TOX # 4491

CASWELL FILE

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

MAY 29 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a pilot teratology study with Bladex (Cyanazine) in Fischer 344 and Sprague Dawley rats.
EPA Reg. No. 201-298
EPA Accession Nos. 256693 thru 256696 Caswell No. 188C

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

FROM: Quang Q. Bui, Ph.D. Quang Q Bui 5/28/85
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THROUGH: Laurence D. Chitlik, D.A.B.T. LDC 5/28/85
Section Head, Section V
Toxicology Branch/HED (TS-769C)

and

Theodore M. Farber, Ph.D. WMF 5/29/85
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant

Shell Oil Company
Washington D.C.

Action Requested

Review of a pilot teratology study with Bladex in Fischer 344 and Sprague Dawley rats (Argus Res. Lab. #619-002P, 11/28/84).

RECOMMENDATION

It is recommended that this study be classified as Core Supplementary Data (dose-range finding study).

Under the conditions of this study, maternal toxicity was demonstrated in both the Sprague-Dawley CD and Fischer 344 rats at all dosage levels tested (lowest dose tested = 10 mg/kg/day) as evidenced by dose-related increases in the incidences of clinical signs and decreases in maternal weight gain during the dosing period (days 6-15 of gestation).

13

Disparity was noted between this pilot Sprague Dawley teratology study (Argus Res. Lab. #619-002P) and the definitive Sprague Dawley teratology conducted at Research Triangle Institute (#31T-2564, Project #61230, dated 5/16/83) previously submitted to the Agency under Accessions No. 071738 and 072838.

Dosage levels of 0, 1, 3, and 30 mg/kg/day were used in the Research Triangle Institute's study. Piloerection (14/30 animals) and decrease in maternal weight gain (19.8% reduction of control values) during the dosing period (days 6-15 of gestation) were associated with the 30 mg/kg dosage level. Litter size, mean resorptions, and mean fetal weights of the 30 mg/kg group were similar to control values. Based upon these submitted data, the maternal NOEL was determined to be 3 mg/kg and the LEL at 30 mg/kg/day (memo of 10/26/84).

In the Argus Research's study recently submitted, compound-related effects relative to clinical signs and maternal body weight gains were noted at all dosage levels tested (10, 50, 100, 150, and 200 mg/kg groups). Effects found at the 10 mg/kg dosage level were characterized by decreased palpebral size (7/8 animals), alopecia (2/8 animals), and decreased maternal weight gain (53.3% reduction of control values) during the dosing period (days 6-15 of gestation). Based upon these findings, the maternal NOEL was not determined but is less than 10mg/kg/day (lowest dose tested).

Consequently, with respect to body weight gain and clinical observations the effects produced at 10 mg/kg/day (Argus Lab.) were more pronounced than those produced at 30 mg/kg/day (Research Triangle Institute). Hence, the findings in these two studies are quite different and the registrant is required to provide explanation and clarification relative to this disparity. Possibly, problems relative to dose preparation may account for the lesser effects noted at the 30 mg/kg dosage level in the Research Trianble Institute's study.

Based upon this new data, it is recommended that the Research Triangle Institute's study (#31T-2564, dated 5/16/83) be re-classified as Core Supplementary Data pending adequate clarification from the registrant.

STUDY REVIEW

Chemical:	Bladex - Cyanazine
Test Material:	Technical Bladex®, SD-15418, 100% purity
Study/Action Type:	Teratology

STUDY IDENTIFICATION:

"Teratology pilot study of Technical Bladex (SD-15418) in Fischer 344 and Sprague-Dawley CD rats".

Testing Facility:	Argus Research Laboratories Inc.,
Final Report No.:	619-002P
Final Report Date:	11/18/84
Study Director:	E.A.Lochry
EPA Accession Nos.:	256693 thru 246696

DISCUSSION AND CONCLUSIONS

Compound-related decreases in body weight gain were noted in both the Sprague-Dawley and Fischer 344 rats at all dosage levels during the dosing period (days 6-15 of gestation). Administration of Technical Bladex also resulted in a dose-dependent increase in the incidences of clinical signs. These toxic signs were characterized by decreased motor activity, decreased palpebral size, impaired righting reflex, hyperpnea, excess salivation, muscle flaccidity, and dyspnea. Under the conditions of this study, no maternal NOEL was demonstrated from the dose levels selected in both Sprague Dawley and Fischer-344 rats (maternal NOEL < 10 mg/kg/day; lowest dose tested)

Significant increases in maternal mortality were observed when both Sprague-Dawley CD and Fischer 344 rats were treated with a dosage level of 100 mg/kg/day and above during days 6-15 of gestation.

Gross examinations of all pups delivered by C-section on day 20 of gestation (Sprague-Dawley rats) or on day 7 post-parturition (Fischer 344 rats) did not reveal any compound-related malformations. However, the teratogenic potential of Bladex in these two strains could not be ascertained since skeletal and soft-tissue examinations were not performed in this dose-range finding study.

It is unclear why a pilot study in Sprague-Dawley rats was conducted since the definitive study was done with Fischer-344 rats.

The Fischer-344 pilot investigation may provide some information relative to the selection of dose levels for the teratology phase of the main study. Selection of 5, 25, and 75 mg/kg as the dose levels for the main study apparently were justified since excessive maternal mortality was demonstrated at 100 mg/kg with slight body weight depression and increased incidences of clinical signs noted at 10 mg/kg in the pilot phase. However, post-natal information was limited since pups in the pilot study were sacrificed on day 7 postnatally instead of at weaning as was performed in the main study.

High incidences of resorptions were observed in all groups including the controls and were attributed to SDA viral infection by the investigators. Due to SDA viral infection, the exact etiology of increased resorptions observed in the treated groups could not be determined with certainty and, hence, this significantly restricts the utility of the Fischer-344 pilot study for dose selection in the primary study.

75

PROCEDURES

Test Material: Technical Bladex (SD-15418), white powder
Vehicle: 0.25% aqueous methyl cellulose
Dosage levels: 0, 10, 50, 100, 150, and 200 mg/kg/day
Period of administration: days 6-15 of gestation by gavage
Species: Fischer 344 and Sprague-Dawley CD rats

The protocol used in this study was previously reviewed by this reviewer (10/12/84). No significant deviations from the originally submitted protocol were noted. A copy of the methods used is appended.

This study was designed to investigate the teratogenic potential of Bladex in rats both after C-section in fetuses as well as post-natally. Therefore, for clarity sake, this study was divided into two sections and reviewed separately.

PILOT TERATOLOGY STUDY WITH SPRAGUE-DAWLEY CD RATS

Mortality

Compound-related deaths were noted in the treated groups. Mortality incidences of 0, 0, 0, 50, 88, and 100% were found in the control, 10, 50, 100, 150, and 200 mg/kg groups, respectively.

Necropsy of the dead animals revealed discoloration and mottling of the liver and lesions in the stomach. These observations apparently were compound-related. No gross lesions were observed in all surviving dams sacrificed on day 20 of gestation.

Clinical observations

Compound-related toxic signs were found in all Bladex-groups and were characterize by decreased palpebral size, decreased motor activity, impaired righting reflexes, labored breathing, ataxia, hyperpnea, and muscle flaccidity. Most of the clinical signs appeared within 5 minutes of dosage but were no longer present within 4 hours after dosage.

Body weight data

The maternal body weight gain data is summarized as follows:

Weight Gain (in grams)	Control	10 mg/kg	50 mg/kg	100 mg/kg	150 mg/kg	200mg/kg
Days 0-6	26.9	18.0	14.4	21.8	24.0	16.1
Days 6-15	33.8	15.8	5.4	-29.7(a)	-45.0(b)	N.D.
Days 16-20	61.9	65.1	70.1	82.3(a)	43.0(b)	N.D.
Days 0-20	132.5	107.8	101.0	64.3(a)	25.0(b)	N.D.

(a): Values averaged from 3 dams
(b): Values obtained from 1 dam which survived
N.D.: No data, all animals died

During the dosing period (days 6-15), body weight reductions were noted in all treated groups with significant differences found in the groups receiving 50 mg/kg/day and above. However, after cessation of dosing (days 16-20), increased body weights were noted in all treated groups suggesting that the body weight reductions found during the dosing period were compound-related.

Reproductive Findings

The following table illustrates the reproductive findings at necropsy:

	Control	10 mg/kg	50 mg/kg	100 mg/kg	150 mg/kg	200 mg/kg
# animals	8	8	8	8	8	8
# pregnant	8	8	8	8	8	7
Pregnancy index	100	100	100	100	100	88
# pregnant and died	0	0	0	4	7	7
# examined	8	8	8	4	1	0
$\bar{X}$ corpora lutea	21.9	17.4	19.1	20.8	21.0	-
$\bar{X}$ implantations	16.1	14.0	14.0	14.8	14.0	-
$\bar{X}$ litter size	14.5	12.5	10.1	9.8	2.0	-
$\bar{X}$ resorptions	1.6	1.5	3.9	5.0	12.0	-
Total fetuses	116	100	81	39	2	-
$\bar{X}$ fetal weight	3.13	3.25	2.99	2.75	2.27	-

The pregnancy index of all groups attained 100% except for the 200 mg/kg group (88%). Compound-related increases in mortality were noted in the 100, 150, and 200 mg/kg groups and thus restricting the number of animals observed at sacrifice for these dosage groups.

No significant differences in the mean number of corpora lutea and implantations were found among the groups. However, a decrease in mean litter sizes was noted in the treated groups. The decreases in litter size apparently resulted from an increase in resorption sites observed in the treated groups. Only the 10 mg/kg group had a comparable incidence of resorptions as the control.

The mean fetal weight of the 50, 100, and 150 mg/kg groups was lower than that of the control despite a smaller litter size suggesting that maternal and fetal toxicity were apparent at 50 mg/kg/day. No differences in fetal weight were noted between the 10 mg/kg and control groups.

Fetal Observations

Only a gross observation of the fetuses was conducted in this preliminary teratology study. One fetus of the 10 mg/kg group was described with "umbilical hernia" and one fetus of a 100 mg/kg group had "swollen and hemorrhagic tail". Since soft tissue and skeletal observations of the fetuses were not performed in this study, the teratogenic potential of Bladex in Sprague-Dawley CD rats could not be ascertained.

PILOT POST-NATAL STUDY WITH FISCHER 344 RATS

Maternal Mortality

During the dosing period (days 6-15), no mortalities were observed in the control, 10, or 50 mg/kg groups. However, compound-induced deaths were noted in 67%, 100%, and 100% of the groups dosed with 100, 150, and 200 mg/kg, respectively. Mottled livers and lesions in the stomachs were observed at gross postmortem necropsy.

Clinical Observations

A dose-dependent increase in the incidences of clinical signs was noted in all treated groups. Decreased palpebral size, decreased motor activity, hyperpnea, impaired righting reflex, bradypnea, excess salivation, muscle flaccidity, and dyspnea were described as the toxic manifestations. All these clinical signs generally appeared within 5 minutes of dosage but disappeared within 4 hours after dosage.

Maternal Body Weight

Maternal body weight was recorded at different stages of gestation and presented as follows:

Body Weight Gain * (grams)	Control	10 mg/kg	50 mg/kg	100 mg/kg
N	4	5	4	9
Days 0-6	10.2	11.8	5.8	9.7
Days 6-15	17.2	6.6	-6.0	-13.0 (a)
Days 6-20	24.0	28.6	33.2	44.0 (a)

(*) Excluding non-pregnant animals and dams with total resorptions
(a) Values taken from one dam which survived
All rats in the 150 and 200 mg/kg groups died prior to the expected day of delivery

Prior to the dosing period (days 0-6), no significant differences in body weight gain were observed among all groups. However, a dose-dependent decrease in body weight gain was noted in all Bladex-treated groups during the dosing period (days 6-15). Signs of recovery were evident in the treated groups after the dosing period (days 16-20) suggesting that the body weight reductions observed during the dosing period were compound-related.

In this study, maternal body weights were also recorded on day 1, 4, and 7 of lactation. No significant differences in body weight gains during the lactation period were found among all groups.

Reproductive Data

The reproductive data is tabulated as follows:

	0 mg/kg	10 mg/kg	50 mg/kg	100 mg/kg	150 mg/kg	200 mg/kg
# assigned	12	12	12	12	12	12
# pregnant	10	9	8	10	9	11
Pregnancy Index	83%	75%	67%	83%	75%	92%
# dead and pregnant	0	0	0	8	9	11
# resorbed	6	4	4	1	-	-
# pregnant and delivering litters	4	5	4	1	0	0
$\bar{X}$ gestation length (in days)	23.0	22.8	23.0	24.0	-	-
$\bar{X}$ implantations	8.8	8.8	8.5	12.0	-	-
$\bar{X}$ litter size delivered	8.0	8.6	7.0	12.0	-	-

A high incidence of dams with total resorptions was noted in all surviving rats including the controls and equaled to 6/10, 4/9, 4/8, and 1/2 of the surviving dams in the groups dosed with 0, 10, 50, and 100 mg/kg, respectively. The investigators indicated that "SDA infection apparently was the cause of the high resorption incidence". Signs of sialodacryoadenitis viral infection are usually marked by "swelling of the neck and a "hunched-up" appearance. In this study, "swelling of the neck" was observed in both control and treated animals.

No significant differences in the pregnancy index and in the mean number of implantations, litter size, and gestational length were observed between the 10 and 50 mg/kg and control groups. Data from the 100 mg/kg was not statistically valid since only one dam delivered offspring.

Pup Data

Pup mortality and body weight data are presented in the next table:

	0 mg/kg	10 mg/kg	50 mg/kg	100 mg/kg
$\bar{X}$ litter/birth	8.0	8.6	7.0	12.0[a]
$\bar{X}$ litter/day 4	8.0	8.6	6.8	11.0[a]
$\bar{X}$ litter/day 7	8.0	8.6	6.8	11.0[a]
Pup Weight/litter (grams)				
$\bar{X}$ day 1	4.8	4.5	5.0	5.0[a]
$\bar{X}$ day 4	7.2	6.4	6.1	6.7[a]
$\bar{X}$ day 7	9.9	9.0	7.9	9.0[a]

(a) data collected from one litter

Significant differences in pup mortality were not found between the control and treated groups up to post-natal day 7. Although, a slight decrease in pup weight was noted in the treated groups by post-natal day 7, no significant differences were found.

Gross malformations were not detected in either control or treated pups.

Litter Observation

No apparent compound-related clinical signs were noted in any treated pups during the 7-day post-natal period. Gross necropsy of all pups was performed on day 7 of lactation and did not reveal any findings.

THE MATERIAL ON PAGES 9 81 THROUGH 20 92 WAS TAKEN DIRECTLY FROM THE REGISTRANT SUBMISSION. THESE PAGES ARE NOT INCLUDED IN THIS REVIEW.