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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR NO. 0051761

DATE: **04/03/003**

MEMORANDUM

SUBJECT: ***Benfluralin:*** - Third Report of the Hazard Identification Assessment Review Committee.

FROM: David G Anderson 4/8/03
Toxicologist.
Reregistration Branch - 2
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Richard Griffin, Risk Assessor
Reregistration Branch - 2
Health Effects Division (7509C)

PC Code: 084301

On January 11, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for benfluralin with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. [RfD was evaluated in 1985 and re-evaluated in 1988]. On December 17, 2002, the HED HIARC re-reviewed issues with benfluralin raised by the reviewer: (1) FQPA assessment according to the OPP 10x Guidance document of 2002, (2) re-evaluated the acute RfD, (3) selection of endpoints for Incidental Oral Exposure, (4) and the request for the additional studies, acute and subchronic neurotoxicity studies and a subchronic inhalation study. Between March 25, 2003, and March 30, 2003, the HIARC reviewed the intermediate term (1 month to several months) incidental oral and inhalation exposure endpoints.

Committee Members in Attendance

Members present were: William Burnam, Elizabeth Doyle, Pam Hurley, Elizabeth Mendez, David Nixon, Ayaad Assaad, John Liccione, Jess Rowland, Jonathan Chen, Brenda Tarplee, Sue Makris

Member(s) in absentia: Steve Knizner

Data evaluation prepared by: David G Anderson, Toxicologist, RRB-2, HED

Also in attendance were:

Data Evaluation / Report Presentation — David G Anderson
Toxicologist

INTRODUCTION

On January 11, 2001, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for benfluralin with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. [RfD was evaluated in 1985 and re-evaluated in 1988]. On December 17, 2002, the HED HIARC re-reviewed issues with benfluralin raised by the reviewer: (1) FQPA assessment according to the OPP 10x Guidance document of 2002, (2) re-evaluated the acute RfD, (3) selection of endpoints for Incidental Oral Exposure, (4) and the request for the additional studies, acute and subchronic neurotoxicity studies and a subchronic inhalation study. The potential for increased susceptibility of infants and children from exposure to benfluralin was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. Between March 25, 2003 and March 30, 2003, the HIARC revisited benfluralin to review the endpoint for "Incidental Oral and Inhalation Exposure: Intermediate-Term (1-6 Months)."

I. FQPA HAZARD CONSIDERATIONS

1. Adequacy of the Toxicity Data base

The toxicity data base is adequate for FQPA consideration. Acceptable rabbit and rat developmental toxicity studies are available in addition to an acceptable 2-generation study on reproduction in the rat.

The HIARC concluded that the acute and subchronic neurotoxicity studies are not required (in spite of the neurotoxicity/neuropathy) because the effects of concern (sciatic nerve degeneration and brain weight decrement) will not likely occur at the doses that would be tested in these studies.

2. Evidence for Neurotoxicity

Sciatic nerve degeneration, muscle atrophy and brain weight decrease have been shown in a chronic rat study only at termination and only at the two highest doses, which were considered excessive. In the combined chronic/oncogenicity study in rats, sciatic nerve degeneration was seen (0 in controls), (2/49) at 10 ppm, (0/50) at 100 ppm, (26/49) at 2500 ppm and (30/50) at 5000 ppm. Corresponding skeletal muscle degeneration also occurred with a frequency similar to sciatic nerve degeneration at 2500 and 5000 ppm by termination. These were the same dose levels where the tumors developed and excess systemic toxicity was shown (increased mortality, body weight depression, liver, kidney toxicity/histopathology and depressed hematological parameters). Absolute brain weight was decreased in males at 5000 (-7%) ppm and in females at 2500 (-6%) ppm and 5000 (-6%) ppm. No other neurotoxic signs were seen in any other studies, including the developmental and reproduction studies. In addition, neither the brain weight decrement nor the sciatic nerve degeneration/muscle atrophy was seen in the 90-day rat study or at the 1-year interim sacrifice for the chronic rat study.

3. Developmental Toxicity Studies

Developmental Toxicity in Rats:

Benfluralin (Technical) was administered to 25 Crl:CD (SD) rats/group by gavage at 0, 50, 225, 475 or 1000 mg/kg/day in 10% acacia from day 6 through day 15 of gestation (MRID# 000147535). Body weights were determined on days 0, 6, 11, 16 and 20, at sacrifice. Food consumption was also measured. On day 20, uterine contents were evaluated. Each fetus was examined viscerally and stained for skeletal evaluation.

A range-finding study at 0, 50, 225, 475, and 1000 mg/kg/day indicated that body weight was decreased in the 475 and 1000 mg/kg/day groups. No fetal effects were noted.

In the main study, maternal body weights were statistically significantly decreased ($p \leq 0.05$) on gestational day (GD) 11 (96% of control) and 16 (96% of control) at ≥475 mg/kg/day and body weight gain was significantly depressed GD 6-11 (48% of control) at the same dose levels. Food consumption was also significantly depressed GD 6-11 (≥85% of control) and 11-16 at these same dose levels. Yellow to orange stained urine was noted at all dose levels. At necropsy, dilated renal pelvises and enlarged hepatic lobes were noted at all dose levels.

There were no significant dose related findings in fetuses. There was one fetus at 50 mg/kg/day with ataila and another fetus at 225 mg/kg/day with a thread-like tail and no anus. These effects were considered incidental. The number of litters examined at all dose levels and control were 24 or 25. At 225 mg/kg/day a statistically significant increased number of male fetuses with unossified sternebrae were found affecting 14, 16, 15, 15 and 21 litters at 0, 50, 225, 475 and 1000 mg/kg/day, respectively. In addition at 225 mg/kg/day, a statistically significant increased number of fetuses were seen with dark brown-red areas on the liver (0, 0, 5, 1, 4 litters at the above respective dose levels). Although these latter effects were not considered to be treatment related, the study was considered supplementary because of discrepancies in numbers of fetuses with abnormalities in two tables. Additional information was requested from the registrant, which satisfied the reviewers (HED Doc# 006693).

The maternal NOAEL is 225 mg/kg/day and the LOAEL is 475 mg/kg/day based on body weight decrement on GD 11 and 16 and body weight gain decrement between 6-11. The developmental NOAEL is 1000 mg/kg/day and the LOAEL was not determined.

The study is **acceptable** for a guideline (870.3700) developmental toxicity study in the rat.

Developmental Toxicity Study - Rabbit

Executive Summary: Benfluralin (Technical) was administered to 20 new Zealand White rabbits/group at 0, 25, 50, 100 or 225 mg/kg/day in by gavage from gestational day (GD) 6 through 18 (MRID# 42039101). Body weights were determined on days 0, 6, 9, 12, 15,9, 24 and 29, at sacrifice. Food consumption was also measured. On day 20, uterine contents were evaluated. Each fetus was examined viscerally and stained for skeletal evaluation.

A range-finding study at 0, 20, 50, 225, and 475 mg/kg/day indicated decreased food consumption and body weight gains through abortions at 475 mg/kg/day. Fetal viability, weight and external morphology were not adversely affected at any dose level. A definitive study initiated at 0, 50, 225 and 475 mg/kg/day was terminated. Severe maternal toxicity, indicated by body weight loss, anorexia, depressed food consumption and abortions at 225 and 475 mg/kg/day, resulted in termination of the study prior to GD 28. No fetal data were collected. Another definitive study (main study) was initiated dropping the 475 mg/kg/day dose.

In the main study, maternal body weight was significantly depressed (6% of control) between GD 6-19 at the 225 mg/kg/day. Body weight gain was nominally reduced during the same period at 100 mg/kg/day. One maternal death and 3 abortions occurred at 225 mg/kg/day. In addition , the incidence of few feces was seen at the two top dose levels (6 and 6 vs. 0 in control). Food consumption was statistically significantly reduced during the same period and may have been related the body weight gain depression as well as the few feces. However, the nominal effect on body weight gain in dams at 100 mg/kg/day was considered insufficient to be considered adverse by the Hazard Identification Assessment Review Committee.

In fetuses, there was a trend to increased sternebrae malaligned, accessory skull bones, and unossified sternebrae, but all were within the historical control range, and none were statistically significant or showed a good dose response.

The maternal NOAEL was determined to be 100 mg/kg/day and the LOAEL is 225 mg/kg/day based on dose related significant body weight gain decrement, few feces and reduced food consumption. The developmental NOAEL is 225 mg/kg/day and the LOAEL was not determined.

The study is **acceptable** for a guideline (870.3700) developmental toxicity study in rabbits.

4. **Reproductive Toxicity**

In this two-generation reproduction study (MRID No.: 43628701), Sprague-Dawley CRL:CD BR rats (30/dose/sex) were fed test diets containing Benefin at 0 (basal diet), 100, 1000 or 5000 ppm [Males (premating), F_0: 0, 7.2, 68.1, or 342 mg/kg/day, F_1: 0, 7.3, 74.4, or 401 mg/kg/day; females (premating), F_0: 0, 8.8, 80.0, 392 mg/kg/day, F_1: 0, 8.6, 87.3, or 437 mg/kg/day] during premating, gestation and lactation periods.

No treatment-related deaths or clinical signs were observed during the study. At 5000 ppm decreases in mean body weights were observed during the premating periods for F_0 (-10%, males; -10.4%, females) and F_1 (-20.5%, males; -15.9%, females) animals, gestation periods for F_0 (-16.7%) and F_1 (-18.5%) females, and lactation periods for F_0 (-13.1%) and F_1 (-12.1%) females. Mean body weight gains were decreased at 5000 ppm during the premating periods for F_0 (-17.2%, males; -27.7%, females) and F_1 (-14.6%, males; -2.0%, females) animals and gestation periods for F_0 (-28.4%) and F_1 (-21.3%) females; during lactation increases were noted in mean body weight gains for F_0 (+18.5%) and F_1 (+394%) females. These decreases in mean body weights were accompanied by significant decreases in food consumption during the premating, lactation and gestation periods.

Mean litter sizes of high-dose, F_0 and F_1 animals were significantly decreased and lower than the mean (range) historical control value of 14.37 (13.46 to 15.67). F_1 females also had a slight, but statistically significant, increase in the mean gestation interval, which was, however, within the historical control range of 21.6 to 22.3 days. All other reproductive parameters of treated animals were comparable to control animals.

At necropsy, treatment-related liver and kidney effects were noted. Liver effects included dose-dependent increases in the absolute and relative weights of livers of 1000 and 5000 ppm parental animals (F_0 and F_1), prominent reticular patter (F_0) and pale areas (F_0 and F_1) and hepatocellular enlargement in 1000 ppm (F_1) and 5000 ppm (F_0 and F_1) animals. Kidney effects observed at 1000 and 5000 ppm included increased incidence of hyaline droplets (graded as minimal) in males and chronic progressive nephropathy (graded as slight and/or moderate) in males and females.

Treatment-related litter effects included deceases in mean litter size of high-dose, F_0 and F_1 maternal animals (compared to both the concurrent control value and historical control range) and weaning index of F_1 pups. An increased incidence of dead/missing pups was also observed in mid- and high-dose F_0 litters and high-dose F_1 litters. All other litter parameters of treated animals were comparable to control values. For both the F_1 and F_2 generations, mid- and high-dose male and female pups had statistically significant decreases in mean body weights throughout the lactation period.

Necropsy examination did not reveal any cervical, thoracic and abdominal visceral abnormalities in F_1 pups; increased incidences of empty/distended stomach and pale colored kidneys were observed in F_2 pups. Gross examination of F_1 pups did not reveal any treatment-related effects; discolored abdominal adipose tissue was noted in high-dose F_2 pups (5/10, males; 2/10, females).

Based on the findings (increased absolute and relative liver weights, liver and kidney histopathology, and decreased mean pup body weight), a LOAEL for systemic/reproductive/developmental toxicity was established at 1000 ppm [Premating: 68.1 mg/kg/day (males) and 74.4 mg/kg/day (females)] and a NOEL, at 100 ppm [Premating: 7.2 mg/kg/day (males) and 8.6 mg/kg/day (females)].

This study is classified as **acceptable** and satisfies the guideline requirements [§83-4] for a two-generation reproduction study in rats.

5. <u>Additional Information from Literature Sources</u>

CNS effects and respiratory depression at lethal doses in animals (Micromedex, 1974-1998) were reported for trifluralin, a structural analog of benfluralin. Also reported were ataxia, weakness, falling and regurgitation from sublethal acute doses in mallard ducks (Fish and Wildlife Service, 1984). Benfluralin is one of the dinitroaniline based pesticides, which the open literature suggests interfere with tubulin among other toxicities. It functions as a herbicide by inhibiting plant root growth (Hartely and Kidd, 1987). Substances interfering with the function of tubulin are generally developmental toxins, but no developmental toxicity was demonstrated in adequately conducted studies with benfluralin. Developmental toxicity has been demonstrated in the trifluralin (skeletal anomalies in mice at unknown dose levels)(Micromedex, 1974-1998). In submitted studies on trifluralin, skeletal effects were noted in rats (HED Doc# 005898), and fetal

weight decrement in rats (HED Doc# 004419), and fetal toxic effects were noted in rabbits (HED Doc# 004419).

6. Pre- and/or Postnatal toxicity

The HIARC concluded that there is not a concern for pre- and postnatal toxicity resulting from exposure to benfluralin.

A. Determination of Susceptibility: There is no evidence (quantitative or qualitative) of increased susceptibility following pre- and/or postnatal studies. No developmental effects were seen at the highest doses tested in rats or rabbits. Similar effects (weight decrement, liver and kidney toxicity) were seen in pups, adult offspring and parents at the two highest doses tested. No effects were seen at the lowest dose tested either in pups or parents.

B. Degree of Concern Analysis and Residual Uncertainties: There are no concerns nor residual uncertainties for pre- and/or postnatal toxicity since there was no evidence of increased susceptibility in rats or rabbits. In the reproduction study, dead and missing pups at the mid dose were neither dose related nor statistically significant.

C. Special FQPA Safety Factor(s): The FQPA Safety factor should be removed [1X] because there is no susceptibility and no residual uncertainty.

The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

7. Recommendation for a Developmental Neurotoxicity Study

The HIARC concluded that there is not a concern for developmental neurotoxicity resulting from exposure to benfluralin. The HIARC believed that the neuropathy seen in the rat chronic study was the indirect effect of normal age related neuropathy exacerbated by excessive doses in the chronic rat study.

A. Evidence That Support a Developmental Neurotoxicity Study:

- sciatic nerve degeneration and muscle atrophy in a rat chronic study at the two highest doses tested. This same chronic study in rats showed an absolute brain weight decrease at the two highest doses.

B. Evidence That Support Not requiring a Developmental Toxicity Study:

- No developmental CNS malformations were seen in the developmental toxicity studies with benfluralin or an analog, trifluralin.

- The sciatic nerve degeneration/muscle atrophy or decreased brain weight seen at the end of the chronic study in rats was not seen in the 90-day subchronic study in rats, or in the interim

sacrifice in the chronic rat study. It would appear doubtful that a 90-day study would detect these effects that are a frequent age related effect in this strain of rat.

• Since there was no dose-related neuropathy until excessive doses were reached, it would appear that the neuropathy resulted from the indirect interaction of a frequent age related effect and/or systemic toxicity from excessive doses. If advanced age interacting with systemic toxicity caused the neuropathy in rats, it seemed unlikely that these effects would be detected by or be relevant in a developmental neurotoxicity test (DNT).

• No brain weight decreases or other neurotoxic signs were reported in the rabbit, dog or mouse studies.

Based on the weight of evidence presented, the HIARC concluded that a developmental neurotoxicity study is not required for benfluralin.

II. HAZARD IDENTIFICATION

1. Acute Reference Dose (RfD) - All populations

Comment:
An endpoint attributable to a single dose was not available from the developmental studies in rabbits or rats or any other appropriate study.

2. Chronic Reference Dose (RfD)

Selected Study: Combined Chronic/Oncogenicity study in rats Guideline # 870.4300

MRID No.: 44050002 & 44545501

Executive Summary: In a chronic toxicity/oncogenicity study, benfluralin was administered to Fischer 344 rats (60/sex/dose) in the diet at dose levels of 10, 100, 2500, and 5000 ppm (0.5, 5.0, 125 and 250 mg/kg body weight/day) for up to 2 years. Of these rats, 10/sex/dose were sacrificed at 12 months.

Survival was significantly reduced ($p<0.05$) in males at 100, 2500, and 5000 ppm to 62, 66, and 64%, respectively, at study termination. However, the mortality rate did not reach statistical significance in males at any dose until the final week of the study. The only treatment-related clinical sign of toxicity was a yellowing of the skin in 97 and 100% of 2500 and 5000 ppm females. Ophthalmoscopic examinations revealed yellow-orange color of the eyes of both sexes at 2500 and 5000 ppm. At study termination body weights were significantly ($p<0.05$) lower than respective controls in males (-8%) and females (-18%) at 2500 ppm and in males (-17%) and females (-28%) at 5000 ppm. Body weight gains were reduced ($p<0.05$) at 2500 and 5000 ppm.

Significant ($p<0.05$) decreases in erythrocyte count (-7% to -12%) were observed in males and females at 2500 and 5000 ppm through 12 months, in 5000 ppm males through 18 months (-11%), and in 5000 ppm females through 24 months. Platelets increased significantly ($p<0.05$)

with respect to controls in 2500 and 5000 ppm males and females at various time points. Hemoglobin and hematocrit were significantly reduced ($p<0.05$) vs controls at the two highest doses in males through 18 months (8-15%) and in females (11-16%) through study termination.

Urea nitrogen was elevated ($p<0.05$) over that in controls by 21-106% at the two highest doses in males and females throughout the study. Creatinine was increased ($p<0.05$) in 2500 and 5000 ppm males and females up to +33% over controls. Increases in total protein, albumin, and globulin at 2500 and 5000 ppm were associated with increased urine volume and mild dehydration. Urinalysis also revealed hyaline and granular casts and dark coloration at these doses. Chemistry and urinalysis results correlated with gross pathology and histological abnormalities, including nephropathy in kidneys of males and females at the two high doses. Total cholesterol was increased significantly ($p<0.05$) compared to controls in 2500 and 5000 ppm males to +81% through 12 months and in 2500 and 5000 ppm females to +101% throughout the study. Bilirubin was increased up to +200% in 2500 and 5000 ppm males and females at various intervals to 18 months. Alkaline phosphatase, alanine AT, and aspartate AT were decreased in both sexes ($p<0.05$) at 5000 ppm during the first year. Alterations in cholesterol, bilirubin, and liver enzymes at the two highest doses correlated with liver enlargement and increased incidence of liver lesions.

Significant increases ($p<0.05$) in absolute (19-43%) and relative (29-104%) liver weights, vs controls, were observed in both sexes at 2500 and 5000 ppm, and toxicity was corroborated by serum chemistry and histopathology findings. Relative thyroid weights were elevated ($p<0.05$) (+33 to +64% over controls) at 2500 and 5000 ppm and correlated with microscopic thyroid abnormalities.

At the 12-month sacrifice a dark yellowing of adipose tissue was observed at 2500 and 5000 ppm. As with other findings of discoloration (eye, skin, urine, etc.) in these groups, the yellow adipose could have been due to jaundice, although deposition of the dark yellow test substance or its metabolite may have been a contributing factor. At terminal sacrifice, gross pathology findings included granular/rough/pitted cortex and darkening of the kidney in both sexes at 2500 ppm. At 5000 ppm, males and females exhibited these same kidney abnormalities as well as pale areas of the lung, enlarged testes, uterine cysts, and darkening of the stomach.

After 12 months (interim sacrifice), noteworthy increases in non-neoplastic lesions were: thyroid follicular cell hypertrophy in the 5000 ppm males and females, hepatocellular hypertrophy in 5000 ppm males and females and in 2500 ppm males, and hepatocellular pigment in 2500 ppm males and females. All rats of both sexes at 100 ppm and above exhibited increased incidences of hyaline droplets in the kidney. Kidney tubule cell karyomegally was observed in all rats of both sexes at 2500 and 5000 ppm, with none in controls. Also observed at the interim sacrifice were pelvic calculi in the kidney in females at 100 ppm, and males and females at 2500 ppm and 5000 ppm. Kidney transitional cell hyperplasia was observed in males and females at 5000 ppm.

Absolute brain weight was decreased in males (-7%) at 5000 ppm and in females (-6%) at 2500 ppm and (-6%) at 5000 ppm only at terminal sacrifice.

At the terminal sacrifice, treatment-related non-neoplastic lesions included the same thyroid, liver, and kidney lesions observed in the interim sacrifice with increasing frequency and severity. In addition, liver sinusoidal pigment was elevated in males at 5000 ppm and necrosis was

increased in males and females at the high dose. Thyroid follicular cell hyperplasia was slightly increased in males and females at 5000 ppm. Skeletal muscle and sciatic nerve degeneration were markedly increased in both sexes at 2500 and 5000 ppm. Chronic lung inflammation appeared to show a treatment-related increase in females at 5000 ppm. Thyroid and liver abnormalities correlated with increased tumor incidences at the high dose.

The NOAEL for chronic toxicity was 10 ppm for males and females (0.5 mg/kg/day and 0.7 mg/kg/day, respectively), based upon an increased incidence of histologic lesions of the kidney at 100 ppm. The LOAEL was 100 ppm (5.4 mg/kg/day for males and 6.8 mg/kg/day for females) based on kidney hyalin droplets in males and females and transition cell hyperplasia and pelvic calculi in males and similar histological effects and peripheral nerve degeneration at 2500 and 5000 ppm in males and females in addition to the liver histology and oncogenicity at 2500 and 5000 ppm.

Under the conditions of this study, benfluralin was oncogenic in both sexes. The test substance induced hepatocellular carcinoma/adenoma in male rats at 2500 and 5000 ppm. The incidence of combined hepatocellular adenoma/carcinoma was 0, 13, and 15% in males at doses of 0, 2500, and 5000 ppm, respectively. The occurrences of hepatocellular adenoma were the major contributor to these findings and malignancy did not appear to increase with dose. In addition, benfluralin caused thyroid follicular adenoma/carcinoma in males and females at the two high doses, with the adenoma slightly predominant. The incidence of combined hepatocellular adenoma/carcinoma was 0, 13, and 15% in males and 0, 10, and 7% in females at doses of 0, 2500, and 5000 ppm, respectively. No evidence of early onset of any tumors was observed.

This chronic toxicity/carcinogenicity study was initially classified as unacceptable (§83-5) because statistical notations for histopathology data were not provided nor was a statistical analysis report submitted. In addition, historical control data were not provided. The study was upgraded to Acceptable following submission of amended data tables for non-neoplastic and neoplastic lesions showing statistical significance of data, a statistical analysis report, and laboratory historical control data.

The above requested information was submitted in MRID# 44545501, and MRID# 44050002 is upgraded to **Acceptable**.

Dose and Endpoint for Establishing RfD: NOAEL = 0.5 mg/kg/day based on kidney lesions.

Uncertainty Factors: 100X (10x for interspecies extrapolation and 10x for intraspecies extrapolation).

Comments about Study/Endpoint/Uncertainty Factor(s): The study is of the appropriate duration.

Chronic RfD = [(0.5 mg/kg/day)/(100)] = 0. 005 mg/kg/day

3. Incidental Oral Exposure: Short Term (1-30 days)

Selected Study: Rabbit Developmental toxicity study § 870.3700

MRID No.: 42039101

Executive Summary: Benfluralin (Technical) was administered to 20 New Zealand White rabbits/group at 0, 25, 50, 100 or 225 mg/kg/day in by gavage from gestational day (GD) 6 through 18 (MRID# 42039101). Body weights were determined on days 0, 6, 9, 12, 15,9, 24 and 29, at sacrifice. Food consumption was also measured. On day 20, uterine contents were evaluated. Each fetus was examined viscerally and stained for skeletal evaluation.

Range-finding studies at 0, 20, 50, 225, and 475 mg/kg/day indicated decreased food consumption and body weight gains through abortions at 475 mg/kg/day. Fetal viability, weight and external morphology were not adversely affected at any dose level. A definitive study was initiated at 0, 50, 225 and 475 mg/kg/day. However, severe maternal toxicity was indicated by body weight loss, anorexia, depressed food consumption and abortions at 225 and 475 mg/kg/day causing termination of the study prior to GD 28. No fetal data were collected. Another definitive study was initiated dropping the 475 mg/kg/day dose.

In the main study, maternal body weight was significantly depressed (6% of control) between GD 6-19 at the 225 mg/kg/day. Body weight gain was nominally reduced during the same period at 100 mg/kg/day. One maternal death and 3 abortions occurred at 225 mg/kg/day. In addition , the incidence of few feces was seen at the two top dose levels (6 and 6 vs. 0 in control). Food consumption was statistically significantly reduced during the same period and may have been related the body weight gain depression as well as the few feces. However, the nominal effect on body weight gain in dams at 100 mg/kg/day was considered insufficient to be considered adverse by the Hazard Identification Assessment Review Committee.

In fetuses, there was a trend to increased sternebrae malaligned, accessory skull bones, and unossified sternebrae, but all were within the historical control range, and none were statistically significant or showed a good dose response.

The maternal NOAEL was determined to be 100 mg/kg/day and the LOAEL is 225 mg/kg/day based on dose related nominal body weight gain decrement, few feces and reduced food consumption. The developmental NOAEL is 225 mg/kg/day and the LOAEL was not determined.

The study is **acceptable** for a guideline (870.3700) developmental toxicity study in rabbits.

Dose and Endpoint for risk Assessment: NOAEL = 100 mg/kg/day based on maternal body weight gain decrement between gestational days 9-12, 12-15, and 15-19.

Comments about Study/Endpoint: The endpoint of concern (decreased body weight gain) is appropriate for the population (infants and children) and the duration of concern.

4. Incidental Oral Exposure: Intermediate-Term (1-6 Months)

Selected Study: Reproduction and fertility effects study § 870.3800

MRID No.: 43628701

In this two-generation reproduction study (MRID No.: 43628701), Sprague-Dawley CRL:CD BR rats (30/dose/sex) were fed test diets containing Benefin at 0 (basal diet), 100, 1000 or 5000 ppm [Males (premating), F_0: 0, 7.2, 68.1, or 342 mg/kg/day, F_1: 0, 7.3, 74.4, or 401 mg/kg/day; females (premating), F_0: 0, 8.8, 80.0, 392 mg/kg/day, F_1: 0, 8.6, 87.3, or 437 mg/kg/day] during premating, gestation and lactation periods.

No treatment-related deaths or clinical signs were observed during the study. At 5000 ppm decreases in mean body weights were observed during the premating periods for F_0 (-10%, males; -10.4%, females) and F_1 (-20.5%, males; -15.9%, females) animals, gestation periods for F_0 (-16.7%) and F_1 (-18.5%) females, and lactation periods for F_0 (-13.1%) and F_1 (-12.1%) females. Mean body weight gains were decreased at 5000 ppm during the premating periods for F_0 (-17.2%, males; -27.7%, females) and F_1 (-14.6%, males; -2.0%, females) animals and gestation periods for F_0 (-28.4%) and F_1 (-21.3%) females; during lactation increases were noted in mean body weight gains for F_0 (+18.5%) and F_1 (+394%) females. These decreases in mean body weights were accompanied by significant decreases in food consumption during the premating, lactation and gestation periods.

Mean litter sizes of high-dose, F_0 and F_1 animals were significantly decreased and lower than the mean (range) historical control value of 14.37 (13.46 to 15.67). F_1 females also had a slight, but statistically significant, increase in the mean gestation interval, which was, however, within the historical control range of 21.6 to 22.3 days. All other reproductive parameters of treated animals were comparable to control animals.

At necropsy, treatment-related liver and kidney effects were noted. Liver effects included dose-dependent increases in the absolute and relative weights of livers of 1000 and 5000 ppm parental animals (F_0 and F_1), prominent reticular patter (F_0) and pale areas (F_0 and F_1) and hepatocellular enlargement in 1000 ppm (F_1) and 5000 ppm (F_0 and F_1) animals. Kidney effects observed at 1000 and 5000 ppm included increased incidence of hyaline droplets (graded as minimal) in males and chronic progressive nephropathy (graded as slight and/or moderate) in males and females.

Treatment-related litter effects included deceases in mean litter size of high-dose, F_0 and F_1 maternal animals (compared to both the concurrent control value and historical control range) and weaning index of F_1 pups. An increased incidence of dead/missing pups was also observed in mid- and high-dose F_0 litters and high-dose F_1 litters. All other litter parameters of treated animals were comparable to control values. For both the F_1 and F_2 generations, mid- and high-dose male and female pups had statistically significant decreases in mean body weights throughout the lactation period.

Necropsy examination did not reveal any cervical, thoracic and abdominal visceral abnormalities in F_1 pups; increased incidences of empty/distended stomach and pale colored kidneys were observed in F_2 pups. Gross examination of F_1 pups did not reveal any treatment-related effects; discolored abdominal adipose tissue was noted in high-dose F_2 pups (5/10, males; 2/10, females).

Based on the findings (increased absolute and relative liver weights, liver and kidney histopathology, and decreased mean pup body weight), a LOAEL for

systemic/reproductive/developmental toxicity was established at 1000 ppm [Premating: 68.1 mg/kg/day (males) and 74.4 mg/kg/day (females)] and a NOEL, at 100 ppm [Premating: 7.2 mg/kg/day (males) and 8.6 mg/kg/day (females)].

This study is classified as **acceptable** and satisfies the guideline requirements [§83-4] for a two-generation reproduction study in rats.

Dose and Endpoint for Risk Assessment: NOAEL is 7.2 mg/kg/day and a LOAEL of 68.1 mg/kg/day based on progressive nephropathy in adult males and females and decreased pup weight.

Comments about Study/Endpoint: Upon reevaluation, the HIARC concluded that hyaline droplet formation alone is not appropriate for endpoint selection since this is a normal occurrence in male rats. Guidance for this determination was obtained from the " Risk Assessment Forum Document titled " Alpha2u-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat" (Purple Book) which states that " Hyaline droplets in the proximal tubules of normal male rats contain alpha2u-g, and their occurrence appears to parallel the variable synthesis of this protein. Thus, hyaline droplets become apparent in male rats at the time of puberty, but they decline progressively with increasing age after 18 months. In female rats, protein droplets in proximal tubules are either absent or considerably less frequent than in males, and they do not contain alpha2u-g.

Therefore, the HIARC selected the dose and endpoint for this exposure scenario based on the NOAEL of 7.2 mg/kg/day in the 2-generation rat reproduction study where increases in absolute and relative liver weights and histopathological lesions of the liver and kidneys were seen at the LOAEL of 68.1 mg/kg/day

5. Dermal Absorption (No dermal absorption studies are available for benfluralin, but an acceptable study is available for ethalfluralin, a close analog of benfluralin.)

Selected Study: Percutaneous absorption ^{14}C-ethalfluralin in monkeys. Guideline #: NG

MRID No.: 132820; & resubmission 92062028; HED Doc# 004090 & 004235

Executive Summary: Four monkeys (2 males and 2 females) were administered 2 mg/kg radio-labeled ethalfluralin in ethanol intravenously or topically to the forearm and the plasma level determined for 120 hours to determine an area under the curve for both types of applications. Two compartments were noted with one-half lives of 1.71 hours for the plasma distributive phase and 79.1 hours for the terminal plasma disappearance phase. After 120 hours label was not detectable in 2 (1 male and 1 female) of the 4 animals studied. Since the 2 animals with undetectable plasma levels at 120 hour yielded the most consistent data, data from these animals were used to calculate the AUCs. The dermal absorption was determined by ratio of the area under the plasma curve AUC; [(AUC-dermal/(AUC-i.v.)] x 100 = 2.84%.

Percentage (%) Dermal Absorption: A 3% dermal absorption factor was determined based on the results of the dermal absorption study for the structurally related compound, Eethalfluralin.

Comments about Dermal Absorption: There are no dermal absorption studies for benfluralin, however, there was an acceptable study conducted in monkeys for ethalfluralin. The structures differ by minor differences in the dialkyl groups substituted in amine of the dinitroaniline group of benfluralin, and the physical constants are similar with melting points being within 2.5°C, solubilities are similar, and the log Kows within 0.18 of each other, i.e., benfluralin, log Kow = 5.29 and ethalfluralin, log Kow = 5.11. Therefore, a dermal absorption percentage for ethalfluralin is probably a more accurate estimation of dermal absorption than assuming 100% dermal absorption for conversion of an oral study to a dermal endpoint for intermediate and long-term occupational exposure. In addition, there are no other studies that could form an adequate basis for dermal absorption.

6. Dermal Exposures (any time period):

Selected Study: None

MRID No.: None

Dose and Endpoint for Risk Assessment; Not applicable

Comments about the Study/Endpoint: The HIARC concluded that risks from dermal exposure can not be adequately quantitated because benfluralin is a dermal sensitizer and a NOAEL for dermal toxicity could not be established from the 21-day dermal study.

In the 21-day dermal toxicity study, rabbits received 15 repeated dermal applications of technical (95.8%) benfluralin in distilled water at dose levels of 0, 100, 500 or 1000 mg/kg/day, 6 hours/day, 5 days/week. Dose-related dermal effects included (epidermal hyperplasia, hyperkeratosis, parakeratosis, chronic-active inflammation, edema and hyperplasia of the sebaceous glands). The erythema and edema were progressively worse in both sexes up to 21 days. Other induced dermal lesions were seen that began as slight skin erythema and edema starting at day 3 of dosing progressing to slight to moderate erythema, edema, necrosis, and sebaceous gland hyperplasia by day 21. For dermal toxicity, the LOAEL was 100 mg/kg/day, the lowest dose tested; a NOAEL was not established. No systemic toxicity was seen at any dose level.

In a modified Buehler topical patch test in 12 Guinea pigs, 7 responded with a typical delayed hypersensitivity reaction to a challenge with technical benfluralin at 5% in 95% ethanol. At 48 hours, 9/12 pigs exhibited slight to moderate erythema and 8/12 pigs exhibited very slight to slight edema (MRID No. 00144283).

The lack of dermal sensitization by formulated products does not completely eliminate the problem associated with benfluralin. Formulated products showed no evidence of sensitization in Beuhler's assays, when tested concentrations range from 19.1% to 60% benfluralin.

The lack of sensitization potential for the formulated products possibly occurred because the tests on the formulations were conducted with water as a vehicle, and/or benfluralin in the formulated product was not of sufficient concentration, did not penetrate the skin or material in formulation interfered with the test. However, it is noted that the skin lesions found in 21-day rabbit dermal study, were with the technical grade and a water vehicle. Therefore potential skin sensitization of products containing benfluralin is not eliminated.

The technical grade caused typical delayed hypersensitivity in guinea pigs. Repeated dermal applications to rabbits resulted in skin lesions that progressed in severity and therefore may have the potential for adverse effects. Because risk can not be quantified, the HIARC also recommends that the products containing benfluralin should be labeled as SENSITIZER and contact should be avoided.

7. **Inhalation Exposure, Short-Term (1-30 days)**

Selected Study: Rabbit Developmental toxicity study. § 870.3700

MRID No.: 42039101.

Executive Summary: (For the Executive Summary see Section II.3.)

Dose and Endpoint for Risk Assessment: Maternal NOAEL = 100 mg/kg/day with a LOAEL of 225 mg/kg/day based on maternal body weight decrement and decreased food consumption.

Comment about the Study/Endpoint/Margin of Exposure: Since an oral study was used, absorption via the inhalation route is presumed to be equivalent to oral absorption.

8. **Inhalation Exposure, Intermediate-Term (1-6 months)**

Selected Study: Reproduction and fertility effects study § 870.3800

MRID No.: 43628701 (See Section II. 4. for the Executive Summary for the Reproduction and fertility effects study)

Dose and Endpoint for Risk Assessment: NOAEL is 7.2 mg/kg/day and a LOAEL of 68.1 mg/kg/day based on progressive nephropathy in adult males and females and decreased pup weight.

Comments about Study/Margin of Exposure: Upon reevaluation, the HIARC concluded that hyaline droplet formation alone is not appropriate for endpoint selection since this is a normal occurrence in male rats. Guidance for this determination was obtained from the " Risk Assessment Forum Document titled " Alpha2u-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat" (Purple Book) which states that " Hyaline droplets in the proximal tubules of normal male rats contain alpha2u-g, and their occurrence

appears to parallel the variable synthesis of this protein. Thus, hyaline droplets become apparent in male rats at the time of puberty, but they decline progressively with increasing age after 18 months. In female rats, protein droplets in proximal tubules are either absent or considerably less frequent than in males, and they do not contain alpha2u-g.

Therefore, the HIARC selected the dose and endpoint for this exposure scenario based on the NOAEL of 7.2 mg/kg/day in the 2-generation rat reproduction study where increases in absolute and relative liver weights and histopathological lesions of the liver and kidneys were seen at the LOAEL of 68.1 mg/kg/day.

9. Inhalation Exposure, Long-Term (> 6 months)

Selected Study: The Combined Chronic/Oncogenicity Rat Feeding Study. §Guideline 870.4300.

MRID No.: 4405002 & Supplementary Study

Executive Summary: **(See Section II 2. for the Executive Summary for the Combined Chronic/Oncogenicity study)**

Dose and Endpoint for risk Assessment: NOAEL of 0.5 mg/kg/day. The LOAEL is 5.4 mg/kg/day based on kidney hyalin droplets in males and females and transition cell hyperplasia and pelvic calculi in males.

Comments about Study/Margin of Exposure: The endpoint is an oral endpoint for inhalation exposure.

10. Margins of Exposure

Summary of target Margins of Exposure (MOEs) for risk assessment.

Route / Duration	Short-Term (1-30 Days)	Intermediate-Term (1 - 6 Months)	Long-Term (> 6 Months)
Occupational (Worker) Exposure			
Dermal[a]	None	None	None
Inhalation	100	100	100
Residential (Non-Dietary) Exposure			
Oral	100	100	100
Dermal[a]	None	None	None
Inhalation	100	100	100

[a] Since the dermal risk can not be quantified, the HIARC recommends that products containing benfluralin should be labeled **SENSITIZER** and that dermal contact should be avoided.

11. Recommendation for Aggregate Exposure Assessments

As per FQPA, 1996, when there are potential residential exposures to the benfluralin, aggregate assessment must consider exposures from three major sources: oral, dermal and inhalation exposures. Since there is no toxicity endpoint could be selected for dermal exposure, the HIARC recommended that products containing benfluralin should carry the label **SENSITIZER** and dermal contact should be avoided. For aggregate exposure risk assessment, the oral and inhalation exposures can be combined since an oral equivalent endpoint was selected for the inhalation route.

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No.: 44050002 & 44545501

Executive Summary: In a chronic toxicity/oncogenicity study, benfluralin was administered to Fischer 344 rats (60/sex/dose) in the diet at dose levels of 10, 100, 2500, and 5000 ppm (0.5, 5.0, 125 and 250 mg/kg body weight/day) for up to 2 years (MRID# 44050002 & 44545501). Of these rats, 10/sex/dose were sacrificed at 12 months.

Survival was significantly reduced ($p<0.05$) in males at 100, 2500, and 5000 ppm to 62, 66, and 64%, respectively, at study termination. However, the mortality rate did not reach statistical significance in males at any dose until the final week of the study. The only treatment-related clinical sign of toxicity was a yellowing of the skin in 97 and 100% of 2500 and 5000 ppm females. Ophthalmoscopic examinations revealed yellow-orange color of the eyes of both sexes at 2500 and 5000 ppm. At study termination body weights were significantly ($p<0.05$) lower than respective controls in males (-8%) and females (-18%) at 2500 ppm and in males (-17%) and females (-28%) at 5000 ppm. Body weight gains were reduced ($p<0.05$) at 2500 and 5000 ppm.

Significant ($p<0.05$) decreases in erythrocyte count (-7% to -12%) were observed in males and females at 2500 and 5000 ppm through 12 months, in 5000 ppm males through 18 months (-11%), and in 5000 ppm females through 24 months. Platelets increased significantly ($p<0.05$) with respect to controls in 2500 and 5000 ppm males and females at various time points. Hemoglobin and hematocrit were significantly reduced ($p<0.05$) vs controls at the two highest doses in males through 18 months (8-15%) and in females (11-16%) through study termination.

Urea nitrogen was elevated ($p<0.05$) over that in controls by 21-106% at the two highest doses in males and females throughout the study. Creatinine was increased ($p<0.05$) in 2500 and 5000 ppm males and females up to +33% over controls. Increases in total protein, albumin, and globulin at 2500 and 5000 ppm were associated with increased urine volume and mild dehydration. Urinalysis also revealed hyaline and granular casts and dark coloration at these doses. Chemistry and urinalysis results correlated with gross pathology and histological

abnormalities, including nephropathy in kidneys of males and females at the two high doses. Total cholesterol was increased significantly ($p<0.05$) compared to controls in 2500 and 5000 ppm males to +81% through 12 months and in 2500 and 5000 ppm females to +101% throughout the study. Bilirubin was increased up to +200% in 2500 and 5000 ppm males and females at various intervals to 18 months. Alkaline phosphatase, alanine AT, and aspartate AT were decreased in both sexes ($p<0.05$) at 5000 ppm during the first year. Alterations in cholesterol, bilirubin, and liver enzymes at the two highest doses correlated with liver enlargement and increased incidence of liver lesions.

Significant increases ($p<0.05$) in absolute (19-43%) and relative (29-104%) liver weights, vs controls, were observed in both sexes at 2500 and 5000 ppm, and toxicity was corroborated by serum chemistry and histopathology findings. Relative thyroid weights were elevated ($p<0.05$) (+33 to +64% over controls) at 2500 and 5000 ppm and correlated with microscopic thyroid abnormalities.

At the 12-month sacrifice a dark yellowing of adipose tissue was observed at 2500 and 5000 ppm. As with other findings of discoloration (eye, skin, urine, etc.) in these groups, the yellow adipose could have been due to jaundice, although deposition of the dark yellow test substance or its metabolite may have been a contributing factor. At terminal sacrifice, gross pathology findings included granular/rough/pitted cortex and darkening of the kidney in both sexes at 2500 ppm. At 5000 ppm, males and females exhibited these same kidney abnormalities as well as pale areas of the lung, enlarged testes, uterine cysts, and darkening of the stomach.

After 12 months (interim sacrifice), noteworthy increases in non-neoplastic lesions were: thyroid follicular cell hypertrophy in the 5000 ppm males and females, hepatocellular hypertrophy in 5000 ppm males and females and in 2500 ppm males, and hepatocellular pigment in 2500 ppm males and females. All rats of both sexes at 100 ppm and above exhibited increased incidences of hyaline droplets in the kidney. Kidney tubule cell karyomegally was observed in all rats of both sexes at 2500 and 5000 ppm, with none in controls. Also observed at the interim sacrifice were pelvic calculi in the kidney in females at 100 ppm, and males and females at 2500 ppm and 5000 ppm. Kidney transitional cell hyperplasia was observed in males and females at 5000 ppm.

Absolute brain weight was decreased in males (-7%) at 5000 ppm and in females (-6%) at 2500 ppm and (-6%) at 5000 ppm only at terminal sacrifice.

At the terminal sacrifice, treatment-related non-neoplastic lesions included the same thyroid, liver, and kidney lesions observed in the interim sacrifice with increasing frequency and severity. In addition, liver sinusoidal pigment was elevated in males at 5000 ppm and necrosis was increased in males and females at the high dose. Thyroid follicular cell hyperplasia was slightly increased in males and females at 5000 ppm. Skeletal muscle and sciatic nerve degeneration were markedly increased in both sexes at 2500 and 5000 ppm. Chronic lung inflammation appeared to show a treatment-related increase in females at 5000 ppm. Thyroid and liver abnormalities correlated with increased tumor incidences at the high dose.

The NOEL for chronic toxicity was 10 ppm for males and females (0.5 mg/kg/day and 0.7 mg/kg/day, respectively), based upon an increased incidence of histologic lesions of the kidney at 100 ppm. The LOEL was 100 ppm (5.4 mg/kg/day for males and 6.8 mg/kg/day for females).

Under the conditions of this study, benfluralin was oncogenic in both sexes. The test substance induced hepatocellular carcinoma/adenoma in male rats at 2500 and 5000 ppm. The incidence of combined hepatocellular adenoma/carcinoma was 0, 13, and 15% in males at doses of 0, 2500, and 5000 ppm, respectively. The occurrences of hepatocellular adenoma were the major contributor to these findings and malignancy did not appear to increase with dose. In addition, benfluralin caused thyroid follicular adenoma/carcinoma in males and females at the two high doses, with the adenoma slightly predominant. The incidence of combined hepatocellular adenoma/carcinoma was 0, 13, and 15% in males and 0, 10, and 7% in females at doses of 0, 2500, and 5000 ppm, respectively. No evidence of early onset of any tumors was observed.

This chronic toxicity/carcinogenicity study was initially classified as unacceptable (§83-5) because statistical notations for histopathology data were not provided nor was a statistical analysis report submitted. In addition, historical control data were not provided. The study may be upgraded to acceptable following submission of amended data tables for non-neoplastic and neoplastic lesions showing statistical significance of data, a statistical analysis report, and laboratory historical control data.

However, the above requested information was submitted under MRID# 44545501, and MRID# 44050002 is upgraded to acceptable.

Discussion of Tumor Data **F-344 male rats** had a significant increasing trend ($p<0.01$), and a significant difference ($p<0.01$) in the pair-wise comparison of the 5000 ppm (275 mg/kg/day) dose group with the controls, for liver adenomas and combined liver adenomas/carcinomas. The incidences of liver adenomas and combined adenomas/carcinomas at 2500 and 5000 ppm exceeded the respective range for the historical controls. The increase in liver carcinomas in males at ≥2500 ppm was not statistically significant, but the incidence was slightly outside the range for the historical controls. There were significant increasing trends in thyroid follicular cell adenomas, carcinomas, and combined adenomas/carcinomas, all at $p<0.01$. There were also significant differences ($p<0.05$ or 0.01) in pair-wise comparisons with the control at 5000 ppm for thyroid follicular cell adenomas, thyroid follicular cell carcinomas and combined adenomas/carcinomas. The incidences of the above tumors at ≥2500 ppm exceeded the respective ranges for the historical controls.

There was no treatment related increase in liver tumors in female rats. However, females had significant increasing trends ($p<0.05$ or 0.01) in thyroid follicular cell adenomas, and combined adenomas/carcinomas. There was a significant difference ($p<0.05$) in the pair-wise comparison of the 2500 ppm (168 mg/kg/day) dose group with the controls for combined thyroid follicular cell adenomas/carcinomas. The incidence of combined thyroid follicular cell adenomas/carcinomas, although not statistically significant at 5000 ppm (331 mg/kg/day), was considered by the CARC to be biologically significant and shared a similar pattern as in the males. The incidences of thyroid follicular cell adenomas (at ≥2500 ppm), carcinomas (at ≥100

ppm) and combined adenomas/carcinomas (at ≥2500 ppm) exceeded the corresponding historical control ranges. **There was some evidence of an increase in thyroid follicular cell tumors in both males and females. However, these tumors occurred at excessive toxic doses and the increase in thyroid tumors in females was statistically significant at the mid dose but was only biologically relevant (statistically not significant) at the highest dose.** The dosing at 100 ppm was considered to be adequate based on decrease in body weight, body weight gain and increased kidney hyaline droplets in both sexes. The dosing at ≥2500 ppm was excessive based on the increased incidence and severity of histopathological lesions (liver hypertrophy, liver necrosis, sciatic nerve and skeletal muscle degeneration, kidney hyaline droplets as well as thyroid hypertrophy/hyperplasia).

Adequacy of the Dose Levels Tested in the rat study: The two top doses were excessive for an adequate dose to test for carcinogenicity in rats. Tumors at these dose levels were discounted.

2. Carcinogenicity Study in Mice

MRID No.: 41021501

Executive Summary: In a mouse oncogenicity study (MRID 41021501), benfluralin (95.25% a.i., Lot/Batch # 231EF4) was administered in the diet to B6C3F$_1$/Crl mice (60/sex/group) for up to two years at 0, 0.005, 0.03, or 0.15% (equivalent to 0/0, 6.0/6.9, 36.4/41.8, and 184.7/223.5 mg/kg/day [M/F], respectively). This mouse study is a data summary of two replicate studies run concurrently (M02785 and M02885) in which 30 mice/sex/group in each study were dosed as stated above.

Mortality, clinical signs, food consumption, and hematology findings for both sexes at all doses were unaffected by treatment with benfluralin. No treatment-related findings were observed in the 0.005% dose group.

Body weights and weight gain were equivocally depressed in males and females of all dosed groups, except that female body weight (-8%) and body weight gain (-11%) was treatment related and statistically significantly decreased in the highest dose group. Male body weight and body weight gain in the highest dose group was less than controls at all time periods. Similarly to female body weights, male body weights in lower dose groups, although statistically significant at many measurement periods, the body weight decreases appeared to random or equivocal and not dose related, except during weeks 44 to 62 in males and 52 to 102 in females, which showed a dose relationship. Only the female body weight and weight gain in the highest dose group was considered toxicologically significant.

Male mice showed a nominal increase in death from urologic syndrome (7/60 vs. 2/60 in controls) at the highest dose. In addition, less severe obstructive urologic syndrome appeared to increase at the highest dose level (18/60 vs. 5/60 in control). The study report indicate that this was a frequent finding in B6C3F1 mice. These findings may be due to an indirect effect of stress from the benfluralin treatment. The only other possible indication of toxicity in males was a nominal increase in multi focal hepatocelluar hyperplasia (8/60 vs. 1/60 in controls) at the

highest dose. Apart from nominally depressed body weight, nominally increased incidence of obstructive urological syndrome, and multi focal hepatocellular hyperplasia in males at the highest dose level, no other indications of toxicity in male mice were seen. However, the weight of evidence from all these nominally increased signs of toxicity at a LOAEL of 0.15% would appear to indicate that a sufficiently high dose was administered to males to test for carcinogenicity.

At 0.03%, increases in absolute liver weights (↑19%), relative to body (↑26%), and relative to brain (↑21.9%) were observed in the females (not statistically significant); the incidence of liver nodules was also slightly elevated (12/60 treated vs 7/60 controls).

At 0.15%, toxicity was observed in the liver of females as follows: at termination, an increase in the levels of alanine aminotransferase (↑276%; $p \leq 0.05$); an increase in alkaline phosphatase (↑32%; $p \leq 0.05$) after exclusion of one outlier from the control animals; increases ($p \leq 0.05$) in absolute, relative to body, and relative to brain liver weights (↑21.2, 30.6, and 22.2%, respectively); an increased incidence of liver nodules (25/59 treated vs 7/60 controls); an increased incidence of minimal to moderate focal hyperplasia (20/59 treated vs 6/60 controls) and an increase in slight to moderate multi focal hyperplasia (6/59 treated vs 1/60 controls). In addition to these liver changes, overall body weight gain was decreased (↓11%; $p \leq 0.05$) in the females. In the males, only minimal increases were observed in the liver weights (↑5.2, 9.6, and 4.8%, respectively; ($p \leq 0.05$)) and slight to moderate multi focal hyperplasia was minimally increased (7/60 treated vs 1/60 controls).

An increased incidence of hepatocellular adenoma was observed in the high-dose females compared to controls (5.1% treated vs 1.7% controls); the incidence was outside of the historical control range (0-3.4%). A nominally increased incidence of hepatocellular carcinoma was observed in the mid-and high-dose females (5.0-5.1%, respectively vs. 0% in controls), but the incidence was within historical control range (0-6.9%). There was also a slightly increased incidence of combined hepatocellular adenomas and carcinomas in the high-dose females (8.5% treated vs. 1.7% in controls) that was at the upper limit of the historical control range (0-6.9%). Peto's Tend Test indicated for onset, $p = 0.018$ and for prevalence, $p = 0.027$. Pair-wise analyses was not reported. Neither the onset nor the prevalence was considered statistically significant by the sponsor who cited, $p = 0.01$ as being an acceptable threshold for these high frequency tumors. The gross and microscopic findings such as increased liver nodules and hypertrophy were observed, demonstrating that the compound is affecting the morphology and growth of hepatocytes. Non-statistically significant nominal increases in combined hepatocellular adenomas and carcinoma occurred in high dose males.

The nitrosoamine content of benfluralin, as measured in 1985 as n-butylnitrosamine, was 0.31 ppm and reported as 0.11 ppm at termination of the current studies.

The LOAEL is 0.03% for females (equivalent to 41.8 mg/kg/day) based on microscopic and macroscopic liver changes. The NOAEL for females is 0.005% (equivalent to 6.9 mg/kg/day). The LOAEL was 0.15% (equivalent to 184.7 mg/kg/day) based on the weight evidence for slight toxicity observed in the males. The NOAEL for males is 0.03% (equivalent to 36.4 mg/kg/day).

The submitted study is classified as **acceptable** (§83-2b) and does satisfy the guideline requirements for a carcinogenicity study in mice.

Discussion of the Tumor Data **In $B6C3F_1$ female mice,** there was a borderline statistically significant increasing trend (p=0.0353) and **a** borderline significant increase (p= 0.0488) by pair-wise comparison of the 224 mg/kg/day dose group with the controls for combined liver adenomas/carcinomas only. The incidence of these tumors was outside the range for the historical controls. Although the incidence of adenomas in females exceeded the range for the historical controls, neither the number of adenomas nor carcinomas in the present study were statistically significantly increased. **Dosing at the highest level for females was considered to be adequate and not excessive based on decreased body weight gain, increased liver enzyme levels, increased incidence of liver nodules, hyperplasia and increased incidence as well as severity of liver foci.** The CARC concluded that the highest dose in males may have approached an adequate dose level to assess the carcinogenic potential of benfluralin based on statistically significant increase in absolute and relative liver weight as well as relative brain weight. Urologic syndrome was stated to be a common cause of death in male $B6C3F_1$ mice. Therefore, the Committee determined that additional data regarding the increased incidence of urologic syndrome and its role in the death of male mice noted in the chronic/carcinogenicity study as well as the results of a 90-day subchronic toxicity study in mice would be required to confirm the adequacy of dosing in male mice.

On Dec 6, 2001, an Ad Hoc Committee reviewed the requested data submitted by the registrant and concluded that the findings of mouse urologic syndrome were not indicative of a compound related effect that showed that the dosing was high enough. It was also determined that 1) the slight decreases in body weight and body weight gain, the small increases in liver weight (both relative and absolute) and minimal increases in liver multifocal hyperplasia were insufficient to determine that dosing was adequate; 2) the results of the 90 day subchronic feeding study indicated that dosing to the males could approach the limit dose of 1000 mg/kg/day rather that the 185 mg/kg used in the cancer study and 3) the metabolism study (in rats) noted no differences in the metabolic profiles between the single low dose of 100 mg/kg/day and the high dose of 500 mg/kg/day. Saturation was not seen at the high dose. The incidence of liver tumors in females at a slightly higher dose also was a consideration in Committee's conclusion that the dosing in the males was not high enough.

The CARC determined that the liver tumors in female mice were treatment-related.

Adequacy of the Dose Levels Tested in the mouse study: The weight of the evidence indicated that the highest dose tested of 223.5 mg/kg/day in female mice appeared adequate to test for oncogenicity/carinogenicity based on statistically significantly increased alanine aminotransferase (276%) and alkaline phosphatase (32%) and increased liver nodules **at** 223.5 mg/kg/day (7/60 in controls and 25/59 at 223 mg/kg/day) in females. The doses in males was not sufficiently high to test for carcinogenicity.

3. Classification of Carcinogenic Potential

According to the Agency's Draft Guidelines for Cancer Risk Assessment (July, 1999), the CARC classified benfluralin into the category **"Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"** based on the occurrence of liver tumors in female mice. "The Committee further recommended that the quantification of human cancer risk is not required."

Contributing factors to the CARC (TXR# 0050378) decision were, (1) the lack of carcinogenic potential in rats, (2) a lack of mutagenic potential in a battery of tests, (3) structurally related pesticides such as trifluralin, ethalfluralin, oryzalin, flumetralin and pendametralin were classified as "C" carcinogens and their respective mutagenicity studies showed no uniform pattern of mutagenicity.

IV. MUTAGENICITY

Adequacy of data base for Mutagenicity: The data base for Mutagenicity is considered adequate based on pre 1991 guidelines. No mutagenic potential was seen in adequately conducted pre 1991 guideline mutagenicity studies on benfluralin. There was a question about a Mouse Lymphoma Test (non activated) showing slight increased mutation frequency (the increase was lower than the accepted spontaneous rate of 10-150 $x10^6$ survivors), which was determined to be due to cytotoxicity. The literature indicates that Trifluralin (a structural analog) was strongly mutagenic in plants (unspecified), producing 3-4 times increase in spontaneous mitosis and chromosomal aberrations (Micromedex 1974-1998). However, no such reports have been seen for benfluralin.

Gene Mutation

Guideline 870.5100, Ames/*Salmonella typhimurium*, reverse mutation MRID 00160863 Acceptable	Assay shows no dose related reverse mutations with any of the 5 strains, +S9 or -S9. At dose ≥ 300 µg/plate +S9 and 750 µg/plate -S9, precipitation (DMSO solvent) occurred . No cytotoxicity and no mutation was shown up to 5000 µg/mL.

Guideline 870.5300, L5178Y TK+/- Mouse Lymphoma cell forward mutation MRID 00160866 Acceptable	Assay shows no dose related increase in mutation frequency up to cytotoxic (excessive) dose levels (<10-20% cell survival) with and without activation. Doses in DMSO solvent & up to 25 µg/mL -S9 and 20 µg/mL + S9.

Cytogenetics

Guideline 870.5375, in vitro chromosomal aberrations in Chinese Hamster ovary (CHO) cells MRID 41031901 Acceptable	Assay shows no dose related increase in clastogenic activity (evidence of mutagenic potential) at precipitating dose levels, +S9 and -S9. Doses in DMSO solvent & up to 40 µg/mL, +S9, and 125 µg/mL, -S9, were tested. Mitotic index was reduced 40% to 60% at top dose. Precipitation occurred at the next higher dose to 40 µg/mL, which was 100 µg/mL.

Other Genotoxicity

Guideline 870.5550, DNA repair in primary rat hepatocytes (UDS) MRID 00160865 Acceptable	No dose related increased number of nuclear grains were seen (neg. for UDS). At doses up to 100 µg/mL were tested with DMSO solvent. Precipitation occurred at ≥500 µg/mL and cytotoxicity was seen at ≥50 µg/mL.

1. **Additional Information from Literature Sources**

No additional literature was found relative to potential developmental or reproductive effects from benfluralin. Some literature was found with regard to the mechanism of the dinitroaniline class of which benfluralin is a member. An analog of benfluralin, trifluralin was studied in the soil. Soil organisms metabolize dinitroanilines by dealkylation of the amino group and reduction of the nitro groups to a substituted aniline (Emmerson, JL and Anderson, RC in Cassarett and Duoll. Toxicology: The Basic Science of Poisons (1975)). The same reference indicates that the 80% of the trifluralin fed was excreted in the feces as parent and the reduced trifluralin and that 20% was excreted in the urine as the dealkylated and reduced nitro compounds. The DER on the metabolism study with trifluralin indicated that 29% to 49% of the dose was excreted in the urine as the dealkylated and reduced dinitroaniline (including their conjugates), leaving 70 to 51% of the dose being excreted (uncharacterized) in the feces. In addition, the trifluralin metabolites in this metabolism study were poorly characterized in the study (HED DER# 007970). Urinary fraction F1 was composed of two main urinary metabolites and one or more minor metabolites comprising 14-15% of dose (one 2.6% and the other 8.9% of the dose; the remaining were <5%) being uncharacterized (the DER stated that they could not be easily be characterized) and none of the fecal metabolites being characterized. Apparently dealkylation of trifluralin is measurable urinary metabolite in rats (percentage of dose is about 1.6% -3.2%), but an insignificant urinary metabolite of benfluralin (about 0.2-0.3%) in rats.

V. HAZARD CHARACTERIZATION

Benfluralin is acutely toxic at high dose levels by the oral (Toxicity category IV), dermal (Toxicity category IV) and inhalation (Toxicity category IV) routes. In addition, Primary skin and eye irritation studies were toxicity category III. As noted in Table 2, the acute oral LD50 in adults is >10 g/kg, in weanling rats the LD50 is > 7 g/kg and in 24 hour old pups the LD50 is 0.7 g/kg. [However, insufficient details were given to determine whether this difference in LD50s of pups and adults in this 1965 study were due to gavage errors, usual variation in acute LD50s, or toxicity.] The technical grade is a skin sensitizer where 7/12 Guinea Pigs were positive in the Buehler test and in a 21-day rabbit dermal study, skin reactions (slight to moderate edema, hyperplasia and/or inflamation) were noted at all dose levels and severe skin reactions (including necrosis and pustules) at the two top dose levels. These studies suggest that benfluralin has little affect on undosed animals, but that it causes severe skin lesions after multiple dosing. Its use for one time during the pre-emergent season may limit multiple exposures except for commercial handlers.

Other dermal sensitizing studies on benfluralin formulations show no sensitization in these formulations at lower concentrations of ≤60% benfluralin. When ≤60% benfluralin was tested in Guinea Pig Beuhler test neat, the test was negative. This suggests that at formulated concentrations, benfluralin does not act as a skin sensitizer, possibly because the concentration of benfluralin in the formulated product was too low. Under these circumstances, the use concentrations are not skin sensitizers.

Chronic studies on benfluralin show liver and kidney pathology in rats, mice and dogs. There was no evidence of tumor induction at adequate doses to test for carcinogenicity in rats. The Cancer Assessment Review Committee (CARC) stated the there was some evidence of treatment related liver tumors in female mice. The committee concluded that there was suggestive evidence of carcinogenicity, but it was not sufficient to assess human carcinogenic potential based on the occurrence of liver tumors in female mice.

In addition, the mutagenicity studies were negative and structurally related dinitroaniline show no uniform pattern of mutagenicity.

There is no increased sensitivity in reproduction studies or developmental toxicity with benfluralin. Analogs of benfluralin are poorly absorbed through the skin, but there are no absorption studies with benfluralin.

Metabolism:
Reports from the literature suggest that benfluralin is a pesticide belonging to the dinitroaniline group, which includes trifluralin. However, the tumors found from trifluralin exposure to rats were bladder tumors and thyroid tumors and exposure to mice produced no tumors. Both benfluralin and trifluralin cause kidney and thyroid pathology. Trifluralin apparently dealkylates and is slightly more readily reduced before being excreted in the urine (Emmerson and Anderson, 1966). The deaklylated unreduced trifluralin was 1.6% to 3.2% of the dose, while the dealkylated unreduced benfluralin was 0.2% of the dose. Parent was noted in the urinary excretion products of neither trifluralin nor benfluralin dosed animals. However, any difference in urinary excretion products may be only superficial, since the urine metabolites of benfluralin and trifluralin were many and less than 5% of the dose and most have not been identified. Urine from trifluralin dosed animals showed mono- and di-reduced products, and urines from trifluralin and benfluralin dose rats showed many other products assumed to be various conjugated reduced degradation products as nearly as could be evaluated from their polarity and chromatographic properties.

The pattern of excretion of benfluralin in the urine is 13.0-16.6% of dose, and for feces, 72.6-74.6% of dose (each contained approximately 100 components). In the feces, 33.5% of the dose was recovered in faction A, of which 33.2% was shown to be parent. The second fraction B (7.8% of the dose) was composed of multiple peaks of which 6.6% was benfluralin reduced at one nitro-group. The third fraction C also composed of multiple fractions of which 0.1% of dose was shown to be the reduced benfluralin at both nitro-groups. The remaining multiple factions were composed of <1% of the dose. Only parent, and the mono- and di-reduced benfluralin were successfully identified by mass-spectra in the feces. An attempt to identify the remaining 100 metabolites was unsuccessful. In the urine no parent or reduced metabolites were found among the approximately 100 metabolites all of which were each <1.1% of the dose. The only metabolite identified in the urine was 2,6-dinitro-4-trifluro-methyl-aniline (0.2% of dose). Mass-spectra failed to identify any of the other metabolites.

In addition, when soil was flooded with water, benfluralin rapidly decomposed. Only 4.6% was detectable after 16 days, the major degradation products were benfluralin with one and two nitro groups reduced; five other polar products were detected, all less than 5% of the total radioactivity (Worthing, CR; The Pesticide Manual. 8th ed.; the Lavenham Press Ltd. Lavenham, Suffolk p. 55 (1987)). The findings in this latter literature report on benfluralin are consistent with the submitted rat metabolism studies on benfluralin. Since only a very small amount benfluralin is dealkylated (0.2%), benfluralin may be less reactive than a typical dinitroaniline that undergoes dealkylation.

VI. DATA GAPS or INFORMATION GAPS:

A subchronic inhalation study is required on a solution of benfluralin. The Agency should be contacted prior to conducting the study.

There is a need to evaluate the potential of benfluralin to cause lung toxicity in an inhalation study; (a) There are acute inhalation studies, but no multi-dose inhalation studies. (b) benfluralin is a dermal sensitizer, and (c) effects in a 21-day dermal study are suggestive of dermal sensitization.

VII. ACUTE TOXICITY

TABLE 2 Acute Toxicity of benfluralin

Guideline No.	Study Type	MRID #(s)/(Date)	Results	Category
81-1	Acute Oral	00024255 (1965)	LD50 >10 g/kg (adults) 0/10 died at 5 and 10 g/kg LD50 > 7 g/kg (weanling rats) (0/10 died at 2-7 g/kg) LD50 = 0.79 g/kg (24 hr old rats) (0/5 died at 0.5 g/kg, 2/5 died at 0.7 g/kg) on day 9&11, 2/5 died at 1.0 mg/kg on day 5&11	IV IV III
81-2	Acute Dermal	41751701 (1990)	LD50 > 5 g/kg	IV
81-3	Acute Inhalation	41613807 (1989) 0024275 (1964)	LC50 >2.3 mg/L LC50 >1.3 mg/L/hr 5% benfluralin in dimethylformamide	IV III
81-4	Primary Eye Irritation	00024265 (1976)	Slightly irritating, reversible within 7 days	III
81-5	Primary Skin Irritation	41751702 (1990)	Moderate erythema and edema at day 7, which cleared by day 11. The study indicates a skin irritation category of III.	III
81-6	Dermal Sensitization	00144283 (1990)	7/12 guinea pig tested positive in the Buehler test	Skin sensitizer

VIII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

Exposure Scenario	Dose used in Risk Assessment, UF (mg/kg/day)	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary	An appropriate endpoint attributable to single dose was not identified. Therefore, an acute RfD was not established		
Chronic Dietary (All populations)	NOAEL = 0.5 mg/kg/day UF = 100 Chronic RfD = 0.005 mg/kg/day	FQPA SF = x1 cPAD = 0.005/1 = 0.005 mg/kg/day	Chronic /carcinogenicity-Rat LOAEL = 5.4 based on increased histopathologic lesions of the kidneys were seen in males (5.4 mg/kg/day and females 6.8 mg/kg/day for females).
Incidental Oral, Short-Term (1-30 days)	NOAEL= 100 mg/kg/day	Residential LOC for MOE = 100 (includes the FQPA SF)	Developmental Toxicity -Rabbits LOAEL = 225 mg/kg/day based on decreases in maternal body weight gain over a 13 day dosing period.
Incidental Oral, Intermediate-Term (1-6 months)	NOAEL= 7.2 mg/kg/day	Residential LOC for MOE = 100 (includes the FQPA SF)	Reproduction and fertility effects-Rats LOAEL = 68.1 mg/kg/day based on Hyaline droplet formation in the kidneys of adult males and progressive chronic nephropathy in adult males and females and pup weight decrement.
Dermal, Short, Intermediate and Long-Term	None	There was no NOAEL for dermal toxicity and no systemic toxicity in the 21-day dermal study and benfluralin has been shown to be a sensitizer. Therefore risk following dermal exposure can not be quantified. The label should indicate that this compound is a skin sensitizer.	
Inhalation, Short-Term (1-30 days)	Oral study NOAEL= 100 absorption rate = 100%	Residential LOC for MOE = 100 (includes FQPA SF) Occupational LOC for MOE = 100	Developmental toxicity - Rabbits LOAEL = 225 mg/kg/day based on decreases in maternal body weight gain over a 13 day dosing period.

Inhalation, Intermediate-Term (1-6 months)	NOAEL= 7.2 mg/kg/day absorption rate = 100%	Residential LOC for MOE = 100 (includes the FQPA SF)	Reproduction and fertility effects-Rats LOAEL = 68.1 mg/kg/day based on Hyaline droplet formation in the kidneys of adult males and progressive chronic nephropathy in adult males and females and pup weight decrement.
Inhalation, Long-Term (>6 months)	Oral study NOAEL= 0.5 mg/kg/day MOE = 100	Residential LOC for MOE = 100 (includes FPQA SF) Occupational LOC MOE = 100	Chronic/carcinogenicity-Rat LOAEL = 5.4 mg/kg/day based on increased histopathologic lesions of the kidneys were seen in males (5.4 mg/kg/day and females 6.8 mg/kg/day for females).
Cancer (oral)	**"Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"** "The Committee further recommended that the quantification of human cancer risk is not required."		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

NOTE: The Special FQPA Safety Factor recommended by the HIARC **assumes** that the exposure databases (dietary food, drinking water, and residential) are complete and that the risk assessment for each potential exposure scenario includes all metabolites and/or degradates of concern and does not underestimate the potential risk for infants and children.

IX. REFERENCES:

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