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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Overview of the teratogenic potential of Bladex (Cyanazine)
Caswell No. 188 C

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Although an overview of the teratogenic potential of Technical Bladex (Cyanazine) was not requested by Registration Division in the recent expedite request, Toxicology Branch nevertheless performed this assessment since we feel that a comprehensive presentation of all of the teratology data was essential.

OVERVIEW OF THE TERATOGENIC AND DEVELOPMENTAL TOXIC POTENTIAL OF BLADEX (CYANAZINE) IN LABORATORY ANIMALS

I. Background Information

Teratology studies are required to support the registration of Bladex (Cyanazine) and § 83.3 of the Subdivision F Guidelines specify that testing should be performed in at least 2 mammalian species, preferrably in rats and rabbits.

The registrant, Shell Oil Company, in an effort to comply with FIFRA requirements has submitted the following teratology studies [1]:

1. Teratology study in Albino Rabbits, IBT #J-238 (invalid study, L. Anderson's memo of 2/4/80)

2. Teratology study in Albino Rabbits, IBT #8580-11112, 3/15/79 (invalid study, Clements Associates 10/8/81, accepted by EPA on 10/9/81)

3. Teratology study in Fischer-344 Rats (Westhollow Research Center RIR-180, Study #61230, dated 12/81) submitted to the Agency under Accession #070584.

4. Teratology study in New Zealand Rabbits (Tunstall Lab., England, Project #221/81, dated 11/82) submitted to the Agency under Accession #071382 on 2/1/83.

5. Teratology study in Sprague Dawley Rats (Research Triangle Institute #31T-2564, Project #61230, dated 5/16/83) submitted to the Agency under Accession #071738 on 7/6/83.

6. Developmental toxicity study in Fischer-344 Rats (Argus Research Lab., #619-002, dated 4/18/85) submitted to the Agency under Accession #257867 on 5/9/85.

II. Study Evaluations

A. New Zealand Rabbits (reviewed by Dr. Mahfouz, memos of 4/29/83 and 11/21/83)

Technical Bladex (98%) was given to pregnant New Zealand rabbits at 0, 1.0, 2.0, and 4.0 mg/kg/day from days 6-18 of gestation.

Anorexia, weight loss, death, and abortion were the main maternal effects noted at the 2 and 4 mg/kg dosage levels. Alterations in skeletal ossification sites, decreased litter size, and increases in post-implantation loss were other effects associated with these two dose levels. Malformations were noted at 4 mg/kg and included domed cranium, dilated brain ventricles, anophthalmia, microphthalmia, and thoracoschisis. These anomalies occurred at dosage levels which produced excessive maternal toxicity.

The maternal and developmental toxicity NOELs in New Zealand rabbits were established at 1 mg/kg/day.

1. Range finding studies not listed.

DISCUSSION AND CONCLUSIONS

In the mothers, administration of Bladex results in body weight reduction during the dosing period in all species and strains studied as well as in food consumption depression in Fischer-344 rats (Argus Res. #619-002). Clinical toxic manifestations were observed in the Fischer 344 rats at dose levels as low as 5 mg/kg/day (lowest dose tested; Argus Res. #619-002), in the Sprague Dawley rats at 10 mg/kg (Argus Res. #619-002P, pilot study) or 30 mg/kg (R.T.I), and in the rabbit at 2 mg/kg (Tunstall Lab.). Due to the disparity in findings in the Sprague Dawley rat relative to clinical signs and body weight gain, the registrant was requested to provide adequate explanation and/or information (Dr.Bui's memo of 5/29/85).

Developmental toxicity was demonstrated in the rabbit at 2 mg/kg (Tunstall Lab.), in the Fischer 344 rat at 1 mg/kg (Westhollow) and 5 mg/kg (Argus Res. #619-002). Based upon the reported findings, three developmental toxic effects are of concern to the Agency: eye-abnormalities, diaphragm abnormalities, and brain-abnormalities.

Anophthalmia and/or microphthalmia was observed in the rabbit at 4 mg/kg (Tunstall Lab.) and in the Fischer rat at 25 mg/kg (Westhollow Res.) and at 25 and 75 mg/kg (Argus Res. #619-002). The presence of anophthalmia and/or microphthalmia is thus confirmed in two species by three studies at maternally toxic levels.

Dilated brain ventricles were found in the rabbit at 4 mg/kg (Tunstall Lab.) and in the Fischer rat at 75 mg/kg (Argus Res. #619-002).

Diaphragm-related abnormalities were found in both Fischer studies. In the Westhollow study, diaphragmatic hernia was observed at all dose levels tested (lowest dose = 1 mg/kg). Findings of diaphragmatic hernia were dose-related except at the lowest dosage level (1 mg/kg) which possibly was erroneously increased by technical errors. Diaphragmatic hernia was not found in the C-section phase of the Argus study but "diaphragm central tendon incomplete fusion with raised area of the liver" was significantly increased in the 25 and 75 mg/kg pups sacrificed at weaning. Only in the first Fischer-344 study (Westhollow) was diaphragmatic hernia observed at dose levels where significant maternal toxicity was <u>not</u> demonstrated.

These data collectively suggest that malformations of the diaphragm, eyes, and brain may be related to Bladex administration.

RECOMMENDATION

Based upon the aforementioned teratology data, it is recommended that Bladex be regulated as a teratogen:

1. Finding of diaphragm-related malformations in two studies (Westhollow and Argus Res. #619-002)
2. Finding of anophthalmia and/or microphthalmia in two species (rabbit and Fischer 344) in three separate investigations (Tunstall Lab., Westhollow, and Argus Res.)

B. Sprague Dawley Rats

Dose levels of 0, 1, 3, or 30 mg/kg/day were used in the Research Triangle Institute study (RTI #31T-2564).

Maternal body weight reductions and increased incidences of piloerection were found at the 30 mg/kg dosage level. No developmental toxicity including teratogenicity was demonstrated at any of the dosage levels tested. Based upon these findings, the maternal NOEL was determined to be 3 mg/kg/day with the developmental toxicity NOEL established at > 30 mg/kg/day.

Data from a recently submitted pilot teratology study (Argus Research Lab., #619-002P) revealed that a dose level of 10 mg/kg/day was associated with significant maternal body weight gain reduction and increased incidences of clinical manifestations. The effects produced at 10 mg/kg were much more extensive than those obtained at 30 mg/kg in the RTI study. These findings trigger the Agency request for additional information and/or clarification relative to this disparity (Dr. Bui's memo of 5/29/85). Possibly, problems relative to dose preparation may account for the lesser effects noted at the 30 mg/kg dosage level (RTI).

C. Fischer-344 Rats

The teratogenic potential of Bladex in this species was investigated by Westhollow Research Center (RIR-180; 12/81) and by Argus Research Center (#619-002; 4/18/85).

In the WRC study, dose levels of 0, 1, 2.5, 10, and 25 mg/kg/day were used and maternal body weight reductions during the dosing period were noted at the two highest dosage levels. Diaphragmatic hernia was noted at all dosage levels tested and anophthalmia/micropthalmia was found at the 25 mg/kg dose level. In the absence of acceptable historical control data, the biological significance of these findings could not be ascertained (see Dr. Dysktra's memo of 1/26/83). To fully evaluate the nature of these findings as well as the survivability of the affected fetuses (Dr. Mahfouz's memo of 11/14/83), a teratology study with a post-natal phase was requested by the Agency and later conducted by Argus Research Laboratory (#619-002).

In the second study, dose levels of 0, 5, 25, and 75 mg/kg/day were tested. Dose-related decreases in maternal body weight gains and food consumption as well as increases in clinical manifestations were noted at all dose levels tested. Alterations in skeletal ossification sites were also found at all dose levels used. Based upon these findings, it was concluded that both the maternal and developmental toxicity NOEL's were not established and were < 5 mg/kg/day (lowest dose tested). Teratogenic effects were demonstrated at the 75 mg/kg dose level as evidenced by the presence of anophthalmia/microphthalmia, dilated brain ventricles, and cleft palate in the fetuses and abnormalities of the diaphragm in pups sacrificed at weanling. The diaphragm-related abnormalities were also significantly increased at the 25 mg/kg dose level. On the basis of these data obtained from a study which included a post-natal phase, it is recommended that the teratogenic NOEL be established at 5 mg/kg/day (see Dr. Bui's memo of 5/30/85).

3. Finding of dilated brain ventricles in two species (rabbit and Fischer 344; Tunstall and Argus Res. Lab.)
4. Close margin between maternal toxicity and developmental toxicity including teratogenic effects
   Rabbit maternal NOEL and developmental NOEL = 1 mg/kg (Tunstall Lab.)
   Rabbit maternal LEL and developmental LEL = 2 mg/kg (Tunstall Lab.)
   Fischer-344 rat maternal NOEL and developmental NOEL < 5 mg/kg (Argus Research Lab.)
   Fischer-344 rat diaphragmatic hernia found at 1, 2.5, 10, and 25 mg/kg but incidence at 1 mg/kg inconsistent (not clearly dose-related); Also, technical errors apparent in this study (Westhollow Research Center)
5. Nature of the major malformations
   Anophthalmia/microphthalmia
   Diaphragm-related malformations: diaphragmatic hernia (Westhollow Res. Center) and "diaphragm central tendon incomplete fusion with raised area of the liver" (Argus Res. Lab.)

Based upon these findings, it is recommended that Bladex be regulated as a teratogen with a NOEL for developmental toxicity (including teratogenicity) established at 1 mg/kg/day based upon findings observed in the rabbit study at 2 and 4 mg/kg/day (maternally toxic levels).

CORE CLASSIFICATION STATUS OF ALL TERATOLOGY STUDIES

1. Teratology, rabbits - IBT #J-238: Invalid
2. Teratology, rabbits - IBT #8580-11112 : Invalid
3. Teratology, Fischer-344 rats - Westhollow Research Center RIR-180: Supplementary Data
4. Teratology, New Zealand rabbits - Tunstall Lab. #221/81: Minimum Data
5. Teratology, Sprague Dawley rats - Research Triangle Institute #31T-2564: Supplementary Data *
6. Teratology with post-natal study, Fischer-344 rats - Argus Research Lab. #619-002: Minimum Data

The registrant has fulfilled the requirements for teratology studies in two species. Although the new Fischer-344 rat study has no NOEL for developmental toxicity, it does provide a NOEL for teratogenic effects (5mg/kg/day). Further, Bladex should be regulated on the basis of the lowest NOEL of 1 mg/kg/day in the rabbit study.

(*) re-classified as of 5/29/85 pending the submission of additional data.

TECHNICAL BLADEX - TERATOLOGY STUDIES

DOSAGE LEVELS

	1 mg/kg	2.0 mg/kg	2.5 mg/kg	3 mg/kg	4 mg/kg	5 mg/kg	10 mg/kg	25 mg/kg	30 mg/kg	75 mg/kg
Maternal Effects (Body weight reductions, Decreased Food consumption, Clinical signs, Mortality,....)										
Rabbit	No	Yes	-	-	Yes	-	-	-	-	-
SD-rats	No	-	-	No	-	-	-	-	Yes	-
F-344a	No	-	No	-	-	-	Yes	Yes	-	-
F-344b	-	-	-	-	-	Yes	-	Yes	-	Yes
Developmental Toxicity (Fetal weight, Fetal crown-rump length, skeletal variations, malformations,...)										
Rabbit	No	Yes	-	-	Yes	-	-	-	-	-
SD-rats	No	-	-	No	-	-	-	-	No	-
F-344a	Yes	-	Yes	-	-	-	Yes	Yes	-	-
F-344b	-	-	-	-	-	Yes	-	Yes	-	Yes
Anophthalmia and/or Microphthalmia										
Rabbit	No	No	-	-	Yes	-	-	-	-	-
SD-rats	No	-	-	No	-	-	-	-	No	-
F-344a	No	-	No	-	-	-	-	Yes	-	-
F-344b	-	-	-	-	-	No	-	No	-	Yes
F-344b*	-	-	-	-	-	No	-	Yes	-	Yes
Diaphragmatic hernia or findings related to diaphragm										
Rabbit	No	No	-	-	No	-	-	-	-	-
SD-rats	No	-	-	No	-	-	-	-	No	-
F-344a	Yes	-	Yes	-	-		Yes	Yes	-	-
F-344b	-	-	-	-	-	No	-	No	-	No
F-344b*	-	-	-	-	-	No	-	Yes	-	Yes

Rabbits: Tunstall Lab., England, Project 221-81
Sprague Dawley rats: Research Triangle Inst. #61230
F-344 a: Westhollow Res. # RIR-180
F-344 b: Argus Res. Lab.#619-002 (Teratology phase)
F-344b*: Argus Res. Lab.#619-002 (Post-natal phase)