CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

005053

APR 23 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of toxicology data of Bladex® (Cyanazine)
EPA No. 100-101 Project No. 1096 and 1596
EPA No. 201-298 Caswell No. 188C

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

James Yowell, PM #25
Registration Division (TS-767C)

FROM: Quang Q. Bui, PhD., DABT., Quang Q Bui 4/22/86
Toxicologist, Section V
Toxicology Branch/HED (TS-769C)

THRU: Laurence D. Chitlik, DABT., W. Testers for L. Chitlik 4-22-86
Section Head, Toxicology Branch
Hazard Evaluation Division (TS-769C)
and
Theodore M. Farber, PhD., DABT.,
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

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

Action Requested:

Special review of four toxicology studies with Bladex (cyanazine).

1. Dermal absorption of Bladex herbicide by rats over 8 days (WRC RIR 427)
2. An expanded final report of WRC-367 including 2 practitioner reports. (see R. Zendzian's separate memo).
3. Pilot dermal rabbit teratology study (WIL 93002 A)
4. Dermal teratology study (WIL 93002)

Background Information:

These studies were previously submitted to the Agency (12/85) as unaudited drafts (EPA No. 100-101, without Accession # assigned, Project No. 1096) and, hence, are not subject for review. Final copies with quality assurance statements of three studies (dermal absorption in rats over 8 days; pilot dermal teratology; dermal teratology study) were recently submitted (3/3/86)

to the Agency (EPA No. 201-298) under Accession No. 261601 and 261602. Data from the draft copies were compared to those of the final reports and discrepancies, if any, are listed in the appropriate data evaluation record.

In this memo, the dermal absorption in rats over 8 days, dermal teratology, and pilot dermal teratology studies are evaluated. The expanded final report of study WRC-367 is evaluated and addressed by Dr. R. Zendzian in a separate memo.

RECOMMENDATION

1. Dermal Absorption Data

It is recommended that the study "dermal absorption of Bladex herbicide by rats over 8 days" (WRC RIR 427 dated 2/86) be classified as Acceptable. Under the conditions of the study design, the investigators demonstrated that dermal absorption of ^{14}C-Bladex in rats over 8 days was minimal, being 2% of the applied dose. However, it should be noted that this study could not be used to fulfill the FIFRA Guidelines requirement of a metabolism study since identification and characterization of the metabolites in the excreta were not performed.

2. Teratology Data

Reviewer's note: As indicated previously (memo of Q. Bui to R. Taylor, 6/3/85), the registrant has already fulfilled the requirements for teratology studies in two species: rat (Argus Res. Lab #619-002) and rabbit (Tunstall Lab #221/81). The developmental toxicity NOEL and LEL for Bladex by the oral route of administration in rabbits (more sensitive than rats) were established at 1 and 2 mg/kg/day, respectively. Therefore, althought the following two teratology studies are each classified as Core Supplementary Data, new studies are not required.

a. It is recommended that the "dermal developmental toxicity study in New Zealand rabbits with the Bladex 4L formulation" (43% a.i. by weight; WIL #93002, 2/86) be classified as Core Supplementary Data. Under the conditions of this study, dermal administration of Bladex 4L at 0.2, 0.6, 1.2, or 2.0 ml/kg (equivalent to approximately 105, 310, 620, and 1050 mg/kg, respectively) during the period of major organogenesis in rabbits (days 6-18) resulted in excessive maternal toxicity observed at all dose levels including the lowest dose used. Therefore, the maternal toxicity NOEL cannot be established (LEL = 0.2 ml/kg, lowest dose used).

Although the number of litters available for evaluation in the treated groups was less than desirable, the available data allow some level of assessment of the developmental toxicity potential of Bladex by the dermal route of administration. Dermal administration of Bladex to rabbits at 0.6 and 1.2 ml/kg during gestational days 6-18 resulted in significant decreases in fetal weights, and significant increases in post implantation loss only higher at the 1.2 ml/kg dosage level. The limited number of litters (2) and fetuses (6) in the highest dose group (2.0 ml/kg) precludes meaningful evaluation of the data associated with this group. Although a statistical significant increase in individual external anomalies was not apparent from the submitted data, a positive trend increase in the fetal and litter incidences of external anomalies was noted in the treated groups. Positive trend increases in both litter and fetal incidences of skeletal variations (27 presacral vertebrae,

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13th full ribs, hyoid body unossified, and unossified sternebrae 5 and/or 6) were observed in the treated groups including the lowest dose tested. Based upon these findings, a dermal developmental toxicity NOEL cannot be established from the submitted data (NOEL < 0.2 ml/kg; lowest dose tested).

It is recommended that this study be classified as Core Supplementary Data due to the following:

i. Insufficient number of litters per group for meaningful statistical analysis.
ii. Lack of a developmental toxicity NOEL.

b. The "developmental pilot study in New Zealand rabbits with the Bladex 4L formulation" (WIL #93002A, 2/86) was primarily designed to investigate the excessive maternal toxicity observed in the main dermal teratology study (WIL #93002). As an ancillary study, it is recommended that this study be classified as Core Supplementary Data due to the following:

i. Only one dose level tested (0.2 ml/kg)
ii. Inadequate number of animals used (9 dams)
iii. No concurrent control group

DEVELOPMENTAL TOXICITY RISK ASSESSMENT

Positive developmental toxicity effects have been noted in rabbits via both routes of administration, oral and dermal. The developmental toxicity NOEL is established at 1 mg/kg/day by the oral route (Tunstall Lab #221/81) and at less than 0.2 ml/kg (approximately 105 mg/kg; WIL #93002 dated 2/86) by the dermal route. From the dermal pharmacokinetic study in rats (WRC RIR 427, 2/86), it was found that dermal absorption of Bladex over 8 days was minimal, being 2% of the applied dose. These data, collectively with the worker exposure data generated by the Exposure Assessment Branch (memo of Curt Lunchick), permit Toxicology Branch to assess the developmental toxicity risk of Bladex.

As per the Agency's "Guideline for the Health Assessment of Suspect Developmental Toxicants" and the Hazard Evaluation Division's "Standard Evaluation Procedure for Teratology", a NOEL from a dermal teratology study would be most appropriate for assessing the developmental potential hazard of dermal exposure by mixer-loader-applicator and an oral NOEL for dietary exposure. However, the dermal teratology study in rabbits (WIL #93002) by being classified as Core Supplementary Data, due to (1) lack of a developmental toxicity NOEL and (2) insufficient number of litters per group, is not useful for regulatory purposes. Therefore, the rabbit oral teratology study (Tunstall Lab. #221/81, Core Minimum Data) with a developmental toxicity NOEL of 1 mg/kg/day and the dermal absorption study in rats (WRC RIR 427; Core Acceptable Data) with a maximal dermal absorption rate of 2% must be used in the calculation of the developmental toxicity risk assessment for workers-loaders-applicators of Bladex.

Developmental toxicity risk assessment for ground boom uses, aerial application, and chemigation is presented in, respectively, Tables I, II, and III.

TABLE I: DEVELOPMENTAL TOXICITY RISK ASSESSMENT (†)

GROUND BOOM USES

	Low Rate Applicator Exposure °° (mg/kg/day)	High Rate Applicator Exposure °° (mg/kg/day)	Margin of Safety (¶) (a)	(b)
CORN				
Protective gloves, enclosed tractor	0.035	0.135	1428	370
Protective gloves, open tractor cab	0.230	0.860	217	58
No protective equipment, open tractor cab	21.000	80.000	2	<1
COTTON				
Protective gloves, enclosed tractor	0.014	0.045	3571	1111
Protective gloves, open tractor cab	0.091	0.290	550	172
No protective equipment, open tractor cab	8.400	27.000	6	2
WHEAT FALLOW				
Protective gloves, enclosed tractor	0.067	0.090	746	555
Protective gloves, open tractor cab	0.440	0.580	114	86
No protective equipment, open tractor cab	40.000	54.000	1	<1
MILO				
Protective gloves, enclosed tractor	0.022	0.045	2272	1111
Protective gloves, open tractor cab	0.150	0.290	333	172
No protective equipment, open tractor cab	13.000	27.000	4	2

(°°) Exposure levels provided by EAB/HED
(†) Developmental toxicity risk assessment using an oral NOEL of 1 mg/kg (Tunstall Lab 221/81; Mimimum data)
(¶) Margin of safety calculated using the equation:

$$\frac{\text{Oral NOEL (mg/kg/day)}}{\text{Exposure level (mg/kg/day)}} \times 50 \text{ (correction factor for 2\% dermal absorption)}$$

(a) MOS for low rate applicator exposure
(b) MOS for high rate applicator exposure

TABLE II: DEVELOPMENTAL TOXICITY RISK ASSESSMENT (†)

	EXPOSURE DURING AERIAL USES			
	Low Rate Applicator Exposure °° (mg/kg/day)	High Rate Applicator Exposure °° (mg/kg/day)	Margin of Safety(¶) (a)	(b)
A. MIXER/LOADER				
GRAIN SORGHUM				
Protective gloves	5.0	24.0	10	2
Without protective gloves	23.0	120.0	2	<1
CORN				
Protective gloves	2.5	54.0	20	1
Without protective gloves	12.0	250.0	4	<1
WHEAT FALLOW				
Protective gloves	8.0	48.0	6	1
Without protective gloves	38.0	230.0	1	<1
B. PILOT				
GRAIN SORGHUM	0.034	0.120	1470	410
CORN	0.017	0.280	2940	178
WHEAT FALLOW	0.069	0.250	725	200

(°°) Exposure levels provided by EAB/HED
(†) Developmental toxicity risk assessment using an oral NOEL of 1 mg/kg (Tunstall Lab 221/81; Minimum Data)
(¶) Margin of safety calculated using the equation:

$$\frac{\text{Oral NOEL (mg/kg/day)}}{\text{Exposure level (mg/kg/day)}} \times 50 \text{ (correction factor for 2\% dermal absorption)}$$

(a) MOS for low rate applicator exposure

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TABLE III": DEVELOPMENTAL TOXICITY RISK ASSESSMENT (†)

EXPOSURE DURING CHEMIGATION

	Low Rate Applicator Exposure °° (mg/kg/day)	High Rate Applicator Exposure °° (mg/kg/day)	Margin of Safety(¶) (a)	(b)
Open Pour - No Protective Gloves	12.0	45.0	4	1
Open Pour - Protective Gloves	2.5	9.7	20	5
Closed System - Protective Gloves	0.06	0.23	830	215

(°°) Exposure levels provided by EAB/HED
(†) Developmental toxicity risk assessment using an oral NOEL of 1 mg/kg (Tunstall Lab. 221/81, Core Minimum Data
(¶) Margin of safety calculated using the equation:

$$\frac{\text{Oral NOEL (mg/kg/day)}}{\text{Exposure level (mg/kg/day)}} \times 50 \text{ (correction factor for 2\% dermal absorption)}$$

(a) MOS for low rate applicator exposure
(b) MOS for high rate applicator exposure

DATA EVALUATION RECORD NO. I

Chemical:	Cyanazine, Bladex
Test Material:	Bladex 4L (Sample No. WRC 828) ^{14}C-Bladex 4L (4.3 uCi/mg)
Study/Action Type:	Dermal absorption study

STUDY IDENTIFICATION:

"Dermal Absorption of Bladex Herbicide by Rats Over Eight Days"

Testing Facility:	Research Triangle Institute
Draft Report No.:	RTI/3442/01F (draft), 11/85
Final Report No.:	WRC RIR 427, 2/86
Study Author:	Jeffcoat, AR.,
EPA Accession No.:	261602 (final report)
Reviewed by:	Quang Q. Bui, PhD., DABT., Toxicologist, Section V Toxicology Branch/HED (TS-769C)
Review Approved by:	Laurence D. Chitlik, DABT., Section Head, Section V Toxicology Branch/HED (TS-769C)

CONCLUSIONS AND CORE CLASSIFICATION

The final copy with quality assurance statements was submitted on 3/3/86 under Accession No. 261602 and was compared with the unaudited draft previously submitted (12/85) and no major discrepancies were detected between the two documents.

Under the conditions of the study design, the investigators demonstrated that dermal absorption of ^{14}C-Bladex in rats was minimal, being 2% of the applied dose. The recovery rate in all groups was incredibly high (98-104% of actual dose) which may reflect unusual attention to detail regarding this aspect of study performance.

It is recommended that this study be classified as Acceptable. However, it should be noted that this study was not designed to fulfill the FIFRA Guidelines requirement of a metabolism study but to investigate the dermal kinetics of Bladex 4L in rats and does not include metabolite identification in either the urine or feces.

MATERIALS AND METHODS

Animals: Male Fischer 344 rats (Harlan Sprague Dawley, Indianapolis)
Average weight = 250 ± 15 g.

Test Chemical: Unlabeled Bladex 4L (Sample No. WRC 828; Code 16-44-101-5) and ^{14}C-Bladex 4L (Sample No. WRC 798; 4.3 uCi/mg).

Dose: 50 mg/rat applied to 12 cm^2 of body surface area.
The dose site was washed at 10 hours after dosing or immediately following sacrifice of the animals at earlier intervals.

Groups: Groups of 4 animals each were sacrificed at time points of 0.5, 2, 4, 10, 24, 48, 72, 120, and 192 hours after dosing.

Control	=	Group VI
0.5 hr.	=	Group VII
2 hr.	=	Group VIII
4 hr.	=	Group IX
10 hr.	=	Group X
24 hr.	=	Group XI
48 hr.	=	Group I
72 hr.	=	Group II
120 hr.	=	Group III
192 hr.	=	Group IV
192 hr.	=	Group V (used in breath collection experiment)

Dose Application:

The dose was applied with an 18 gauge ball-tipped gavage needle attached to a 250 ul glass syringe with a teflon-tipped plunger. The dose formulation was mixed for 15 seconds with a vortex stirrer before each dose was withdrawn. The actual dose (in uCi) administered to each animal was determined by the equation:

$$D = (S_B - S_A)\ A_D - NW$$

S_B = weight of the syringe before dosing
S_A = weight of the syringe after dosing
A_D = specific activity of the dosing formulation
NW = Kimwipe swab

Urine and Feces Collection:

After dosing, all animals were housed individually in metabolic cages to allow urine and feces collection. For animals sacrificed at less than 24 hours post dose, excreta were collected prior to dosing and at sacrifice. For animals sacrificed at a later time, urine and feces were collected at 0, 24, 48, 72, 96, 120, 144, 168, and 192 hours. After the animals were sacrificed, the chamber pan was rinsed and the radiolabel measured was added to that in the urine.

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Breath Collection:

Group V animals were housed in sealed metabolism chambers. Air drawn from the chambers was determined for radiolabeled content.

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Exposure:

The maximum exposure time was 10 hours. For animals sacrificed prior to the maximum exposure time, an initial wash (wash A) of the testing area was performed immediately following sacrifice (groups sacrificed at 0.5, 2, and 4 hours). For animals sacrificed at or after the maximum exposure time (groups sacrificed at 10, 24, 48, 72, 120 and 198 hours post-dosing), washing of the skin was conducted at 10 hours post-dosing (wash A). The dose site was then covered with a new protective device and all animals returned to their metabolic cages until the scheduled sacrifice. A second wash was performed at necropsy (wash B).

RESULTS

A. General Observations

No observations relative to systemic toxicity or dermal irritation were mentioned by the investigators.

B. Pharmacokinetics

Data concerning absorption, non-absorption, and total recovery are presented in the table below:

Table I: Kinetic Data with ^{14}C-Bladex 4L
(expressed as actual dose: uCi)

TIME (hr)	Actual Dose[a]	Absorbed[b]	Non-absorbed[c]	Total Recovery[d]
0.5	35.18	0.063	36.78	36.84
2	31.60	0.097	32.45	32.55
4	33.68	0.233	34.00	34.23
10	31.63	0.374	31.83	32.20
24	29.75	0.596	29.38	29.98
48	50.65	0.182	49.76	49.94
72	53.15	0.149	52.23	52.38
120	53.78	0.312	53.28	53.59
192	56.10	0.481	54.65	55.13
192(†)	46.20	0.513[e]	44.78	45.29

All values are the mean of 4 animals
(a) : Bladex expelled from dosing syringe minus needle wipe
(b) : Sum of ^{14}C in urine, feces, skin, carcass, and blood
(c) : Total of ^{14}C in protective devices and skin washes
(d) : Sum of ^{14}C absorbed and non-absorbed
(e) : Including ^{14}C excreted in breath
(†) : Group used in breath assay

A noticeable difference in the actual dose (expressed in uCi) was found between the groups sacrificed within and after 24 hours. This difference was due to the fact that two dosing solutions with different specific activities were used. For the groups sacrificed at 48-192 hours, a dosing solution with a-

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specific activity of 260 uCi/g was used whereas a dosing solution of 152 uCi/g was given to the groups sacrificed at 0.5-24 hours. The kinetic data expressed as % of the actual dose are presented in the table below:

Table 2: Kinetic Data with ^{14}C-Bladex 4L
(expressed as % of actual dose)

TIME (hr)	Absorbed[a]	Absorbed thru skin[b]	Non-absorbed[c]	Total Recovery[d]
0.5	0.18	<0.01	104.5	104.68
2	0.31	0.06	102.7	103.01
4	0.69	0.13	100.9	101.59
10	1.18	0.16	100.6	101.78
24	2.00	0.11	98.8	100.80
48	0.36	0.10	98.2	98.56
72	0.28	0.16	98.3	98.58
120	0.58	0.27	99.1	99.68
192	0.86	0.76	97.4	98.26
192(†)	1.11[e]	0.81	96.9	98.01

(a) : Sum of ^{14}C in urine, feces, skin, carcass, and blood
(b) : Total absorbed minus ^{14}C found in skin
(c) : Total of ^{14}C in protective devices and skin washes
(d) : Sum of ^{14}C absorbed and non-absorbed
(e) : Including ^{14}C excreted in breath
(†) : Group used for breath assay

A time-dependent increase in the absorption of radioactive Bladex was noted and reached a peak of 2% of the administered dose at the 24 hour sacrifice. However, the total amount of ^{14}C absorbed for the groups sacrificed at 48, 72, 120, and 192 hours post-dosing was lower than those of the 10 and 24-hour groups. The total amount of ^{14}C absorbed through the skin reached a maximum of approximately 0.8% of the actual dose; this amount was found for the breath assay group sacrificed at 192 hours.

The total recovery obtained in all groups was phenomenal and varied from 98 to 104% of the dose administered.

C. Excretion in breath

The actual amount of radiolabel excreted in breath was minimal (mean = 0.0025 uCi) and accounted for less than 0.01% of the dose applied.

D. Amount in blood

The amount of radiolabel in the blood was extremely low (< 0.01%) in all groups sacrificed at different time intervals.

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DISCUSSION

Inspection of the data in Table I revealed a difference in the actual dose (expressed in uCi) between the groups sacrificed within and after the 24-hour period. This was due to differences in the dosing-solution concentration. Groups sacrificed after the 24-hour period were dosed on September 10 with a preparation containing approximately 260 uCi/g whereas those sacrificed before and at the 24-hour period were dosed on September 19 with a preparation containing approximately 152-157 uCi/g.

An apparent time-dependent increase in the radiolabel absorbed into the skin (expressed as % of actual dose) was noted during the first day after dosing. Approximately 0.18, 0.31, 0.69, 1.18, and 2% of the actual dose was absorbed by 0.5, 2, 4, 10, and 24 hours sacrifice. Thereafter, a decline in the percentage of radiolabel absorbed, as compared to the 10 and 24-hour groups, was observed. Since the total amount absorbed into the skin was calculated as the sum of radiolabel in the urine, feces, skin, carcass, and blood, the amount of radiolabel absorbed in the groups sacrificed at 24, 48, 72, 120, and 192 hours, would be expected to be equal to that of the 10-hour group. No concise explanation was given by the study authors. In order to clarify this disparity, a careful examination of individual animal data was conducted. It was found that the mean $\pm$ S.D. of total ^{14}C in the 24-hour group was 0.596 $\pm$ 1.69 uCi. This large S.D. was attributed to the findings in animal #725 (amount absorbed = 1.39 uCi or 4.6% of actual dose). If data from this animal were deleted from those of the 24-hour group, a mean of 0.331 uCi was calculated, which corresponded to 1.12% of the actual dose. This new percentage apparently was more in line with the data presented but still could not explain the apparent disparity in amount absorbed between animals of the 10-hour group and those sacrificed at a later time. However, due to the small numerical values of the amount absorbed (< 2%), the variations noted in the groups 48-192 are considered by this reviewer as experimental errors and would not have any impact on the interpretation of the study data.

When the amount of radiolabel absorbed through the skin (amount absorbed minus amount in skin) was calculated (Table 2), the maximum absorption was approximately 0.8% of the actual dose applied. The amounts of radiolabel in the blood or exhaled were minimal (< 0.01% of the actual dose applied).

Although several parameters in the conduct of the study were unjustified, [i.e., two dosing solutions with two different specific activities, two dosing days, and uses of animals older than those of the previous kinetic study (250 g in this study versus 180 g in WRC RIR-367)], the data still indicated that dermal absorption of Bladex 4L in the rat was minimal, reaching a maximum of 1-2% of the applied dose. The recovery rate in all groups was incredibly high (98-104% of the applied dose) which may reflect unusual attention to detail regarding this aspect of study performance.

DATA EVALUATION RECORD No. II

Chemical: Cyanazine, Bladex
Test Material: Bladex 4L (43% a.i. by weight), Lot WRC 795
Study/Action Type: Developmental toxicity

STUDY IDENTIFICATION:

"A dermal developmental toxicity study in New Zealand White Rabbits with the Bladex 4L formulation"

Testing Facility: Wil Research Lab.,
Final Report No.: WIL 93002
WRC RIR-425
Date: 2/86
Study Director: Rodwell, D.
EPA Accession No.: 261601

Reviewed by: Quang Q. Bui, PhD., DABT.,
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Review Approved by: Laurence D. Chitlik, DABT.,
Head, Section V
Toxicology Branch/HED (TS-769C)

CONCLUSIONS AND RECOMMENDATION

It is recommended that this study be classified as Core Supplementary Data, but a new study is not required.

Dermal application of 0.2, 0.6, 1.2, or 2.0 ml/kg of Bladex 4L (approximately 105, 310, 620, and 1050 mg/kg of a.i.) to rabbits during the period of major organogenesis (days 6-18) resulted in significant decreases in maternal body weight gains and food consumption associated with increased mortality and abortions. Due to a high incidence of maternal loss (death, nonpregnant, and abortion) in the treated groups, the number of litters available for examination at laparotomy was substantially reduced for the 0.2, 1.2, and 2.0 ml/kg groups (8, 6, and 2 litters, respectively). Therefore, the maternal toxicity NOEL cannot be established (LEL = 0.2 ml/kg; lowest dose tested).

Compound and dose-related decreases in fetal weights were noted in the treated groups with statistical significances noted at the 0.6 and 1.2 ml/kg dosage levels. Although a dose-related increase in individual external anomalies was not apparent, the litter incidences of the 0.6, 1.2, and 2.0 ml/kg groups were, respectively, 41.7, 50.0, and 50.0% as compared to 23.1% in the control. However, the limited number of litters (2) and fetuses (6) in the highest dose group (2.0 ml/kg) precluded meaningful evaluation of the data associated with this group. Soft tissue examinations did not reveal any evidence of a treatment-related effect. Positive trend increases in fetal and litter incidences of skeletal variations (13th full ribs, 27 presacral vertebrae, hyoid body unossified, unossified sternebrae 5 and/or 6) were noted in all treated groups with statistical significant differences found at the 1.2 ml/kg dosage level. Based upon these findings, a dermal developmental toxicity NOEL cannot be established from the submitted data (NOEL < 0.2 ml/kg; lowest dose tested).

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MATERIALS AND METHODS

A copy of the study procedures is appended. The study investigators indicated that this study was designed and conducted in compliance with both the Good Lab Practice Regulations and the 1982 FIFRA Guidelines. The following highlights and comments are noted:

1. Artificial insemination was conducted with semen collected from seven males obtained from the same supplier. The authors stated that semen from each male was used to inseminate an equal number of females in each group. However, insemination data were not appended to substantiate the authors' statement.

2. Each day, neck collars were affixed to all rabbits for 6 hours during the exposure period. However, on 8/31, 9/1, and 9/2/85 (weekend and Labor Day), all rabbits wore the neck collar continously for 72 hours. This extended period of unneccessary stress may have had an impact on the behavior, food consumption, body weight, as well as gestational development in all dams.

3. Fetal brain was examined only by a mid-coronal slice. It would have been better to examine the intra-cranial structures in serial slices.

Animals: New Zealand white rabbits (Hazleton-Dutchland, Denver) weighing approximately 3.5 - 4.0 kg at study initiation

Groups: Each group consisted of 20 inseminated females.

Dose levels: Control (formulation blank), 0.2, 0.6, 1.2, and 2.0 ml/kg/day [equal to approximately 105, 310, 620, and 1050 mg/kg using a specific gravity of 1.2 as given by Mr. Harrison, Chemistry Information, Shell, Houston, Texas)

Treatment: Dermal application from days 6 to 18 of gestation

RESULTS

1. Stability Data

Appendix II of the final report indicated that the Bladex 4L formulation analyzed 18 months after storage still shows over 99% stability.

2. Clinical Observations and Mortality

Six animals died during this investigation: 1, 1, 1, and 3 in the 0.2, 0.6, 1.2, and 2.0 ml/kg groups, respectively. These deaths apparently were compound-related. One animal in the 1.2 ml/kg group was sacrificed in extremis on gestation day 15 due to prolapsed rectum. Twenty one females aborted: 1, 6, 0, 6, and 8 in the control, 0.2, 0.6, 1.2, and 2.0 ml/kg groups, respectively. The authors also reported that treatment-related reduction in defecation and urination was found in the treated groups.

3. Maternal Loss

The following table illustrates the incidence of maternal loss (non-pregnant, dead, and aborted dams) in all groups:

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Incidences of Maternal Loss

	Control	0.2 ml/kg	0.6 ml/kg	1.2 ml/kg	2.0 ml/kg
# inseminated	20	20	20	20	20
# dead	0	1	1	1	3
# sacrificed	0	0	0	1	0
# aborted	1	6	0	6	8
# non-pregnant	4	2	2	1	3
# maternal loss	5	9	3	9	14
(%)	25%	45%	15%	45%	70%
# gravid	15	11	17	11	6
pregnancy rate°	80%	90%	90%	95%	85%

(°) Number of pregnancies/ Number of animals inseminated x 100

Pregnancy rate of 85-95% was obtained in this study and is considered as acceptable. The incidence of maternal loss in the treated groups was relatively high in the 0.2, 1.2, and 2.0 ml/kg groups being 45, 45, and 70%, respectively. These high incidences of maternal loss in the treated groups undoubtedly restrict the number of animals available at laparotomy and limit the validity of the statistical analysis of the data.

4. Dermal Irritation

Dermal irritation as characterized by slight erythema, slight edema, fissuring, and desquamation was observed in all groups including the formulation blank control group. However, moderate edema was noted only in one animal of the highest dose group.

Dermal Observations (expressed as Total incidence/No. animals)

	Control	0.2 ml/kg	0.6 ml/kg	1.2 ml/kg	2.0 ml/kg
Slight erythema	135/20	123/19	141/20	117/20	110/20
Slight edema	8/2	0/0	3/3	5/2	10/2
Moderate edema	0/0	0/0	0/0	0/0	3/1
Fissuring	9/5	8/3	20/7	13/5	37/11
Desquamation	20/9	1/1	16/6	2/1	4/3

(Note: The number of dams (N) changed over time due to maternal death and abortion)

5. Body Weight Data

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

Mean Body Weight Gains (in grams)

	Control	0.2 ml/kg	0.6 ml/kg	1.2 ml/kg	2.0 ml/kg
Days 0-6	133	169	132	158	174
Days 6-18	-71	-392*	-628*	-804*	-985*
Days 18-29	195	334	453*	510*	279
Days 0-29	249	214	-31*	-122*	-522*

(*) Significantly different from controls, $p < 0.05$

Prior to the treatment period (days 0-6), all does gained weight. However, during the treatment period (days 6-18), negative body weight gains were noted in all groups including the control. Statistically significant differences and dose-related decreases in body weight gains were found in all treated groups including the lowest dose tested (0.2 ml/kg). A rebound increase in weight gain was observed after the treatment period (days 18-29) with statistically significant increases found at the 0.6 and 1.2 ml/kg dosage levels. Throughout the entire investigation, only the control and the lowest dose group (0.2 ml/kg) gained weight whereas dose-related decreases in body weight gains with statistically signiticant differences were calculated for the 0.6, 1.2, and 2.0 ml/kg groups.

6. Food Consumption

The mean food consumption is tabulated as follows:

Mean Food Consumed (in grams/animal/day)

	Control	0.2 ml/kg	0.6 ml/kg	1.2 ml/kg	2.0 ml/kg
Days 0-6	185	190	163	185	195
Days 6-18	153	90*	41*	30*	20*
Days 18-29	150	153	120	126	121
Days 0-29	157	140	96*	101*	97*

(*) Statistically significant different from controls, $p < 0.05$

No differences in food consumption were noted among all groups prior to treatment (days 0-6). Statistically significant decreases in food consumed were noted in all treated groups during the treatment period (days 6-18), but no changes were detected after the treatment period (days 18-29) which reflected a compensatory effect after withdrawal of the test chemical exposure. Throughout the entire investigation (days 0-29), significant reductions in food consumed were found at the 0.6, 1.2, and 2.0 ml/kg dosage levels.

The reductions in food consumption noted in the treated groups may have resulted, therefore, in the significant decreases in body weight gains discussed previously.

7. Reproductive Data

The following table represents the reproductive data of all does at laparotomy:

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Reproductive Data

	Control	0.2 ml/kg	0.6 ml/kg	1.2 ml/kg	2.0 ml/kg
# sacrificed	19	13	19	12	9
# gravid	15	11	17	11	6
# does with resorptions only	2	3	5	5	4
# does with viable fetuses	13	8	12	6	2
$\bar{X}$ implantations	5.7 (6.2)°	8.6 (8.5)	7.1 (7.3)	7.3 (8.3)	4.7 (11.0)
$\bar{X}$ resorptions	1.8 (1.7)	3.3 (1.1)	2.8 (1.4)	4.4* (2.9)*	3.7 (8.0)
$\bar{X}$ viable fetuses	3.9 (4.5)	5.4 (7.4)	4.3 (6.1)	2.9 (5.3)	1.0 (3.0)
$\bar{X}$ dead fetuses	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.2)	0.0 (0.0)

(*) Statistically significant from controls, $p < 0.05$
(°) data excluding dams with resorptions only

Since only 2 does of the 2.0 ml/kg group had viable fetuses at laparotomy, data obtained from this group were excluded from statistical analyses.

Treatment-related increases in resorptions were noted in all treated groups with a statistical significance found at the 1.2 ml/kg dosage level when data from all pregnant dams, including those with resorptions only, were considered. Excluding dams with resorptions only, the incidence of post implantation loss in the 1.2 ml/kg group still remains statistically significant as compared to controls (2.9 vs 1.7 for controls). However, the mean numbers of viable fetuses (litter size) in the treated groups were not significantly different from control values. Respective means of viable fetuses of 4.5, 7.4, 6.1, and 5.3 were found for the control, 0.2, 0.6, and 1.2 ml/kg groups.

8. Developmental Toxicity

Compound and dose-related decreases in fetal weights were noted in the treated groups with statistical differences found at the 0.6 and 1.2 ml/kg groups. Respective fetal weights of 44.9, 41.0, 37.7, and 32.0 were found at the 0, 0.2, 0.6, and 1.2 ml/kg dosage levels.

a) External Observations

External observations of interest are summarized as follows:

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External Observations

	Control	0.2 ml/kg	0.6 ml/kg	1.2 ml/kg	2.0 ml/kg
Number examined	59 (13)[a]	59 (8)	73 (12)	32 (6)	6 (2)
Omphalocele	0	0	2 (2)	1 (1)	0
Carpal/tarsal flexure	0	0	1 (1)	2 (1)	2 (1)
Open eyelid	0	0	1 (1)	0	0
Ectrodactyly	1 (1)	0	0	0	0
Spina bifida	1 (1)	0	0	0	0
Multiple anomaly	0	0	0	1 (1)	0
Short tail	1 (1)	0	0	0	0
Hydrocephaly	0	1 (1)	1 (1)	0	0
# with external findings (litters)	3 (3)	1 (1)	5 (5)	4 (3)	2 (1)
% fetuses with external findings (litters)	5.1 (23)	1.7 (13)	6.8 (42)	12.5 (50)	33.3 (50)

(a) litter incidence

External anomalies that were observed in the treated fetuses but not controls included omphalocele, carpal and/or tarsal flexure, and open eyelid. Although the incidence of individual external anomalies was not dose-related, the total litter and fetal incidences of all external anomalies combined are highly suggestive of a treatment-related effect.

b) Soft tissue examinations

The findings of hydrocephaly in one fetus each in the 0.2 and 0.6 mg/kg groups were confirmed by soft tissue examinations. Additionally, a retroesophageal aortic arch was found in one control fetus.

c) Skeletal examinations

Skeletal findings of interest are summarized as follows:

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Skeletal Observations

	Control	0.2 ml/kg	0.6 ml/kg	1.2 ml/kg	2.0 ml/kg
# fetuses exam.	59	59	73	32	6
# litters exam.	13	8	12	6	2
27 presacral vert.	23.7[a]	47.5	47.9	34.4	83.3
	(38.5)[b]	(75.0)	(83.3)	(83.3)	(100.0)
13th full ribs	32.2	59.3	67.1	59.4	100.0
	(61.5)	(100.0)	(100.0)	(83.3)	(100.0)
Hyoid body unossif.	0.0	1.7	11.0	9.4	0.0
	(0.0)	(12.5)	(33.3)	(33.3)	(0.0)
Sternebrae 5 and/or 6 unossified	3.4	6.8	8.2	12.5	0.0
	(15.4)	(37.5)	(41.7)	(33.3)	(0.0)
Vert.centra malform.	0.0	1.7	1.4	0.0	0.0
	(0.0)	(12.5)	(8.3)	(0.0)	(0.0)
Stern. misaligned	0.0	0.0	1.4	3.1	0.0
	(0.0)	(0.0)	(8.3)	(16.7)	(0.0)

(a) fetal incidence
(b) litter incidence

Increases in both fetal and litter incidences of 27 pre-sacral vertebrae and 13th full ribs were noted in the treated groups. Unossified hyoid body was found only in the treated groups affecting 12.5, 33.3, 33.3, and 0.0% of the litters in the 0.2, 0.6, 1.2, and 2.0 ml/kg groups, respectively. The lack of findings in the highest dose group may have resulted from the extremely limited number of fetuses and litters available for examination. This group is actually too small for any meaningful comparisons. The litter incidence of unossified sternebrae 5 and/or 6 was also increased in the treated groups. Misaligned sternebrae occurred in 8.3 and 16.7% of the litters in the 0.6 and 1.2 ml/kg groups, respectively.

DISCUSSION

Dermal application of 0.2, 0.6, 1.2, or 2.0 ml/kg of Bladex 4L to rabbits throughout the period of organogenesis (days 6-18) resulted in significant maternal toxicity observed at all dosage levels. Significant decreases in body weight gains and food consumption were noted in all treated groups concomittant with increased mortality and abortions. Maternal mortality was noted in all groups being 1, 1, 2, and 3 in, respectively, the 0.2, 0.6, 1.2, and 2.0 ml/kg dose groups. Twenty-one dams aborted: 1, 6, 0, 6, and 8 in the respective groups. In addition, maternal loss (death, abortion, and reduced pregnancy rates) was relatively high in the treated groups being 45, 45, and 70% in the 0.2, 1.2, and 2.0 ml/kg groups, respectively. These high incidences of maternal loss resulted in a limited number of dams available for examination at laparotomy. Data could only be collected from 13, 8, 12, 6, and 2 litters in the control, 0.2, 0.6, 1.2, and 2.0 ml/kg groups, respectively. From a statistical standpoint, data from the 2.0 ml/kg group could not be used in the evaluation process.

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Based upon the above findings, a maternal NOEL cannot be established with decreased body weight gain and food consumption observed at 0.2 ml/kg (lowest dose tested) during the treatment period (days 6-18 of gestation).

Compound and dose-related decreases in fetal weights were found in the treated groups with statistical differences noted at the 0.6 and 1.2 ml/kg dosage levels. Increased resorptions were noted at the 1.2 ml/kg dosage level but the litter size in all treated groups was unaffected. Although a dose-related increase in individual external anomalies was not apparent from the submitted data, the total fetal and litter incidences of external anomalies were increased in the treated groups. External anomaly litter incidences of 23.1, 12.5, 41.7, 50.0, and 50.0% were associated with the 0, 0.2, 0.6, 1.2, and 2.0 ml/kg groups, respectively. Soft tissue examinations did not reveal any evidence of a treatment related effect. Positive trend increases in fetal and litter incidences of skeletal variations were found in the treated groups (27 presacral vertebrae, 13th full ribs, unossified hyoid body, and unossified vertebrae 5 and/or 6) with noticeable differences noted at the 1.2 ml/kg dosage level.

Although the number of litters available for evaluation in the treated groups was less than desirable, there still are sufficient data to perform an assessment of the developmental toxicity potential of Bladex. Dermal administration of Bladex 4L to rabbits during gestational days 6-18 resulted in significant decreases in fetal weights at the 0.6 and 1.2 ml/kg dosage levels, and significant increases in resorptions, and litter and fetal incidences of skeletal variations at the 1.2 ml/kg dosage level. It should be re-emphasized that the low number of litters (2) and fetuses (6) in the 2.0 ml/kg group precluded meaningful evaluation of the data associated with this group. Positive trend increases in skeletal variations were noted in all treated groups including the lowest dose tested (0.2 ml/kg). Based upon the above findings, a dermal developmental toxicity NOEL cannot be established from the submitted data (NOEL < 0.2 ml/kg; lowest dose tested).

It is recommended that this study be classified as Core Supplementary Data due to the following:

a. Insufficient number of litters per group for meaningful statistical analysis.
b. Lack of a developmental toxicity NOEL.

THE MATERIAL ON PAGES 20 139 THROUGH 27 146 WAS TAKEN DIRECTLY FROM THE REGISTRANT SUBMISSION. THESE PAGES ARE NOT INCLUDED IN THIS REVIEW.

DATA EVALUATION RECORD NO. III

Chemical: Cyanazine, Bladex
Test Material: Bladex 4L (43% a.i. by weight), Lot No. WRC 795
Study/Action Type: Developmental toxicity

STUDY IDENTIFICATION:

"A method developmental pilot study as an adjunct to the main dermal developmental toxicity study in New Zealand rabbits with the Bladex 4L formulation".

Testing Facility: WIL Tox. Lab.
Ashland, Ohio
Final Report No.: WIL #93002A
Date: 2/86
Study Author: Rodwell D.E.
EPA Accession No.: 261601

Reviewed by: Quang Q. Bui, PhD., DABT.,
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Toxicology Branch/HED (TS-769C)

Review Approved by: Laurence D. Chitlik, DABT.,
Section Head, Section V
Toxicology Branch/HED (TS-769C)

CONCLUSIONS AND RECOMMENDATION

It is recommended that this study be classified as Supplementary Data.

This ancillary study was designed to clarity the extensive maternal toxicity noted in the main dermal developmental study with Bladex 4L (WIL #93002). This study does not fulfill the Guideline requirements for a teratology study due to (1) insufficient number of animals used (9 rabbits used), (2) one dose level tested (0.2 ml/kg), and (3) no concurrent control group.

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MATERIALS AND METHODS

A copy of the study procedures is appended. Although the investigators indicated that this study was designed in compliance with the Good Laboratory Practice Regulations and the 1982 FIFRA Guidelines, the following comments and highlights are noted:

1. No concurrent control rabbits were used.
2. Only one dose level (0.2 ml/kg) was investigated with 9 animals.
3. Although the investigators stated that artificial insemination was conducted with semen collected from 2 males of the same supplier, data related to the artificial insemination procedures were not appended with this final report.
4. The fetal brain was examined by one mid-coronal slice. It would have been better to examine the intra-cranial structures by serial slices.
5. Statistical analysis of the data could not be performed since only one dose group was tested.
6. After the exposure period (6 hours), collars were affixed to the neck of each rabbit until the commencement of dosing the next day.

Animals: New Zealand white rabbits (Hazleton-Dutchland, Denver, Pennsylvania) weighing approximately 4.0 kg at study initiation.

Treatment: Dermal application from days 6-18 of gestation.

Group: One group consisting of 9 inseminated animals.

Dose Level: 0.2 ml/kg (approximately 105 mg/kg)

RESULTS

1. Clinical and Dermal Observations

Decreased urination and defecation were noted in 5/9 animals. Slight to moderate erythema and edema, fissuring, and desquamation were observed in all animals.

No deaths were reported but one doe aborted on day 28 of gestation

2. Body Weight Data

Maternal body weights were recorded at different intervals throughout the investigation. During the treatment period (days 6-18), a mean weight gain of 56 grams was found and throughout the entire study (days 0-29), a positive weight gain of 218 grams was noted.

For comparison purposes, data from this study are tabulated as compared to those of the 0.2 ml/kg group in the main study (WIL # 93002) and presented as follows:

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BODY WEIGHT GAIN DATA (in grams)

	Study 93002 A 0.2 ml/kg†	Study 93002 0.2 ml/kg¶
Days 0-6	34	169
Days 6-18	56	-392
Days 18-29	165	334
Days 0-29	218	214

(†) neck collar worn after the exposure period, 18 hours/day
(¶) neck collar worn during the exposure period, 6 hours/day
tabulated for comparison purposes

Does from study 93002 A gained weight in all three phases of gestation whereas those of the main study lost weight during the treatment period. However, throughout gestation (days 0-29) the body weight gains in both groups were statistically comparable (218 and 214 grams).

3. Food Consumption

Data on food consumption of both groups are summarized as follows:

FOOD CONSUMPTION (in grams/animal/day)

	Study 93002 A 0.2 ml/kg	Study 93002 ¶ 0.2 ml/kg
Days 0-6	135	190
Days 6-18	123	90
Days 18-29	82	153
Days 0-29	104	140

(¶) data tabulated for comparison purposes

In study 93002 (does wearing neck collar during the exposure period), reduced food consumption was noted during the treatment period (days 6-18) with a rebound increase noted after chemical exposure withdrawal (days 18-29). No significant reductions in food consumption were noted in does of study 93002A during the period of treatment (days 6-18) but a slight decrease was observed, however, from days 18-29. The overall mean food intake (days 0-29) in study 93002 A was lower than that seen in the main study (104 gm versus 140 gm).

4. Reproductive Data

Eight out of nine rabbits inseminated became pregnant and one doe aborted on day 28 of gestation. The reproductive data collected at laparotomy is presented in the next table.

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REPRODUCTIVE DATA

	Study 93002 A 0.2 ml/kg	Study 93002¶ 0.2 ml/kg
Number pregnant	8/9 (89)†	18/20 (90)
Number dead	0	1
Number aborted	1/8 (13)	6/17 (35)
Number with resorptions only	0 (0)	3/17 (18)
Number with viable fetuses	7/8 (88)	8/17 (47)
$\overline{X}$ implantations	6.1	8.5
$\overline{X}$ resorptions	0.7	1.1
$\overline{X}$ viable fetuses	5.4	7.4
$\overline{X}$ fetal weight (gm)	39.8	41.0

(†) number in parenthesis = %
(¶) data tabulated for comparison purposes

Maternal toxicity was also demonstrated in study 93002A as characterized by 1/8 dams aborted (13%). The means of implantation, resorption, litter size, and fetal weight) were not biologically different as compared to the historical control data.

5. Developmental Toxicity

External, soft tissue, and skeletal examinations of 38 fetuses did not reveal any evidence of teratogenicity.

The incidences of skeletal variations are given in the next table:

SKELETAL FINDINGS

	Study 93002 A 0.2 ml/kg	Study 93002 ¶ 0.2 ml/kg
Number of fetuses [litters] examined	38 [7]	59 [8]
27 presacral vertebrae	18.4 (28.8)	47.5 (75.0)
13th full ribs	26.3 (71.4)	59.3 (100.0)
Stern. 5 and/or 6 unossif.	5.3 (28.6)	6.8 (37.5)
Vertebrae centra malformed	0.0 (0.0)	1.7 (12.5)
Hyoid body unossified	0.0 (0.0)	1.7 (12.5)
Sternebrae misaligned	2.6 (14.3)	0.0 (0.0)

(¶) data tabulated for comparison purposes

From the above data, it is suggested that higher developmental toxicity was noted in fetuses of study 93002 as characterized by increased litter and fetal incidences of 27 presacral vertebrae and 13th full ribs.

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DISCUSSION

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High incidences of maternal mortality and abortion were previously noted in animals exposed dermally to Bladex 4L and wearing a neck collar during the exposure period (6 hours per day, WIL # 93002). This study (WIL # 93002A) was then designed to see whether gradual acclimation of rabbits to being in stocks for six hours per day (exposure period) and then to wearing collars for the remainder of the day (18 hours/day) rather than only during the exposure period (6 hours/day) would be a better approach to study the dermal developmental toxicity of Bladex 4L in rabbits.

For comparison purposes, data collected from this study (WIL #93002A) were tabulated by this reviewer with those of the 0.2 ml/kg group in study WIL 93002 since the same dose level was administered dermally during the period of major organogenesis (days 6-18) to the same species and strain of animal used.

Higher incidences of maternal toxicity and abortion were noted in animals of study WIL # 93002 (neck collar during exposure period ; 6 hours/day) which also gained less weight than those of study WIL # 93002 A during the treatment period. However, the overall weight gain (days 0-29) between the two groups was almost identical (218 vs 214 gm). Does wearing the neck collar for 18 hours/day (study 93002A) ate less than those wearing neck collars for only 6 hours/day (104 gm vs. 140 gm). No significant differences relative to implantations, resorptions, viable fetuses, and fetal weights were noted.

Developmental toxicity was noted in both studies as characterized by the presence of 27 presacral vertebrae and 13th full ribs. However, no frank teratogenic effects were evident in either study.

This study (WIL #93002 A) does not fulfill regulatory purposes due to the following:

a. Absence of a concurrent control group.
b. Insufficient number of animals used (9 rabbits)
c. Only one dose level tested (0.2 ml/kg)

It is recommended that this study be classified as Supplementary Data (Ancillary study).

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THE MATERIAL ON PAGES 33 152 THROUGH 39 158 WAS TAKEN DIRECTLY FROM THE REGISTRANT SUBMISSION. THESE PAGES ARE NOT INCLUDED IN THIS REVIEW.