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

005331

AUG - 6 1986

MEMORANDUM

SUBJECT: Expedite review of a dermal developmental toxicity study with Bladex 4L in rabbits.
EPA No. 201-208
Project No. 2115 Laswell No. 166C

TO: Joanna Dizikes, PM #64
Special Review Branch
Registration Division (TS-767C)

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Registration Division (TS-767C)

FROM: Quang Q. Bui, PhD., DABT., 8/5/86
Toxicologist, Section V
Toxicology Branch/HED (TS-769C)

THRU: Laurence D. Chitlik, DABT.,
Head, Section V
Toxicology Branch/HED (TS-769C)
and
Theodore M. Farber, PhD., DABT.,
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C) 8/6/86

Registrant: Shell Oil Company
1025 Connecticut Ave., N.W.
Washington D.C. 20036

Action Requested: Expedite review a dermal developmental toxicity study with Bladex 4L in rabbits.

Background Information

A dermal developmental toxicity study with Bladex 4L in rabbits (MIL #83002, 2/86), was previously submitted by the registrant, Shell Oil Company, to the Agency under Accession No. 261601. The study was reviewed by the Agency (memo of Q. Bui to J. Dizikes, 4/23/86) and was classified as Core Supplementary Data.

In this action, the registrant submitted a new dermal developmental toxicity study with Bladex 4L in rabbits (WRC RIR-481, MIL #83003, 6/20/86) for review.

RECOMMENDATION

It is recommended that this study (WIL #93003, 6/26/86) be classified as Core Minimum Data.

Dermal application of 0.2, 0.6, 1.2, and 2.0 ml/kg (96, 286, 573, and 955 mg/kg, respectively) of Bladex 4L to rabbits during the period of major organogenesis (days 6-18 of gestation) resulted in dermal irritation (slight erythema, fissuring, and desquamation). A positive trend decrease in body weight and food consumption was also found in all treated groups. Under the conditions of this investigation, the maternal NOEL is established at less than 96 mg/kg (lowest dose tested).

Evidence of a compound-related increase in frank teratogenic effects was not apparent from the submitted data. However, a slight decrease in skeletal ossification centers was found in fetuses of the 955 mg/kg (highest dose tested). Under the conditions of this study, a developmental toxicity NOEL is established at 573 mg/kg and the LEL at 955 mg/kg.

By the dermal route of exposure, the calculated A/D ratio for Bladex 4L is less than 1 suggesting that developmental toxicity occurs at dose levels associated with maternal toxicity.

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DATA EVALUATION RECORD

Chemical: Cyanazine, Bladex
Test Material: Bladex 4L, Off-white liquid, WRC sample No.
Study/Action Type: Developmental toxicity

Study Identification:

"A dermal developmental toxicity study in New Zealand white rabbits with the Bladex 4L formulation"

Testing Facility: Mil Research Lab. Inc.,
Report No.: MRL #93003
WRC RTR-451
Report Date: 5/26/86
Study Authors: J.P. Rose et al.,
EPA Accession No.: 263813

Reviewed by: Quang X. Bui, Ph.D.
Section V, Toxicology Branch
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Review approved by: Laurence D. Chitlik, D.A.B.T.
Head, Section V
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CONCLUSIONS AND RECOMMENDATION

Dermal administration of Bladex 4L to rabbits during the period of major organogenesis (days 6-18) produced dermal irritation (slight erythema, fissuring, and desquamation) at all dosage levels tested (LOT = 96 mg/kg). A positive trend decrease in body weight gains and food consumption was noted in all treated groups with statistical significances found at the 286, 573, and 955 mg/kg dosage levels. No dose-related effects on reproductive indices were found in the treated groups. Under the conditions of this study, the maternal toxicity NOEL is established at less than 96 mg/kg/day (lowest dose tested).

Evidence of a compound-related increase in frank teratogenic effects was not apparent from the reported data. No statistical significant differences in structural variations were noted among all groups. However, a slight increase in the litter incidences of 7th sternebrae, 27th presacral vertebrae, and hyoid body unossified was noted in the 955 mg/kg group (HDT). A slight decrease in fetal weight was found at the 573 and 955 mg/kg dosage levels but a statistical difference was not attained.

Under the conditions of this study, the developmental toxicity NOEL is established at 573 mg/kg and the LEL at 955 mg/kg (highest dose tested).

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

PROCEDURES

A copy of the study procedures is appended. The following highlights and comments are noted:

1. Animals were restrained in stock during the exposure period (6 hours/daily from gestational days 6-18) followed by wearing a collar for the remainder of each day (18 hours/daily).

2. Two control groups were used: naive and vehicle. These two control groups were run concurrently and were exposed to the same conditions (stock, collar, environment, etc.,) as the Bladex-animals.

3. The authors stated that artificial insemination was used in this study with semen collected from 20 males of the same strain obtained from the same commercial supplier. Diluted semen from each male was then used to inseminate an equal number of females in each group. However, insemination data were not appended with this final report to substantiate the authors' statement.

4. Fetal brain was examined by a mid-coronal slice. This approach apparently is the usual examination conducted by the testing facility (WIL) and not a design of the study protocol. It is this reviewer's opinion that it would have been better to examine the intra-cranial structures in serial slices.

5. The authors indicated that live fetuses were "killed by T-61® injection". No description of the site of injection (intracardiac? intravenous?), volume of injection, and nature of the T-61® solution were given.

6. The dosage levels selected for this study were 0.2, 0.6, 1.2, and 2.0 ml of 4L Bladex formulation (40 % a.i. by weight). These dosage levels equalled to 76, 228, 573, and 955 mg/kg (information provided by Dr. R. Hull of Shell Oil Company, Houston, Texas).

7. Historical control data which covered a period from 11/80 to 3/85 for this species and strain were provided by the testing facility.

RESULTS

I. MATERNAL TOXICITY

1. Clinical Observations:

Scattered incidences of alopecia, clear ocular discharge, soft stool, decreased urination, and decreased defecation were noted in all groups including the controls. These clinical signs apparently were incidental and not treatment-related.

Four animals died during this investigation: 1 in the 296 mg/kg group and 3 in the highest dose tested (955 mg/kg). The authors indicated that one death in the 955 mg/kg group was related to the animal's escape from the restraining stock and ingestion of the test material from the application site. The cause of death for the other three animals could not be determined by necropsy findings.

2. Dermal Irritation

Dermal irritation as characterized by slight erythema and very slight edema was noted in all treated groups and the vehicle control. However, slight edema, moderate erythema, desquamation, and fissuring were found only in the Bladex groups.

Dermal Observations (expressed as # of animals with findings)

	Naive	Vehicle	96 mg/kg	286 mg/kg	573 mg/kg	955 mg/kg
No. of dams	40	20	20	20	20	20
Slight erythema	4	4	12*	17*	19*	18*
Moderate erythema	0	1	1	5*	11*	11*
Very slight edema	0	1	4	9*	14*	11*
Desquamation	0	0	19*	20*	20*	19*
Fissuring	0	0	13*	14*	16*	13*

(*) Statistically different from vehicle control at 0.05 level

3. Body Weight Data

Maternal body weights were recorded at different intervals throughout the investigation. Non-gravid females were excluded from the data analysis.

Body Weight Data (in grams)

	Naive	Vehicle	96 mg/kg	286 mg/kg	573 mg/kg	955 mg/kg
Day 6	3701	3667	3644	3660	3700	3677
Day 8	3716	3711	3704	3700	3702	3684
Day 18	3869	3793	3741	3597	3519*	3496*
Day 29	4220	4089	4048	4033	3869	3867
Body weight gain (grams)						
Days 6-18	153	91	96	-103*	-226*	-355*
Days 18-29	325	302	307	437	350	313*

(*) Significantly different from vehicle control at 0.05 level

During the treatment period (days 6-18), compound- and dose-related decreases in body weight gains were noted in all Bladex-treated groups with statistical significances ($p < 0.05$) found at all dosage levels except the lowest dose group (96 mg/kg). A rebound increase in body weight gain was observed in all treated groups after cessation of exposure (days 18-29) with a statistical significance noted at the highest dose tested (955 mg/kg).

4. Food Consumption Data

The mean food consumed is tabulated in the next table:

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Mean Food Consumed (grams/kg body weight/day)

	Naive	Vehicle	96 mg/kg	286 mg/kg	573 mg/kg	955 mg/kg
Days 0-6	41	41	41	42	41	40
Days 6-18	39	38	38	30*	25*	20*
Days 18-29	38	37	39	35	31	39

(*) Statistically different from vehicle control at 0.05 level

Prior to the treatment period (days 0-6), no differences in food consumed were noted among all groups. However, dermal application of Bladex (days 6-18) significantly reduced the mean food consumed at the 286, 573, and 955 mg/kg dosage levels. No changes in food consumed were noted after cessation of Bladex exposure (days 18-29). The reductions in food consumption noted during the exposure period (days 6-18) may have resulted, therefore, in the significant decreases in the body weight gains discussed previously.

5. Reproductive Data at Necropsy

The following table summarizes the findings at necropsy:

	Naive	Vehicle	96 mg/kg	286 mg/kg	573 mg/kg	955 mg/kg
# dams assigned	40	20	20	20	20	20
# dams pregnant	38	18	19	18	19	18
Pregnancy rate (%)	95	90	95	90	95	90
# died	3	3	0	1	0	3
# aborted	2	1	2	1	3	1
# litter resorbed	-	-	2	0	2	0
# dams with fetuses	33	16	15	16	14	14
$\bar{X}$ corpora lutea	10.1	10.4	9.6	11.2	10.7	10.0
$\bar{X}$ implantations	7.1	8.1	7.6	8.5	8.3	7.4
$\bar{X}$ preimplant. loss	3.0	2.3	2.0	2.7	2.4	2.6
$\bar{X}$ viable fetuses	6.6	7.6	6.4	7.5	6.3	6.3
$\bar{X}$ postimplant. loss	1.4	0.5	1.2	1.0	2.0	1.1

The pregnancy rates of both the control and treated groups were within the acceptable range of the historical control data (94.9%) for artificial insemination. No compound-related effects were evident relative to the number of dead and/or aborted animals. All groups, including the highest dose tested (955 mg/kg), had an adequate number of litters for examination (more than 12 litters required by the 1982 FIFRA Guidelines). No significant deviations in the means of corpora lutea, implantation sites, or preimplantation loss were noted among the groups. No statistically significant differences in the number of viable fetuses were found when data from the treated groups were compared to either concurrent or historical control data (mean viable fetuses = 7.0). A trend increase (not statistically significant) in the mean postimplantation loss was noted in all treated groups as compared to either naive or vehicle control data. However, a dose-response relationship was not noted.

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I. DEVELOPMENTAL TOXICITY

1. Fetal Weight

As mentioned previously, no significant differences in litter size were noted among the groups. Fetal data at maternal final sacrifice are as follows:

	Naive	Vehicle	96 mg/kg	286 mg/kg	573 mg/kg	955 mg/kg
# dams with viable fetuses	35	16	15	16	14	14
# viable fetuses	239	129	109	120	100	88
# males	114	78	57	63	44	44
# females	125	51	52	57	56	44
Sex ratio (M:F)	0.91	1.53	1.10	1.11	0.78*	1.00
Fetal weight (gm)	44.3	42.1	45.8	42.6	38.3	40.3

(*) Significantly different from vehicle control at 0.05 level

No evidence of a dose-related effect on fetal weight was apparent from the data submitted. Although the fetal weight of the 573 and 955 mg/kg groups was slightly less than the vehicle control values, a statistical significance was not attained and these findings are considered by this reviewer as coincidental. The historical control fetal weight was 39.6 grams. An unlikely compound-related alteration in the the sex ratio was found at the 573 mg/kg dosage level and this finding apparently resulted from the unusually high male:female ratio of the concurrent vehicle control group (1.53). Crown-rump length was not measured in this study.

2. External Observations

All fetuses were examined externally. Carpal and/or tarsal flexure was found in one fetus each in the 286 and 955 mg/kg groups. One fetus was reported with short tail in the 96 mg/kg group.

3. Soft Tissue Examinations

Scattered incidences of hydrocephaly, heart and/or great vessel anomaly, recto-vestibular fistula, gallbladder absent or small, and retrocaval ureter were found in both the control and treated groups. Findings that were not observed in either naive or vehicle controls included distended ureter and hemorrhagic ring around the iris. Soft tissue findings are summarized in the next table.

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SOFT TISSUE FINDINGS

	Naive	Vehicle	96 mg/kg	286 mg/kg	573 mg/kg	955 mg/kg
# fetuses (litter) examined	239(35)	129(16)	109(15)	120(16)	100(14)	88(14)
[a]Hydrocephaly	0	0	0	0	1(1)	0
[a]Rectovestibular fistula	0	0	0	0	0	1(1)
[a]Retrocaval ureter	1(1)	4(4)	0	4(3)	1(1)	0
[a]Hemorrhagic ring around the iris	0	0	0	0	0	2(2)
[a]Gall bladder small or absent	1(1)	0	2(2)	0	1(1)	1(1)
[a]Distended ureter or renal papilla(e) not developed	0	0	1(1)	0	2(2)	1(1)
[a]Left carotid arises fr. brachiocephalic trunk	15(10)	3(2)	2(2)	4(3)	1(1)	2(2)
[a]Retroesophageal aortic arch	1(1)	0	0	2(2)	0	0
[a]Retroesophageal right subclavian arises independently from aortic arch	0	0	0	1(1)	0	1(1)
[a]Bulbous aortic arch	0	2(2)	0	0	0	0
[a]Common truncus arteriosus; interventricular septum incomplete	0	0	1(1)	0	0	0

4. Skeletal Findings

Skeletal findings of interest are summarized in the next table:

SKELETAL FINDINGS

	Naive	Vehicle	36 mg/kg	286 mg/kg	573 mg/kg	955 mg/kg
# fetuses (litter) examined	239(35)	129(16)	109(15)	120(16)	100(14)	38(14)
°Fused sternebrae	0	1(1)	1(1)	0	0	0
°Vertebral anomaly with/without rib anomaly	8(7)	1(1)	0	2(1)	0	2(2)
°Vertebral centra anomaly	2(2)	0	0	0	0	2(2)
°25 presacral vertebrae	0	0	0	1(1)	1(1)	2(2)
°27 presacral vertebrae	56(21)	23(8)	30(9)	37(12)	22(8)	36(11)
°7th sternebrae	2(1)	1(1)	0	0	0	5(4)
°Hyoid body/arches unossified	0	0	0	1(1)	0	2(2)
°Sternebrae 5 and/or 6 unossified	24(11)	7(4)	13(5)	8(5)	4(3)	12(5)
°Accessory skull bones	0	2(2)	2(1)	2(2)	3(2)	0

No statistically significant differences were found in any of the skeletal findings. Scattered incidences of fused sternebrae, vertebral anomaly with or without associated rib anomaly, vertebral centra anomaly, sternebrae malaligned, etc., were found in both control and treated groups. Skeletal incidences that were found in the treated groups but not in the controls included 25th presacral vertebrae and hyoid body and/or arches unossified. Slight increases in the litter incidences of 7th sternebrae (18.0%), hyoid body and/or arches unossified (14.3%), 25 presacral vertebrae (7.1%), and 27th presacral vertebrae (7.0%) were noted in the 955 mg/kg group (highest dose group) as compared to both the vehicle and naive controls.

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DISCUSSION

Dermal administration of Bladex 4L formulation to rabbits during the period of major organogenesis (days 6-18 of gestation) at 0.2, 0.6, 1.2, and 2.0 ml/kg (96, 286, 573, and 955 mg/kg, respectively) produced slight erythema, desquamation, and fissuring at all dosage levels tested. The severity of the injury was proportional to the dosage level used. Slight erythema was also observed in animals treated with the formulation blank. Compound-related decreases in body weight gains were noted in all treated groups with statistical significances observed at the 286, 573, and 955 mg/kg dosage levels. Significant reductions in food consumption were also found at the 286, 573, and 955 mg/kg dosage levels.

No dose-related effects on abortion and mortality were noted. Pregnancy rates as well as the means of corpora lutea, implantations, viable fetuses and resorptions were not statistically different from control values. A slight increase in postimplantation loss was noted in all treated groups as compared to both the concurrent naive and vehicle control data but evidence of a dose-response relationship was not found.

Under the conditions of this study, the maternal NOEL is determined to be less than 96 mg/kg (lowest dose tested) based upon dermal irritation and systemic toxicity.

Evidence of a compound-related increase in frank teratogenic effects was not observed in this study. Scattered incidences of hydrocephaly, recto-vestibular fistula, carpal and/or tarsal flexure, and short tail were noted among the groups. The incidences of skeletal variations were not statistically different among all groups. No dose-related increases in the frequency of each variation and in the incidences of litters and fetuses with variations were found with the exception of slight increases in 7th sternebrae, 27th presacral vertebrae, and hyoid body unossified in the 955 mg/kg group (highest dose tested). Slight decreases in fetal weight were found at the 573 and 955 mg/kg dosage levels but a statistical difference was not attained.

Under the conditions of this study, the developmental toxicity NOEL is established at 573 mg/kg and the LEL at 955 mg/kg (highest dose tested).

By the dermal route of administration, the calculated A/D ratio for Bladex 4L was less than 1 suggesting that developmental toxicity occurred at dose levels associated with maternal toxicity.

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

Cyanazine

RIN 3332-96

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