014304

DATA EVALUATION RECORD

BENFLURALIN (BENEFIN)

Study Type: §83-2 (b) Oncogenicity Study in Mice

Work Assignment No. 2-01-69A (MRID 41021501)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/30/00

Secondary Reviewer
Guy R. Beretich, Ph.D.

Signature:
Date: 6/30/00

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 6/30/00

Quality Assurance
Steve Brecher, Ph.D.

Signature:
Date: 6/30/00

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

HED reviewer: David G Anderson, PhD David G Anderson 8/7/2000
Reregistration Branch 2/HED (7509C)

Work Assignment Manager: Sanjivani Diwan, Ph.D. Sanjivani Diwan 8/8/2000
Reregistration Branch 4/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Study in Mice
OPPTS Number: 870.4200 OPP Guideline Number: §83-2b

DP Barcode: D268004 SUBMISSION: S5883395
P.C. CODE: 084301 TOX. CHEM. NO.: 130

TEST MATERIAL (PURITY): Benfluralin (95.25% a.i.)

SYNONYMS: Benefin; EL-110; Compound 54521; N-(n-butyl)-N-ethyl-2,6-dinitro-∝,∝,∝, trifluoro-p-toluidine

CITATION: 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 Unpublished.

SPONSOR: Lilly Research Laboratories, Greenfield, IN

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 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. 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 multifocal 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 multifocal 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 (1.8% each vs 0% 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 (6.8% treated vs 1.7% 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.

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 of slight toxic evidence observed in the males. The NOAEL for males is 0.03% (equivalent to 36.4 mg/kg/day).

Under the conditions of this study, the carcinogenic potential of benfluralin is equivocal.

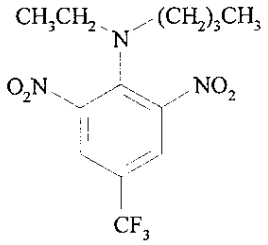
The submitted study is classified as **acceptable (§83-2b)** and does satisfy the guideline requirements for a carcinogenicity study in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Benfluralin (Benefin)
   Description: Not provided
   Lot/Batch #: 231EF4
   Purity (w/w): 95.25% a.i. (nitrosamine content at termination of the current studies was reported to be 0.11 ppm, page 28 of MRID# 41021501)
   Stability of compound: The test substance was stable in the diet for up to 4 weeks stored at 25 or 37°C
   CAS #: 1861-40-1 (from MRID 44050001)
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Mouse
   Strain: B6C3F1/Crl
   Age and mean weight at start of dosing: 6-7 weeks old; For the M02785 study, 20.2±1.8 g (males), 16.7±1.3 g (females). For the M02885 study, 19.7±1.6 g (males), 17.0±1.1 g (females)
   Source: Charles River Laboratories, Inc., Wilmington, MA
   Housing: Three/cage in stainless steel cages with wire mesh floors
   Diet: Standard mash diet (Purina Certified Rodent Chow No. 5002), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 22.2±1.6°C
   Humidity: ≥40%
   Air changes: Information not provided
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: 7 days

B. STUDY DESIGN:

1. In life dates: start: 6/10/85 (M02785) and 6/25/85 (M02885)
   end: 6/15/87 (M02785) and 6/26/87 (M02885)

2. Animal assignment: The mice were randomly assigned (stratified by weight) to the test

groups shown in Table 1.
Table 1. Study design [a]

Test Group	Dietary Concentration (%)	Mean Achieved Dose (mg/kg/day)[b] (M/F)	Number of Animals	
			Males	Females
Control	0	0	60	60
Low	0.005	6.0/6.9	60	60
Mid	0.03	36.4/41.8	60	60
High	0.15	184.7/223.5	60	60

a Data obtained from the study report, page 17.
b Achieved doses obtained from the study report, Table 14, page 167.

3. Dose selection rationale - The doses chosen for the current study were based on the results of a 3-month study in which benfluralin was administered to $B6C3F_1$ mice at dietary concentrations of 0.01, 0.03, 0.1, 0.3 or 1.0% (equivalent to approximately 15, 46, 150, 464, or 1547 mg/kg/day in males and females). Increases in alkaline phosphatase and alanine transaminase were observed in the 0.3 and 1.0% males. Increases in p-nitoanalsole O-demethylase activity were observed in both sexes at 1.0% and in the 0.1 and 0.3% females. Absolute and relative liver (to body) weights were increased in the 0.3 and 1.0% males and females and increased relative liver weights were observed in the 0.03 and 0.1% females. An increased incidence of centrolobular hepatocellular hypertrophy was observed in the males at ≥0.01%.

   Based upon the results of this 3-month study, the doses summarized in Table 1 were selected for the oncogenicity study.

4. Dose preparation, administration, and analysis - The appropriate amount of test substance was mixed with the diet. The formulations were prepared every two weeks and stored at room temperature prior to use. Prior to the study, homogeneity and stability were determined. Homogeneity was determined on samples from the 0.005 and 0.01% formulations. Stability was determined at concentrations of 0.01% over a period of up to 4 weeks at 25 or 37° C. Concentration analyses were performed on the 0.005, 0.03, and 0.15% formulations from samples collected at the start of the study and every four months throughout the study.

   Results:
   Homogeneity (range as mean % of nominal): 98.0-98.6%
   Stability (range as mean % of day 0):

87.3% after 4 weeks of storage at 25° C.
101% after 4 weeks of storage at 37° C.
Concentration (range as mean % of nominal): 86.7-104%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

5. Statistics - The Dunnett's test was applied to the body weight and body weight gain data. The Bartlett test followed by the Kruskal-Wallis test were applied to the hematology, blood chemistry, and organ weight data. The Cochran-Armitage and Peto tests were applied to the neoplastic findings.

C. METHODS:

1. Observations - Changes in clinical condition or behavior were recorded daily. Detailed clinical observations were recorded weekly.

2. Body weight - All animals were weighed weekly up to week 14, then every other week until study termination. Body weights were also recorded at scheduled termination. Weekly and overall body weight gains were also reported.

3. Food consumption and efficiency - Food consumption in three cages/sex/dose was calculated (g/mouse/day) for a two week interval at 1, 3, 6, 12, 18, and 24 months. Cages containing three mice were used when possible. Information concerning food utilization was not provided.

4. Blood analyses - Blood from non-fasting control and high-dose animals (10/sex/dose) was collected via the tail on study days 367 (M02885) and 382 (M02785) and 538 (M02885) and 548 (M02785) and hematological parameters determined. At scheduled termination (study days 724-735), all surviving animals were fasted overnight and bled from the orbital sinus for determination of hematological parameters; blood samples from these animals was also obtained by cardiac puncture to determine clinical chemistry parameters. The checked (X) hematological and clinical chemistry parameters below were examined:

a. Hematology:

X	Hematocrit (HCT)	X	Leukocyte-total and differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
	Leukocyte count (WBC)	X	Mean corpuscular HGB conc.(MCHC)
	Corrected leukocyte count (Cor WBC)	X	Mean corpuscular volume (MCV)
X	Erythrocyte count (RBC)		Reticulocyte count
	Platelet count	X	Erythrocyte morphology
	Blood clotting measurements		Packed cell volume
	(Prothrombin time)		
	(Activated partial thromboplastin time)		

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
	Calcium		Albumin
	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Phosphate		Total Cholesterol
	Potassium		Globulins
	Sodium	X	Glucose
		X	Total bilirubin
	ENZYMES		Total serum protein
X	Alkaline phosphatase (AP)		Triglycerides
	Plasma cholinesterase (PL-ChE)		Albumin/globulin ratio
	Erythrocyte cholinesterase (RBC-CHE)		
	Brain cholinesterase (BR-CHE)		
	Creatine phosphokinase		
	Lactate dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT)		
	Serum aspartate aminotransferase (AST)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GLDH)		

7. Sacrifice and Pathology - All animals were subjected to a gross pathological examination. The following checked (X) tissues were collected from all animals sacrificed at scheduled termination, animals that died prematurely, and animals sacrificed *in extremis;* the spinal cord and sciatic nerve were collected only from the animals that were sacrificed at termination; all tissues were examined microscopically. Additionally, the (XX) organs were weighed at the scheduled termination.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT		NEUROLOGIC
	Tongue	X	Aorta	X	Brain (3 levels)
X	Salivary glands	X	Heart	X	Periph. nerve [a]
X	Esophagus	X	Bone marrow	X	Spinal cord [a]
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus	X	_____
X	Ileum	X	_____		GLANDULAR
X	Cecum	X	UROGENITAL		Adrenal gland
X	Colon		Kidneys	X	Lacrimal gland
	Rectum		Urinary bladder		Mammary gland
X	Liver	X	Testes	X	Parathyroids
X	Pancreas	X	Epididymides	X	Thyroids
X	Gall bladder	X	Prostate	X	_____
	_____	X	Seminal vesicle		OTHER
	RESPIRATORY	X	Ovaries		Bone
	Trachea		Cervix	X	Skeletal muscle
X	Lung	X	Uterus	X	Skin
X	Nose			X	All gross lesions and
	Pharynx	X		X	masses
	Larynx	X			Preputial gland
					Harderian gland
		X			Nasopharyngeal cavity
		X			Oral cavity

a Spinal cord and sciatic nerve obtained only from animals killed at termination.

II. RESULTS

A. Observations:

1. Toxicity - No adverse treatment-related clinical signs were observed. Very bright yellow urine was observed in all of the high-dose animals starting on study weeks 5-7. During the last two months of the study, a yellow urine stain was observed in the genital area of all high-dose males. Although these effects are considered to be treatment-related, they are not adverse.

2. Mortality - No differences in mortalities were observed in either sex of the treated groups throughout the study when compared to the respective control groups. Percentage survival in all dose groups of mice after two years was 75.0-86.7%.

B. Body weight:

Overall body weight gain was minimally decreased (↓11%; $p \leq 0.05$) in the high-dose females.

No adverse treatment-related differences in body weight or body weight gain were observed in the other treated groups when compared to the respective control groups. Decreases ($p \leq 0.05$ or 0.01) in body weight were observed in the low- (↓4-7%), mid-(↓4-6%), and high-dose males (↓3-8%) and high-dose females (↓2-10%) (Table 2a). Decreases ($p \leq 0.05$ or 0.01) in body weight gain were observed in the low- (↓7-10%), mid-(↓7-12%), and high-dose males (↓7-15%) and mid-(↓8-13%) and high-dose females (↓9-16%) (Table 2b). The decreases in body weight and body weight gain of males and females appeared to be consistently greater in the highest dose groups than in controls, and especially between weeks 44 and 62 in males and between 52 and 102 in females and at termination. All dosed groups appear to be affected. These differences from controls in body weight and body weight gain were intermittent, only weakly dose-dependent during some weeks, and/or minor and were therefore considered to be possibly treatment-related in males in the highest dose group. Neither the contract reviewer nor the study authors believed the statistically significant effects on body weight of dosed groups were adverse.

This reviewer believes that females showed a significant treatment related decreased body weight and body weight gain. In the highest dose group in females, the decreased body weight (-8%) and body weight gain (-11%) was treatment related and statistically significant throughout most of the study. Furthermore no decrement in food consumption throughout the study was noted in males or females. Male body weights and weight gains in the highest dose group could be considered a treatment related effect only in conjunction with other toxic effects at the same dose level.

Table 2a. Mean body weight (g) at selected intervals in mice fed benfluralin for up to two years.[a]

Week	Dose (%)			
	0	0.005	0.03	0.15
	Males			
0	20.1	19.5	20.2	20.0
1	22.8	21.9**(↓4)	22.6	22.7
22	37.2	35.4**(↓5)	36.5	35.9*(↓3)
30	39.7	37.4**(↓6)	38.0*(↓4)	37.9**(↓5)
44	42.5	39.7**(↓7)	39.9**(↓6)	39.0**(↓8)
52	43.2	41.1*(↓5)	41.1*(↓5)	40.2**(↓7)
62	44.6	42.1**(↓6)	42.0**(↓6)	41.2**(↓8)
104	41.3	40.5	41.6	39.4
	Females			
0	17.0	17.0	16.7	16.7
1	18.8	18.4	18.4	18.4
3	21.5	21.0	20.9*(↓3)	20.8*(↓3)
9	24.8	24.5	24.4	24.2*(↓2)
52	35.9	35.5	34.4	33.2**(↓8)
56	36.8	35.6	35.1	33.3**(↓10)
102	38.6	37.1	35.7*(↓8)	35.7*(↓8)
104	37.5	36.8	35.7	35.4

a Numbers listed parenthetically represent the percent difference from controls. These data were obtained from Tables 5, 8.1, and 8.2 in the study report, pages 65, and 68 through 99.

* or ** Significantly different from controls $p \leq 0.05$ or 0.01, respectively.

Table 2b. Mean weekly body weight gains (g) at selected intervals in mice fed benfluralin for up to two years.[a]

Week	Dose (%)			
	0	0.005	0.03	0.15
	Males			
1	2.7	2.4	2.4	2.7
18	15.3	14.3*(↓7)	14.8	14.1*(↓8)
22	17.1	15.9*(↓7)	16.2	15.9*(↓7)
26	18.5	16.8**(↓9)	16.3**(↓12)	16.5**(↓11)
44	22.4	20.1**(↓10)	19.8**(↓12)	19.0**(↓15)
52	23.1	21.5*(↓7)	20.9**(↓10)	20.2**(↓13)
64	24.4	22.7*(↓7)	21.6**(↓11)	21.5**(↓12)
74	24.1	23.4	22.3*(↓7)	22.2*(↓8)
104	21.5	20.9	21.3	19.3
Overall body weight gain	21.1	20.8	20.9	19.2
	Females			
1	1.9	1.5*(↓21)	1.6	1.7
3	4.5	4.0*(↓11)	4.2	4.1
26	13.1	12.3	12.0	11.9*(↓9)
30	14.4	13.4	13.2*(↓8)	12.5**(↓13)
46	18.1	17.2	16.3*(↓10)	15.2**(↓16)
52	18.9	18.6	17.6	16.4**(↓13)
102	21.6	20.1	18.9*(↓13)	19.0*(↓12)
104	20.7	19.9	19.0	18.7
Overall body weight gain	20.7	19.7	18.7	18.4*(↓11)

a Numbers listed parenthetically represent the percent difference from controls. These data were obtained from Tables 5, 8.1, and 8.2 in the study report, pages 65, and 68 through 99.

* or ** Significantly different from controls $p \leq 0.05$ or 0.01, respectively.

C. Food consumption:

No treatment-related effects were observed in food consumption after 24 months of treatment with benfluralin.

E. Blood analyses:

1. Hematology - No treatment-related differences from concurrent controls were observed in any hematological parameter. Leucocyte counts were decreased (↓28%; $p \leq 0.05$) in the high-dose males at the 12 month interval and eosinophil counts were increased (↑126%, $p \leq 0.05$) in the high-dose females at termination. These findings were considered incidental since they occurred at only one interval. At the 12 month interval in the high-dose animals, monocyte counts were increased ($p \leq 0.05$) in the males (↑1100%) and the females (↑600%) and were decreased ($p \leq 0.05$) at the 18 month interval in both sexes (↓85-86%); these inconsistent differences from controls were considered not to be treatment-related. Other minor differences (<8%; $p \leq 0.05$) from controls, such as hemoglobin, packed cell volume, and red blood cell count were also considered to be not of toxicological concern.

2. Clinical chemistry - An increase was observed at termination in the high-dose females in the levels of alanine aminotransferase (ALT) (↑276%; $p \leq 0.05$) (Table 3). An increase in alkaline phosphatase (ALP) (↑32%; $p \leq 0.05$) was also observed in this group after exclusion of one outlier from the control animals. Additionally, blood urea nitrogen was increased ($p \leq 0.05$) in the mid- and high-dose females compared to controls (↑41 and 15%, respectively); because these changes were not dose-dependent, they were considered to be unrelated to treatment.

Table 3. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) values (mean ± s.d.; IU/L) observed at termination in high-dose female mice fed benfluralin for up to two years.[a]

Clinical Chemistry Parameter (IU/L)	Dietary Level (%)			
	0	0.005	0.03	0.15
ALT	25 ±14	26±24 [b]	49±74	94±182*(↑276)
ALP	405±225 [b]	387±198	461±258	535±260*(↑32)

a Numbers listed parenthetically represent the percent difference from controls. These data were extracted from the study report, Table 33.2, page 205.

b Means and standard deviation were calculated by the reviewers after exclusion of 1 outlier for each parameter. Data were obtained from Tables 70.1 and 70.2, pages 533, 534, 541, and 542.

* Significantly different from controls at $p \leq 0.05$.

G. Sacrifice and pathology:

1. Organ weights - At the high-dose, increases ($p \leq 0.05$) in absolute, relative to body, and relative to brain liver weights were observed in the females (↑21.2, 30.6, and 22.2%, respectively); minimal increases were observed in the high-dose males (↑5.2, 9.6, and 4.8%, respectively) (Table 4). In the mid-dose females, increases in absolute (↑19%), relative to body (↑26%) and relative to brain (↑21.9%) liver weights were observed, but the differences were not statistically significant.

   The following differences ($p \leq 0.05$) from controls were considered not to be treatment-related because they were minor, not dose-dependent, and/or there were no corroborating histopathological or gross pathological data: (I) in the high-dose males, increased absolute and relative to body heart weight (↑6 and 9%, respectively); (ii) in the 0.003 and 0.15% females, increased absolute kidney weight (↑8% each); and (iii) in the 0.15% females, increased relative brain weight (↑6%). No differences from controls were observed in organ weights in the low-dose males and females.

   Brain weight was unaffected in males and females.

Table 4. Mean liver weights (g) in mice fed benfluralin for up to 2 years.[a]

Liver Weight	Males				Females			
	Dietary Level (%)							
	0	0.005	0.03	0.15	0	0.005	0.03	0.15
Absolute	1.823	1.650	1.783	1.917* (↑5.2)	1.564	1.578	1.867	1.895* (↑21.2)
Relative to body	453.0	417.5	437.8	496.3* (↑9.6)	417.7	434.9	528.0	545.5*(↑30.6)
Relative to brain	3.934	3.568	3.828	4.124* (↑4.8)	3.222	3.247	3.928	3.937*(↑22.2)
Terminal body weight	41.0	40.3	41.2	39.2	37.7	36.6	35.5	35.1*(↓7)
Terminal brain weight	466.0	464.5	466.9	465.3	485.7	486.2	482.7	481.1

a Data were obtained from study report Tables 36.1, 39.1. and 42.1, pages 210 and 211, 216 and 217, and 222 and 223; n=45-52. Percent difference from controls listed parenthetically.

* Significantly different from controls at $p \leq 0.05$.

2. Gross pathology - In the females, an increased incidence of liver nodules was observed in the high-dose group (25/59 treated vs 7/60 controls) (Table 5); this finding was significantly different from controls at $p<0.0005$ as analyzed by the reviewers using the Fisher's Exact test. The incidence of liver nodules was slightly elevated in the mid-dose females (12/60 treated) and similar to controls in the low-dose females and in all of the dosed males. There were no other macroscopic finding of toxicological concern.

Table 5. 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
# 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.

3. Microscopic pathology:

a) Non-neoplastic: When all animals were combined including those sacrificed on schedule and those found dead or sacrificed *in extremis*, an increased incidence of minimal to moderate focal hyperplasia (20/59 treated vs 6/60 controls) and slight to moderate multifocal hyperplasia (6/59 treated vs 1/60 controls) were observed in the

liver of high-dose females (Table 6). Slight to moderate multifocal hyperplasia was increased in the high-dose males (7/60 treated vs 1/60 controls).

In the males, dose-dependent decreases in the incidence of renal tubular epithelial cytoplasmic vacuolization (31, 23, 11, 2, in the control, low-, mid- and high-dose, respectively; n=60) and cortical tubular epithelial regeneration (20, 15, 4, 3, in the control, low-, mid- and high-dose, respectively; n=60) were observed. The decreases in kidney lipid deposition may be related to the intermittent decreases in body weight in these animals. In addition, an increased incidence of obstructive uropathy was observed in the high-dose males (18 treated vs 5 controls)(Table 7). In the mice that died, the urologic pathology was reported as a cause of death more frequently in the high-dose males than in the controls (7 treated vs 2 controls)(Table 7). This urologic syndrome is a common cause of death in male $B6C3F_1$ mice. It appears that the lesions in the kidneys of the dosed mice may be an indirect toxic effect (i.e. added stress) of treatment with benfluralin.

Table 6. 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
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.

Table 7: Urologic pathology in male mice[a]				
Finding/Dose level	0%	0.005%	0.03%	0.15%
Obstructive urologic syndrome, all severities	5/60	13/60	12/60	18/60
Death by urologic syndrome	2/60	2/60	4/60	7/60

[a] = Data obtained from pages 308 & 310 of Table 49, and 316-318 of Table 50 from MRID# 4021501.

b) Neoplastic: No treatment-related neoplastic changes were observed. An increased incidence of hepatocellular adenoma was observed in the high-dose females compared to controls (5.1% treated vs 1.7% controls) (Table 8); the incidence was outside of the historical control range (0-3.4%); however, this is a non-malignant lesion. An increased incidence of hepatocellular carcinoma was observed in the mid-and high-dose females (5.1% each vs 0% controls), but the incidence was within historical control range (0-6.9%). There was also an increase incidence of combined hepatocellular adenomas and carcinomas in the high-dose females (10.2% treated vs 1.7% controls) that was beyond the historical control range (0-6.9%), but the difference ($p \leq 0.018$) was not considered statistically significant by the sponsor stating that $p<0.01$ is the currently accepted threshold for common tumors.

Mesothelioma of the mediastinum and mesentery was observed in two separate low-dose males, but there were no tumors of these types observed in the higher doses and therefore these findings are considered not treatment-related.

Table 8. Incidence (# of animals) of hepatocellular adenomas and carcinomas in female mice dosed with benfluralin for up to 2 years. [a]

Observation	Dose (%)				Historical controls (%)
	0	0.005	0.03	0.15	
Males					
Hepatocellular adenoma	2 (3.3)	1 (1.7)	3 (5.0)	5 (8.3)	NR
Hepatocellular carcinoma	1 (1.7)	1 (1.7)	1(1.7)	2(3.3)	NR
Total	3 (5.0)	2 (3.3)	4 (6.7)	7 (11.7)	NR
Females					
Hepatocellular adenoma	1 (1.7)	1 (1.7)	1 (1.7)	3 (5.1)	0-3.4
Hepatocellular carcinoma	0	1 (1.7)	1 (1.7)	1 (1.8)	0-6.9
Total	1 (1.7)	2 (5.1)	4 (6.7)	4 (6.8)*	0-6.9

a Data were obtained from study report Tables 52 and 53, pages 334 and 341; n=60 for control, low-dose and mid-dose males and females, and 60 & 59 for the high-dose males & females, respectively. Percent incidence listed parenthetically. NR = Historical control data for males not reported. Historical control data for females extracted from Appendix I, Table 11, page 427 of study report. * $p = 0.018$ for onset and $p = 0.027$ for prevalence by Peto's Trend Test.

III. DISCUSSION

A. Investigators conclusions - Treatment with benfluralin for up to two years had no affect on survival. An increased incidence of hepatocellular hyperplasia, increased levels of alanine transaminase and alkaline phosphatase, and increased liver weights were observed in the high-dose females. Increased liver weights and increased incidence of mouse urologic

syndrome were observed in the high-dose males. A decreased incidence of renal tubular epithelial vacuolization in the mid- and high-dose males was observed. It was concluded that there was no evidence of carcinogenicity in mice of both sexes; the NOAEL was 0.005% and the LOAEL was 0.03%.

B. Reviewer's discussion/conclusions - In this mouse oncogenicity study (MRID 41021501), benfluralin (95.25% a.i., Lot/Batch # 231EF4) was administered in the diet to B6C3F$_1$ 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/sex/group mice were dosed as stated above. Dietary analyses at select study intervals confirmed that nominal diet concentrations of benfluralin were achieved.

Mortality, clinical signs, food consumption, and hematology findings for both sexes at all doses were unaffected by treatment with benfluralin.

At 0.03%, toxicity was observed in the liver of females as follows: non-significant increases in absolute (↑19%), relative to body (↑26%), and relative to brain (↑21.9%) liver weights were observed; the incidence of liver nodules was slightly elevated (12/60 treated vs 7/60 controls).

At 0.15%, toxicity was observed in the liver of females as follows: an increase at termination in the levels of ALT (↑276%; $p \leq 0.05$); an increase in ALP (↑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 multifocal hyperplasia (6/59 treated vs 1/60 controls). In addition to these liver changes, overall body weight gain was minimally 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) and slight to moderate multifocal 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%). An increased incidence of hepatocellular carcinoma was observed in the mid-and high-dose females (5.1% each vs 0% controls), but the incidences were within historical control range (0-6.9%). There was also an increase incidence of combined hepatocellular adenomas and carcinomas in the high-dose females (6.8% treated vs 1.7% controls) that was at the upper end of the historical control range (0-6.9%); the difference ($p=0.018$) was not considered statistically significant by the sponsor, who cited $p<0.01$ as the currently accepted threshold for significance that is being used to prevent false positive results for common tumors in carcinogenicity screens. However, three arguments can

be made against disregarding this finding as a positive carcinogenic response. First, although the incidence of liver tumors in these mice is not rare, the historical control incidences are low, suggesting that liver tumors in these studies should not necessarily be viewed as high-frequency tumors, and therefore the argument for setting $p<0.01$ as the criteria for significance may not be valid. Second, as the incidence of liver tumors in $B6C3F_1$ male and female mice has been shown to be positively correlated with the 12 month body weight of the animals (Seilkop, S.K. Fundam Appl Toxicol, 24, 247-259 [1995]) and the female mice had diminished body weights at these times (↓8% at 52 weeks and ↓10% at 56 weeks), the incidence of tumors in the high-dose females may have been reduced by this diminished body weight. Thus, the incidence of tumors may have been masked by the protective effect of diminished body weight.

However, this argument that a beneficial effect from body weight decrement in females may not be valid, since the beneficial effect refers to food restriction. It is problematic to ascribe a beneficial effect from a body weight decrement in females due to toxicity. This beneficial effect is especially difficult to understand when most liver tumors in mice in carcinogenicity studies occur at toxic dose levels.

Finally, gross and microscopic findings such as increased liver nodules and increased liver hypertrophy were observed, demonstrating that the compound is affecting the morphology and growth of hepatocytes.

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 of slight toxic evidence observed in the males. The NOAEL for males is 0.03% (equivalent to 36.4 mg/kg/day).

Under the conditions of this study, the carcinogenic potential of benfluralin is equivocal.

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

C. Study deficiencies -None noted.

014304