

CASWELL FILE



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

JUN 5 1985

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a developmental toxicology study and a subchronic study with Bladex (Cyanazine)  
EPA Reg. No. 201-298  
EPA Accession No. 257867 & 257868 Caswell No. 188 C

TO: Robert Taylor, PM #25  
Registration Division (TS-767C)

FROM: Quang Q. Bui, Ph.D. *Quang Bui 5/30/85*  
Section V, Toxicology Branch  
Hazard Evaluation Division (TS-769C)

THROUGH: Laurence D. Chitlik, D.A.B.T. *LDC 5/31/85*  
Section Head, Section V  
Toxicology Branch/HED (TS-769C)

and

Theodore M. Farber, Ph.D. *TFB 6/3/85*  
Chief, Toxicology Branch  
Hazard Evaluation Division (TS-769C)

Registrant:

Shell Oil Company  
Washington D.C. 20036

Action Requested:

Expedite request for review of a developmental toxicology study with a post-natal segment (Argus Research Lab.) and a sub-chronic dermal study (Stanford Research Institute) with Bladex (Cyanazine).

RECOMMENDATION

1. It is recommended that the Teratology study with Post-natal investigation in Fischer-344 rats (Argus Research Lab. #619-002) be classified as Core Minimum Data with the following findings:

- a. Under the conditions of this study, maternal toxicity was demonstrated at all dosage levels including the lowest dose (Maternal NOEL < 5 mg/kg/day) as characterized by significant body weight depressions and dose-related increases in clinical manifestations during the dosing period.

b. Significant alterations in skeletal variations were found at all dosage levels including the lowest dose tested (5 mg/kg/day). Significant decreases in lumbar vertebrae ossification sites and concomitant increases in extra ribs and thoracic centra ossification sites were noted at all dosage levels tested. Dose-related increases in post-implantation loss were found at the 25 and 75 mg/kg dose levels with significant increases in the incidences of fetuses and litters with malformations (dilated brain ventricles, microphthalmia, cleft palate) noted at the 75 mg/kg dose level.

Pup body weight, mortality, eye-opening, pupil constriction, and necropsy findings were similar between the control and 5 mg/kg/day group. However, significant increases in the incidences of fetuses and litters with diaphragm-related findings were found at the 25 and 75 mg/kg dosage levels.

Based upon these findings, it is concluded that no NOEL for developmental toxicity was demonstrated at the dose levels selected (Developmental Toxicity NOEL < 5 mg/kg/day). Teratogenic effects are demonstrated by the presence of abnormal development of the diaphragm at the 25 and 75 mg/kg dosage levels, by anophthalmia/microphthalmia at the 25 and 75 mg/kg dose levels, and by cleft palate, exencephaly, and dilated brain ventricles at the 75 mg/kg dosage level. It is concluded that the Teratogenic NOEL be established at 5 mg/kg/day.

#### c. Data Evaluation

It is recommended that:

Maternal NOEL < 5 mg/kg/day (LDT)  
Developmental NOEL < 5 mg/kg/day (LDT; as demonstrated from the C-section derived data)  
Teratogenic NOEL = 5 mg/kg/day (as demonstrated from both the C-section and post-natal data)

2. The sub-chronic dermal study in rabbits (Stanford Research Institute Inc. #868-19) is classified as Core Supplementary Data due to several deficiencies in the study conduct as listed on page 20 of this memo.

## STUDY REVIEW

Chemical: Bladex, Cyanazine  
Test Material: Technical Bladex  
Study/Action Type: Developmental Toxicology (Teratology and Post-natal study)

### STUDY IDENTIFICATION:

"Study of the developmental toxicity of Technical Bladex herbicide (SD-15418) in Fischer-344 rats".

Testing Facility: Argus Research Laboratory Inc.,  
Horsham, PA  
Final Report No.: 619-002  
Final Report Date: 4/18/85  
Study Directors: E.A. Lochry, A.M. Hoberman, M.S. Christian  
EPA Accession No.: 257867

Study Reviewed by: Quang Q. Bui, Ph.D.  
Section V, Toxicology Branch  
Hazard Evaluation Division

Study Approved by: Laurence D. Chitlik, D.A.B.T.  
Section Head, Section V  
Toxicology Branch/HED

## BACKGROUND

The teratogenic potential of Bladex in Fischer-344 rats was investigated in a study conducted by Westhollow Res. Center, Project #61230, December 81 and submitted to the Agency for review under Accession No. 070584. Dr. Dykstra in his review of 2/3/82 indicated that the presence of eyes small or absent and diaphragmatic hernia are suggestive of teratogenic effects. Dr. Dykstra concluded that in order to adequately assess the teratogenic potential of Bladex a new study was requested.

The registrant submitted an addendum to the study on 7/6/83 (Accession No. 071739). Included in that addendum was a review of Dr. Christian who served as a consultant to the registrant. These additional data and rebuttals were reviewed by Dr. Mahfouz (memo of 11/14/83). Dr. Mahfouz indicated that the study remained classified as Core Supplementary Data and suggested that a new teratology study with a post-natal phase needed to be conducted in Fischer-344 rats to ascertain the nature and incidence of diaphragmatic hernia and anophthalmia/microphthalmia apparently induced in this strain as well as to determine the survivability of the affected fetuses.

In this action, a new teratology study with a post-natal phase in Fischer-344 rats conducted at Argus Research Laboratory was submitted by the registrant (4/23/85).

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## CONCLUSION

Under the conditions of this study, maternal toxicity was demonstrated at all dosage levels tested including the lowest dose (Maternal NOEL < 5 mg/kg/day). Significant body weight depressions were noted in all Bladex-groups during the dosing period as well as throughout gestation. Administration of Bladex at 25 and 75 mg/kg/day during gestational days 6-15 were also associated with decreased food consumption, increased resorptions, increased clinical adverse effects, decreased implantation efficiency, and increased post-implantation loss. Besides excessive maternal lethality observed at 75 mg/kg, this dosage level also resulted in significant increases in the number of dams with complete resorptions and a persistent depression on food intake up to post-natal day 21.

Although the means of litter size, post-implantation loss, fetal weight, fetal length, and survival rates of the lowest dose group (5 mg/kg) were not exceptionally different from those of the controls, this dosage level was still associated with significant alterations in skeletal variations. Significant decreases in "lumbar vertebrae" ossification sites and concomitant increases in "extra ribs" and "thoracic vertebrae" ossification sites were found at all dosage levels tested (Developmental Toxicity NOEL < 5 mg/kg/day)

In the fetuses, frank teratogenic effects were noted only in the 75 mg/kg group as characterized by significant increases in the litter and fetal incidences of external and visceral malformations. Microphthalmia, cleft palate, and dilated brain ventricles were found only at this dosage level.

Examination of the pups at post-natal day 21 revealed the presence of microphthalmia and/or anophthalmia and some unusual findings relating to the diaphragm. Increases in the incidences of microphthalmia and/or anophthalmia were noted in the 25 and 75 mg/kg groups with statistical significance found at the 75 mg/kg/day dosage level. "Diaphragm, central tendon incomplete fusion with raised area of the liver" was also found in the 25 and 75 mg/kg groups with significant increases noted at both dosage levels. Although this finding could not be regarded as a diaphragmatic hernia due to the absence of diaphragmatic perforation, it should still be regarded as a malformation of the diaphragm and/or liver due to (1) absence in the controls, (2) dose-response increases, (3) compound-induced findings.

No significant delays in eye-opening and eye-constriction reflex were noted in the post-natal study.

From the previous data, two malformations were of concern to the Agency, namely small or absent eyes and diaphragmatic hernia. Microphthalmia and/or anophthalmia were present in this study and apparently were increased at the 25 and 75 mg/kg groups as compared to controls. Although diaphragmatic hernia per se was not confirmed in this study, the presence of abnormal development of the diaphragm at the 25 and 75 mg/kg dosage levels is considered a teratogenic effect.

Based upon the reported findings in this study, it is concluded that the Teratogenic NOEL be established at 5 mg/kg/day.

## RECOMMENDATION

It is recommended that this study be classified as Core Minimum Data.

## PROCEDURES

Test Material:	Technical Bladex (SD-15418)
Vehicle:	0.25% aqueous methyl cellulose
Dosage levels:	0, 5, 25, and 75 mg/kg/day by gavage
Period of administration:	days 6-15 of gestation
Species:	Fischer-344 rats

The protocol used in this study was previously evaluated by this reviewer (memo of 10/12/84). No significant deviations from the originally submitted protocol were noted. However, due to high mortality, the number of dams in the 75 mg/kg group of the post-natal study was reduced to eight instead of approximately 30 as originally planned.

In summary, this investigation was designed to evaluate the teratogenic potential of Bladex both at term and post-natally in Fischer-344 rats dosed with the test material during the period of major organogenesis (a copy of the study procedures is appended).

## RESULTS

### Maternal Mortality

A high incidence of maternal deaths was observed in the 75 mg/kg group (18.6%; 13/70) and apparently was compound related. Necropsy of the dead animals indicated that 12/13 dead animals were pregnant. Lesions in the GI tract, thinning of the stomach wall, and ulcerations of the GI tract were the most common observations.

No other rats died during the course of the study.

### Clinical Observations

Compound-related toxic signs were noted in all treated groups and included: lacrimation, decreased palpebral size, excess salivation, chromodacryorrhea, soft feces, alopecia, clear rectal discharge, decreased motor activity, ataxia, and ptosis. The severity of these clinical signs were proportional to the dosage levels used. Only the 5 mg/kg group had an incidence similar in frequency and severity to the controls.

### Pregnancy Rates

The pregnancy rates for the 0, 5, 25, and 75 mg/kg groups were respectively 80%, 80%, 88.6%, and 75.7%. These rates were within the acceptable ranges for rats.

Pregnant animals were divided into two sections: those sacrificed on day 20 of gestation and those allowed to deliver. For the first section of this investigation (conventional teratology study), 25, 25, 25, and 21 dams were sacrificed on day 20 of gestation for the 0, 5, 25, and 75 mg/kg groups, respectively. The rest of the pregnant animals were reserved for the second phase (31, 30, 29, and 8 dams for the 0, 5, 25, and 75 mg/kg groups, respectively).

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## A. TERATOLOGY PHASE

### Maternal Body Weight Data

Maternal body weights were recorded daily throughout gestation. The body weight gain data at different periods of gestation are presented in Table 1

Table 1: Maternal Body Weight Data/Gestation

<u>Body Weight Gain (grams)</u>	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
# of dams	56	56	62	53
Days 0-6	17.1 ± 4.3	16.4 ± 4.7	17.1 ± 4.5	16.1 ± 4.9
Days 6-15	33.3 ± 5.0	24.4 ± 5.5*	7.7 ± 12.0**	-15.3 ± 9.1**
Days 16-20	36.1 ± 9.4	36.4 ± 9.4	35.4 ± 17.6	22.4 ± 16.8**
Days 0-20	92.8 ± 13.9	83.5 ± 14.3*	66.4 ± 29.7**	26.8 ± 24.8**

(\*) Significantly different from controls, P < 0.05

(\*\*) Significantly different from controls, P < 0.01

Prior to the dosing period (days 0-6), no differences in body weight gain were noted among the groups. However, during the dosing period (days 6-15) compound-induced body weight reductions were found in all treated groups. These effects were statistically different from control values at the 0.05 level for the 5 mg/kg group and at the 0.01 level for both the 25 and 75 mg/kg groups. After the termination of dosing (days 16-20), compensatory effects were noted for all treated groups but significant differences (P<0.01) in body weight gains were still evident for the 75 mg/kg group. Throughout the gestation period (days 0-20) dose-related depressions in body weight gains were noted in all Bladex-treated dams with significant differences found at all dosage levels including the lowest dose tested (5 mg/kg; P<0.05).

To determine whether the body weight reductions noted in all treated groups were also associated with decreases in food intake, the mean food consumption values (gm/kg/day) were calculated for the gestation period and presented in Table 2.

### Maternal Food Consumption Data

Comparable food consumption data were found among the groups prior to the dosing period. Compound-related reductions in food consumption were noted in all treated groups during the dosing period (days 6-15 of gestation) attaining significant differences at the 25 and 75 mg/kg dosage level. After cessation of dosing, all treated groups exhibited a rebound increase in food consumption. These findings suggested that administration of Bladex at all dosage levels (5, 25, and 75 mg/kg) resulted in decreased food consumption which was found to be significant at the 25 and 75 mg/kg/day dose levels.

The decreases in body weight gains discussed earlier may be associated with the reductions in food intake induced by the administration of Bladex.

Table 2: Maternal Food Consumption Data/Gestation

<u>Food Consumption †</u> <u>(gm/kg/day)</u>	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
# of dams	56	56	62	53
Days 0-6	70.8	68.8	70.4	70.0
Days 7-16	76.5	70.7	61.4*	54.5*
Days 17-21	66.2	68.0	71.4	84.8*
Days 0-21	72.4	69.5	66.4	66.2

(†) calculated by this reviewer from data in Table A10

(\*) Significantly different from controls,  $P < 0.05$

Reproduction Data at C-Section

Table 3 illustrates the reproductive status of all dams sacrificed on day 20 of gestation.

Table 3: Reproductive Data from Dams Sacrificed on Day 20 of Gestation

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
# pregnant and sacrificed	25	25	25	21
# dams with complete resorption	0	0	3	10
# dams with live fetuses (%)	25(100)	25(100)	22(88)	11(52)**
$\bar{X}$ corpora lutea/dam	12.2	12.7	13.6*	14.5**
$\bar{X}$ implantations/dam	11.0	11.9	11.8	11.1
Implantation Efficiency†	90.2%	93.7%	86.8%	76.6%
$\bar{X}$ resorptions/dam	0.4	0.3	1.8	7.0**
$\bar{X}$ live fetuses/dam	10.7	11.6	10.0	4.1**
Total dead fetuses	0.0	0.0	1.0	1.0
Postimplantation loss %††	3.3	2.3	15.2*	62.8**
# dams with resorptions(%)	7(28)	6(24)	11(44)	20(95)

(†) Total implantations/total corpora lutea X 100; calculated by this reviewer

(††) Total resorptions + Dead fetuses/Total implant.X 100;calculated by this reviewer

(\*) Significantly different from controls,  $P < 0.05$

(\*\*) Significantly different from controls,  $P < 0.01$

Respectively, 25, 25, 25, and 21 pregnant animals of the 0, 5, 25, and 75 mg/kg groups were sacrificed on day 20 of gestation. Necropsy data revealed that 3 and 10 dams of the 25 and 75 mg/kg groups respectively had their litters completely resorbed. These findings apparently were compound-related.

A significant increase in the mean number of corpora lutea was noted in the 25 and 75 mg/kg groups. It is doubtful whether there is any biological significance associated with this finding. The number of mean implantations was similar among control and treated groups. When the implantation efficiency was calculated by this reviewer, significant decreases in this index were noted at the two highest dosage groups. The decrease in implantation efficiency in the 25 and 75 mg/kg groups was probably unrelated to test material administration but rather as a consequence of the unexpected increase in corpora lutea noted in these treated groups.

Compound-related increases in the mean number of resorptions per litter were found in the 25 and 75 mg/kg groups with significant differences observed at the highest dosage level. Due to resorptions, the mean number of live fetuses per dam in these two highest dosage groups was also less than that of controls with statistical differences noted at the 75 mg/kg group. No dose-related and statistically significant increase in the number of dead fetuses was observed.

Signs of embryo-fetotoxicity were evidenced by a dose-response increase in the number of dams with 1 or more resorptions. These incidences were 28, 24, 44, and 95% for the groups receiving 0, 5, 25, and 75 mg/kg, respectively.

#### Fetal Data/Teratology Phase

The fetal data collected at C-section were summarized in table 4

Table 4: Fetal Data at C-Section

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
# pregnant dams examined	25	25	25	21
# dams with live fetuses	25	25	22	11
Total live fetuses	267	290	250	86
Total dead fetuses	0	0	1	1
$\bar{X}$ male weight (g)	3.1	3.2	3.2	2.4**
$\bar{X}$ female weight (g)	2.9	2.9	3.0	2.2**
$\bar{X}$ crown-rump length (cm)	3.3	3.3	3.3	3.0**

(\*\*) Significantly different from controls,  $P < 0.01$

As indicated earlier, the litter size was statistically reduced in the 75 mg/kg group. No variations in fetal sex ratio were detected. Significant decreases in mean male and female fetal weights and crown-rump length were noted only at the 75 mg/kg dosage level.

Table 5 summarizes the reported gross, visceral, and skeletal findings of all treated and control fetuses.

(NOTE: Table 5 includes both malformations and variations since no distinctions are made by the investigators in the tables of the final report. However, distinctions between malformations and variations are mentioned in the text summary and discussion (page 1-4)).



Table 5: Fetal Observations

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
<u>EXTERNAL OBSERVATIONS</u>				
# fetuses (litters)				
examined	267(25)	290(25)	251(22)	87(11)
Exencephaly	0(0)	0(0)	0(0)	1(1)*
Eyes, bulged depressed	0(0)	0(0)	0(0)	6(3)*
Cleft palate	0(0)	0(0)	0(0)	4(1)*
Micrognathia with				
cleft palate, hypoglossia	0(0)	0(0)	0(0)	1(1)*
Total fetuses (%)†	0(0)	0(0)	0(0)	12(13.8)*
Total litters (%)†	0(0)	0(0)	0(0)	6(54.5)*
<u>SOFT-TISSUE EXAMINATIONS</u>				
# fetuses (litters)				
examined	125(25)	138(25)	123(22)	40(10)
Cleft palate	0(0)	0(0)	0(0)	4(1)*a
Micrognathia with				
cleft palate, hypoglossia	0(0)	0(0)	0(0)	1(1)*a
Microphthalmia	0(0)	0(0)	0(0)	6(3)*a
Brain ventricles dilated	2(2)	1(1)	0(0)	13(4)*b
Hydronephrosis associated				
dilatation of ureters	0(0)	0(0)	1(1)	1(1)
Total fetuses (%)†	2(1.6)	1(0.7)	1(0.8)	19(47.5)*
Total litters (%)†	2(8.0)	1(4.0)	1(4.5)	7(70.0)*
<u>SKELETAL FINDINGS</u>				
# fetuses(litters)				
examined	142(25)	152(25)	128(22)	46(10)
Skull, sphenoid hole	0(0)	0(0)	0(0)	4(3)*
Skull, bones unossified	0(0)	0(0)	0(0)	1(1)*
Vertebrae, centra bifid	4(4)	4(4)	4(4)	2(2)
Manubrium, unil.ossified	0(0)	0(0)	2(2)	6(5)*
Manubrium, bifid	1(1)	0(0)	0(0)	3(3)*
Fused sternebrae	1(1)	0(0)	1(1)	0(0)
Manubrium, irregular				
shaped	0(0)	0(0)	0(0)	2(2)*
Manubrium, asymmetric	0(0)	0(0)	1(1)	0(0)
Sternebrae, unil.ossified	0(0)	0(0)	1(1)	1(1)
Sternebrae, centra unossi.	0(0)	0(0)	0(0)	4(4)*
Sternebrae, centra asymmetric	0	0(0)	0(0)	1(1)*
Total fetuses (%)†	5(3.5)	4(2.6)	8(6.3)	20(43.4)*
Total litters (%)†	5(20.0)	4(16.0)	8(36.4)	9(90.0)*

(a) : Confirmed the external observations

(†) : Calculated by this reviewer

(\*) : Significantly different from controls,  $P < 0.05$

(b) : Grading from slight to moderate

External observations of the fetuses revealed increased incidences of eyes, bulge depressed unilateral or bilateral and cleft palate in the highest dosage group. One pup of this group was described with micrognathia and another pup with exencephaly. The litter and fetal incidences of external findings were significantly increased in the 75 mg/kg group.

Soft-tissue examinations confirmed the external findings. The "eyes, bulge, depressed" observed externally were described as microphthalmia. The incidence of microphthalmia attained significance at the 75 mg/kg dosage level with three litters (27%) and 6 fetuses (7%) affected. Dilation of the brain ventricles was found at a low incidence in the control (2 fetuses/2 litters) and in the 5 mg/kg group (1 fetus/1 litter). However, the number of fetuses (32.5%) and litters (40%) with this finding was relatively high in the 75 mg/kg group as compared to control values. Significant increases in fetal and litter incidences of soft-tissue findings were observed only at the 75 mg/kg dosage level.

Skeletal abnormalities were found in all groups. Skeletal findings that were observed in the treated animals but not the controls included: skull, sphenoid contains a hole, manubrium - unilateral ossification, manubrium - irregularly shaped, manubrium - asymmetric, and centrum sternbrae - incompletely or not ossified. Most of these findings were restricted to the 75 mg/kg group. The fetal incidences for skeletal findings were 3.5, 2.6, 6.3, and 43.5% for the groups receiving respectively 0, 5, 25, and 75 mg/kg. Respective litter incidences of skeletal findings were 20, 16, 36, and 90%. Statistical significances in fetal and litter incidences were attained only at the 75 mg/kg dosage level.

#### Fetal Ossification Site Averages

In this study, fetal ossification site averages were calculated (per fetus and per litter) with findings of interest summarized in table 6.

Table 6: Fetal Ossification Site Averages

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
# fetuses examined	142	152	127	45
# litters examined	25	25	22	10
<u>Ossification Sites</u>				
Thoracic Vertebrae	13.04	13.14**	13.83**	13.80**
Lumbar Vertebrae	5.96	5.84**	5.17**	5.20**
Ribs	13.02	13.10**	13.71**	13.77**
Sternal	3.62	3.72	3.85**	2.58**
Xiphoid	0.91	0.94	0.90	0.23**
Metacarpals	3.96	3.96	3.98	3.34**
Phalanges (forepaw)	5.36	5.47	5.39	2.56**
Metatarsals	4.00	4.00	4.00	3.81**
Phalanges (hindpaw)	4.89	4.90	4.75	1.14**

(\*\*) Significantly different from controls,  $P < 0.01$

Data extracted from Table A18, p.A-41

Decreased ossification sites of the sternal, xiphoid, metacarpals, and phalanges (both hindpaws and forepaws) attained statistical significances only at the 75 mg/kg dosage level. The incidences of decreased lumbar vertebrae ossification sites apparently were compound-related and attained statistical significances at all dosage levels tested.

Significant increases in the incidences of rib and thoracic vertebrae ossification sites were noted at all dosage levels tested including the lowest dose tested.

#### B. POST-NATAL STUDY

Animals that were not selected for sacrifice on day 20 of gestation were allowed to deliver and raise their offspring up to day 21 of lactation. The number of dams which delivered for the groups receiving 0, 5, 25, and 75 mg/kg were respectively 31, 30, 29, and 8.

Table 7 summarizes the reproductive status of all animals used in this study:

Table 7: Reproductive status of dams

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
Number dams pregnant	56	56	62	53
# dams sacrificed on day 20 of gestation	25	25	25	21
# pregnant dams that died	0	0	0	12
# dams delivered	31	30	29	8
# dams sacrificed on day 25 of gestation	0	1	8	12

All dams not delivering were sacrificed on day 25 of gestation and were described as having total litters resorbed. Table 3 (page 5) of this memo shows that, in the teratology phase, 3 and 10 dams of the 25 and 75 mg/kg groups were found to have their litters completely resorbed at cesarean. Therefore, the total number of dams with complete resorptions was 0(0%), 1(1.7%), 11(17.7%), and 22(41.5%) for the control, 5, 25, and 75 mg/kg groups, respectively.

### Maternal Body Weight/Lactation Period

The maternal body weight data is tabulated as follows:

Table 8: Maternal Body Weight/Lactation Period

<u>Body Weight (grams)</u>	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
Day 1	212.3	203.8**	194.6**	183.0**
Day 21	237.6	234.3	229.9*	238.8
Body weight gain	25.3	30.6	35.3	56.2**
% increase(a)	11.9	15.0	18.1	30.7

(a): Percent increase of day 1 lactation body weight

(\*) Significantly different from controls,  $P < 0.05$

(\*\*) Significantly different from controls,  $P < 0.01$

On day 1 of lactation, significant differences in maternal weights were noted in all treated groups as compared with controls. These differences were due to the effects of Bladex administration during days 6-15 of gestation which still persisted up to the day of delivery.

A rebound effect increase in body weight was observed in all treated groups during lactation. On day 21 of lactation, all treated groups had comparable maternal body weight with the controls except for the mid-dose group. However, all treated groups had higher body weight gains during lactation than controls. The body weight gains for the control, 5, 25, and 75 mg/kg groups were respectively 25.3, 30.6, 35.3, and 56.2 grams.

### Maternal Food Consumption/Lactation Period

Throughout lactation (days 1-21), the average daily food consumption (g/kg/day) was calculated by this reviewer to be 164.1, 165.7, 177.0, and 117.0 for the 0, 5, 25, and 75 mg/kg groups, respectively. No differences in food consumption were found between the control, 5 and 25 mg/kg groups. However, statistically significant differences ( $P < 0.05$ ) were still evident at the 75 mg/kg dosage level suggesting persistent effects of Bladex.

### Delivery Data

Table 9 summarizes the data collected from dams which delivered naturally

Table 9: Maternal Natural Delivery Data

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
# dams delivery	31	30	29	8
Gestation length (days)	23.1	23.1	23.1	23.5*
# dams delivering 1 or more live pups	31	30	29	8
# dams with stillborn pups (%)	1(3.2)	0(0)	0(0)	2(25.0)**
Total pups delivered	334	312	318	68
X litter size	10.8	10.4	11.0	8.5*
Total pups alive	332	312	318	65
X live pups/litter	10.7	10.4	11.0	8.1*
X pup weight/litter (at birth; grams)	5.4	5.5	5.5	5.1

(\*) Significantly different from controls,  $P < 0.05$

(\*\*) Significantly different from controls,  $P < 0.01$

A significant increase in gestational length was found in the 75 mg/kg group.

One and two litters of the control and 75 mg/kg groups respectively delivered stillborn pups. Due to the small number of dams in the 75 mg/kg group (8 dams), the two litters with stillborn represented 25% of the number of dams in that group and reached statistical significances from control values at the 0.05 level.

Compound-related effects were noted in the 75 mg/kg group as evidenced by a decrease in the mean number of pups born/litter and live pups/litter. Pup weights at birth were comparable among all groups although a slight decrease was noted only in the 75 mg/kg group. This decrease was not significantly different from controls.

#### Pup Data

The decrease in the mean number of live pups/litter observed in the 75 mg/kg group observed at birth (Table 9) persisted up to day 4 and 21 of lactation (Table 10). When the viability and lactation indices were calculated by this reviewer, significant differences in these two indices were found in the 75 mg/kg group as compared with control values. The low and mid-dose group indices were not exceptionally different from controls.

The slight pup weight depression noted in the 75 mg/kg group at birth attained statistical differences by day 4 of lactation and remained lower than control values throughout lactation. No differences in pup weights were found between the 5, 25 mg/kg and control groups up to post-natal day 21. Overall, pups from the 0, 5, 25, and 75 mg/kg groups gained an average of 23.4, 23.5, 23.1, and 18.8 grams throughout lactation.

Table 10: Pup Body Weight and Mortality Data

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
Pup Weight/litter (grams)				
Day 1 lactation	5.4	5.5	5.5	5.1
Day 4 lactation	7.4	7.3	7.2	5.9**
Day 21 lactation	28.8	29.0	28.6	23.9
Weight Gain	23.4	23.5	23.1	18.8*
Viability Data				
# pups live day 1	332	312	318	65
# pups live day 4	319	308	312	35
Viability Index <sup>a</sup>	96.0	98.7	98.1	53.8**
# pups live day 21	316	299	308	23
Lactation Index <sup>b</sup>	99.0	97.0	98.7	65.7**
$\bar{X}$ live pups/litter				
Day 1	10.7	10.4	11.0	8.1**
Day 4	10.3	10.3	10.8	4.4**
Day 21	10.2	10.0	10.6	2.9**
Cummulative mortality <sup>c</sup> (postnatal days 1-21)	4.8%	4.2%	3.1%	64.6%**

(\*) Significantly different from controls,  $P < 0.05$

(\*\*) Significantly different from controls,  $P < 0.01$

- (a) % Number of pups live day 4/Number of pups live day 1; calculated by this reviewer  
 (b) % Number of pups live day 21/Number of pups live day 4; calculated by this reviewer  
 (c) % Number of pups dead/Number of pups born alive; calculated by this reviewer

No significant differences in viability index, lactation index, and cummulative mortality were noted in the 5 and 25 mg/kg groups as compared to controls. However, the pups from the 75 mg/kg groups apparently could not recover from the toxic effects of Bladex as evidenced by significant increases in mortality and significant decreases in viability index and lactation index.

#### Pup Observations

The investigators indicated that the litter incidence of pups with chromodacryorrhea was higher than controls in the 25 and 75 mg/kg groups. In general, pups from the 75 mg/kg groups appeared thin or weak in appearance. These findings may well be related to the low pup weights observed with this dosage level.

### Pup Eye Opening and Pupil Constriction

In this study, a total of 316, 299, 308, and 23 pups of the 0, 5, 25, and 75 mg/kg groups were examined for eye opening and pupil constriction. These maturation and functional developmental data were tabulated as follows:

Table 11: Eye Opening and Pupil Constriction Data

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
Number of pups examined (litters)	316(30)	299(30)	308(29)	23(5)
<u>Eye Opening (<math>\bar{X}</math> in days)†</u>				
(a)	17.0 $\pm$ 0.7	17.1 $\pm$ 0.6	17.0 $\pm$ 0.6	18.2 $\pm$ 1.1*
(b)	17.6 $\pm$ 0.6	17.9 $\pm$ 0.8	17.9 $\pm$ 0.8	18.8 $\pm$ 1.5
(c)	18.1 $\pm$ 0.7	18.2 $\pm$ 0.8	18.5 $\pm$ 1.0	19.2 $\pm$ 1.3
<u>Pupil Constriction (%)††</u>				
	99.4 $\pm$ 2.2	99.2 $\pm$ 2.6	98.5 $\pm$ 5.1	87.6 $\pm$ 21.6

(†): Criterion = the first break in the membrane covering the right and/or left eye

(a): Average day that at least 1% of all pups in a litter had opened eyes

(b): Average day that at least 50% of all pups in a litter had opened eyes

(c): Average day that 100% of all pups in a litter had opened eyes

(††): Criterion = Constriction of the pupil of each eye following light stimulation of the same eye and of the contralateral eye.

(\*): Significantly different from controls,  $P < 0.05$

Respectively 3, 1, 5 and 7 pups of the 0, 5, 25, and 75 mg/kg groups could not be tested for eye opening or pupil constriction. Necropsy of these pups indicated that they were affected with either microphthalmia or anophthalmia (see "Necropsy" section).

A slight delay in eye opening was observed in the 25 and 75 mg/kg groups. However, only the 75 mg/kg group exhibited a slightly lower percentage of pups with pupil constriction reflex. The pupil constriction reflex recorded in pups of the 5 and 25 mg/kg groups was comparable to controls.

### Pup Necropsy

Table 12 summarizes the necropsy findings. As indicated in the previous section, tests for eye-opening and eyeconstriction could not be performed on several pups of the control and treated groups. Necropsy of these pups indicated that in the control group 1 pup had anophthalmia and 2 pups had microphthalmia. Anophthalmia was also found in 3 and 6 pups of the 25 and 75 mg/kg group, respectively. Two pups of the 25 mg/kg group and 1 pup of the 75 mg/kg group had microphthalmia. The litter and pup incidence of anophthalmia and/or microphthalmia in the 75 mg/kg group was statistically different from control values ( $P < 0.01$ ).

Table 12: Pups Necropsy Data

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
# pups evaluated	316	299	308	23
# litters evaluated	30	30	29	5
Anophthalmia				
Pups (%)	1(0.3)	0(0)	3(1.0)	6(26.1)**
Litters (%)	1(3.3)	0	3(10.3)	3(60.0)**
Microphthalmia				
Pups (%)	2(0.6)	0	2(0.6)	1(4.3)**
Litters (%)	2(6.7)	0	2(6.9)	1(20.0)
Total pups with anophthalmia and/or microphthalmia				
Pups (%)	3(6.9)	0	5(1.6)	7(30.4)**
Litters (%)	2(6.7)	0	4(13.8)	4(80.0)**
Chromodacryorrhea				
Pups (%)	0	4(1.3)	16(5.2)**	1(4.3)**
Litters (%)	0	2(6.7)	5(17.2)	1(20.0)
Diaphragm, central tendon incomplete fusion with raised area of liver				
Pups (%)	0	0	4(1.3)*	3(13.0)**
Litters (%)	0	0	4(13.8)**	2(40.0)**
Diaphragm, central tendon raised area				
Pups (%)	3(0.9)	4(1.3)	9(2.9)	6(26.1)**
Litters (%)	3(10.0)	3(10.0)	6(20.7)	4(80.0)**
Diaphragm, any finding†				
Pups (%)	3(0.9)	4(1.3)	13(4.2)*	9(39.1)**
Litters (%)	3(10.0)	3(10.0)	9(31.0)	5(100.0)**

(\*): Significantly different from controls,  $P < 0.05$

(\*\*): Significantly different from controls,  $P < 0.01$

(†): Calculated by this reviewer

In pups born from mothers treated with 75 mg/kg, the incidences of anophthalmia and/or microphthalmia, "central tendon incomplete fusion with raised area of liver", and "central tendon raised area" were significantly increased in the 75 mg/kg group. "Central tendon incomplete fusion with raised area of liver" were also significantly increased at the 25 mg/kg group.

Apparently two findings were of concern in this study, namely, anophthalmia/microphthalmia and "Central tendon incomplete fusion with raised area of liver". A summary of these incidences found in both fetuses and pups is presented as follows:



Table 13: Summary of Ophthalmologic and Diaphragmatic Variations Observed in Fetuses and Pups

	<u>Control</u>	<u>5 mg/kg</u>	<u>25 mg/kg</u>	<u>75 mg/kg</u>
Number of pups and fetuses evaluated	583	589	559	110
Number of litters evaluated	55	55	51	16
<u>Microphthalmia and/or anophthalmia</u>				
Fetal/pups (%)	3(0.5)	0	5(0.9)	13(11.8)**
Litters (%)	2(3.6)	0	4(7.8)	7(43.8)**
<u>Central tendon incomplete fusion with raised area of liver</u>				
Fetal/pups (%)	0	0	4(0.7)*	3(2.7)**
Litters (%)	0	0	4(7.8)**	2(12.5)**
<u>Central tendon raised area</u>				
Fetal/pups (%)	3(0.5)	4(0.7)	9(1.6)	6(5.4)**
Litters (%)	3(5.4)	3(5.4)	6(11.8)	4(25.0)*

(\*) Significantly different from controls,  $P < 0.05$

(\*\*) Significantly different from controls,  $P < 0.01$

When the findings in fetuses and pups were combined, significant increases in litter and fetal incidences of "central tendon incomplete fusion with raised area of liver" were evident at the 25 and 75 mg/kg dosage levels. The highest dose tested was also associated with significant increases in both litter and fetal incidences of microphthalmia and/or anophthalmia and in the fetal incidence of "central tendon raised area".

## DISCUSSION

Administration of Technical Bladex to Fischer-344 rats from days 6-15 of gestation was associated with several manifestations of maternal toxicity. Significant decreases in body weight gains during the dosing period as well as throughout gestation were noted at all dosage levels tested (5, 25, and 75 mg/kg/day). Concomitant reductions in food intake were noted in all treated groups during the dosing period and attained significant differences at the 25 and 75 mg/kg dosage levels. The food intake depression persisted in the 75 mg/kg group up to post-natal day 21.

Compound-related clinical manifestations such as decreased palpebral size, excess salivation, chromodacryorrhea, soft feces, alopecia, decreased motor activity, etc...., were found at the 25 and 75 mg/kg groups. Dosing at 75 mg/kg/day was also associated with excessive maternal lethality (18%), significant increases in the number of dams with complete resorptions (41.5%), significant decreases in implantation efficiency and mean litter size, as well as increased gestational length. Evidence of a compound-related effect was also noted at the 25 mg/kg dosage level as characterized by an increase in the number of dams with complete resorptions (17.7%).

It can be concluded that a maternal NOEL could not be demonstrated from the submitted data as indicated by significant decreases in body weight gains during the dosing period as well as throughout gestation noted in all groups including the lowest dosage level tested.

Significant increases in post-implantation loss were found at the 25 and 75 mg/kg dosage levels. However, fetal weight and length as well as pup survival indices (viability index and lactation index) were significantly reduced only at the 75 mg/kg dosage level. Although the means of litter size, post-implantation loss, fetal weight, and fetal length in the 5 mg/kg group were not exceptionally different from controls, several indications of compound-related fetotoxic effects were still evident at this lowest dosage level. Significant increases in the ossification process of ribs and thoracic vertebrae as well significant decreases in the ossification sites of lumbar vertebrae were found at all dosage levels tested. Consequently, a developmental toxicity NOEL could not also be demonstrated from the dosage levels selected (NOEL for developmental toxicity < 5 mg/kg/day).

In this study, the investigators tabulated all fetal findings as variations since increased incidences of both malformations and variations were considered as indications of developmental toxicity. However, the investigators did attempt to some degree to differentiate between these two findings in the text summary and discussion (page 1-4). From a regulatory stand-point, all findings should not be treated as of equal importance and differentiation should be made between a malformation and a variation based upon the severity, frequency of occurrence, and reversibility of the adverse effect.

Teratogenic effects in the cesarean obtained fetuses were characterized in this study by exencephaly, cleft palate, micrognathia, anophthalmia and/or microphthalmia, and dilated brain ventricles with most of these findings restricted to the 75 mg/kg group. Significant increases in both litter and fetal incidences of malformations were found only at the 75 mg/kg dosage level. The incidences of malformations in the 5 and 25 mg/kg groups were within the normal range: one fetus in the 5 mg/kg group had dilated brain ventricles and one fetus in

the 25 mg/kg group was described with hydronephrosis associated with dilated ureters. These two findings were considered by this reviewer as spontaneous occurrences unrelated to treatment in the absence of a dose-response relationship.

During the post-natal phase, pupil-eye opening and pupil-constriction reflex were recorded and used as indices of growth development. A slight delay in eye-opening and pupil constriction was noted in the 25 and 75 mg/kg groups. Necropsy of the pups at weaning revealed that both the fetal and litter incidences of pups with anophthalmia and/or microphthalmia increased at the 25 mg/kg and became statistically significant from controls at the 75 mg/kg dosage level.

Findings with respect to the diaphragm were also found in pups sacrificed at post-natal day 21 but not in fetuses collected on day 20 of gestation. The diaphragmatic findings were classified by the investigators as:

- a) Diaphragm, central tendon raised area
- b) Diaphragm, central tendon incomplete fusion with raised area of liver

The "diaphragm, central tendon raised area" was found in all groups including the control whereas the "diaphragm, central tendon incomplete fusion with raised area of the liver" was observed only in pups of the 25 and 75 mg/kg groups (Table 12, page 14). The investigators described the latter finding as "the liver protruded between and separated the ligamentous fibers of the central tendon of the diaphragm". The investigators concluded that these observations "represent a continuum in a variation in development of the liver" and "cannot be interpreted as the malformation, diaphragmatic hernia".

It is this reviewer's opinion that since the liver protrudes but does not perforate the diaphragm nor invade the thoracic cavity, this finding may not be interpreted as diaphragmatic hernia per se and on that basis this reviewer concurs with the investigators. However, its presence cannot be regarded as a normal variation in development but rather as a compound-induced malformation. It is recognized that any significant increase in a normal background developmental incidence may well indicate teratogenicity. In the absence of similar findings in the control group, the occurrences of "diaphragm, central tendon incomplete fusion with raised area of the liver" should be interpreted as compound-induced malformations and these findings suggest that administration of Bladex at 25 and 75 mg/kg may alter the normal development and/or function of the diaphragm and/or liver at least in Fischer-344 rats. To some extent, therefore this study confirms involvement of the diaphragm which was observed in the earlier Fischer-344 study (WRC RIR-180).

Based upon the findings in this study, it is concluded that no NOEL for Developmental Toxicity was demonstrated (NOEL for Developmental Toxicity < 5 mg/kg/day) while a NOEL for teratogenic effects was found to be 5 mg/kg/day with malformations of the diaphragm and/or liver noted at 25 and 75 mg/kg/day.

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## STUDY REVIEW

Chemical: Bladex, Cyanazine, SD 15418  
Test Material: 80% WP and 4 lbs/gal Water Dispersible Liquid (WDL)  
Study/Action Type: Subchronic dermal study in rabbits

## STUDY IDENTIFICATION

"Repeated Dermal Toxicity Studies with Formulations of SD 15418"

Testing Facility: Stanford Research Institute  
Menlo Park, CA. 94025  
Final Report No.: Project No. 868-19, Report No. 2  
Final Report Date: May 5, 1970  
Study Author: G.W. Newell  
EPA Accession No.: 257868

Study Reviewed by: Quang Q. Bui, Ph.D.  
Section V, Toxicology Branch  
Hazard Evaluation Division

Study Approved by: Laurence D. Chitlik, D.A.B.T.  
Section Head, Section V  
Toxicology Branch/HED

## BACKGROUND INFORMATION

In addition to the developmental study in Fischer-344 rats, the registrant also submitted a subchronic dermal study in rabbits conducted by Stanford Research Institute in 1970. The data from this dermal study were referred to by the registrant (E.L. Hobson, letter of 4/23/85) and enclosed with this action (EPA # 201-198).

Although this study was initially submitted by the registrant in 1970 and was previously reviewed by the Agency (Dr. Greenman, memo of 11/2/70), a re-review of this study was performed for the purpose of Core Classification.

## DISCUSSION AND CONCLUSIONS

The toxicity resulting from dermal exposure to Bladex in rabbits, if any, could not be fully demonstrated from the limited data provided. Findings of hyperkeratosis and acanthosis occurred in both control and treated animals and may have been related to the vehicles used in the preparation of the formulations. Although, a slight increase in "spermatogenesis, maturation arrest" was noted in the abraded animals, its biological significance could not be assessed with certainty due to the limited number of animals used in each test group.

Furthermore, the absence of clinical observation, chemistry, hematological, and food consumption data limits the scientific merit of this study and precludes an assessment of systemic toxicity.

Other deficiencies were also noted in this study such as the use of only two dosage levels and the lack of detail relative to the procedures used (dosing calculation, identification of dead animals, skin surface preparation,...).

RECOMMENDATION-

It is recommended that this study be classified as Core Supplementary Data for the following reasons:

- a. At least 3 dose levels should be used
- b. Lack of clinical observation data
- c. Lack of clinical chemistry data
- d. Lack of individual animal body weight data
- e. Lack of food consumption
- f. Dead animals could not be identified
- g. Inadequate description of the procedures used (preparation of skin, unknown surface area of exposure, application volume, dosing calculation)

## PROCEDURES

Test Material: SD 15418, 80% WP at 500 and 2000 mg/kg/day  
 SD 15418, 4 lb/gal WDL at 500 and 2000 mg/kg/day  
 Species: Rabbits (strain unknown)  
 Duration of treatment: 5 days/week for 3 weeks (15 applications)  
 6 hours/day

The two formulations used (80% WP and 4 lb/gal WDL) were applied to intact or abraded-skin of 5 males and 5 females each at 500 and 2000 mg/kg/day. Five abraded-skin males and females receiving the wettable powder base or the water dispersible liquid (WDL) without SD 15418 served as controls. All animals were treated for 5 days/week for 3 consecutive weeks.

The study organization is presented as follows:

	<u>Number of Males</u>	<u>Number of Females</u>
Control, WP		
abraded skin	5	5
80% WP		
abraded skin, 500 mg/kg	5	5
abraded skin, 2000 mg/kg	5	5
intact skin, 500 mg/kg	5	5
intact skin, 2000 mg/kg	5	5
Control, WDL		
abraded skin	5	5
4 lbs/gal WDL		
abraded skin, 500 mg/kg	5	5
abraded skin, 2000 mg/kg	5	5
intact skin, 500 mg/kg	5	5
intact skin, 2000 mg/kg	5	5

All animals were housed individually. Body weights were recorded weekly and all animals were sacrificed after 15 dermal exposures. Gross necropsy was performed and selected organs (liver, kidneys, spleen, heart, gonads) were removed and weighed. All animals were subjected to a complete histopathologic observation.

## RESULTS

The investigators indicated that all animals were treated with sulfaquinolone during the acclimatization period and with Tetracycline during the first week of treatment to minimize complications of pasteurellosis and of acute diarrhea.

Consequently, confounding effects and the pharmacological impacts of antibiotics on this study results could not be neglected.

Four animals in the 4 lbs/gal WDL groups died during this investigation: one control female, one 500 mg/kg abraded female, one 2000 mg/kg intact male and one 2000 mg/kg intact female. However, these dead animals could not be identified from the report. The cause of death could not be determined by this reviewer and was not mentioned by the authors.

Two animals of the 80% WP groups died: one control female and one intact-skin female in the 500 mg/kg group. Furthermore, two males (one each in the 500

and 2000 mg/kg intact-skin groups) were inadvertently considered as females until necropsy.

Body weights were recorded at study initiation and thereafter at weekly intervals. Body weight data are presented in the next table:

Body Weight Change †

	<u>MALE</u>			<u>FEMALE</u>		
	<u>Week 1</u>	<u>Week 2</u>	<u>Week 3</u>	<u>Week 1</u>	<u>Week 2</u>	<u>Week 3</u>
<u>SD 15418 80% WP</u>						
Control (abraded)	+13.7	+14.8	+33.7	+ 8.8	+13.9	+22.8
500 mg/kg (abraded)	+ 5.4	+ 9.9	+20.6	+10.2	+11.2	+15.4
2000 mg/kg (abraded)	+ 1.0	+ 1.3	+12.0	- 2.6	+ 2.4	+13.8
500 mg/kg (intact)	+ 4.8	+ 7.6	+15.9	+ 5.1	+ 7.0	+19.4
2000 mg/kg (intact)	+ 2.0	+ 2.5	+ 8.1	+ 0.9	+ 9.4	+19.5
<u>SD 15418 4 lbs/gal WDL</u>						
Control (abraded)	+ 5.0	+ 8.9	+11.1	+ 4.1	+ 7.9	+ 8.2
500 mg/kg (abraded)	+ 6.1	+ 9.9	+16.1	+10.2	+13.6	+17.1
2000 mg/kg (abraded)	-16.4	- 8.8	- 6.0	- 8.1	- 1.6	+11.6
500 mg/kg (intact)	+ 0.1	+ 4.2	+ 5.2	+ 4.8	+ 6.3	+16.0
2000 mg/kg (intact)	- 7.0	+ 0.6	+ 5.4	-11.6	- 9.4	+ 3.3

(†) All values expressed as % changes of initial body weight; calculated by this reviewer.

In the males, applications of SD 15418 80% WP resulted in compound-related depressions in body weight gain in both intact and abraded groups. Females apparently were less affected except for the 2000 mg/kg abraded group.

Compound-induced depressions in body weight gains were noted in both intact and abraded males and females treated with 2000 mg/kg of SD 15418 4 lbs/gal WDL. No significant changes were noted at the 500 mg/kg dosage level for both intact and abraded males and females.

The average organ weights of the liver, kidneys, spleen, heart, and gonads were presented by the authors with no significant differences found among the treated and control groups exposed to either 80% WP or 4 lbs/gal WDL. However, individual organ weights were not available and, hence, the relative organ weights reported could not be confirmed.

Histopathologic examinations of all animals were performed by an independent pathologist (DR. R. Shaken). Individual histopathologic sheets were available to confirm the reported findings.

Two findings of interest were: skin hyperkeratosis and spermatogenesis maturation arrest in the gonads.

Skin Hyperkeratosis

Gonads, Maturation Arrest

4 lbs/gal WDL

Males

Controls, abraded	4/5(a)	1/5
500 mg/kg, abraded	3/5	2/5
2000 mg/kg, abraded	3/5	1/4
500 mg/kg, intact	1/5	0/5
2000 mg/kg, intact	2/5	2/5

Females

Controls, abraded	3/4
500 mg/kg, abraded	3/4
2000 mg/kg, abraded	1/4
500 mg/kg, intact	1/4
2000 mg/kg, intact	3/4

80% WP

Males

Controls, abraded	3/5	1/5
500 mg/kg, abraded	2/5	1/5
2000 mg/kg, abraded	2/5	2/5
500 mg/kg, intact	1/6	0/6
2000 mg/kg, intact	4/6	2/6

Females

Controls, abraded	2/4
500 mg/kg, abraded	1/5
2000 mg/kg, abraded	4/5
500 mg/kg, intact	1/3
2000 mg/kg, intact	2/4

(a) Number affected/Number examined

Hyperkeratosis and acanthosis occurred in control animals as well as in unabraded and abraded rabbits exposed to both formulations. Therefore, the skin reaction may have been related to the vehicles used in the preparation of the formulation.

"Spermatogenesis, maturation arrest" was found in 2/10 control animals. However, its incidence was slightly higher in abraded males exposed to 4 lbs/gal WDL (3/9 = 33.3%) and 80% WP (3/10 = 30%).

No other abnormal histopathologic findings were noted between the treated and control groups.



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