11-14-83

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

NOV 14 1983

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Robert Taylor, PM#25
Registration Division (TS-767)
and
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

THRU: William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: PP#9F2239; Bladex in/on Soybeans; EPA Reg.#201-279 and 201-281. A New Teratology Study in SD CD Rats, Accession #071738; and an Addendum to the Teratology Study in F344 Rats, Accession #071739. CASWELL#188C

This review confines itself to the evaluation of the teratogenic potential of Bladex in the Sprague Dawley and Fischer 344 rat strains. Any tolerances, i.e., soybeans, should be addressed by the Registration Standard reviewer in light of the newly re-evaluated toxicity data base.

Recommendations:

1. Teratology Study In SD CD Rats:

°This study is classified Core Supplementary because the maximum tolerated dosage was not tested and because individual animal data were not included in the submitted report. Potentially, once individual animal data is received and if further assessment demonstrates maternal toxicity, this study may be upgraded.

°Bladex at dosages of 0.0, 1.0, 3.0 and 30.0 mg/kg/day did not cause any significant maternal or fetal toxicity or teratogenic effects when administered by gavage from day 6-15 of gestation to 10-12 week old CD rats.

2. Teratology Study in F344 Rats:

°This study remains classified Core Supplementary as previously determined in Dykstra's review of 1/26/83. The control and the high dose level should be repeated in order to evaluate the nature and incidence of diaphramatic hernia. A postnatal phase should also be included to determine the survivability of the affected fetuses.

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It is assumed that if these new data reflect a life threatening fetal defect, a new study with this strain of rats should be performed at the lower dosage levels to determine a NOEL. If, on the other hand, the limited study requested (high dose and control) confirms the contention presented by the registrant in his latest submission, a study to determine a NOEL for diaphragmatic hernia will obviously not be necessary.

° It is concluded that Bladex is teratogenic in rats. An elevated incidence of anophthalmia/microphthalmia is noted in this study at 25 mg/kg/day(HDT),i.e. 15% incidence in the high dose litters as compared to 5% in the control and 7% in the historical data. Maternal toxicity is not considered severe enough at this dosage level to cause this defect (only a 6% body weight reduction on days 12-15 gd as compared to the control).

REVIEW

1. Teratologic Evaluation of Bladex in SD CD® Rats.

Accession No.: 071738

Sponsor : Shell Development Company, a Division of Shell Oil Company; West Hollow Research Center, P.O. Box 822, Houston, Texas 77001

Project No. : 61230

Date Submitted to EPA: 7/6/83

Testing Laboratory: Research Triangle Institute (RTI); POB 12194, Research Triangle Park, N.C. 27709.

RTI Study No.: RTI Project #31T-2564 (the study code number was reported as Rt83-BDX on page 5 of the report and as Code #Rt82-BDX on page 8); 5/16/83

Study Period: February 9, 1983 to April 1, 1983

Study Director: Rochelle Wolkowski - Tyl, Ph.D. (RTI).

Study Coordinator: Cheryle C. Lu, Ph.D. (Shell Company).

The study was conducted according to Shell's protocol #WTP-209, and RTI's master protocol #RTI-113/31T-2564.

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Test Article: Bladex technical, 98.5%. Code Number SD 15418, 16-16-0-0, WRC Tox Sample No. 107B. Nuclear magnetic reasonance (NMR) analysis reflected a purity of 98.5% (2/2/82); and gas chromatographic analysis reflected a purity of 96.5% (1/21/83). The test substance was stable from 2/2/83 to 4/18/83 as determined by chemical analysis. The test substance was supplied by the Sponsor in preformulated dose groups (see Vehicle and Dosage sections below).

Vehicle: A solution of 0.2% w/v of METHOCEL in distilled water was used as a vehicle. The Bladex dosages were prepared by the Sponsor as suspensions in this vehicle and were reported to be stable for 22 days when stored at 5°C.

Dosages: The following dosages of Bladex were administered daily by gavage from day 6 through 15 of gestation:

Dosages mg/kg b.w.	Vehicle ml/kg b.w.	Number of Dams
0.0	5	30
1.0	5	30
3.0	5	30
30.0	5	30

The above dosages were equivalent to 0.0, 0.02, 0.06 and 0.60% w/w of Bladex in aqueous solution. The control group received only the appropriate volume of the vehicle.

The dose volume was based on the dams body weight on day 6 of gestation. The Sponsor indicated that the actual dosages were within 15% of the nominal concentrations.

A range-finding study was initially performed to determine the doses used in this study. However, data for this study were not included in the submitted report.

Test Animals: Sprague-Dawley albino rats were used. Hundred male, 10-12 weeks old and 200 female 8-10 weeks old substrain CD® rats (COBS(SD)BR) were obtained on 2/9/83 from the Charles River Breeding Laboratories, Inc., Kingston, N.Y.

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All animals were ear tagged for identification and quarantined for 14 days upon arrival. Afterward, individual females in oestrus or proestrus were placed overnight with a male for insemination. Day 0 of gestation was thus designated for sperm-postive females. At gestation day(gd) 0, the males weighed 300-400g and the females, 202.43-268.87g. Five days were needed to produce 120 sperm-positive females which were distributed into 4 dosage groups (30 dams/group). Equal number of animals inseminated on a specific day were assigned in each group. The dams were individually housed and were assigned to dosage groups on the basis of matched weights.

No male was used for insemination more than once per dosage group.

All animals were maintained at constant room temperature (19.5 to 22.5°C), relative humidity (33%-64%) and 12 hours light/dark cycle; the electronic hydrothermograph was reported to be accurate within 2°C and 3% RH.

Feed (Purina Rodent Chow #5002) and water (deionized/filtered) were available ad libitum.

The dams were monitored daily for general health. The body weights were recorded on days 0, 6, 12, 15 and 20 p.c. (immediately after sacrifice).

Food consumption data were not reported and apparently were not measured.

On day 20 p.c. the animals were anesthetized by CO_2 and sacrificed by cervical dislocation. At necropsy, after determination of the pregnancy status of all dams, the first 20 pregnant females per dose group were examined for internal abnormalities; the remaining dams were discarded (we note that this is not a procedure suggested by the Agency; all pups from all dams should have been examined). The liver was dissected and weighed. Affected tissues were also removed and placed in buffered neutral 10% formalin for storage.

The uteri of the above mentioned 20 females/group were removed, weighed and examined for evidence of fetal toxicity or teratogenic response. The following parameters were reported:

1. Number of corpora lutea
2. Number of implantation sites
3. Number of resorptions
4. Number of live or dead fetuses
5. Fetal sex, weight, and crown to rump length
6. Malformations (external, visceral and skeletal) and variations.

For the evaluation of fetal malformations, half the live fetuses were decapitated before dissection for visceral examination, and the heads were fixed in Bouin's solution for free hand sectioning and examination (Wilson, 1965). All live fetuses were examined for visceral malformations using Staples method (1974); and the remaining carcasses were stained with Alizarin Red S for examination of the skeletal malformations (Peltzer and Schardain, 1966; Crary, 1962).

Statistical analyses were performed. All analyses were evaluated at the 5% probability level.

RESULTS:

Maternal Data

No mortality occurred in this study. Piloerection was reported only in treated animals during the compound adminition period, (gd 6 to 15). This effect was not noted after day 15 of gestation and it appeared to occur at a negligible rate in the low and mid dose groups (two animals were affected on day 11 at the low dose; at the mid dose, one animal was affected on day 10 and two animals on day 11). At the high dose level, 1 to 3 animals were affected throughout the dosing period with the exception of day 11 were 6 animals were affected. However it is not possible from the data as submitted to identify the animals affected because the identification numbers of these animals were not reported. It is also not possible to verify if these animals were included among the 20 dams examined at necropsy in this group because individual animal data were not submitted.

All groups showed a body weight gain during pregnancy (only one high-dose animal showed a slight weight loss on day 12 and 15 of gestation).

No abortions occurred in this study and only a few animals in the Bladex treated groups were found non-pregnant at termination; the following table reflects these findings as presented in table 2 (p. 28) of the submitted report:

	Dosage (mg/kg/day)			
	0.0	1.0	3.0	30.0
Total No. of sperm-positive dams initiated in the study	30	30	30	30
Number dead	0	0	0	0
Number aborted	0	0	0	0
Number non-pregant at sacrifice	0	2	3	3
Number pregnant at sacrifice (%)	30 (100)	28 (93)	27 (90)	27 (90)
Number examined*	19	21	19	21

*Note that 20/30 dams per group were to be examined at necropsy according to the protocol. However one dam at 1.0 and one at 30.0 mg/kg/day were inadvertantly entered on the sacrifice sheet in error as belonging to 0.0 & 3.0 mg/kg/day group respectively.

All animal groups reflected an increase in the mean maternal body weights during pregnancy (day 0 to 20 post coitus), during dosing (day 6 through 15 p.c.), and at sacrifice in the mean absolute body weight (mean body weight at sacrifice minus mean corrected gravid uterine weight). However, these increases were statistically significantly ($p < 0.05$) lower than the control group at the high dose level for both the mean corrected absolute body weight gain and the mean body weight gain during dosing, see table below:

	Dosages mg/kg/day			
	0.0	1.0	3.0	30.0
1. Mean b.w. at gd 0 g.	236.01	233.70	238.92	238.49
2. Mean b.w. at gd 6 g.	263.10	258.95	266.01	263.59
3. Mean b.w. gain at gd 0-20 g.	131.82	128.33	145.29	135.59
4. Mean b.w. gain at gd 6-15 g.	47.54	47.56	48.06	38.1*
5. Mean b.w. at gd 15 g.	310.64	306.51	314.07	301.69
6. Mean corrected ab b.w. gain g.	62.30	56.52	61.33	50.91*
7. Mean absolute b.w. g.	298.3	290.2	300.2	289.4

*$p < 0.05$

It was not clear if the above discussed reductions ($p < 0.05$) in the mean body weight gains at the high dose level were directly related to the test substance or occurred because of a lower rate of food consumption; these data were not submitted. In addition, these reductions in the mean weight gains, although statistically significant, appear not to be biologically significant in light of the fact that the mean body weight during the dosing period and the mean corrected absolute body weight for this group were not statistically significantly or biologically different from the control data, i.e., 3% lower than the control group in both cases.

No additional effects were noted at necropsy. The mean absolute and relative liver weights were not affected at any dose level.

In conclusion, maternal toxicity was not clearly demonstrated at the highest dosage tested. The significant ($p < 0.05$) reduction in the mean body weight gain reported at this level may have been indicative of some maternal toxicity; however in the absence of additional significant toxicity data, it appears that the maximum tolerated dosage level was not tested in this study. Further evaluation of this issue will await the submission of individual animal data.

Cesarean Data:

The following table reflects the results of the uterine examinations as reported in table 4 (p. 33 to 36) of the final report:

	Bladex Dosages (mg/kg/day)				Historical Data
	0.0	1.0	3.0	30.0	Mean (lower and upper 95% confidence intervals)
No. of litter	19	21	19	21	
Mean No. of corpora lutea per dam	15.00	14.71	15.33	17.29	16.74 (15-18)
% preimplantation loss per dam	22.14	16.08	8.36	18.08	16.56 (7-25)
Mean No. of implantations per dam	11.95	12.48	14.26	13.62	12.43 (12.0-12.8)
Mean No. of resorptions* per dam	0.58	0.52	0.47	0.38	0.57 (.4-.8)
Mean No. of dead fetuses per dam	0	0	0	0	0.01 (.002-.02)
Mean No. of live fetuses** per dam	11.32	11.95	13.79	13.24	12.0 (11.6-12.4)
% male/female ratio	44/56	48/52	46/54	50/50	51/49 (48/52-52/48)
Mean male fetal weight (g)	4.0	4.0	4.0	4.2	3.7 (3.6-3.7)
Mean female fetal weight (g)	3.9	3.8	3.8	3.9	3.5 (3.4-3.5)
Mean crown-rump length (mm)	37.38	36.91	37.05	37.18	———

*No dam had total resorption; the number of dams with resorptions were 7, 7, 5 and 3 in the control, low, mid and high dose groups respectively.

**The mean uterine weights increased significantly ($p < 0.05$) at both the mid and high dose groups i.e. 69.53 g, 71.81 g, 83.96* g and 84.66* g in the control, low, mid and high dose groups respectively as compared to 67.38 g in the historical data. This increase at both dose levels may be associated with the above noted increase in the number of fetuses/dam.

No adverse effects are noted in the reproduction parameters listed above. In fact the control group appears to reflect data slightly different from the historical data. Some parameters in this control group appear to be more affected than in the treatment groups, see discussion below:

°The control group had a lower value for the mean number of implantations/dam and for the mean number of live fetuses/litter than any of the treated groups; and it had a higher mean number of resorptions/dam than the treated groups. Individual animal data were not available in the submitted report, hence a comprehensive assessment of these data cannot be provided; for example, it was discovered on page 43 of the submitted report (table 7: only dams with one or more malformed fetuses were listed in this table) that control animal #89 had only one live fetus. Obviously the high postimplantation loss in this control dam cannot be explained(note that in the mid and high dose groups, the mean number of implantations/dam and the mean number of live fetuses/dam were slightly higher than the 95% upper confidence interval for the mean historical value).

°No effect was noted on the mean fetal weight or on the mean crown-rump length between the treatment groups and the control group. However the mean fetal weight in this study was slightly higher in all groups including the control group than the historical values(we note that the mid and high dose groups had higher number of pups per litter; however, it is our experience that an increase in litter size is usually accompanied by a decrease in fetal weight and it is rather unusual in this study to note that the fetal weight increased at the high dose despite of a larger litter size).

Fetal Malformations and Variations

No significant malformations (external, visceral or skeletal) or variations were noted in this study. Bladex did not cause any significant teratogenic activity at any dose level as compared to the control group or to the historical data. The following table reflects the major malformations and some significant variations reported in this study:

Major Malformations and some variations	Dosage in mg/kg/day 0.0	1.0	3.0	30.0	Historical Data
No. Fetuses (litters) examined	216(9)	251(21)	262(19)	278(21)	2808(234)
No. Fetuses (litters) with malformations	4(4)*	6(5)	5(5)	1(1)	7(7)
Gross Malformations					
low set ears & small nose	1[a]	0	0	0	1.
Tip of tail 7 tread like	0	0	1	0	___
Visceral Malformations					
Hydroureter (see below)	1[a]	3(3)	1	1	17+ 9 left & 4 right.
Hydronephrosis (see below)					7 bilateral + 8 right
Hydroureter & Hydronephrosis	2(2)	0	2(2)	0	(see above)
Missing subclavia	1	0	0	0	___
Aorta behind trachea and or/ esophagus	0	1	0	0	___
Reversed aorta and pulmonary arch	0	0	1[b]	0	1
Skeletal Malformations					
Frontals, parietal fused,	0	2(1)	0	0	2
Scrambled vertebra	0	0	1[b]	0	___
Significant Variations in Fetuses					
Bipartite vertebral centra [c](%)	5(2.3)	27(11)	25(5.7)	19(5.8)	92(3.2)

*Four fetuses of four dams apparently seemed to be affected; however one dam had only one fetus.

a: Control fetus # 1 of litter #89 had gross and visceral malformations. We also note that this fetus is the whole litter for this dam.

b: Fetus #81 (litter #60) was affected with both visceral and skeletal malformations.

c: The variation was located in the thoracic region except for one fetus which was affected in the lumber region.

The above table reflects no significant increases in the incidence of malformations which can be clearly associated with Bladex treatments. However, the incidence of malformations in this study in the control, low and mid dose groups is slightly higher than the historical data; but this does not appear to be biologically meaningful.

It is noted that incidences of hydroureter and hydronephrosis are within the same range in the control, low and mid-dose groups but much lower at the high dose.

It is also noted that the aortal position was affected in 1/251 low-dose fetus and 1/262 mid-dose fetus; however this effect is not seen at the high dose level and is not considered compound related. In addition 1/2808 fetuses is reported with this finding in the historical data.

Slightly increased incidences of bipartite vertebral centra are noted in all Bladex dosage groups as compared to both the control group and the historical data. However, in the absence of other significant variations in the treatment groups, and in the absence of other significant fetotoxic effects, the increase in this variation cannot be explained at the present time.

Conclusions:

No significant fetotoxic or teratogenic effects are noted in this study. We note that litters of the first 20 of 30 dams which were found pregnant at necropsy, were examined while the remaining dams were discarded. The Agency does not suggest that any available litters be discarded and the registrant should be informed that such a procedure should not be used in the future.

At the high dose level a slight decrease in the mean maternal body weight (3% lower than the mean control value) was noted during the dosing period (day 6 through 15 of gestation) and in the mean corrected asolute body weight (also 3% lower than the control value) at termination. Also piloerection was noted at this dose level during the dosing period with a maximum of 6/30 animals affected on day 11 gd. However, the mild and questionable nature of these maternal toxicity data do not firmly demonstrate that the maximum tolerated dose level was tested in this study. In addition, it is not possible to identify if these affected dams were discarded at necropsy or further examined. Thus, The maternal toxicity issue will be examined more carefully once individual animal data are submitted; but tentatively it has been concluded that no significant maternal toxicity has been demonstrated in this study.

Classification: Supplementary Data

°Maximum tolerated dose apparently not tested.
°Individual animal data not reported.

2. Addendum to the Teratology Study with F344 Rat strain (WRC RIR-180). Accession #071739.

Background

1. The teratology study with technical Bladex (SD 15418) in Fischer 344 Rats (Westhollow Research Center, Project No. 61230, December, 1981; Accession No. 070584) was reviewed on 2/3/82 by W. Dykstra for PP#9F2232 (copy of review attached). The review indicated that groups of 30 mated female rats were dosed with Bladex at a rate of 0, 1.0, 2.5, 10.0 and 25.0 mg/kg/day during gestation days 6-15. At necropsy (day 20 gd) litters of the first 20 females/group were examined; the remaining dams were killed and discarded. A positive control group (vitamin A) was also used.

The study reflected a significant reduction ($p < 0.05$) in body weight gains in the 10 mg/day dosage group (on gestation day 12) and in the 25.0 mg/kg/day group (on gestational days 12 and 15) and in the positive control group (on gestation days 12, 15 and 20) when compared to the vehicle control group. The carcass weight was also significantly ($p < 0.05$) reduced in the 25.0 mg/kg/day group and in the positive control group.

No significant differences were seen between the Bladex treated groups and the vehicle control group in the number of corpora lutea, implantations, resorptions, live or dead fetuses, fetal body weights, fetal crown-rump length and sex ratio. However, several litters in the various dose groups consisted of a single fetus.

In this study the reviewer noted several malformations: small or absent eyes; displaced esophagus, diaphragmatic hernia, small liver protrusion, undescended testis and displaced testes. The reviewer indicated that the presence of small or absent eyes and of diaphragmatic hernia are suggestive of teratogenic effects, and he requested that the registrant submit the historical data for this rat strain.

Also, a significantly increased incidence of a skeletal variation, lumber spur, was observed and was considered as an indication of fetotoxicity.

2. The registrant submitted several sets of historical data from Shell, Dow, and USAF laboratories which were reviewed by W. Dykstra in 1/26/83 (copy of review attached).

The following discrepancy in the incidence of diaphragmatic hernia for the 1.0 mg/kg/day dosage group was reported by Dykstra (page 3 and table 2) and later corrected by the registrant:

% Incidence of Incomplete Hernia (Number affected)	Correction	Mistake
Fetus	1.3 (1)	5.00 (3)
Litter	5.3 (1)	15.79 (3)

(The above correction was performed by hand in the attached copy of Dykstra's review).

Two major potential teratogenic effects, namely eye absent (anophthalmia) or small (microphthalmia) and diaphragmatic hernia were thoroughly discussed:

a. Anophthalmia & Microphthalmia

°Increased incidences of anophthalmia and combined anophthalmia and microphthalmia were noted in the Bladex study at 25 mg/kg/day dose level (HDT) as compared to both the study control group and to Shell historical data, see discussion #1 on page 1, 2 and 3, and table #1 on pages 4 and 5 of Dykstra's review of 1/26/83.

b. Diaphragmatic Hernia

°Increased incidence of diaphragmatic hernia (complete hernia) was clear in the Bladex study in all dosage groups as compared to the study control and to Shell historical data, see table 2 page 6 of the 1/26/83's review.

°Increased incidences of combined incomplete and complete hernia in the 10 and 25 mg/kg/day dosage levels were noted relative to the study control. However Shell historical data reflected even a much higher incidence for this lesion (3x in the litters data) than the study control, see table 2 on page 6 as mentioned previously; this was and is considered of serious concern in the study. The overall incidence of this finding in this study was considered extremely high and at very unusal incidences relative to our experience.

Dykstra concluded that in order to adequately assess the teratogenic potential of Bladex "the study needs to be repeated" and classified it as Supplementary Data.

3. The registrant submitted the addendum at hand for review (Accession #071739, 7/6/83) in order to discuss the above findings and to demonstrate that Bladex is not a teratogen. Dr. Mildred Christian served as a consultant in this endeavor.

Discussion:

1. Anophthalmia and Microphthalmia

Table #2 page 8 (copy attached) of the submitted addendum reflects Shell's reassessment of anophthalmia and microphthalmia findings. This table is almost identical to table #1 on page 4 of the 1/26/83 review with minor changes in the numerical values of the fetal data at 25 mg/kg. Dr. Christian indicated that incidences of anophthalmia previously reported by Shell were not completely accurate because according to her examination "the eyes were not missing, but were very small due to agenesis of the lens"; in her opinion the incidence of these two defects should be combined.

We accept the above recommendation by Dr. Christian. However, the increase in the incidence of anophthalmia/microphthalmia at the high dose level, 25 mg/kg/day, reflects a Bladex induced teratogenic effect. The extent of maternal toxicity noted at this dosage level (only a 6% reduction in the mean body weight on day 12 of gestation as approximated from figure#1 on page 10 of the submitted addendum) does not justify the registrant conclusion that the noted anophthalmia/microphalmia in this study are manifestations of maternal toxicity.

2. Diaphragmatic (liver) Hernia, Complete or Incomplete

The use of an improved Wilson's technique which involves pulling the liver away from the diaphragm to permit a better view of the liver, diaphragm and viscera, contributed to the observation of this lesion in Shell's Laboratory. This is the registrant's explanation as to why this finding is not well documented in other laboratories. However, in this reviewer's opinion, it does not explain the 3x fold increase in the occurrence of this lesion in Shell's historical data as compared to the study control group. The submitted addendum does not provide an explanation for this rather significant discrepancy.

However, the addendum provided a discussion concerning the difference between this kind of lesion and the well known lateral diaphragmatic defect, which usually includes herniation of the stomach and intestine into the thoracic cavity and causes fetolethality. The submitted addendum described the liver protusion that was associated with the reported incidence of diaphragmatic hernia as a spontaneous diaphragmatic/liver developmental variation in the Fischer 344 rat, a variation which causes no embryolethality. This variation "consists of a thinning of the fibrous central tendon of the diaphragm so that it appears translucent in the thinnest cases and, at first glance, absent in part".

This reviewer notes that the submitted addendum indicates on page #4 that the classification of this lesion in the previously submitted study reports was based on the fact that the tendon appeared partially torn or missing and that the developing liver had taken advantage of the weak tendon and had slightly bulged into the central tendon area. However, on page #5 of the submitted addendum, the registrant indicated that upon further examination of the fetal specimens by Shell's teratologist and pathologist, the occasional apparent perforation seen in the central tendon and classified as diaphragmatic hernia is an artifactual tear associated with the manipulations of fetuses for examination. The registrant added "However, we recognized that absolute verification of the artifactual nature of the opening in the thin tendon is difficult at this time". Table #1 on page 6 of this addendum (copy attached) reflect the same data reported in table #2 page 6 of the 1/26/83 review.

We note that the above explanation by the registrant is a retroactive assumption. Bladex may, however, have caused complete diaphragmatic hernia at all dosages tested in the Fischer 344 rat. Although the overall high incidences reported in the control as well as the test groups for combined complete and incomplete hernia were much lower(3x) than the historical values, these incidences in both the study and the historical data must be questioned, see the attached table #1 of the addendum and table #2 of the 1/26/83 review.

Thus, it is not clear at the present time whether this finding is a strain-specific variation or a lifetreatening malformation; it is also not possible to know whether the complete hernia is due to artifact (mechanical examination of the fetuses causing the tear of the central tendon) or whether the tendon was penetrated due to a teratogenic (herniation) effect.

Hence, the study remains classified Core Supplemental. It is suggested that the control and the high dose group, 25 mg/kg/day, should be repeated in order to evaluate the nature and incidence of diaphramatic hernia in this strain of rats. A postnatal phase should also be included to ascertain the survivability of potentially affected fetuses.

11/4/83

Amal Mahfouz, Toxicologist
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769)

TS-769:th:TOX/HED:AMahfouz:10-27-83:card #7

Table 2. Tabulation of Classification and Incidence of the Eye Defects Seen in Fischer 344 Rats

Classification used			Mean Percent Affected (no. affected)			Range of Incidence (Attachment III.4.)		
RIR-180	Attachment III.4. Supplemental Information to EPA	Addendum to WRC RIR-180	Shell Historical Control	Shell BLADEX® Control	Shell 25 mg/kg BLADEX	DOW[a] Laboratory	RTI[a] Laboratory	USAF[a] Laboratory
eye small	microphthalmia		F) 0.4(5) L) 5.1(5)	1.25(1) 5.00(1)	0.83(1) 5.00(1)	0-0.8 0-7.4	0-0.6 0-4.6	0 0
eye absent	anophthalmia		F) 1.29(4) L) 4.08(4)	0 0	3.67(4) 15.00(3)	0-0.5 0-5.0	0-0.6 0-4.6	0-3.9 0-14.8
	combined	microphthalmia[b] with agenesis of the lens	F) 1.62(8) L) 7.14(7)	1.25(1) 5.00(1)	4.50(5) 15.00(3)	0-1.3 0-12.4	0-1.2 0-9.2	0-3.9 0-14.8

a) range of per cent incidence of the eye defect in total fetuses examined from several study groups.
b) eye defects as reported in the Shell Fischer 344 rats are microphthalmia with partial to complete agenesis of the lens. The 5 fetuses noted in the 25 mg/kg BLADEX group were from 3 litters, 4 fetuses with unilateral microphthalmia, 1 with bilateral microphthalmia.
F) is fetus
L) is litter

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TABLE 1

INCIDENCE OF ANOPHTHALMIA AND MICROPHTHALMIA IN FETUSES OF BLADEX® TREATED AND HISTORICAL CONTROL FISCHER 344 RATS

Laboratory Anomalies	Fetuses Affected/ Fetuses Exam.	Percent Affected	Total #Lit. Affected/ #Lit. Exam.	Percent Affected	Tab Number
SHELL DEVELOP. (Dec. 1981) (Houston, TX)					1
Control					
Anophthalmia	0/82	0	0/20	0	
Microphthalmia	1/82	1.2	1/20	5	
Combined	1/82	1.2	1/20	5	
BLADEX 1,2.5 & 10 mg/kg					
Anophthalmia	0/259	0	0/56	0	
Microphthalmia	0/259	0	0/56	0	
BLADEX 25 mg/kg					
Anophthalmia	4/97	4.12	3/20	15	
Microphthalmia	1/97	1.03	1/20	5	
Combined	5/97	5.15	3/20	15	
SHELL DEVELOP. (August-October, 1982) (Houston, TX)					1
Anophthalmia	4/1025	0.39	4/98	4.1	
Microphthalmia	5/1025	0.49	5/98	5.1	
Combined	8/1025	0.78	7/98	7.1	

Table 1. Tabulation of Classification and Incidence of the Spontaneous Diaphragmatic/Liver Developmental Variation in Fischer 344 Rats

Classification used				Incidence					
RIR-180	Attachment III.2. Letter to EPA	Attachment III.4. Supplemental Information to EPA	Addendum to WRC RIR-180	Dose Groups, mg/kg BLADEX® 0	1	2.5	10	25	Shell Historical Controls
diaphragmatic hernia	a. diaphragmatic hernia	complete hernia		F) 0	L 5.00(3)	1.05(1)	2.78(2)	3.00(3)	0.56(4)
				L) 0	15.79(3)	5.26(1)	11.11(2)	15.00(3)	4.08(4)
liver protrusion, small	b. incomplete hernia (small liver protrusion)	incomplete hernia		F) 8.50(5)	*1.32(1)	1.05(1)	4.54(4)	7.50(7)	10.56(102)
				L) 20.00(4)	*5.26(1)	5.26(1)	16.66(3)	20.00(4)	63.27(62)
	a. & b. combined	combined	diaphragmatic/ liver developmental variation	F) 8.50(4)	6.3(4)	2.1(2)	7.3(6)	10.5(10)	11.11(106)
				L) 20.00(4)	21.1(4)	5.3(1)	27.8(5)	35.0(7)	64.29(63)

F)mean percent fetuses affected (number fetuses affected).
L)mean percent litters affected (number litters affected).
*Incorrectly reported as 5.0(3) for fetus and 15.8(3) for litter in Supplemental Information to EPA (Attachment III.4.).

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TABLE 2

INCIDENCE OF DIAPHRAGMATIC HERNIA
MEAN PERCENT AFFECTED (Number Affected)

Variable		BLADEX® Teratology Study (WRC RIR-180) 0	1	2.5	10	25	Shell Historical Data
Number of litters		20	19	19	18	20	98
Number of fetuses		82	76	93	90	97	1025
Incomplete hernia	- Fetus	8.50 (5)	5.00 (3)	1.05 (1)	4.54 (4)	7.50 (7)	10.56 (102)
	- Litter	20.00 (4)	15.79 (3)	5.26 (1)	16.66 (3)	20.00 (4)	63.27 (62)
Complete hernia	- Fetus	0 (0)	5.00 (3)	1.05 (1)	2.78 (2)	3.00 (3)	0.56 (4)
	- Litter	0 (0)	15.79 (3)	5.26 (1)	11.11 (2)	15.00 (3)	4.08 (4)
Combined	- Fetus	8.5 (5)	6.3 (4)	2.1 (2)	7.3 (6)	10.5 (10)	11.11 (106)
	- Litter	20.0 (4)	21.1 (4)	5.3 (1)	27.8 (5)	35.0 (7)	64.29 (63)