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

SEP 21 1987

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

Subject: Benefin 1471-71 (Record Number 201457): Review of the Interim Report of Gross Pathology Findings in A Two-Year Oncogenic Mouse Study with Benefin Caswell Number 130

From: John H.S. Chen, D.V.M. John H S Chen 9/14/87
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C)

To: Robert J. Taylor, Product Manager (25)
Herbicide-Fungicide Branch
Registration Division (TS-767C)

Thru: Robert B. Jaeger, Section Head 9/16/87
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C) 9/14/87

Petitioner:

Eli Lilly and Company
Greenfield, Indiana 46140

Action Requested:

Review of the interim report of gross pathology findings in a two-year oncogenic mouse study with Benefin (EL-110, Compound 54521). Lilly Research Laboratories Studies MO2785, MO2885 and MO 2965. August 7, 1987.

Recommendation:

Toxicology Branch acknowledges receipt of information from Eli Lilly and Company pertaining to preliminary evidence of adverse effects in a two-year oncogenic mouse (B6C3F1) study with Benefin recently terminated. The final tabulated report of this study is scheduled to be submitted in January 1989. An increased incidence of gross liver nodules was reported for high dose (dietary concentration of 0.15%) females in this interim report. No classification of these noted liver nodules (i.e. malignant and/or benign) was provided by the registrant at this time. Toxicology Branch will await the complete report for a full evaluation. In the interim, any additional new use of Benefin should be carefully weighed against the potential adverse effect demonstrated in mice.

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Note, only Supplementary Information

83-1 & 83-2 - Rat - Chronic Toxicity and Oncogenicity

Reviewed by: John H.S. Chen — John H.S. Chen 11/11
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DATA EVALUATION REPORT

Study Type: 2-Year Feeding and Oncogenic Study in Rats — Tox.

MRID No.: 37675 — EPA

Test Material: Technical Benefin (Lot No. X-11424; 95.6% Pu

Synonyms/CAS No.: Benfluralin (1861-40-1)

Study Number(s): A-0295

Sponsor: Elanco Products Co., Indianapolis, Ind.

Testing Facility: Toxicology Division Lilly Research Labora

Title of Report: A Study of the Effects on Rats from Ingest Benefin for Two Years

Author(s): Glen C. Todd, D.V.M., Ph.D.

Report Issued: August 5, 1976

Conclusions:

Oncogenic NOEL - Not determined (poor survival in the 1% dose

Systemic NOEL - Not determined (Incomplete study)

Levels tested: 0.1, 0.3, and 1% of benefin in t

Classification of Data: Supplementary without the possibilit

Title of Study: A Study of the Effects on Rats from Ingestion of Benefin for two Years. Study No. R-0295

I. Materials and Methods:

1. Test Material: Technical benefin (Lot No. X-11424, 95.6% Purity)

2. Test Animals: The study consisted of 200 Harlan weanling rats. Number of animals in each dose group was given below:

Dose Group (%)	No. of Animals Male	Female
0	26	24
0.1 (1000 ppm)	25	25
0.5 (5000 ")	24	26
1.0 (10000 ppm)	26	24
Total	101	99

3. Preparation of Test Diets: Test diets were prepared monthly. The test material, benefin, was ground and rubbed in a mortar until it was finely devided. The required amounts of feed were added to this material for each dose level and mixed in a twin-shell blender.

4. Statistical Analysis: The significance of differences of data between control and treated groups were analyzed by the Dunnett's t test ($P<0.05$ or $P<0.01$).

II. Reported Results:

1. Clinical Observations

All rats were observed daily for mortality and signs of toxic effects throughout the pretreatment period, the treatment period and at necrop

Results: There was a significantly progressive increase in mortality rate (i.e., decrease in percentage of survival) in males and females receiving 1% benefin in the diet from 18 months to study termination (i.e., percentage of survival for males: 18 mo., 65%; 21 mo., 23%; 22 mo., 15%; 24 mo., 4%; Percentage of survival for females: 18 mo., 46%; 21 mo., 13%; 22 mo., 4%; 24 mo., 0%). No effect on survival of animals fed 0.1% and 0.5% dose levels was observed in this study. There were no other compound-related clinical observations reported (Table 1 attached).

2. Body Weights

Individual bodyweights were recorded weekly throughout the pre-treatment period, the treatment period and at necropsy.

Results: No data were given in this report.

3. Food Consumption

Food consumption was measured weekly throughout the study.

Results: No data were given in this report.

4. Ophthalmology

Ophthalmological examinations were not performed in this study.

5. Hematology

At 3, 6, and 24 months, blood was collected from 5 rats/dose/sex for hematological analyses. The following parameters (X) were examined:

*(X) Hematocrit (HCT)
*(X) Hemoglobin (HGB)
*(X) Erythrocyte count (RBC)
*(X) Leukocyte count (WBC)
*(X) Leukocyte differential count
(X) Red cell morphology
*( ) Platelet count
*(X) Prothrombin time

*Recommended by Subdivision F (October 1982) Guidelines

Results: The mean values of HGB for the 0.5% and 1% dose males and females at month 3 were significantly lower from that of the respective control groups ($P<0.01$). However, after 6 months on test, only males in the middle dose group (0.5%) had a reduction in HCT and HGB values ($P<0.05$), whereas females on the middle and high (1%) dose treatments had only HGB depression ($P<0.05$ and $P<0.01$). These results at the termination period were not used for statistical analysis because of single survivor found in males and no survivor in females in the high dose treatment groups. No changes in any of the other hematological parameters were observed in this study (Tables 2, 3, and 4 attached).

6. Clinical Chemistry

At the last month (24), blood was collected from 5 rats/dose/sex for biochemical analyses. The following parameters (X) were examined:

Electrolytes	Enzymes	Other
*( ) Calcium	*(X) Serum alanine aminotransferase	*(X) Glucose
*( ) Chloride	*( ) Serum aspartate aminotransferase	*(X) Blood urea nitrogen
*( ) Magnesium		*( ) Total protein
*( ) Phosphorus		*( ) Albumin
*( ) Potassium		*( ) Cholesterol
*( ) Sodium		*( ) Total bilirubin
		*( ) Creatinine

*Recommended by Subdivision F (October 1982) Guidelines

6. Clinical Chemistry - continued

Although the mean values of blood urea nitrogen and serum alanine aminotransferase for 1% dose group males were significantly different from that of the corresponding control male group ($P<0.05$), statistical analysis of these data from single survivor in this group is not considered appropriate and reliable. Complete results for the recommended parameters were not reported in this study.

7. Urinalysis

Results: No data were given in this report.

8. Sacrifice and Pathology: interim death 156; terminal kill 44

All animals that died and that were sacrificed on schedule were subject to gross examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

Respiratory System	Urogenital	Neurologic
*(X) Lung	*(XX) Kidney	( ) Brain
(X) Pleura	*(X) Urinary bladder	( ) Eyes
*( ) Trachea	(X) Seminal vesicle	(X) Pituitary
Digestive System	(XX) Prostate	*( ) Peripheral (sciatic) nerv
*(X) Stomach	(XX) Testes	**Other**
*(X) Colon	(XX) Uterus	( ) Bone (sternum)
*(X) Jejunum	(XX) Ovaries	*( ) Skeletal muscl
*(X) Ileum	**Cardiovasc./Hemat.**	(X) Skin
*(X) Colon	*(XX) Heart	(X) Ear
*(X) Salivary gland	*(X) Aorta	
*( ) Retum	*( ) Bone marrow	
(X) Intestine	*(X) Lymph nodes	
(X) Mesentery	*(X) Thymus	
*(XX) Liver	*(XX) Spleen	
*(X) Pancreas	**Glandular**	
*(X) Gall bladder	*(XX) Adrenals	
	*(XX) Thyroids	
	*(XX) Parathyroids	
	*(X) Mammary glands	

*Recommended by Subdivision F (October 1982) Guidelines.

(a) Organ Weights

Results: As shown in Table 5, the mean values of liver weights were significantly higher ($P<0.05$ or $P<0.01$) in the 0.5% dose males and females whem compared to that of the corresponding control males and females. The thyroid, uterus, and ovary weights of middle-dose (0.5%) females were also significantly increased ($P<0.05$ or $P<0.01$) at this time. Changes in organ weights found in those fed at the high-dose level (1%) are not considered appropriate and reliable because the results were based on data from single survivor.
(Table 5 attached)

(b) Gross Pathology

Results: The incidence of yellow discoloration of fat deposits was found in both middle and high -dose males and females (Table 8); this was reported by the study author to be the only compound-related effect. Other macroscopical changes observed sporadically in the lung, heart, liver, and kidney of dosed animals were of types commonly occuring spontaneously in laboratory-maintained rats at their old age.

(c) Microscopic Pathology:

Selected non-neoplastic and neoplastic findings in rats fed benefin for 24 months are summarized in the following tables:

(1) Selected Non-Neoplastic Findings:

	Dietary Level % Male				Dietary Level % Female			
	0	0.1	0.5	1	0	0.1	0.5	1
No. Started	26	25	24	26	24	25	26	24
No. Unsuitable for evaluation	3	4	6	5	5	3	6	10
No. used for Evaluation	23	21	18	21	19	22	20	14
Organ/Findings								
Ear								
Inner middle ear infection	0	1	0	0	0	1	2	0
Lung								
Bronchitis, purulent bronchiectasis, bronchiolitis, abscesses	9	4	3	7	0	8	9	4
Pneumonia, bronchopneumonia	1	8	5	0	4	1	1	2

(i) Selected Non-Neoplastic Findings: continued

Organ/Findings:	Dietary Level % Male 0	0.1	0.5	1	Dietary Level % Female 0	0.1	0.5	1
Heart								
Pericarditis, abscesses	3	5	1	0	0	1	1	2
Liver								
Slight fatty metamorphosis of liver	2	5	5	1	5	5	5	2
Kidney								
Slight progressive glomerulonephritis (PGN)	1	4	6	10	5	5	6	0
Moderate PGN	4	3	2	4	1	0	0	0
Severe PGN	4	6	2	2	1	1	0	0
Total	9	13	10	16	7	6	6	0
Slight fatty degeneration of kidney	1	5	5	0	2	4	4	5

Results: Major non-neoplastic changes were found in the ear, lung, heart, liver, and kidney of the animal groups fed benefin in this study. The changes that were observed in ear, lung, and heart of both control and treated rats were primarily caused microbial infection. There were no non-neoplastic findings in tissues of rats which were considered to be treatment-related responses during the two years of study.

(ii) Selected Neoplastic Findings:

		Dietary Level % Male 0	0.1	0.5	1	Dietary Level % Female 0	0.1	0.5	1
No. Started		26	25	24	25	24	25	26	24
No. Unsuitable for evaluation		3	4	6	5	5	3	6	10
(A) No. Intrim death		17	17	12	20	11	13	12	14
(B) No. Survivor for 2 years		6	4	6	1	8	9	8	0
Organ/Findings:									
Mammary Gland									
Fibroadenoma	(A)	0	0	1	0	0	1	0	1
	(B)	0	0	1	0	3	2	2	1
	Total	0	0	2	0	3	3	2	2
	%	0	0	11.1	0	15.8	13.6	10	7.1
Adenocarcinoma	(A)	0	0	0	0	0	1	0	0
	(B)	0	0	0	0	0	1	0	0
	Total	0	0	0	0	0	2	0	0
	%	0	0	0	0	0	9.1	0	0

(ii) Selected Neoplastic Findings: continued

Organ/Findings:		Dietary Level % Male 0	0.1	0.5	1	Dietary Level % Female 0	0.1	0.5	1
Thyroid									
Light cell carcinoma	(A)	0	0	0	0	0	1	0	0
	(B)	1	0	0	0	3	1	1	0
Total		1	0	0	0	3	2	1	0
%		4.3	0	0	0	15.8	9.1	5	0
Papillary adenoma and carcinoma	(A)	0	0	0	1	0	0	0	0
	(B)	0	0	1	0	0	0	0	0
Total		0	0	1	1	0	0	0	0
%		0	0	5.6	4.8	0	0	0	0
Pituitary									
Chromophobe adenoma and pituitary adenoma	(A)	1	0	0	0	0	0	0	0
	(B)	0	0	1	0	0	3	3	0
Total		1	0	1	0	0	3	3	0
%		4.3	0	5.6	0	0	13.6	15	0
Hematopoietic									
Lymphosarcoma and reticulum cell sarcoma	(A)	1	1	0	2	0	1	1	1
	(B)	0	0	0	0	1	0	2	0
Total		1	1	0	2	1	1	3	1
%		4.3	4.8	0	9.5	5.3	4.5	15	7.1

Results: Major neoplastic changes were found in the mammary gland, thyroid, pituitary, and hematopoietic system of the animal groups fed benefin during the study. However, there were no neoplastic findings in tissues of rats which were considered to be treatment-related responses during the two years of study.

III. Study Author's Conclusion:

" Associated with old age of the rats were many chronic inflammatory and degenerative tissue changes, and some hyperplastic and neoplastic lesions. Theses changes were present in varied organs of both control and treated rats. The most common cause of death was probably related to the frequent occurance of chronic lung and kidney diseases. Rats fed for 2 years on mash diet containing 0.1% benefin (1000 ppm) were similar to the untreated control animals. The no effect level was 0.1%. "

IV. Reviewer's Assessment of Results:

1. Test Material Analysis: Chemical analysis for the assayed concentrations of benefin in the diet was not given. The findings for the homogeneity and stability of test diets were not provided.

2. The study design was incomplete and the conduct and reporting of specific areas were deficient as follows:

   i. Inadequate numbers of animals of each sex/dose used. fifty animals of each sex per dose group are required.

   ii. Statistical analysis of data based on the Dunnett's "t" test only is not considered adequate.

   iii. The study records of examination for the clinical signs of toxicity for individual animals were not provided. No data in body weight, food consumption, ophthalmology, and urinalysis were given for the test animals in this study.

   iv. Inadequate numbers of animals used for hematology and clinical chemistry studies. The following parameters were not examined: platelet count, chloride, magnesium, phosphorous, potassium, sodium, serum aspartate aminotransferase, protein, albumin, cholesterol, bilirubin abd creatinine.

   v. Inadequate data for histopathologic findings presented. The following parameters were not examined: trachea, rectum, bone marrow, brain, eye, peripheral nerve, bone, skeletal muscle.

3. Classification of Data: Supplementary without the possibility of upgrading

5031-12-020

TABLE 4

Percentages of Survival of Rats Fed Benefin
in the Diet for 2 Years

Study R-0895

Time	Sex	Dietary Concentration 0	0.1%	0.3%	1.0%
Start	M	100	100	100	100
12 mo	M	100	92	75	96
18 mo	M	73	68	54	69
21 mo	M	38	52	33	23
22 mo	M	31	32	29	15
24 mo	M	23	16	25	4
Start	F	100	100	100	100
12 mo	F	96	100	88	83
18 mo	F	83	88	69	46
21 mo	F	58	48	42	13
22 mo	F	50	44	35	4
24 mo	F	33	36	31	0

(10)

TABLE 5

Rat Organ Weights Benefin Two Years Feeding Study R-0295

Percent in Diet	Body wt. g	Mean Organ Weights per 100 Grams Body Weight and Standard Err: Liver g	Kidney g	Heart g	Spleen g	Thyroid mg	Adrenal mg	Prostate g	Te:
Males									
0.0	418.3 ±44.2	2.945 0.164	0.749 0.071	0.339 0.021	0.183 0.028	6.94 1.01	13.60 2.05	0.177 0.017	0. 0.
0.1	458.0* ±37.3	3.387 0.140	0.922 0.063	0.418 0.038	0.204 0.016	9.99 1.30	19.15 2.63	0.174 0.043	0. 0.
0.5	543.0 ±32.0	3.756** 0.206	0.770 0.034	0.315 0.019	0.162 0.013	7.86 1.77	17.79 3.33	0.181 0.013	0. 0.
1.0	488.0a/ ±0.0	3.445 0.0	0.839 0.0	0.337 0.0	0.195 0.0	13.52 0.0	18.65 0.0	0.113 0.0	0. 0.
Females									
								Uterus g	Ova mg
0.0	327.9 ±44.0	3.347 0.247	0.750 0.078	0.431 0.055	0.465 0.262	8.35 0.70	30.00 5.38	0.208 0.022	29. 2.
0.1	358.3 ±25.5	3.650 0.346	0.823 0.075	0.404 0.039	0.288 0.116	9.74 0.71	37.88 10.50	0.229 0.027	24. 2.
0.5	279.9** ±19.3	4.426* 0.264	0.927 0.054	0.429 0.028	0.208 0.020	14.58** 1.73	28.27 2.60	0.309* 0.025	40. 5.
1.0	No survivors								

a/ Since these values were from a lone survivor, statistical analyses were not deemed appropriat
The differences from the control appear to be significant.

* Statistically different from the control at the .05 level, using a Dunnett t.

** Statistically different from the control at the .01 level, using a Dunnett t.

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