular stomach was also higher than to the glandular stomach and to some of the major organs.

We suggest that a possible reason for stomach lesions in the nonglandular tissue may be due to preferential uptaken and retention by this type of tissue. We cannot accept the conclusion by the applicant the ulcerogenic activity by 1,3-DCP in the stomach is a result of stress rather than a compound-related effect. In fact, data from animal studies submitted by the applicant supports the suggestion that the stomach lesions in rats is more likely due to preferential accumulation and retention by tissue in nonglandular regions of the stomach.

Another site of tissue damage which affected the majority of all males and females of the F_1-exposed and virtually all of the F_2 exposed adult rats was the nasal tissue. Most of them had "slight" hyperplasia of the respiratory epithelium but some had inflammatory responses and a number had "very slight" degeneration of olfactory epithelium. It is claimed that the respiratory epithelial damage occurred in the nasal turbinate section at the level of the first ruga palantina, involving about 1 percent of

the respiratory mucosa. The lesions consisted of disorganization of respiratory epithelium characterized by "slight" hyperplasia and uniform thickening which occasionally produced a pseudostratified appearance. Olfactory epithelium damage was characterized by "a focal degenerative process in the neuronal layer and loss of sustenticular cell cytoplasm growth or survival.. ..." The data presented supports this conclusion.

There are discrepancies in the data of Tables 18 and 19 on pages 50 and 51 of the submitted report. Values for N (number of litters are surprisingly low for body weight and body weight gain of dams on day 21 of gestation. This requires an explanation because there is no indication of any loss of litters. No gross or histopathological damage due to high dose treatment was seen in the reproductive organs of males (testes, prostate, seminal vesicles, epididymides, and coagulating glands) or females (ovaries, uterus, oviducts, cervix, and vagina). Also, no effects due to high dose treatment was seen in a few major organs including kidney and liver. Unfortunately, weight measurements of major organs and reproductive organs were not obtained at necrospy. In 2 studies previously reviewed at EPA, liver, and kidney weight increases were observed after only 12 weeks of inhalation exposure at a dose as low as 5 ppm in 1 study and 50 ppm in the second study. Also, "cloudy swelling of renal tubular epithelium" was observed in male rats exposed by inhalation for 6 months. Weights of reproductive organs and major organs would have been particularly useful since it was already well known from 2 previous studies that Telone caused increases in weight of two major organs and damage to renal tubular epithelium in a third study. We also believe it was unfortunate that organ weights on weaned pups and at least a little more pathology was not performed on weanling rats killed on about 28 days of age after exposure of the mothers to Telone during most of pregnancy and lactation. Only 10 weanlings per sex in each dose group were examined for gross pathology in the F_{1b} and F_{2b} litters and none at all in the F_{1a} and F_{2a} litters. No histopathology was included.

Tentatively, the NOEL for reproductive effects is considered as being greater than 90 ppm.

Although toxic effects to the F_0 and F_1 exposed adults (decreased weight gain, stomach, and nasal mucosa damage) were seen at 90 ppm but not usually seen at 30 ppm, we believe it is not possible to accurately assign a meaningful NOEL or LOEL for systemic effects to exposed adults in the absence of organ weight data. This is based on the previous observations by the applicant of increased kidney

and liver weights and renal histopathology damage at even lower dose levels and duration of time following inhalation exposure, discussed above.

Core Classification: Supplementary