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EVALUATION OF THE CARCINOGENIC POTENTIAL OF
BENFLURALIN
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EXECUTIVE SUMMARY

On September 26, 2001, the Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of benfluralin. The Committee evaluated the combined chronic/carcinogenicity studies in F344 rats and $B6C3F_1$ mice.

In a study in F-344 rats, benfluralin (95.8% a.i.) was administered to 60 rats/sex/dose at dietary levels of 0, 10, 100, 2500 or 5000 ppm (equivalent to 0, 0.5, 5.4, 136.3 or 275 mg/kg/day in males and 0, 0.7, 7, 168 or 331.3 mg/kg/day in females, respectively) for 24 months. In another study, B6C3F1/Crl mice (60/sex/dose) received diets containing benfluralin at dose levels of 0, 6, 36.4 or 185 mg/kg/day for males and 0, 7, 42 or 224 mg/kg/day for females for up to 2 years.

The Committee concluded that benfluralin caused liver tumors in female B6C3F1 mice. Although there was <u>some</u> evidence of an increase in liver tumors in male F-344 rats and thyroid follicular cell tumors in male and female F-344 rats, these tumors occurred only at excessively toxic doses. These conclusions were based on the following findings:

- **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 <u>some evidence</u> 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).

- **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.

The studies regarding the mode of action of thyroid tumor induction were not available and therefore, the mechanism of tumor induction in rats and mice could not be determined.

- Benfluralin was nonmutagenic in *in vitro* Salmonella, mouse lymphoma, Chinese hamster ovary cell and unscheduled DNA synthesis assays.

- Structurally-related compounds, trifluralin, ethafluralin, oryzalin, flumetralin and pendamethalin were classified as group "C" carcinogens with no uniform pattern of mutagenicity.

According to the Agency's Draft Guidelines for Cancer Risk Assessment (July, 1999), the Committee 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.

I. INTRODUCTION

On September 26, 2001, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of benfluralin. Dr. David Anderson from Reregistration Branch 2 of the Health Effects Division (HED) presented the results of the combined chronic and carcinogenicity studies in F344 rats and $B6C3F_1$ mice with benfluralin. The presentation included details of the experimental design, survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of dose levels tested, and other information related to the weight of the evidence for the carcinogenicity of benfluralin.

II. BACKGROUND INFORMATION

Benfluralin (Benefin®; CAS #:1861-40-1) is a selective pre-emergence herbicide used to control annual grasses (including crabgrass) and broadleaf weeds. There is occupational exposure through food and non-food uses, and non-occupational exposure from lawn and ornamental use.

In plants, benfluralin inhibits several hormone-induced enzymes and uncouples oxidative-phosphorylation in roots.

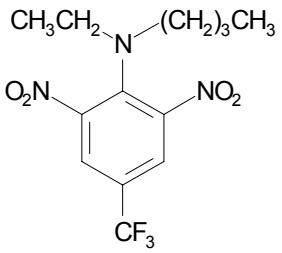


Figure 1: Benfluralin

A Registration Standard was issued for benfluralin in 1988. Earlier, the RfD was set at 0.30 mg/kg/day by Office Research Development (ORD) based on the results of a chronic dog study. In this study, the LOAEL of 125 mg/kg/day was based on decreased hemoglobin and

hematocrit as well as increased relative liver weight. The NOAEL was 25 mg/kg/day. Office of Pesticide Programs (OPP) verified the RfD in 1985, and reassessed it again in 1988. Additional toxicity data have been submitted since then. The current RfD is 0.005 mg/kg/day based on kidney lesions in the combined chronic/oncogenicity study in rats reported in the Hazard Identification Assessment Review Committee (HIARC) report on Benfluralin (HED Doc. # 014534).

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with Benfluralin in F-344 Rats

References:

Moore, M.R. (1996) Benefin: Two-Year Dietary Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats. Corning Hazleton, Inc. (CHV), Midland, Michigan. Laboratory Study Identification: DR-0097-3397-005, July 1, 1996. MRID 44050002. Unpublished.

Moore, Michael R (1998) Benefin: Two-Year dietary Chronic Toxicity/Oncogenicity Study in Fischer 344 Rats. Testing Lab. Covance Labs. Inc., VA, (April 22, 1998) Lab No. CHV174-133, Sponsors ID DR-0097-3397-005. Supplementary Information. MRID# 44545501. Unpublished.

A. Experimental Design

Benfluralin (95.8% a.i.) was administered in the diet to Fischer 344 rats (60/sex/dose) at dose levels of 0, 10, 100, 2500 or 5000 ppm (equivalent to 0, 0.5, 5.4, 136.3 or 275 mg/kg/day in males and 0, 0.7, 7, 168 or 331.3 mg/kg/day in females, respectively) for 24 months.

B. Discussion of Tumor Data

Table 1 summarizes the tumor data in male and female rats.

Male rats had a significant increasing trend, and significant differences in the pair-wise comparison of the 5000 ppm dose group with the controls, for liver adenomas and combined adenomas/carcinomas, all at $p < 0.01$. There were significant increasing trends in thyroid follicular cell adenomas, carcinomas, and combined adenomas/carcinomas, all at $p < 0.01$. There was a significant difference in the pair-wise comparison of the 5000 ppm dose group with the controls for thyroid follicular cell adenomas at $p<0.05$. There were also significant differences in the pair-wise comparisons of the 2500 and 5000 ppm dose groups with the control for thyroid follicular cell carcinomas and combined thyroid follicular cell adenomas/carcinomas, at $p < 0.01$ or $p<0.05$..

Historical control data on tumors in the Fisher 344 rat (1989 to 1995) submitted by the testing laboratory (Table 1) indicate that the incidence of male liver adenomas and combined adenomas/carcinomas at 5000 ppm was higher than the historical control data. 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. The incidences of male thyroid follicular cell adenomas at 5000 ppm, as well as carcinomas and combined adenomas/carcinomas at $2500 were outside the corresponding historical control ranges. These combined thyroid tumors in males, which were statistically significantly increased at $2500 ppm, exceeded their respective historical control range.

There was no treatment related increase in liver tumors in female rats. Females had significant increasing trends in thyroid follicular cell adenomas at $p < 0.05$, and combined adenomas/carcinomas at $p < 0.01$. There was a significant difference in the pair-wise comparison of the 2500 ppm dose group with the controls for combined thyroid follicular cell adenomas/carcinomas, at $p < 0.05$. However, the incidence of combined thyroid follicular cell adenomas/carcinomas was not significant at 5000 ppm. The incidences of thyroid follicular cell adenomas (at $2500 ppm), carcinomas (at $100 ppm) and combined adenomas/carcinomas (at $2500 ppm) were outside the corresponding historical control ranges.

Although the incidences of thyroid adenomas, carcinomas or combined adenomas/carcinomas were not statistically significant at 5000 ppm in females, **the CARC believed that they were biologically significant. Thus, increases in thyroid tumors in females at 2500 and 5000 ppm were considered to be treatment-related.**

Table 1: Liver and thyroid tumor rates[+] in male and female rat [The statistical analyses of the female rats was based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. The statistical analyses of the male rats was based upon the Peto's Prevalence test.] (Brunsman, 2001).

Tumor/doses (ppm)	0	10	100	2500	5000	Historical control (range)
Male liver tumors						
Liver adenomas (%) p =	1/46 (2) 0.0001**	2/49 (4) 0.2510	1/45 (2) 0.4631	3[a]/46 (7) 0.2675	9/46 (20) 0.0007**	3.2% (3-6%)
Liver carcinomas (%) p =	1/44 (2) 0.0568	0/43 (0) -	0/38 (0) -	2/38 (5) 0.2142	2[b]/39 (5) 0.5000	0.8% (0-4%)
Combined (%) p =	2/46 (4) 0.0000**	2/49 (4) 0.4275	1/45 (2) -	5/46 (11) 0.1551	11/46 (24) 0.0017**	4% (3-8%)
Male thyroid tumors						
Thyroid adenomas (%) p =	1/46 (2) 0.0051**	1/49 (2) -	1/45 (2) 0.4257	3[a]/46 (7) 0.1763	5/46 (11) 0.0445*	1.8% (0-8.6%)
Thyroid carcinomas (%) p =	0/44 (0) 0.0016**	0/44 (0) -	0/41 (0) -	4[c]/39 (10) 0.0194*	3/39 (8) 0.0123**	0.3% (0-1.7%)
Combined (%) p = 0/50	1/46 (2) 0.0001**	1/49 (2) -	1/45 (2) 0.4257	7/46 (15) 0.0154*	8/46 (17) 0.0030**	(1.7-8.6%)
Female thyroid tumors						
Tumor/doses (ppm)	0	10	100	2500	5000	Historical control (range)
Thyroid adenomas (%) p =	0/49 (0) 0.0307*	0/49 (0) 1.0000	0/50 (0) 1.0000	3[d]/50 (6) 0.1250	2[d]/49 (4) 0.2474	0.7% (0-2.9%)
Thyroid carcinomas (%) p =	0/49 (0) 0.0558	0/49 (0) 1.0000	1[e]/50 (2) 0.5051	2/50 (4) 0.2525	2/49 (4) 0.2474	0.2% (0-1.4%)
Combined (%) p =	0/49 (0) 0.0048**	0/49 (0) 1.0000	1/50 (2) 0.5051	5/50 (10) 0.0296*	4/49 (8) 0.0587	(0-4%)

[+] Number of tumor bearing animals/number of animals examined, excluding those that died before week 54. Animals that were sacrificed at week 53 are also excluded from analyses (MRID# 44050002 & 44545501).
[a] First adenoma not in an interim sacrifice animal observed at week 88, dose 2500 ppm. [b] First carcinoma observed at week 100, dose 5000 ppm. [c] First carcinoma not in interim sacrifice animal observed at week 98, dose 2500 ppm. [d] First adenoma not in an interim sacrifice animal observed at week 105 in final sacrifice animals, simultaneously in the 2500 and 5000 ppm dose groups. [e] First carcinoma observed at week 105 in the final sacrifice animal, dose 100 ppm.

Note: Interim sacrifice animals are not included in this analysis. However, there was one male liver adenoma in an interim sacrifice animal in the 2500 dose group and one male thyroid follicular cell adenoma in an interim sacrifice animal in the 5000 ppm dose group. There was one thyroid follicular cell carcinoma in an interim sacrifice animal in the 2500 ppm dose group. There was one female thyroid follicular cell adenoma in the interim sacrifice animal in the 2500 ppm dose group.

Significance of trend denoted at control; Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Lesions

The hepatocellular hypertrophy, pigment and individual cell necrosis, occasionally with coagulation necrosis, seen at 2500 and 5000 ppm are consistent with the liver adenomas and carcinomas seen in males at the same dose levels (Table 2). Although, no dose related increase in liver necrosis and hepatocellular tumors were seen, there was dose related increase in thyroid tumors in females at $2500 ppm (Table 1).

The hepatocellular hypertrophy seen in males at the $2500 ppm dose levels supported the P450 enzyme induction seen in the subchronic study (MRID# 44050001) at comparable dose levels.

Kidney lesions were seen in males and females at $100 ppm dose levels. Kidney hyaline droplet formation was seen as low as 100 ppm in males and females, and transition cell hyperplasia and pelvic calculi in males $100 ppm. Female kidneys showed tubule cell karymegally, transition cell hyperplasia and pelvic calculi at $2500 ppm in addition the hyaline droplets at 100 ppm.

Females showed no dose-related increase in hepatocellular adenomas or carcinomas or combined tumors. The incidence of hepatocellular hypertrophy in females was similar to males at $2500 ppm.

Table 2: Summary of statistically significant non-neoplastic lesions as reported in the supplement (MRID# 44545501) to the two-year rat study (MRID# 44050002).

Site & lesion/Dose levels in ppm, mg/kg/day (mkd), %	Control	10 ppm 0.5 mkd 0.001%	100 ppm 5.4mkd 0.01%	2500 ppm 136 mkd 0.25%	5000 ppm 275 mkd 0.50%
Males					
Liver, hepatocellular hypertrophy hepatocellular pigment sinusoidal pigment individual cell necrosis, occasionally with coagulation necrosis	0/48* 0/48* 2/48* 2/48*	2/50 0/50 2/50 6/50	3/50 0/50 5/50 5/50	27/50* 18/50* 2/50 15/50*	31/50* 30/50* 12/50* 25/50*
Kidney, hyaline droplets tubule cell karymegally transition cell hyperplasia calculi, pelvis	10/48* 1/48* 0/48* 16/48*	9/50 2/50 0/50 1/50	33/50* 25/50 49/50* 37/50*	43/50* 50/50* 49/50* 47/50*	48/50* 49/50* 50/50* 47/50*
Lung chronic inflammation	13/48*	7/50	8/50	10/49	20/50
Skeletal muscle degeneration (Historical control =0.5%; 0-3.3%))	1/48*	2/50	0/50	33/50*	35/50* [a]
Sciatic nerve degeneration (Historical control data not submitted)	0/48*	2/49	0/50	26/49*	30/50* [b]
Thyroid, follicular cell hypertrophy [a] focal follicular hyperplasia [a]	0/48* 1/48	0/50 1/50	0/48 0/48	0/50 3/50	3/50 3/50
Nasal turbinates, chronic inflammation	7/48	11/50	10/49	9/50	16/50*
Female					
Site & lesion/Dose levels in ppm, mg/kg/day (mkd), %	Control	10 ppm 0.7 mkd 0.001%	100 ppm 6.80mkd 0.01%	2500 ppm 168 mkd 0.25%	5000 ppm 331 mkd 0.50%
Liver, hepatocellular hypertrophy hepatocellular pigment sinusoidal pigment necrosis	1/50 5/50 7/50 7/50	1/50 6/50 5/50 5/50	6/50 6/50 6/50 11/50	34/50* 44/50* 2/50 5/50	42/50* 41/50* 3/50 9/50
Kidney, hyalin droplets tubule cell karymegally transition cell hyperplasia calculi, pelvis	5/50* 0/50* 0/50* 6/50*	12/50 0/50 0/50 7/50	47/50* 1/50 1/50 8/50	49/50* 49/50* 41/50* 30/50*	47/50* 49/50* 47/50* 26/50*
Lung chronic inflammation	7/27*	11/39	11/37	33/47*	37/44*
Skeletal muscle degeneration (Historical control =0.5% (0-2.0%))	0/50*	0/50	0/50	32/50*	44/50* [a]
Sciatic nerve degeneration (Historical control data not submitted)	0/50*	1/50	0/50	26/50*	41/50* [b]
Thyroid, follicular cell hypertrophy focal follicular hyperplasia	0/50 0/50*	0/50 0/60	0/50 1/50	0/50 2/50	0/50 4/50
Nasal turbinates, chronic inflammation	7/50	7/50	9/50	4/50	13/50

* = in control indicates positive trend; * = in dose groups indicates statistically significant at # 0.05; [a] = Skeletal muscle degeneration in historical controls submitted in MRID# 44545501 indicated the range of incidence is 0-3.3% in males and 0-2.0% in females. [b] = No historical control data for sciatic nerve degeneration was included in MRID# 44545501.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

A significant increase in male mortality occurred at 100, 2500 and 5000 ppm, along with a significant increasing trend, but no differences among groups were seen in female mortality. Male mortality was 19%, 18%, 40%($p<0.05$), 38%($p<0.05$) and 36%($p<0.05$) from 0, 10, 100, 2500 and 5000 ppm, respectively. The historical control data for F-344 rats showed a mean of 22% mortality. Mortality rates in females were 28%, 18%, 26%, 8% and 18%, respectively from 0 to 5000 ppm. The CARC discounted the increased mortality in males; noting that there was no dose relationship even though there was a 50- fold spread in dose level between 100 ppm and 5000 ppm.

The body weights were statistically significantly lower than control for males and females at 2500 (-8% for males and -18% for females) and 5000 ppm (-17% for males and -28% for females). No effect on body weight was seen in males or females at 100 ppm. **Thus, the CARC concluded that in males and females, the two top dose levels were excessive for carcinogenicity testing and that the dose level of 100 ppm for males and females was adequate to test for the carcinogenic potential of benfluralin in the Fisher 344 rat.** At this dose level in males and females, there was evidence of toxicity based on clinical chemistry and histological findings, such as kidney hyaline droplet formation (males and females), kidney transition cell hyperplasia and pelvic calculi (males) and at 2500 and 5000 ppm by addition kidney tubule cell karymegally, transitional cell hyperplasia and pelvic calculi (males and females). These latter findings at 2500 and 5000 ppm were supported by increased urea nitrogen and creatinine levels (males and females). Increased hepatocellular hypertrophy and hepatocellular pigment were seen in males and females at 2500 and 5000 ppm. In addition, individual cell necrosis, occasionally with coagulating necrosis was seen in males at the latter dose levels. Increased sinusoidal pigment was seen in males and females at 5000 ppm.

Hematological parameter values (erythrocyte count) were lower than controls for males and females at 2500 and 5000 ppm for the first 12 months. Hemoglobin and hematocrit were decreased in males and females at the two highest dose levels. Urinalysis showed hyaline and granular casts and dark coloration in males and females at 2500 and 5000 ppm. Urea nitrogen (21 - 102%) and creatinine (33%) were increased over control values in males and females at these same dose levels. The clinical chemistry and urinalysis correlated with gross and histological abnormalities including pathology in the kidneys of males and females at the two highest dose levels. Clinical chemistry values were elevated for cholesterol (up to 81% in males and 101% in females during the study), and bilirubin (up to 200% in males and females during the study) at the two top dose levels. Increased urine output was seen at the two top dose levels and may have been related to dehydration and increased plasma protein, albumin, and globulin concentrations, which were also seen at the two top dose levels.

2. <u>Carcinogenicity Study in Mice</u>

<u>*Reference*</u>*:* Koenig, G.R., Jordan, W.H., (1998). A Chronic Toxicity and Oncogenicity Study in B6C3F$_1$ Mice given Benefin (EL-110, Compound 54521) in the Diet for Two Years. Lilly Research

Laboratories, Greenfield, IN. Laboratory Project Id. M02785 and M02885. December 14, 1988. MRID 41021501. Unpublished.

A. Experimental Design

B6C3F1/Crl mice (60/sex/dose) were fed diets containing benfluralin at dose levels of 0, 0.005, 0.03 or 0.15% (0, 6, 36.4 or 185 mg/kg/day in males and 0, 7, 42 or 224 mg/kg/day for females, respectively) for up to 2 years. The data presented are a summary of two replicate studies (MO2785 and (M02885) in which 30 mice/sex/group were dosed as stated above.

B. Discussion of Tumor Data

Benfluralin is carcinogenic in female mice at the highest dose level (224 mg/kg/day) tested, but not in male mice (at 185 mg/kg/day). Female mice had a significant increasing trend and a significant difference in the pair-wise comparison of the 224 mg/kg/day dose group with the controls, for combined liver adenomas/carcinomas (11% vs 2% in controls; Table 3), both at $p < 0.05$. The incidence of combined adenomas/carcinomas exceeded the corresponding range in historical control values (0%-6.9%). Neither adenomas nor carcinomas were significantly increased, but the incidence of adenomas (5.1%) in females exceeded the range in historical control values (0-3.4%).

Table 3: Benfluralin - 18 month B6C3F1mouse study (MRID# 41021501). The statistical analyses of mouse tumors were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons (Brunsman, 2001).

Male liver tumors					
Tumor/Dose (mg/kg/day)	0	6.0	36.4	184.7	Historical control (range, %)
Adenomas (%) p =	2[a] /60 (3) 0.0536	1/59 (2) 0.5064	3/56 (5) 0.4671	5/59 (8) 0.2125	NA
Carcinomas (%) p =	7/60 (12) 0.3234	7/59 (12) 0.5984	5[b]/56 (9) 0.4306	8/59 (14) 0.4859	NA
Combined (%) p =	9/60 (15) 0.0908	8/59 (14) 0.5151	8/56 (14) 0.5618	13/59 (14) 0.2262	NA
Female liver tumors					
Tumor/Dose (mg/kg/day)	0	6.9	41.8	223.5	Historical control (range, %)
Adenomas (%) p =	1/58 (2) 0.1024	1/59 (2) 0.7479	1/56 (2) 0.7434	3[c]/55 (5) 0.2893	0-3.4%
Carcinomas (%) p =	0/58 (0) 0.1137	2/59 (3) 0.2521	3/56 (5) 0.1153	3[d]/55 (5) 0.1121	0-6.9%
Combined (%) p =	1/58 (2) 0.0353*	3/59 (5) 0.3157	4/56 (7) 0.1711	6/55 (11) 0.0488*	0-6.9%

[+]Number of tumor bearing animal/number of animals examined, excluding those died before week 53. NA = Not available. [a] First adenoma observed at week 84, dose 0 mg/kg./day. [b] First carcinoma observed at week 74, dose 36.4 mg/kg/day. [c] First carcinoma observed at week 104 sacrifice animal, dose 223.5mg/kg/day. [c] First carcinoma observed at week 67, dose 223.5 mg/kg/day. [d] An adenoma and carcinoma occurred in one animal. This animal was included in the carcinoma incidence.
Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, Then $p < 0.05$. If **, then $p < 0.01$.

C. Non-Neoplastic Lesions in mice

Liver nodules and hyperplasia were seen in females at the highest dose (Table 4). There was an increase in liver nodules (25/59 versus 7/60 in control), liver-focal hyperplasia (20/60 versus 6/59 in control) and multifocal hyperplasia (6/59 versus 1/60 in control) in females only at the highest dose tested. Females also showed an increase over control values in alanine aminotransferase (276%) and in alkaline phosphatase (32%) activity.

In males, only liver-multifocal hyperplasia was increased at the highest dose (7/60 versus 1/60 in control); there was also a treatment related increase in male mortality from urologic syndrome (7/60

versus 2/60 in control). In addition, males showed an increase in obstructive urologic syndrome (18/60 versus 5/60 in control) at the highest dose.

Table 4. Incidence of liver nodules (# animals) observed in female mice treated with benfluralin for up to 2 years.[a]

Dose (%)	0	0.005	0.03	0.15
Dose (mg/kg/day)	0	6.9	41.8	223.5
# Females examined	60	60	60	59
Liver nodules	7	6	12	25****

[a] Data obtained from the study report, Tables 45 and 46, pages 230 and 244. **** Significantly different from controls at p<0.0005 as analyzed by the reviewers using Fisher's Exact test.

Table 5. Incidence (# of animals) of selected hepatocellular microscopic findings in mice dosed with benfluralin for up to 2 years. [a]

Observation	Males				Females			
	Dietary Level (%)							
	0	0.005	0.03	0.15	0	0.005	0.03	0.15
	Dietary Level (mg/kg/day)							
	0	6.0	36.4	184.7	0	6.9	41.8	223.5
Liver-Focal hyperplasia								
Minimal	4	1	3	4	0	1	0	2
Slight	9	2	9	10	4	3	4	7
Moderate	7	9	9	6	2	1	2	11
Total	20	12	21	20	6	5	6	20
Liver-Multifocal hyperplasia								
Slight	1	1	0	4	0	0	0	2
Moderate	0	0	0	3	1	0	0	4
Total	1	1	0	7	1	0	0	6

a Data were obtained from study report Tables 47 and 48, pages 255, 256, and 283; n=60 except for the high-dose females where n=59.

*** Significantly different from controls at p<0.005 as analyzed by the reviewers using Fisher's Exact test.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The statistical evaluation of mortality indicated no statistically significant incremental changes with increasing doses of benfluralin in male or female mice (Brunsman, 2001). The adequacy of the dose levels to assess the carcinogenicity in male mice was based on multiple factors. Both deaths by urologic syndrome and severity of urologic syndrome were elevated at the highest dose level. Deaths by urologic syndrome in males were 2/60 (3%), 2/60 (3%), 4/60 (7%), and 7/60 (12%), respectively, at 0, 6, 36.4, and 185 mg/kg/day dose levels. The incidence of urologic syndrome (with severities) at these

same dose levels was 5/60 (8%), 12/60 (20%), 11/60 (18%) and 18/60 (30%), respectively. Urologic syndrome was stated to be a common cause of death in male B6C3F1 mice. Benfluralin appeared to exacerbate the effect at the high dose. Other kidney lesions such as kidney tubular epithelial cytoplasmic vacuolation and cortical tubular epithelial regeneration showed a dose related decreased incidence with increasing dose level. The body weight of males was periodically nominally depressed (3-8%) at all dose levels and sometimes the depression was statistically significant for the first 62 weeks, but the body weight decrement was only nominally depressed (5%) at the highest dose tested at termination at week 104. The study report authors did not believe this body weight decrement in males was treatment related. Absolute (+5%) and relative liver weight (10%) and relative brain weight (5%) were statistically significantly increased at the highest dose tested only. **Therefore, the CARC concluded that the highest dose level in males may have approached an adequate dose to assess the carcinogenic potential of benfluralin based on statistically significant increase in absolute and relative liver weight as well as relative brain weight. However, additional data regarding the increased incidence of urologic syndrome noted in the chronic/carcinogenicity study and the results of a 90-day subchronic toxicity study in mice would be required to confirm the adequacy of dosing in male mice.** The highest dose in females was clearly adequate to assess the carcinogenicity, and was not excessive. Body weight (-8%) and body weight gain were significantly decreased (-11%) at the highest dose level. Alanine aminotransferase (276%) and alkaline phosphatase levels were significantly elevated at the highest dose level. The incidence of liver nodules was elevated at the highest dose (25/59 verus 7/60 in control)(Table 4). There was an increased incidence and severity of liver-focal (20/59 verus 6/60 in control) and multifocal hyperplasia (6/59 verus 1/60 in control) at the highest dose in females (Table 5).

At the CARC meeting of Sept 26, the dosing was an issue that was not totally resolved since more information about the mouse urologic syndrome (MUS) as it relates to benfluralin treatment was needed. Following the CARC meeting an additional information was submitted by the registrant. On Dec 6, 2001, Marion Copley, William Burnam, Dave Anderson and Karl Baetcke met to discuss the adequacy of dosing in males in the benfluralin mouse cancer study in light of the newly submitted data. The Ad Hoc Committee agreed on the following regarding the male dosing:

1. In the mouse study, there were no dose related increases in mortality. There were no specific gross or histopathology findings that appeared to be related to mouse urologic syndrome (MUS). In this strain of mouse, the incidence of MUS is variable and all the dose groups appear to be within the background values. The Committee concluded that the findings of MUS were not indicative of a compound related effect that showed that dosing was high enough.

2. It was also determined that 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.

3. 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.

4. 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. Saturation was not seen at the high dose.

5. 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.

6. The Agency should be consulted about the doses used in the new male mouse cancer study.

IV. TOXICOLOGY

1. Metabolism

In rat metabolism studies (MRIDs 40693201 through 40693207), [^{14}C-phenyl]benfluralin ($99.0 % radiochemical purity) was administered to F-344 rats (4-6/sex/dose) as either a single oral (gavage) dose at 100 or 500 mg/kg or as a single oral dose at 100 mg/kg following a 14-day pretreatment with benfluralin at 100 mg/kg/day. In addition, 5-10 bile-duct cannulated rats/sex were administered a single oral dose of [^{14}C]benfluralin at 100 or 500 mg/kg. Two additional groups of normal rats (5/sex) were dosed once orally (gavage) with [^{14}C]benfluralin at target doses of 100 or 500 mg/kg to examine plasma/blood kinetics. To examine the accumulation and elimination of residual ^{14}C-residues in tissues and organs, another group of normal rats (5/sex/time point) was dosed once orally with [^{14}C]benfluralin at a target dose of 100 or 500 mg/kg.

Dosed radioactivity was quantitatively recovered from the single oral low-, single oral high-, and repeated oral low-dose groups, with 85.5-94.9% of the dosed radioactivity being recovered in urine, feces, and tissues within 168 hours of dosing. Absorption of [^{14}C]benfluralin from the G.I. tract of rats was apparently limited based upon the high levels for fecal excretion (64.6-78.8% dose) and the low levels of renal (11.8-22.6% dose) and biliary (5.7-12.9% dose) excretion. Excretion of radioactivity was similar between the sexes within each dose group, although females from the single dose groups excreted slightly higher (1.3-1.8x) amounts of radioactivity in the urine than males from the same groups. The pattern of excretion was also similar between the dose groups. For the single low- and high-dose groups, male and female rats excreted 64.6-78.9% of the dose in the feces and 11.8-22.6% of the dose in the urine. Repeated dosing at 100 mg/kg/day had no effect on the pattern of excretion (urine, 13.0-16.6% dose; feces, 72.6-74.6% dose). Increasing the dose level delayed excretion slightly, but had no effect on overall excretion and repeated dosing resulted in only slightly lower (0.7x) renal excretion in females.

Maximum concentrations (C_{max}) of radioactivity in plasma were observed at 5 hours post-dose in the low-dose males, 10 hours post-dose in the low-dose females, and at 24 hours post-dose in high-dose males and females. Concentrations in plasma declined steadily thereafter in both sexes from both dose groups. Plasma C_{max} values were slightly higher (1.5x) in low-dose females (13.43 µg/mL) than males (9.23 µg/mL), but were similar for the high-dose males and females (34.2-36.0 µg/mL). AUC values for plasma were 300-451 µg·hr/mL for low-dose rats and 1739-2286 µg·hr/mL for high dose rats, an increase of 5.1-5.8x that approximated the 5-fold increase in dose level. At both dose levels females had slightly higher (1.3-1.5x) AUC values than males. Although increasing the dose proportionally increased the level of radioactivity in plasma, the half-life ($t_{½}$) for elimination of radioactivity from plasma was similar between sexes and dose levels

(54.3-62.6 hrs) indicating that elimination of radioactivity was not saturated at the higher dose level. At both dose levels, the $t_{½}$ was slightly longer (1.1x) for females than males. At 168 hours post-dose, radioactivity remaining in the carcass/tissues of both sexes accounted for 0.8-1.3% dose at the low-dose level, 0.7-1.0% dose at the high-dose level, and 0.4-0.6% dose in the repeated dose animals. Within each dose group, levels of radioactivity remaining in the carcass and tissues averaged 1.2-8.3x higher in females than males, with the largest differences occurring in the blood (2.0-2.3x), plasma (2.1-3.0x), fat (2.6-3.7x), and spleen (8.3x). The relative distribution of radioactivity between tissues were the same within each dose group; ^{14}C-residues were highest in liver, kidney, and fat and lowest in spleen in males, bone, and testes. Increasing the dose level from 100 to 500 mg/kg (5x) increased the concentration of radioactivity in tissues by 3.7x on average for both sexes, with the greatest increases occurring in fat (4.2-4.9x), testes (4.6x), plasma (4.1-4.6x), ovaries (4.8x), and uterus (5.3x). Compared to the single oral low-dose group, ^{14}C-residues were on average 0.6x lower in tissues of repeated low-dose animals, with the greatest relative decreases in the plasma (0.4x) and liver (0.3x) of males, and liver, fat, and spleen (0.4x each) of females.

^{14}C-Residues in tissues of males and females from the low and high-dose groups showed a steady decline from the time of maximum plasma concentrations (T_{max}) to 168 hours post-dose and the general distribution of ^{14}C-residues between tissues was similar at T_{max} and at 48 and 168 hours post-dose. At T_{max}, ^{14}C-residues were highest in the liver (males, 24.59-32.68 µg/g; females, 33.39-42.70 µg/g), fat (males, 15.46-25.42 µg/g; females, 16.37-49.09 µg/g), kidney (males, 13.29-24.38 µg/g; females, 14.36-28.56 µg/g), and plasma (males, 8.30-16.81 µg/g; females, 9.36-25.63 µg/g). Higher residues were also noted in the ovaries (14.31 µg/g) and uterus (9.57 µg/g) of high-dose females. Tissues with the lowest ^{14}C-residues were spleen, muscle, and brain (2.32-4.78 µg/g) in both dose groups. By 48 hours post-dose, ^{14}C-residues declined in all low-dose tissues and were 0.03-1.75 µg/g in males and 0.43-3.31 µg/g in females, except in liver from both sexes (4.37-7.13 µg/g) and in fat from females (6.76 µg/g). For the high-dose rats, ^{14}C-residues in tissues at 48 hours post-dose had declined to 0.80-11.33 µg/g in males and 0.55-9.26 µg/g in females, except in liver (17.89-21.29 µg/g) and fat (14.63-22.59 µg/g) from both sexes.

At both dose levels, concentrations of radioactivity were higher (1.2-3.9x) on average in females than males, with the largest differences at T_{max} occurring in the bone (2.3x), fat (1.9x), blood (1.8x), and spleen (1.7x), and at 48 hours post-dose in the fat (1.5-3.9x), muscle (2.6x), and plasma (2.5x).

For the single low- and high-dose groups, 84.4-93.8% of the administered dose was adequately characterized. In feces, the major fraction of radioactivity (Fraction A, 30.6-43.9% dose of all groups) was identified in low-dose males as being comprised almost totally of parent (33.2 % dose). Other compounds identified in the fecal extract of low-dose males included Metabolite #36 (N-butyl-N-ethyl-α,α,α-trifluro-5-nitro-p-toluene-3,4-diamine)(6.6% dose) and Metabolite #37 (N-butyl-N-ethyl-α,α,α-triflurotoluidine-3,4,5-triamine)(0.1% dose). All other individual unknown fecal components (approximately 100) isolated by TLC each accounted for <1% of the dose.

The majority of radioactivity in urine was recovered in Fraction H (4.3-7.6% dose) from low- and high-dose animals and in Fraction I for the low- and high-dose females (5.2-6.1% dose). The remaining 8 fractions each accounted for #3.7% of the dose. TLC analyses of urinary column fractions from the high-dose males detected approximately 100 unknown components, each accounting for #1.1% of the dose. Metabolite #3 (2,6-dinitro-4-trifluro-methyl-aniline) was the only metabolite identified in the urine (0.2% of dose), and only in the urine.

2. Mutagenicity:

The data base for mutagenicity is considered adequate to fulfill the pre-1991 guideline requirements. No mutagenic potential was seen in adequately conducted guideline mutagenicity studies on benfluralin. The literature indicates that trifluralin, a structural analog, was strongly mutagenic in plants (species unspecified), producing 3-4 fold 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 at insoluble doses ($ 300 Fg/plate +S9 and 750 Fg/plate -S9). No cytotoxicity was shown up to 5000 Fg/plate.

Guideline 870.5300, L5178Y TK+/- Mouse Lymphoma cell forward mutation MRID 00160866 Acceptable	Assay shows no dose related increase in mutation frequency up to severely cytotoxic doses (<10% cell survival)($30 Fg/mL -S9; $20 Fg/mL +S9).

Cytogenetics

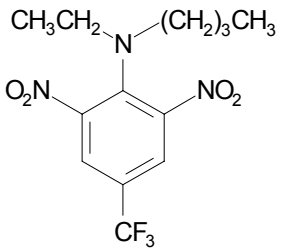
Guideline 870.5375, in vitro chromosomal aberrations in Chinese Hamster ovary (CHO) cells MRID 41031901 Acceptable	Assay shows no dose related increase in clastogenic activity (evidence of mutagenic potential) up to precipitating doses. Doses in DMSO (solvent) & up to 40 Fg/mL, -S9, and 125 Fg/mL, +S9,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

Other Genotoxicity

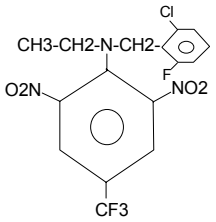
Guideline 870.5550, DNA repair in primary rat hepatocytes (UDS) MRID 00160865 Acceptable	No dose related increased number of net nuclear grains were seen (neg. for UDS). Doses up to 100 Fg/mL were tested with DMSO solvent. Precipitation occurred at $500 Fg/mL and cytotoxicity was seen at $50 Fg/mL.

3. <u>Structure-Activity Relationship</u>

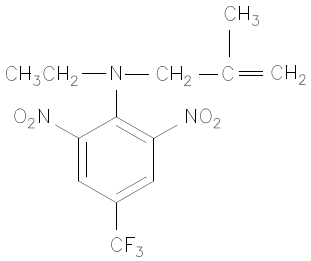
Benfluralin is structurally similar and its mode of action in plants is similar to trifluralin, ethalfluralin, flumetralin, profluralin, and oryzalin. These compounds are dinitro-,trifluro methyl-, alkylated anilines, and all differ only by the substituents on the aromatic amino group, except oryzalin. Oryzalin, a dinitroaniline compound, differs by having a sulfonoamido group instead of the trifluromethyl group in the para position. The structures were chosen for a common mechanism of action in the plants and/or common structural features and not for common tumors. A common characteristic among the analogs that they are weakly mutagenic or non-mutagenic. There is some commonality in tumor type, i.e., trifluralin, oryzalin and pendimethalin cause thyroid tumors in rats and profluralin causes hepatoma B tumors in male mice. Among the remaining analogs, two cause mammary adenomas and fibromas, one causes bladder and kidney tumors, one causes skin tumors, and one causes adrenal pheochromocytomas. However, trifluralin, ethalfluralin, oryzalin, flumetralin, pendimethalin are classified a “C” carcinogens. For some of these compounds, the human cancer risks were quantified using a linear low dose extrapolation approach (Q*) while an RfD approach was used for others. There is some evidence from the literature that oryzalin causes thyroid tumors by inhibiting the forma-tion of thyroxin resulting in a positive feedback in the pituitary and an increased thyroid stimulating hormone (TSH). No dose related tumors were produced in B6C3F1 mice. The structures are shown below.



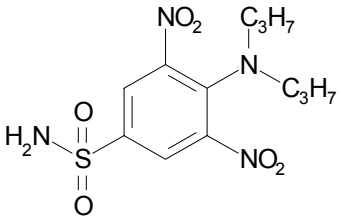
Benfluralin



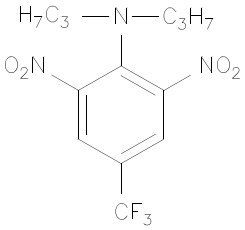
Trifluralin



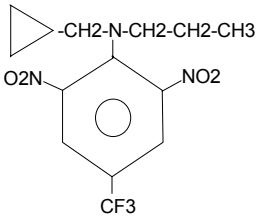
Ethalfluralin



Oryzalin



Flumetrin (Prime®)



Profuralin

Trifluralin (PC 036101)(N,N-dipropyl-α,α,α-trifluromethyl-2,6-dinitro-p-toluidine) causes thyroid tumors (follicular cell adenomas and carcinomas), neoplasms of the renal pelvis in male and benign urinary bladder tumors in female Fisher 344 rats. Trifluralin was classified as a group "C" carcinogen with a Q* of 7.7 E^{-3} $(mg/kg/day)^{-1}$ in human equivalents. It was not carcinogenic in mice. The mutagenicity studies conducted were negative. The literature indicates that Trifluralin was strongly mutagenic in plants (species unspecified), producing a 3-4 fold increase in spontaneous mitosis and chromosomal aberrations..

Ethalfluralin (PC 113101)[N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluromethyl) benzenamine] causes mammary gland adenomas and adenomas/fibroadenomas in females at two dose levels, and a suggestive evidence of bladder tumors in females and kidney tumors in male and female Fisher 344 rats. No treatment related tumors were seen in mice, but the doses were not adequate to make a

determination. Ethalfuralin is positive in two Salmonella assays, negative in a mouse lymphoma assay and unscheduled DNA Synthesis assay. However, it is positive for chromosomal aberrations with activation, but negative without activation. The mutagenicity studies support a carcinogenicity concern for ethalfluralin.

Oryzalin (PC 104201)[3,5-dinitro-N,N-dipropylsulfanilamide] causes thyroid and mammary tumors in male and female Fisher 344 rats. It was classified as a group "C" carcinogen with a Q* of 1.3 E^{-1} (mg/kg/day in human equivalents)$^{-1.}$ Oryzalin was not found to be mutagenic in tests for reverse gene mutation in the Ames test, and in a chromosomal aberration test (dominant lethal study in Wistar rats). It was negative for DNA synthesis (unscheduled DNA synthesis in primary rat hepatocyte cultures). It was both positive and negative for DNA damage/repair. It was positive for sister chromatid exchange by the intraperitoneal route in Chinese hamster bone marrow cells, but negative by the oral route.

For structure activity of oryzalin was compared to another para-substituted aniline, 2,4-diaminoanisole, which causes malignant tumors of the thyroid and skin of both rat sexes. There is some evidence from the literature that oryzalin causes thyroid tumors by inhibiting the formation of thyroxin resulting in a positive feedback in the pituitary and an increased thyroid stimulating hormone (TSH). No dose related tumors were produced in B6C3F1 mice.

Flumetralin (Prime®)(123001)[2-chloro-N-{(2,6-dinitro-4-(trifluromethyl)-phenyl}-N-ethyl-6-flurobenzenmethanamine] caused significant increasing trend for mammary adenocarcinoma and combined adenomas/adenocarcinomas in female rat studies at the 30 ppm dose level. Significant increase in adrenal pheochromocytomas in male rats at the 30 ppm dose level. It is not carcinogenic in mice. No mutagenicity was seen in one test system assayed. It was negative in the mouse micronucleus assay, but positive in an Ames test with and without activation.

Profluralin (PC 106601)[N-cylopropylmethyl-N-propyl-2,6-dinitro-4-(trifluromethyl) benzenamine] caused a significant ($p< 0.05$) increase in hepatoma B in male mice. It is not carcinogenic in rats. No genotoxicity studies are available for review. It has no registered uses in U.S.A .

The following dinitrotoluidines may be structurally less relevant to benfluralin. Fluchloralin's structure is relevant, but there is less information about its carcinogenic potenial.

Pendimethalin (PC108501) (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) is a dinitro alkyl substituted aniline, but it has a para and meta methyl groups instead of a para substituted trifluromethyl group (structure not shown). However, it causes thyroid follicular cell adenomas in male and female Sprague Dawley rats. It was classified a "C"

carcinogen with use of a RfD approach for quantification of human risk. The thyroid tumors were believed to cause disruption of the thyroid-pituitary hormone balance. No

mutagenicity was evident in most studies but pendamethalin showed equivocal mutagenicity in a Salmonella assay (positive in strains TA98 and TA1538 only).

Other structures that are relevant are **butralin** and **fluchloroalin**, but there is very little information on the carcinogenic potential of these pesticides. Butralin (PC 106501)[(N-sec-butyl-4-tert-butyl-2,6-dinitrobenzamine)] has a tertiarybutyl group instead of a trifluromethyl group at the para position. The carcinogenicity studies were not adequate for an assessment of the carcinogenic potential and mutagenicity studies gave positive and negative responses. Butralin is not used on food. Fluchloralin (PC 108701)[N-(2-chloroethyl)-α,α,α-trifluro-2,6-dinitro-N-propyl-p-toluidine] has different substituents on amino-group, which are n-propyl and 2-choloroethyl groups (No further information could be found and the structure is not available).

4) Subchronic/chronic Toxicity

a) **Subchronic Toxicity**

Rats

In three separate subchronic toxicity studies (MRID 44050001), benefin ($95.6% a.i., Lot #231EF4) was administered to rats in the diet at concentrations ranging from 0.005 to 0.75% for 3 months. In **Study #R33989**, 15 rats/sex/dose were fed benefin at dietary concentrations of 0, 0.025, 0.11 or 0.5% (0, 17, 74 or 341 mg/kg/day for males and 0, 20, 94 or 395 mg/kg/day for females) for 3 months. In **Study #R44089**, 15 rats/sex/dose were fed diets containing 0 or 0.75% benefin (0 or 522 mg/kg/day for males and 0 or 605 mg/kg/day for females) for 3 months. In **Study #R29990**, 15 males/dose were fed diets containing benefin at concentrations of 0, 0.005, 0.05 or 0.5% (0, 3, 32 or 322 mg/kg/day) for 3 months.

Concentration-dependent hepatic changes observed at $0.025% included increased absolute and relative liver weights and minimal to slight hepatocellular hypertrophy. The absolute and relative liver weights in both sexes were increased at the 0.025%, the 0.11%, and the 0.5%, and the 0.75% treatment levels. At 0.025, 0.11, 0.5 or 0.75%, mild diffuse centrilobular hypertrophy of liver was evident. Enlarged livers were noted in the 0.11% group males, in both sexes treated at 0.5%, and 0.75%. Hepatic microsomal enzyme activities were increased in both sexes treated at 0.11%, 0.5% or 0.75%. Hepatic lesions were reversible in females in the 0.75% treatment groups following a 6-week recovery phase. Concentration-dependent nephrotoxic effects included hyaline droplet formation at $0.005%; bilateral chronic nephrosis and/or cortical tubular epithelial pigment deposition at $0.025%; increased absolute and relative kidney weights at $0.11%, and increased urine volumes and enzyme activities at $0.5%. Absolute and/or relative kidney weights were increased in the 0.025% group females and in both sexes in the 0.11, 0.5, and 0.75% treatment groups. After 3 months of treatment, minimal cortical tubular

epithelial regeneration was observed in the 0.05% and the 0.5% males. In the 0.75%

treatment groups, kidney lesions were reversible in females following a 6-week recovery period. Increased urinary AST activities were observed in the 0.05% group males, in the 0.5% group males, and in the 0.75% males. The 0.5 and 0.75% group males also exhibited increased urinary LDH activities. In the 0.5% treatment groups, males exhibited increased total bilirubin, and females exhibited decreased A/G ratios and increased gamma glutamyl transferase (GGT) activities, and globulin. In the 0.75% treatment groups, males exhibited increased neutrophil counts, and females had decreased monocyte counts. Both sexes had increased total bilirubin, globulin, and cholesterol, and GGT activity was increased in females. Body weights were suppressed at 0.75%, and food consumption was depressed at $0.5%.

Under the conditions of these studies, the subchronic LOAEL for these studies is 3.23 mg/kg/day (0.005% benefin), based on hyaline droplet formation in the kidneys of males. A NOAEL was not established. These subchronic toxicity studies are acceptable and satisfy the guideline requirement for a subchronic oral toxicity study in rodents (§82-1a).

Mice

Benfluralin was administered in the diet to B6C3F$_1$ mice (15/sex/group) at 0, 0.01, 0.03, 0.10, 0.30 or 1.00% (0, 100, 300, 1000, 3000 or 10,000 ppm) (Males: 0, 13.5, 40.3, 132.8, 420.8 or 1364 mg/kg/day; Females: 0, 17.4, 51.1, 168.2, 506.5 or 1730 mg/kg/day) for 90 days. The study was conducted according to the guidelines for a 90-day dietary study. Clinical chemistry data were collected at the end of the dosing period, histopathological examination was conducted, organ weights were measured, and hepatic p-nitroanisole O-demethylase activity was determined. No urinalysis was conducted.

Body weights were periodically statistically significantly increased at the mid-dose levels, but no dose related body weight changes were seen at any dose level in males or females. Hepatic p-nitroanisole O-demethylase was statistically elevated at 1.0% dose level in males (by 44%)) and at 0.10, 0.30 and 1.00% in females (37%, 60% and 46%, respectively). In males, absolute liver weights were elevated 21% at 0.30% and 38% at 1.00%. In females, absolute liver weights were elevated 28% at 0.30% and 36% at 1.00%. In addition, the weight of uterus and ovary combined was significantly decreased 21% at 0.30% and 20% at 1.00%. Relative organ weights were changed at the same dose levels, except that there was a dose related relative liver weight increase in females at 0.03%, 0.10% and 1.00%.

The only histological findings seen were mild centrilobular hypertrophy in 14/15 males, multifocal necrosis in 2/15 males and nodular hyperplasia in 1/15 males, all at 1.00%. No histological findings were seen in the kidneys or the remaining urogenital tract of males. No dose related findings were seen in females.

Hematology showed slightlay lower hemoglobin at 1.00% and slightly lower PCV and MCV and slightly elevated MCHC in males at he 1.00% dose. Although all these values were statistically significant, only the lower level of hemoglobin may have been real. Females showed similar depressions in the same hematology parameters as the males, except MCHC was unchanged.

The clinical chemistry examination revealed that in males, BUN was elevated 81% at the 1.00% dose and alkaline phosphatase was elevated 30% at 0.30% and 42% at the 1.00% does level and alanine aminotransferase was elevated 119% at the 1.00% dose level. Females showed no significant elevations or depressions in any of the parameters studied.

The 90-day study in mice adds support to the BUN increase in the male mouse chronic studies and the plausibility of male mouse urologic syndrome at the HDT in the chronic mouse study. The study supported enzyme induction in the livers of female mice with a NOAEL/LOAEL= 0.01%/0.03%. The centrilobular hypertrophy in males and the enzyme induction in females are generally not considered adverse effects. However, the multifocal necrosis in 2/15 males and the nodular hyperplasia in 1/15 males and the 119% increase in alanine aminotransferase all at the HDT of 1364 mg/kg/day may have indicated the beginning of adverse effects in males. The alkaline phosphatase increase at two highest dose levels in males may have been from the liver. Thus, the NOAEL/LOAEL is 0.10%/0.30% or 132.8/420.8 mg/kg/day based on male alkaline phosphatase elevation, accompanied at 1364 mg/kg/day by alanine aminotransferase elevation and few (3/15 versus 0/15 in control) animals with liver pathology.

b) Chronic Toxicity

Rats

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 & Supplement 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.

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). The study is acceptable for a Guideline study 870.4300 (83-5).

It was noted that the skeletal muscle degeneration and sciatic nerve degeneration occurring a 2500 and 5000 ppm was secondary to the nerve lesions (MRID# 44545501; Supplement). These age related nerve lesions were not a neurotoxic effect since they were not accompanied by axonopathy. It is noted that the skeletal muscle degeneration was well above historical control data (no historical control data were submitted for sciatic nerve degeneration) and the 43% to 73% incidence in both effects at 2500 and 5000 ppm was also well above concurrent controls at 0% incidence. Thus these effects were treatment related. This study is classified as Acceptable and satisfies the guideline requirements [§83-5] for a combined chronic and carcinogenicity study in rats.

Mice

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, 6.0/7, 36.4/42, and 185/224 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.

The decreases in body weight (-8%) and body weight gain (-11%) in females in the highest dose group were statistically and toxicologically significant.

Male mice had a nominal increase in mortality 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 multifocal 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 multifocal hepatocellular hyperplasia in males at the highest dose level, no other indications of toxicity in male mice were seen. Based on the weight of evidence from all these nominally increased signs of toxicity the LOAEL was 0.15%. At 0.03%,

increases in absolute liver weights (819%), relative to body (826%), and relative to brain (821.9%) 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 (8276%; p#0.05); an increase in alkaline phosphatase (832%; p#0.05) after exclusion of one outlier from the control animals; increases (p#0.05) in absolute, relative to body, and relative to brain liver weights (821.2, 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 multifocal hyperplasia (6/59 treated vs 1/60 controls). In addition to these liver changes, overall body weight gain was decreased (911%; p#0.05) in the females. In the males, only minimal increases were observed in the liver weights (85.2, 9.6, and 4.8%, respectively; (p#0.05)) and the incidence of slight to moderate multifocal hyperplasia was minimally increased (7/60 treated vs 1/60 controls). Gross and microscopic findings such as increased liver nodules and hypertrophy were observed, demonstrating that the compound affected the morphology and growth of hepatocytes.

The LOAEL is 0.03% for females (equivalent to 42 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 185 mg/kg/day) based on the weight of slight toxic evidence 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 satisfies the guideline requirements for a carcinogenicity study in mice.

Dog

In this chronic toxicity study, male and female beagle dogs (4/sex/group) were dosed (capsule) with Benefin (95.8%) at 0, 5, 25, or 125 mg/kg body weight/day for one year. With the exception of one high-dose female, which was sacrificed in moribund condition, all animals survived to terminal sacrifice without any adverse clinical signs. The mean body weights, food consumption, hematology, urinalysis, gross pathology, and organ weights of treated animals were comparable to control values.

ALT activities of mid- and high-dose females and high-dose males were significantly decreased. Although this effect appears to be real, the toxicological and biological significance of the effect is not clear. Histopathology observations at terminal sacrifice revealed an increase in the incidence of sinusoidal cell pigment in the livers of mid- and high-dose females and high-dose males. Based on these findings (liver histopathology), LOAELs of 125 mg/kg/day in males and 25 mg/kg/day in females were established and the NOAELs were 25 mg/kg/day in males and 5 mg/kg/day in females. This study is classified as Acceptable and satisfies the guideline requirements [§83-1(b)] for a chronic oral study in dogs.

5. Mode of Action Studies

No mode of action studies were submitted. However, hepatic microsomal enzyme activity (as measured by p-nitroanisole O-demethylase activity) was shown to be significantly elevated at 79 and 341 mg/kg/day in males and at 94 an 395 mg/kg/day in female rats. The enzyme activity was conducted on liver sections taken at termination of the 90-day subchronic study. This would suggest that mixed function oxidases were in induced in the livers of rats at about the same and lower dose levels than those showing hepatocellular and thyroid adenomas and carcinomas in the chronic/carcinogenicity rat study at 125 an 250 mg/kg/day.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The Committee concluded that benfluralin caused liver tumors in female B6C3F$_1$ mice. Although there was <u>some</u> evidence of an increase in liver tumors in male F-344 rats and thyroid follicular cell tumors in male and female F-344 rats, these tumors occurred at excessively toxic doses.

- **F-344 male rats** had significant increasing trends (p<0.01), and significant differences (p<0.01) in pair-wise comparisons of the 5000 ppm (275 mg/kg/day) dose group with the controls, for liver adenomas (9/46; 20%; p< 0.01 vs 1/46; 2% in controls) and combined adenomas/carcinomas 11/46; 24%; p< 0.01 vs 2/46; 4% in controls). The incidence of liver adenomas (20%) and combined adenomas/carcinomas at 5000 ppm (24%) was higher than the historical control data (range: 3%-6% and 3%-8%, respectively). The increase in liver carcinomas in males at 2500 and 5000 ppm (2/38, 5% and 2/39, 5%, respectively, vs 1/44, 2% in controls) was not statistically significant, but was slightly outside the historical control range (0%-4%).

  There were significant increasing trends (p<0.01) for thyroid follicular cell adenomas, carcinomas, and combined adenomas/carcinomas in males. There were also significant differences(p<0.05) in pair-wise comparisons of the $2500 ppm ($136 mg/kg/day) dose groups with the controls for thyroid follicular cell carcinomas and combined thyroid follicular cell adenomas/carcinomas. The incidences in the 2500 and 5000 ppm dose groups, respectively, were as follows:

  i) <u>adenomas:</u> 3/46, 7% and 5/46, 11% p<0.05 vs 1/46, 2% in controls;
  ii) <u>carcinomas:</u> 4/39, 10% , p<0.05, and 3/39, 8%, p<0.01 vs 0/44, 0% in controls; and
  iii) <u>combined adenomas/carcinomas</u>: 7/46, 15%, p<0.05, and 8/46, 17%, p<0.01, vs 1/46, 2% in controls

  There were no treatment related increases in liver tumors in female rats. **The CARC concluded that the liver tumors in males occurred at a dose that was excessively toxic.** Female rats had significant increasing trends for thyroid follicular cell adenomas (p<0.05), and combined adenomas/carcinomas (p<0.01).

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 (5/50, 10%, $p<0.05$ vs 0/49, 0% in controls). The incidence of combined thyroid follicular cell adenomas/carcinomas (4/49, 8% vs 0/49, 0% in controls) although not statistically significant at 5000 ppm (331 mg/kg/day), was considered by the CARC to be biologically significant and shared similar pattern as in males. The incidences of thyroid follicular cell adenomas (at 2500 and 5000 ppm; 6% and 4%, respectively), carcinomas (at 100, 2500 and 5000 ppm or 7 , 41.8 and 224 mg/kg/day, respectively; 2%, 4% and 4%, respectively) and combined adenomas/carcinomas (at 2500 and 5000 ppm;10% and 8%, respectively) exceeded the corresponding historical control range (adenomas: 0%-2.9%; carcinomas: 0%-1.4%) and combined adenomas/carcinomas: 0%-4%). **Therefore, the Committee determined that there was <u>some evidence</u> of an increase in thyroid follicular cell tumors in both males and females. However, these tumors in males and females occurred at excessively toxic doses and the increase in thyroid tumors in females was statistically significant at the mid dose but was only biologically significant (statistically not significant) at the highest dose.** The dosing at 100 ppm was considered to be adequate based on decrease in body weight and body weight gain and increased kidney hyaline droplets in both sexes. However, the dosing at $2500 ppm was excessive based on increased incidence and severity of histopathological lesions (liver hyperplasia and necrosis, sciatic nerve and skeletal muscle degeneration, kidney hyaline droplets as well as thyroid hypertrophy/hyperplasia) in both sexes.

- **In B6C3F$_1$ female mice,** there was a statistically significant increasing trend ($p=0.0353$) and a borderline significant increase ($p= 0.0488$) by pair-wise comparisons of the 224 mg/kg/day dose group with the controls for combined liver adenomas/carcinomas 6/55, 11% vs 1/58, 2% in controls). The incidence of these tumors (11%) was outside the range for the historical controls (0%-6.9%). Although the incidence of adenomas (5%) in females exceeded the historical control range (0%-3.4%), <u>neither the number of adenomas nor carcinomas in the present study were statistically significantly increased</u>. The dosing at the highest dose for females was considered to be adequate and not excessive based on decrease in body weight gain, increased liver enzyme levels, increased incidence of liver nodules, liver 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.

2. Mutagenicity

Benfluralin has been tested in an adequate battery of pre-1991 mutagenicity assays which satisfies the guideline requirements. All the *in vitro* mutagenicity studies were negative, including the *Salmonella typhimurium* gene mutation assay, mouse lymphoma cell forward gene mutation test, Chinese Hamster Ovary (CHO) cell cytogenetic assay and DNA repair assay in primary rat hepocytes.

3. Structure Activity Relationship

Benfluralin is a structurally related to the dinitro, trifluromethyl, alkylated pesticides, one of which (trifluralin) has the potential to be a carcinogen, but it is mutagenic in studies submitted to the Agency. The literature indicates, however, that trifluralin (a structural analog) was strongly mutagenic in plants (species unspecified), producing 3-4 fold increase in spontaneous mitosis and chromosomal aberrations (Micromedex 1974-1998). Other analogs cause thyroid tumors, mammary tumors, kidney tumors and urinary bladder tumors in rats with little or no mutagenicity concern. Most of the analogs (trifluralin, ethalfluralin, oryzalin, flumetralin) are not carcinogenic in mice, however, profluralin caused an increase in hepatoma B in male mice. Trifluralin, ethafluralin, oryzalin, flumetralin and pendamethalin were classified as group "C" carcinogens.

4. Mode of Action

No mode of action studies were conducted with benfluralin.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the Agency's *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee 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.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended that the quantification of human cancer risk is not required.

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